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Medical Genetics Summaries

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Medical Genetics Summaries (MGS) is an international point-of-care resource for clinicians. It describes the impact that specific DNA sequence variations have on health. The chapters in MGS form a growing collection of peer-reviewed articles that synthesize pharmacogenomic evidence. These articles provide practical information about genetic testing to guide drug therapy and include therapeutic recommendations based on genotype from medical and professional societies. Each chapter is standardized and includes a summary; a description of drug use, metabolism, and action; the enzymatic activity status of significant alleles; the assignment of likely metabolic phenotype based on genotype; and the translation of allele terms used in published literature to terms used in the laboratory, including HGVS expression, star allele, and dbSNP number. Additional MGS articles review genetic variants that underlie inherited conditions or affect the risk of developing a disease in the future. The primary focus of MGS is on genetic variations that influence how an individual may respond to a specific drug.

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Introduction

Laura Dean, MD[1] and Megan Kane, PhD[2]

Created: September 15, 2016; Updated: June 28, 2024.

Medical Genetics Summaries (MGS) is a collection of articles featuring phenotypes with a genetic component, for which information useful at the point of care is limited. The main focus of *MGS* is pharmacogenomics, but there are also chapters about diseases.

The intended audience of *MGS* is clinicians who seek practical, evidence-based information to use in clinical care settings. The summaries are guideline-driven, drawn from authoritative sources, undergo a formal review process, and are regularly updated.

Genetic Variants and Drug Responses

There can be variability in how different individuals respond to standard doses of the same drug. This is because a drug response can be influenced by age, gender, drug-drug interactions, drug-food interactions, comorbidity, liver and renal function, pregnancy, and genetic factors. For an increasing number of drugs, genetic testing (also known as pharmacogenetic testing) can be used to optimize drug therapy.

Currently, about 10% of drug labels approved by the U.S. Food and Drug Administration (FDA) contain pharmacogenetic information. However, actionable information on genetic variants can be hard to find, and sources often differ in their recommendations. To provide actionable information at the point of care, *MGS* draws together information from different authoritative sources to one place and includes a summary.

Chapters in *MGS* use generic drug names. Nomenclature tables include both the official and commonly used terms for alleles with links to molecular resources, including ClinVar and dbSNP. Phenotypes that encompass how an individual responds to a drug are termed "drug responses", such as omeprazole drug response. Finally, each summary links to the NIH Genetic Testing Registry®, which provides information about laboratories that offer genetic tests and details about the tests, including ordering information.

Genetic Testing to Ensure the Drug Has a Therapeutic Target

A small number of drugs are prescribed after genetic testing has been performed. One reason for this is that the drug is effective only for specific genotypes. These drugs include trastuzumab, a chemotherapy agent only indicated for specific tumors that overexpress HER2, and maraviroc, an antiviral agent indicated only for a specific strain of the HIV virus (CCR-5 trophic HIV-1). Additionally, specific variants or conditions that indicate a lack of efficacy are discussed, such as dabrafenib and *BRAF* variant colorectal cancer or *BRAF* wild-type malignancies.

Genetic Testing to Avoid Idiosyncratic Drug Reactions

Another reason for genetic testing is to avoid severe and potentially fatal drug reactions. Some drug reactions are idiosyncratic—they are unpredictable, severe, and not related to the dose or duration of the drug therapy.

The FDA recommends that all individuals be screened for the *HLA-B*57:01* allele before starting treatment with abacavir, a drug used in the treatment of HIV. Around 6% of Caucasians of European origin carry this variant allele, placing them at high risk of abacavir-induced hypersensitivity reaction, with symptoms including fever,

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rash, and acute respiratory symptoms. Similarly, individuals with dihydropyrimidine dehydrogenase deficiency are at risk of fatal reactions with fluoropyrimidines like capecitabine and fluorouracil.

An Individual's Ancestry May Be Important

For the epilepsy drug carbamazepine, the FDA states that patients with ancestry in "genetically at-risk populations" should be screened for the presence of *HLA-B*15:02* prior to initiating treatment. Carriers of this variant, most commonly found in individuals of Han Chinese descent, are at a high risk of developing Stevens-Johnson syndrome and toxic epidermal necrolysis—both potentially fatal conditions—during carbamazepine therapy.

The *HLA-B*58:01* allele, also common in individuals with Han Chinese ancestry, is strongly associated with severe cutaneous adverse reactions triggered by allopurinol therapy, which is used to treat gout.

A Wide Range of Gene Variants Are Associated with Idiosyncratic Drug Reactions

Idiosyncratic drug reactions are not limited to variant *HLA-B* alleles. For the antibiotic gentamicin, genetically predisposed individuals carrying a variant in a mitochondrial gene (*MT-RNR1*) may suffer irreversible hearing loss after just a single dose of gentamicin. For individuals requiring treatment with thiopurines (for example, azathioprine), the FDA recommends thiopurine S-methyltransferase (*TPMT)* genotyping or phenotyping prior to treatment because patients with 2 non-functional *TPMT* alleles experience life-threatening myelosuppression when treated with thiopurines.

Genetic Testing to Optimize Drug Dose

Drug labels always provide standard dosing information, but a growing number of labels also include recommendations for adjusting the dose or selecting an alternative drug based on a patient's genotype (if known). Generally, dose adjustment is recommended for variants in genes known to influence drug metabolism, leading to altered plasma levels of active drugs and metabolites. Decreased activity of the liver enzyme SLCO1B1 (a drug transporter) can lead to increased exposure and adverse muscle symptoms with statins like simvastatin, prompting experts to recommend lower doses or alternative medications.

Cytochrome P450 (CYP) Genes Influence Drug Levels

The "CYP" gene family encodes enzymes that metabolize over a quarter of commonly prescribed drugs. One of these genes, *CYP2D6*, is particularly complex, with over 100 known variants, many encoding enzymes with different activity levels. Depending on the level of CYP2D6 activity, individuals may respond poorly to analgesics like codeine and tramadol. A standard dose of codeine may provide inadequate pain relief in some, and severe toxicity, such as respiratory depression, in others.

Additionally, standard doses of a wide range of drugs (for example, atomoxetine for Attention-Deficit/Hyperactivity Disorder, venlafaxine as an antidepressant, clozapine as an antipsychotic, and tamoxifen for breast cancer) can lead to higher than expected active drug plasma levels in individuals with low or absent CYP2D6 activity, increase the risk of side effects and potentially lead to non-compliance and treatment failure.

Barriers to Genetic Testing

At this time, there is a lack of recommendations from authoritative professional societies for indication for pharmacogenetic testing. The field is rapidly evolving, as evidenced by an increasing number of available pharmacogenetic tests. There are potential legal concerns, such as liability in cases where the optimal dose of a drug was not given. Education and training are needed.

More prospective randomized trials are needed to investigate the clinical outcomes when drug therapy or specific doses are selected based on genotype. Effectiveness data can be used for cost-effectiveness analysis and

summarized into actionable clinical guidelines with prescribing recommendations. Similarly, the breadth of evidence for various ancestral groups may be limited, and guidelines may not exist for variants that are rare.

Sometimes, genetic testing is not possible due to the acute nature of the clinical scenario (such as gentamicin and neonatal sepsis). However, as technology improves and turnaround time is reduced, the use of genetic testing is expected to increase.

For example, clopidogrel, an antiplatelet agent used in patients with acute coronary syndrome who may undergo percutaneous intervention, must be metabolized by CYP2C19 before becoming effective. In 3% of Caucasians and 15–20% of Asians with low or absent CYP2C19 activity, clopidogrel will have a smaller or no effect on platelet function. The advent of "bedside testing" and faster turnaround times means that more patients can be identified and offered alternative antiplatelet agents.

The Use of Genetic Testing Is Often Not Clear-Cut

In the case of warfarin, the FDA-approved drug label provides a dosing table for adjusting initial doses based on *CYP2C9* and *VKORC1* genotypes. Warfarin is an anticoagulant given to prevent the formation of blood clots. If the dose is too low, the risk of thrombosis remains; if too high, there is an increased risk of bleeding, both of which can cause strokes.

Despite the drug label's dosing table, it is thought that less than 1% of patients commence warfarin therapy with their *CYP2C9* and *VKORC1* genotypes known. Recent evidence suggests that *CYP2C9* and *VKORC1* variants may have less effect on warfarin levels than previously thought, with many other clinical factors having a more significant impact.

The Future

Genetic testing is important—it can help avoid drug toxicity and optimize drug efficacy. As the number of genetic tests grows, *Medical Genetics Summaries* will expand to help ensure that healthcare providers have the information they need to provide evidence-based care.

Genetic Variants and Disease

Pitt-Hopkins syndrome has a clear genetic component. A variant in the TCF4 gene results in the syndrome, and genetic testing of the *TCF4* gene confirms the diagnosis. For many other diseases, the underlying genetics is complex. For example, although schizophrenia is highly heritable, many genes contribute to the disease, and genetic testing is not currently available.

A person's blood group is determined by genetics—the four common blood groups (A, B, AB, and O) are encoded by *ABO* alleles. Serological testing is commonly used to determine an individual's blood type before receiving a blood transfusion. However, in other settings, genetic testing may determine an individual's ABO genotype, such as in research investigating associations between ABO blood groups and the risk of diseases like pancreatic cancer and thromboembolic disease.

These chapters focusing on genetic diseases are maintained for legacy use and reference but are no longer the primary focus of MGS. Readers are encouraged to check other sources for information on specific genetic disorders including MedGen which provides links to search PubMed for recent review articles, professional practice guidelines, and links to the Bookshelf at NCBI for additional resources.

Genetic variants and drug responses

Abacavir Therapy and *HLA-B*57:01* Genotype

Laura Dean, MD[1]

Created: September 1, 2015; Updated: April 18, 2018.

Introduction

Abacavir (brand name Ziagen) is used in the treatment of human immunodeficiency virus (HIV) infection. Abacavir is a nucleoside (and nucleotide) reverse transcriptase inhibitor (NRTI), and is used in combination with other medications as part of highly active antiretroviral therapy (HAART) (1).

Hypersensitivity reactions associated with abacavir can be severe and potentially fatal. Symptoms include fever, rash, vomiting, and shortness of breath. They typically appear within the first 42 days of treatment (11 days median onset).

*HLA-B*57:01* significantly increases the risk of hypersensitivity reactions when abacavir is administered. Approximately 6% of Caucasians and 2-3% of African Americans carry this allele in the human leukocyte antigen B (*HLA-B*) gene. The *HLA-B* gene plays an important role in how the immune system recognizes and responds to pathogens, and mediates hypersensitivity reactions. *HLA-B*57:01* has been found to be associated with abacavir hypersensitivity across different ethnicities, including Caucasians, Hispanics, and individuals of African origin (2, 3).

Screening for the *HLA-B*57:01* allele before starting abacavir therapy is recommended for all patients according to the FDA drug label for abacavir (Table 1). Even if previously tolerated, screening should happen before restarting abacavir therapy if *HLA-B*57:01* status is unknown. Abacavir is contraindicated in *HLA-B*5701*-positive patients, and in patients with a prior hypersensitivity reaction to abacavir. Dosing guidelines from the professional societies, Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), also recommend that *HLA-B*57:01* screening should be performed prior to initiation of abacavir therapy and an alternate drug be administered for patients with the allele (Table 2, Table 3)(1, 3-5).

Table 1. FDA (2017) Drug Label for Abacavir. Therapeutic Recommendations based on *HLA-B*57:01* Genotype. Warnings and Precautions.

Genotype	Hypersensitivity reactions
*HLA-B*5701*-positive patients	Due to the potential for severe, serious, and possibly fatal hypersensitivity reactions with abacavir sulfate: All patients should be screened for the *HLA-B*5701* allele prior to initiating therapy with abacavir tablets or reinitiation of therapy with abacavir tablets, unless patients have a previously documented *HLA-B*5701* allele assessment. Abacavir tablets are contraindicated in patients with a prior hypersensitivity reaction to abacavir and in *HLA-B*5701*-positive patients.

Please see 2017 Statement from the US Food and Drug Administration (FDA) for more information from the FDA. Table adapted from (1).

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Table 2. CPIC (2014) Recommended Therapeutic Use of Abacavir in relation to *HLA-B* Genotype[a]

Genotype	Implications for phenotypic measures	Recommendations for abacavir	Classification of recommendations[b]
"Negative" Noncarrier of *HLA-B*57:01*	Low or reduced risk of abacavir hypersensitivity	Use abacavir per standard dosing guidelines	Strong
"Positive" Carrier of *HLA-B*57:01*	Significantly increased risk of abacavir hypersensitivity	Abacavir is not recommended	Strong

HLA-B, *human leukocyte antigen B.*
[a] The 2014 update states that the recommendations shown in the table from the 2012 guideline remain the same. This table has been adapted from the 2012 guideline (3, 4).
[b] Rating scheme described in supplementary data online (3, 4).
Please see 2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC) for more information from CPIC. Table adapted from (3).

Table 3. DPWG (2017) Recommendations for Abacavir based on *HLA-B* Genotype

Genotype	Recommendation
*HLA-B*57:01*-positive	Abacavir is contraindicated for *HLA-B*57:01*-positive patients. Advise the prescriber to prescribe an alternative according to the current guidelines.

Please see 2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) for more information from DPWG. Table adapted from (5).

Drug: Abacavir

Abacavir is an antiretroviral drug that belongs to the drug class of nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs). The NRTIs, also known as nucleoside (or nucleotide) analogs, were the first type of drug available to treat HIV infection, and they remain effective today. In addition to abacavir, NRTIs include drugs such as AZT/zidovudine, emtricitabine, tenofovir, and lamivudine. Abacavir is always used in combination with other drugs.

Antiretroviral drugs, like abacavir, inhibit the activity of retroviruses, such as HIV. To replicate, retroviruses must convert their RNA genome into a DNA copy, which can then be inserted into the host cell's genome. Abacavir inhibits the conversion of viral RNA to DNA, preventing viral replication.

Abacavir is a pro-drug and its antiviral activity is facilitated by the drug's phosphorylation by intracellular enzymes to form carbovir triphosphate, a nucleoside analog. Carbovir triphosphate competes with the natural substrate of the HIV reverse transcriptase enzyme, to be incorporated into viral DNA. Once incorporated, the nucleoside analog terminates DNA chain elongation, preventing further synthesis of viral DNA (6).

Abacavir started to be used in the late 1990s, as part of a combination of therapies to treat HIV. However, the use of abacavir in the US was limited by a severe hypersensitivity reaction that occurred in approximately 5-8% of patients. Symptoms occurred during the first 6 weeks and included a constellation of symptoms presenting as rash, fever, fatigue, gastrointestinal symptoms (e.g., nausea, vomiting, abdominal pain), and acute respiratory symptoms (e.g., cough and dyspnea) (7). Life-threatening skin diseases, Stevens-Johnson syndrome and toxic-epidermal necrolysis, can occur in severe reactions.

Data from the PREDICT-1 study suggest that 100% of individuals with immunologically confirmed (abacavir patch test positive) abacavir hypersensitivity present within 3 weeks of initial dosing. The median onset of symptoms is 9-11 days (1, 7, 8).

Abacavir can trigger a hypersensitivity reaction in people who have the *HLA-B*57:01* allele. The frequency of the *HLA-B*57:01* allele varies by population; for example, approximately 6% of Caucasians, and 2-3% of African-American and admixed American populations carry at least one copy of this high-risk *HLA-B* allele (Table 4). *HLA-B*57:01*-postitive individuals have an increased risk of a hypersensitivity reaction to abacavir compared to *HLA-B*57:01*-negative individuals (8).

Table 4. CPIC (2014) Assignment of likely *HLA-B* Phenotypes based on Genotype

Likely phenotype	Genotype	Examples of diplotype
Very low risk of hypersensitivity (constitutes ~94%[a] of patients)	Absence of **57:01* alleles (reported as "negative" on a genotyping test)	*X/*X[b]
High risk of hypersensitivity (~6% of patients)	Presence of at least one **57:01* allele (reported as "positive" on a genotyping test)	*57:01/*X[b] *57:01/*57:01

HLA-B, human leukocyte antigen B.
[a] See supplementary data online for estimates of genotype frequencies among different ethnic/geographic groups.
[b] *X = any *HLA-B* genotype other than *57:01.
Table adapted from (3).

The FDA-approved label for abacavir states that all patients should be screened for the *HLA-B*57:01* allele prior to initiating therapy with abacavir, or when reinitiating therapy with abacavir, unless patients have a previously documented *HLA-B*57:01* allele assessment. The FDA also warns that abacavir must be discontinued immediately if a hypersensitivity reaction is suspected, regardless of *HLA-B*57:01* status and even when other diagnoses are possible (1).

Several studies have shown that routine genetic screening for *HLA-B*57:01* significantly reduces the incidence of abacavir-induced hypersensitivity, and is cost-effective. Because it is rare for individuals who do not carry the high-risk *HLA* variant to develop hypersensitivity, adhering to the screening guidelines can reduce the incidence of immunologically confirmed cases of abacavir hypersensitivity to nearly zero (9-12).

HLA Gene Family

The *HLA* genes are members of the major histocompatibility complex (*MHC)* gene family, which includes more than 200 genes. The *MHC* family has been subdivided into 3 subgroups based on the structure and function of the encoded proteins: class I, class II, and class III. The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in antigen presentation. The HLA region also contains a variety of other genes, including genes involved in immunity and genes not known to be involved in immune function.

An important role of HLA class I molecules is to present peptides (processed fragments of antigens) to immune cells ($CD8^+$ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented, e.g., from a pathogen, $CD8^+$T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the *HLA* genes are both numerous and highly polymorphic. More than 4,700 *HLA-B* alleles have been identified (6, 13).

HLA Allele Nomenclature

HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a four (or six) digit number that corresponds to the assigned allele number (14). For example, the *HLA-B*15:02:* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular HLA gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have been added to the nomenclature to discriminate between alleles that do not differ in the protein amino acid sequence but differ in their genetic sequence (i.e., due to synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in susceptibility to autoimmune disease and infections. These variations are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

HLA variants have also been associated with susceptibility to Type B adverse drug reactions. For example, as noted above, an *HLA-B* variant has been associated with severe hypersensitivity reactions to abacavir. Other *HLA-B* variants have been associated with severe reactions to allopurinol (used to treat gout), and carbamazepine and phenytoin (used to treat epilepsy).

Gene: *HLA-B*

The *HLA-B*57:01* allele is associated with an increased risk of hypersensitivity reaction to abacavir. Studies across ethnicities have reported that in immunologically confirmed cases of abacavir hypersensitivity, 100% of cases occurred in patients who were carriers of this HLA variant (7).

Other immune factors are also involved, however. For example, not everyone who carries the high-risk *HLA* allele will develop abacavir hypersensitivity - approximately 39% of individuals who are positive for *HLA-B*57:01* will tolerate abacavir treatment (8).

Cytotoxic (CD8+) T cells mediate the hypersensitivity reaction to abacavir. Abacavir is thought to form a non-covalent complex with *HLA-B*57:01* (15-18). Several theories have been proposed for how this drug peptide-HLA complex activates the T cell receptor, which then releases inflammatory cytokines, signaling the start of the hypersensitivity response (19-23). More than one immune mechanism may be involved (7). It has been shown that abacavir occupies a space below the region of HLA that presents peptides. This leads to altered peptide presentation (including the presentation of self-peptides to which the host has not been tolerized) and triggers an autoimmune-like reaction (19, 24).

The hypersensitivity reaction to abacavir is thought to be maintained over the lifetime of an individual. The reintroduction of abacavir to a sensitized individual may be fatal, presumably due to a rapid activation of a memory T cell population. Therefore, abacavir is contraindicated in individuals with a prior hypersensitivity reaction to abacavir (1, 25).

*HLA-B*57:01* also has an important role in HIV infection. In Caucasians with HIV, *HLA-B*57:01* has been linked to a lower viral load set point (the amount of viral RNA detected in blood during the asymptomatic phase of HIV infection) (26). In addition, *HLA-B*57:01* is overrepresented in a small group of individuals who have HIV which has not progressed to AIDs, despite lack of treatment with antiretroviral therapy. These individuals are known as “long-term non-progressors” (27).

The frequency of the *HLA-B*57:01* allele varies significantly by population. The allele is most common in Northern Thai and Indian populations (up to 20%). It is relatively common in European populations (6–7%), and is present but less common in African Americans, admixed American populations, and Middle Eastern populations (2-3%). *HLA-B*57:01* is uncommon in homogenous South-Asian and African populations, being mostly absent in the Japanese, and some African populations (2, 3, 28).

Genetic Testing

Pharmacogenetic testing is now routine in HIV clinical practice (28).The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for abacavir hypersensitivity, and the *HLA-B* gene.

The genotype results for an *HLA* allele such as *HLA-B*57:01* can either be "positive" or "negative". There are no intermediate phenotypes because the *HLA* genes are expressed in a codominant manner.

Abacavir is contradicted in patients with a "positive" result, and only one copy of the **57:01* allele is required for a positive result. Therefore, the positive result is either "heterozygous" or "homozygous", depending upon whether the patient is carrying one or 2 copies of the **57:01* allele, respectively.

A negative result indicates that the patient does not carry the *HLA-B*57:01* allele. However, a negative result does not rule out the possibility of a patient developing abacavir hypersensitivity. Therefore, clinicians should carefully monitor all patients according to standard practices (3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Serious and sometimes fatal hypersensitivity reactions have occurred with abacavir sulfate. These hypersensitivity reactions have included multi-organ failure and anaphylaxis and typically occurred within the first 6 weeks of treatment with abacavir sulfate (median time to onset was 9 days); although abacavir hypersensitivity reactions have occurred any time during treatment. Patients who carry the *HLA-B*57:01* allele are at a higher risk of abacavir hypersensitivity reactions; although, patients who do not carry the *HLA-B*57:01* allele have developed hypersensitivity reactions. Hypersensitivity to abacavir was reported in approximately 206 (8%) of 2,670 patients in 9 clinical trials with abacavir-containing products where *HLA-B*57:01* screening was not performed. The incidence of suspected abacavir hypersensitivity reactions in clinical trials was 1% when subjects carrying the *HLA-B*57:01* allele were excluded. In any patient treated with abacavir, the clinical diagnosis of hypersensitivity reaction must remain the basis of clinical decision making.

Due to the potential for severe, serious, and possibly fatal hypersensitivity reactions with abacavir sulfate:

- All patients should be screened for the *HLA-B*57:01* allele prior to initiating therapy with abacavir tablets or reinitiation of therapy with abacavir tablets, unless patients have a previously documented *HLA-B*57:01* allele assessment.
- Abacavir tablet is contraindicated in patients with a prior hypersensitivity reaction to abacavir and in *HLA-B*57:01* -positive patients.
- Before starting abacavir tablets, review medical history for prior exposure to any abacavir-containing product. NEVER restart abacavir tablets or any other abacavir-containing product following a hypersensitivity reaction to abacavir, regardless of *HLA-B*57:01* status.
- To reduce the risk of a life-threatening hypersensitivity reaction, regardless of *HLA-B*57:01* status, discontinue abacavir tablets immediately if a hypersensitivity reaction is suspected, even when other

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

diagnoses are possible (e.g., acute onset respiratory diseases such as pneumonia, bronchitis, pharyngitis, or influenza; gastroenteritis; or reactions to other medications).
- If a hypersensitivity reaction cannot be ruled out, do not restart abacavir tablets or any other abacavir-containing products because more severe symptoms which may include life-threatening hypotension and death, can occur within hours.
- If a hypersensitivity reaction is ruled out, patients may restart abacavir tablets. Rarely, patients who have stopped abacavir for reasons other than symptoms of hypersensitivity have also experienced life-threatening reactions within hours of reinitiating abacavir therapy. Therefore, reintroduction of abacavir tablets or any other abacavir-containing product is recommended only if medical care can be readily accessed.
- A Medication Guide and Warning Card that provide information about recognition of hypersensitivity reactions should be dispensed with each new prescription and refill.

Please review the complete therapeutic recommendations that are located here: (1).

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

We agree with others that *HLA-B*57:01* screening should be performed in all abacavir-naive individuals before initiation of abacavir-containing therapy (see Table 2); this is consistent with the recommendations of the FDA, the US Department of Health and Human Services, and the European Medicines Agency. In abacavir-naive individuals who are *HLA-B*57:01*-positive, abacavir is not recommended and should be considered only under exceptional circumstances when the potential benefit, based on resistance patterns and treatment history, outweighs the risk. *HLA-B*57:01* genotyping is widely available in the developed world and is considered the standard of care prior to initiating abacavir. Where *HLA-B*57:01* genotyping is not clinically available (such as in resource-limited settings), some have advocated initiating abacavir, provided there is appropriate clinical monitoring and patient counseling about the signs and symptoms of HSR [hypersensitivity reaction], although this remains at the clinician's discretion.

Please review the complete therapeutic recommendations that are located here (3, 4).

2017 Summary of Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

*HLA-B*57:01*-positive patients have a strongly increased risk of a hypersensitivity reaction to abacavir.

Recommendation:

Abacavir is contraindicated for *HLA-B*57:01*-positive patients.

1 Advise the prescriber to prescribe an alternative according to the current guidelines.

Background information

Mechanism:

Although the mechanism of hypersensitivity reactions to abacavir is not fully known, experimental data suggest the following mechanism.

Abacavir metabolites (aldehydes and acids) form a covalent bond with cellular proteins. Peptides derived from these modified proteins bind to *HLA-B*5701* and are recognised on the cell surface as foreign by the immune cells, which triggers an immune response against cells containing abacavir. For more information about the

*HLA-B*57:01* genotype: see the general background information about HLA on the KNMP Knowledge Bank or on http://www.knmp.nl/ (search for HLA).

Other considerations:

If tests are performed for *HLA-B57* instead of *HLA-B*57:01*, some patients will incorrectly be denied treatment with abacavir. This is primarily the case in patients of African descent, where *HLA-B*57:03* is the most common *HLA-B57* sub-type and to a lesser extent for Caucasian patients, where *HLA-B*57:01* is the most common *HLA-B57* sub-type. If there are enough alternatives, it is not a problem that the patient is being denied abacavir incorrectly.

Clinical consequences:

*HLA-B*5701*-positive patients have a strongly increased risk of a hypersensitivity reaction to abacavir (OR [odds ratio] 7 to 960 for clinically diagnosed hypersensitivity reactions and 900 to 1945 for immunologically confirmed hypersensitivity reactions).

Exclusion of *HLA-B*5701*-positive patients from abacavir therapy reduced the number of clinically diagnosed hypersensitivity reactions in predominantly white populations by 56-96% and the number of immunologically confirmed hypersensitivity reactions by 100%.

Hypersensitivity reactions to abacavir generally disappear spontaneously after stopping abacavir, but can be fatal in severe cases.

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature

Nomenclature of Selected *HLA-B* alleles

Allele name	dbSNP reference identifier for allele location
*HLA-B*57:01*	rs2395029 is a tag SNP for *HLA-B*57:01*

For the MHC region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*57:01* allele is defined by its sequence (GenBank: AF196183.1) rather than single coding or protein variants. If there is strong linkage disequilibrium between one or more SNPs, the presence of these SNPs (tag SNPs) may be used for *HLA* typing (29). In the case of *HLA-B*, the presence of the rs2395029 allele (a SNP in the HLA complex P5 gene) is 99.9% predictive of the presence of an *HLA-B*57:01* allele (30).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (4).
Guidelines on the naming of *HLA* genes are available from *HLA* Nomenclature.

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Version History

To view the 2015 version of this summary (Created: September 1, 2015) please click here.

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Allopurinol Therapy and *HLA-B*58:01* Genotype

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Introduction

Allopurinol (brand names Zyloprim, Aloprim) is a xanthine oxidase inhibitor that decreases the production of uric acid. It is most commonly used to manage gout, tumor lysis syndrome, and symptomatic hyperuricemia (high levels of uric acid). It is not indicated for use in asymptomatic hyperuricemia (1).

The human leukocyte antigen B (*HLA-B*) plays an important role in how the immune system recognizes and responds to pathogens. The variant *HLA-B*58:01* allele is strongly associated with severe cutaneous adverse reactions (SCAR) during treatment with allopurinol. This allele is most common among Asian subpopulations, notably in individuals of Korean, Han-Chinese, or Thai descent.

At this time, the FDA-approved drug label for allopurinol does not discuss *HLA-B* genotype (Table 1) (1). However, the American College of Rheumatology (ACR) conditionally recommends testing *HLA–B*5801* before starting allopurinol for individuals of Southeast-Asian descent (for example, Han-Chinese, Korean, Thai) and African-Americans (Table 2) (2). For individuals who are positive for the *HLA-B*58:01* variant, an alternative drug is recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) (Table 3 and 4). While CPIC states allopurinol is contraindicated in carriers of *HLA-B*58:01*, both DPWG and ACR state that a possible option is allopurinol desensitization (3, 4, 5).

Table 1. The FDA Allopurinol Dosage and Administration (2019)

Drug	Dosage
Allopurinol	The minimal effective dosage is 100–200 mg daily and the maximal recommended dosage is 800 mg daily. To reduce the possibility of gout flares, it is recommended that the individual start with a low dose of allopurinol tablets (100 mg daily) and increase at weekly intervals by 100 mg until a serum uric acid level of 6 mg/dL or less is attained but without exceeding the maximal recommended dosage.

This FDA table is adapted from (1). Dosage information given is for individuals with normal renal function.

Table 2. The ACR Recommendations for Individuals Taking Allopurinol (2020)

Genotype	Testing
*HLA–B*58:01*	We conditionally recommend testing *HLA–B*58:01* before starting allopurinol for individuals of Southeast-Asian descent (for example, Han-Chinese, Korean, Thai) and African-American individuals, who have a higher prevalence of *HLA–B*5801*. We conditionally recommend against *HLA–B*58:01* testing in all others. For individuals with a prior allergic response to allopurinol who cannot be treated with other oral urate-lowering therapies, we conditionally recommend using allopurinol desensitization.

Note: certainty of evidence is 'Very low'.
This ACR table is adapted from (2). ACR, American College of Rheumatology

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Table 3. The DPWG Allopurinol Dosing based on *HLA-B*58:01* Genotype (2017)

Genotype	Dosing recommendations
Positive for *HLA-B*58:01*	Choose an alternative, such as febuxostat Another option is to induce allopurinol tolerance first: the allopurinol dose is increased every 3 days until a dose of 100 mg/day has been achieved on day 28. The consecutive daily doses in the induction protocol are 50 µg, 100 µg, 200 µg, 500 µg, 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, and 100 mg.

This DPWG table is adapted from (5). DPWG, Dutch Pharmacogenetics Working Group

Table 4. CPIC Recommended Therapeutic Use of Allopurinol by *HLA-B* Genotype (2015)

Genotype test results	Example of diplotypes	Phenotype	Therapeutic recommendations
Negative for *HLA-B*58:01*	*(*X/*X)*[b]	Low or reduced risk of allopurinol-induced SCAR	Use allopurinol per standard dosing guidelines
Positive for *HLA-B*58:01*	*58:01/(*X)*[b] *58:01/*58:01*	Significantly increased risk of allopurinol-induced SCAR	Allopurinol is contraindicated

The strength of therapeutic recommendations is "strong" (3, 4).
SCAR, severe cutaneous adverse reaction, CPIC, Clinical Pharmacogenetics Implementation Consortium
[b] *X, any *HLA-B* genotype other than *HLA-B*58:01*
This table adapted from (4) with standardized terminology (6).

Drug: Allopurinol

Allopurinol is a commonly prescribed drug for the management of gout, tumor lysis syndrome, or for individuals with recurrent calcium oxalate calculi with daily uric acid excretions above 800 mg/day for men and 750 mg/day for women (1, 7). It is not recommended for preventative treatment of asymptomatic and non-severe hyperuricemia (8). Uric acid is produced by the breakdown of purine nucleotides, and high concentrations of uric acid can lead to gout and uric acid kidney stones.

Allopurinol is an analogue of the purine hypoxanthine. Allopurinol decreases the production of uric acid by inhibiting xanthine oxidase, which catalyzes the conversion of hypoxanthine and xanthine to uric acid. In addition, allopurinol facilitates the incorporation of hypoxanthine and xanthine into DNA and RNA, and the resulting increase in nucleotide concentration leads to a feedback inhibition of *de novo* purine synthesis, which in turn leads to a decrease in uric acid levels (9).

Allopurinol is rapidly oxidized in the liver to the active metabolite oxypurinol, which is the primary inhibitor of xanthine oxidase. Allopurinol has a short plasma half-life of ~1–2 hours, whereas oxypurinol has a half-life of ~15 hours in individuals with normal renal function. After the rapid oxidation of allopurinol, any remaining drug is promptly filtered and excreted by the kidneys. Thus, oxypurinol clearance correlates with kidney function, and individuals with reduced renal function will have much longer plasma half-lives (10). However, after oxypurinol is filtered by the kidneys, it is reabsorbed in a manner similar to how uric acid is reabsorbed. Therefore, it is thought that the effective inhibition of xanthine oxidase over a 24-hour period after a single dose of allopurinol is largely brought on by the effects of oxypurinol (1). It has been shown that oxypurinol can be removed by hemodialysis in individuals with end stage renal disease (11).

In general, allopurinol is well tolerated; however, allopurinol is one of the most common causes of SCAR, and the *HLA-B*58:01* allele is strongly associated with allopurinol-induced SCAR.

Allopurinol-induced Adverse Drug Reactions

In general, there are 2 categories of adverse drug reactions. Type A reactions account for up to 85–90% of all adverse drug reactions (12). They are predictable based on the known properties of the drug, and they can affect

any individual if their exposure to the drug is high enough. For allopurinol, one of the most common type A adverse effects is a gout flare after starting allopurinol therapy.

Type B reactions account for the remaining 10–15% of all adverse drug reactions (12). These include hypersensitivity reactions that occur in susceptible individuals. Such idiosyncratic hypersensitivity reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug. For this reason, it is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur.

Allopurinol-induced SCARs are examples of type B reactions, which include Stevens-Johnson syndrome (SJS), or the more severe toxic epidermal necrolysis (TEN); as well as drug reaction with eosinophilia and systemic symptoms (DRESS), and allopurinol hypersensitivity syndrome (AHS). Both SJS and TEN are disorders on the same spectrum, differentiated by the extent of skin detachment. Detachment on less than 10% of the total body surface area is classified as SJS, and on over 30% is classified as TEN; 10–30% detachment is SJS-TEN overlap. In contrast, DRESS has significantly less (or no) skin detachment or mucocutaneous involvement, maculopapular exanthema is the most common presentation. As the name implies, DRESS is also characterized by common multisystemic involvement that may include hematologic, renal, hepatic, cardiac, pulmonary, neurologic, gastrointestinal, and endocrine abnormalities. (13)

Allopurinol is one of the most common causes of SJS/TEN in Europe and Israel with similar reports from Singapore, Korea, and China (14, 15, 16, 17). Both are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucus membranes (severe erosions). Both conditions are also associated with fever, raised white cell count, hepatitis, and acute renal failure.

The underlying mechanisms for allopurinol-induced SCARs remain unclear, but cytotoxic T-cells (CD8+ T-cells) are involved. In the case of allopurinol, although the presence of *HLA-B*58:01* substantially increases the risk of SCAR, it is not an absolute requirement, indicating that other variables also contribute to its etiology (3, 18). Although allopurinol-induced-SCAR is rare (the risk is estimated to be 0.1–0.4%), allopurinol is one of the most serious causes of SCAR, which has a mortality rate of up to 25% (3, 4).

One theory, known as the p-I concept, is that there is a direct pharmacological reaction of the drug (for example, allopurinol) with the immune receptors (activated drug-specific T-cells) and this provides an initial signal to induce T-cell activation and trigger a T-cell-mediated hypersensitivity reaction. The signal may be strengthened by the additional interaction with HLA molecules (for example, *HLA-B*58:01*) (18, 19, 20, 21, 22).

The FDA-approved dose of allopurinol for the management of gout or hyperuricemia is to start with a daily dose of 100 mg and titrate the dose to a maximum daily dose of 800 mg, until the uric acid concentrations are less than 6.0 mg/dl. It has been suggested that titrating the starting dose based on kidney function can reduce the risk of adverse drug reactions (ADR). One proposed dosage model is starting allopurinol at a dose of 1.5 mg per unit of estimated glomerular filtration rate (23). Allopurinol is often prescribed in doses that may be too low to achieve a therapeutic goal, an approach taken in part to reduce the risk of drug hypersensitivity (24). One study has found that a lower starting dose of allopurinol may reduce the risk of allopurinol hypersensitivity syndrome (23). An additional retrospective database study similarly found that older individuals prescribed higher allopurinol starting doses (≥300 mg/day versus <200 mg/day) had a higher hazard ratio of an adverse drug reaction (25). The DPWG guidelines recommend a gradual titration regimen to support allopurinol tolerance in individuals with ADR-associated genotypes (5). There is emerging evidence supporting a gradual dose escalation approach to achieve target serum urate levels in most individuals, including those with chronic kidney disease. This approach depends upon appropriate monitoring and should be limited to individuals who do not experience adverse effects to allopurinol therapy (26, 27).

The HLA Gene Family

The HLA genes are members of the major histocompatibility complex (MHC) gene family, which includes more than 200 genes. The MHC family has been subdivided into 3 subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the genes encoding the HLA molecules, *HLA-A*, *HLA-B*, and *HLA-C*. These molecules are expressed on the surfaces of almost all immune cells and play an important role in processing and presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T-cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented (for example, from a pathogen), CD8+ T-cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen or foreign body (28).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 *HLA-B* alleles have been identified. Each HLA allele has a name that is prefixed by HLA, followed by the gene name, an asterisk and a 2 digit number that corresponds to antigen specificity, and the assigned allele number (29). For example, the *HLA-DRB1*13:01* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- *DRB1*: the *DRB1* gene (a particular HLA gene in this region)
- 13: the allele group (historically determined by serotyping, namely, a group of alleles that share the same serotype)
- 01: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (namely, due to synonymous and noncoding genetic variants).

Variation in the HLA genes plays an important role in the susceptibility to autoimmune disease and infections and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible (3, 4). More recently, specific HLA variants have been associated with susceptibility to adverse drug reactions, including allopurinol-induced hypersensitivity reactions.

Gene: *HLA-B*

The *HLA-B*58:01* allele is associated with an increased risk of severe hypersensitivity reactions to allopurinol, such as SJS/TEN. The allele is codominant, so an individual needs to have only one copy of the *HLA-B*58:01* allele to be at increased risk.

The association between *HLA-B*58:01* and allopurinol-induced adverse effects was first discovered in the Han-Chinese population, where a study found that all individuals who had allopurinol-induced SJS/TEN (51/51, 100%) carried *HLA-B*58:01*, compared with only 15% of the allopurinol-tolerant individuals (20/135, 15%) (30).

Further studies also found an association with *HLA-B*58:01* and severe allopurinol-induced adverse effects in other populations, including Thai, Korean, European, and Japanese populations (31, 32, 33). The association is stronger in the Han-Chinese than in European and Japanese populations, which is most likely due to differences in *HLA-B*58:01* allele frequencies between racial and ethnic populations (34).

The *HLA-B*58:01* allele is most common in individuals of Asian descent, with a frequency of ~10–15% in the Han-Chinese, ~12% in Koreans, and ~6–8% among individuals of Thai descent (35, 36, 37, 38, 39, 40). The risk allele is less common among Europeans and Japanese with a frequency of only ~1–2% (41, 42).

Although the risk of SCAR due to allopurinol is generally low (0.1–0.4%) and certain populations have a low frequency of the *HLA-B*58:01* risk allele (for example, Europeans), the risk of allopurinol-induced SCAR is substantially elevated in *HLA-B*58:01* carriers.

Linking *HLA-B* Genetic Variation with the Risk of Side Effects and Treatment Response

The relationship between *HLA-B*58:01* and allopurinol-induced SJS/TEN continues to be reported in many ethnicities, including in Taiwanese, Japanese, Korean, Thai, and Malaysian individuals (2, 43, 44, 45, 46, 47, 48, 49).

While *HLA-B*58:01* is the most well-known risk factor, other genetic risk factors may include *HLA-B75*, *DR13* homozygosity, and *DR14*, especially in individuals with chronic kidney disease (50). Non-genetic risk factors include kidney impairment, allopurinol starting dose, and concomitant diuretic use (10). Experts caution against reliance of the *HLA-B*58:01* as a sole predictor for development of allopurinol-induced adverse drug reactions (51). Both genetic and non-genetic risk factors contribute to adverse effect risks, and tolerance induction protocols now exist for individuals at higher risk (regardless of genotype) (10).

Genetic Testing

Genetic testing is available for several *HLA-B* alleles, including *HLA-B*58:01*, and for allopurinol response. The genotype results are either "positive" (*HLA-B*58:01* being present in one or both copies of the *HLA-B* gene) or "negative" (no copies of *HLA-B*58:01* are present). There are no intermediate phenotypes because *HLA-B* is expressed in a codominant manner (3, 4).

The ACR and CPIC recommend *HLA-B*58:01* screening for select populations before initiation of allopurinol therapy. However, *HLA-B*58:01* testing has not been approved by the FDA for this indication, and screening in select populations is underutilized (52, 53, 54). The ACR 2020 guidelines recommend *HLA-B*58:01* testing for Southeast-Asian and African-American descent individuals, but discourage use of this test in other ethnic groups unless the individual and their medical provider agree to proceed with testing (2) (see Therapeutic Recommendations based on Genotype). The rationale likely stems from the rarity of this allele outside of those specific populations and lack of cost-effectiveness of the testing.

Both *HLA-B*58:01* screening and avoidance of allopurinol when testing positive has shown to be, or estimated to be, cost-effective in several ethnic groups (for example, Chinese, Taiwanese, Korean, and in the US-Asians and African-Americans). Screening may not be cost-effective in other groups for example, Malaysians (43, 48, 55, 56, 57); however, routine testing for *HLA-B*58:01* is expected to become cost-effective with reductions in genotyping cost and the costs of alternative treatments for gout (for example, cheaper, generic febuxostat) (58, 59).

A potential alternative to costly HLA genotyping may be to test for single nucleotide variants that are tightly associated with *HLA-B*58:01*. A number of variants have been found to be in linkage disequilibrium with *HLA-B*58:01*, for example, the rs9263726 variant in the *PSORS1C1* gene is strongly associated with *HLA-B*58:01* in the Japanese population (34); however, the sensitivity and specificity of these linked variants may not be adequate in different ancestral populations.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

The dosage of allopurinol tablets to accomplish full control of gout and to lower serum uric acid to normal or near-normal levels varies with the severity of the disease. The average is 200 to 300 mg/day for individuals with mild gout and 400 to 600 mg/day for those with moderately severe tophaceous gout. The appropriate dosage may be administered in divided doses or as a single equivalent dose with the 300-mg tablet. Dosage requirements in excess of 300 mg should be administered in divided doses. The minimal effective dosage is 100 to 200 mg daily and the maximal recommended dosage is 800 mg daily. To reduce the possibility of flare-up of acute gouty attacks, it is recommended that the individual start with a low dose of allopurinol tablets (100 mg daily) and increase at weekly intervals by 100 mg until a serum uric acid level of 6 mg/dL or less is attained but without exceeding the maximal recommended dosage.

Please review the complete therapeutic recommendations that are located here (1).

2020 Statement from the American College of Rheumatology (ACR)

Testing for the *HLA–B*58:01* allele prior to starting allopurinol is conditionally recommended for individuals of Southeast Asian descent (e.g., Han Chinese, Korean, Thai) and for African American individuals, over not testing for the *HLA–B*58:01* allele.

Universal testing for the *HLA–B*5801* allele prior to starting allopurinol is conditionally recommended *against* in individuals of other ethnic or racial background over testing for the *HLA–B*5801* allele. [Conditional recommendations are those "which would warrant provider-individual shared medical decision-making discussion."]

As noted above, starting allopurinol in daily doses of ≤100 mg (and lower doses in individuals with CKD [chronic kidney disease]) is strongly recommended over starting at a higher dose.

The *HLA–B*58:01* allele is associated with a markedly elevated risk for AHS. The prevalence of *HLA–B*58:01* is highest among persons of Han Chinese, Korean, and Thai descent (7.4%), lower among African Americans (3.8%), and even lower among whites and Hispanics (0.7% each). Testing for this allele among Asians and African American individuals was reported to be cost-effective (incremental cost-effectiveness ratios <$109,000 per quality-adjusted life years). Asian and African American individuals taking allopurinol both have a 3-fold increased risk of AHS compared with white individuals taking allopurinol (for recommendations for ULT medications, see Table 4 and Supplementary Figure 3, available [online]).

Please review the complete therapeutic recommendations that are located here (2).

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

Individuals with the *HLA-B*58:01* genetic variation have a strongly increased risk of developing the life-threatening cutaneous side effects Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and DRESS.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

The risk of an allopurinol-induced life-threatening cutaneous side effect in these individuals is 1.6-13% in the case of a normal or slightly reduced renal function and 12-100% in the case of a severely reduced renal function.

Recommendation:

- Choose an alternative, such as febuxostat.

Another option is to induce allopurinol tolerance first:

To induce allopurinol tolerance, the allopurinol dose is increased every 3 days until a dose of 100 mg/day has been achieved on Day 28. The consecutive daily doses in the induction protocol are 50 µg, 100µg, 200µg, 50 µg, 1mg, 5mg, 10mg, 25 mg, 50mg and 100mg.

Please review the complete therapeutic recommendations that are located here (5).

2015 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Given the high specificity for allopurinol-induced SCAR, allopurinol should not be prescribed to individuals who have tested positive for *HLA-B*58:01*. Alternative medication should be considered for these individuals to avoid the risk of developing SCAR. For individuals who have tested negative, allopurinol may be prescribed as usual. However, testing negative for *HLA-B*58:01* does not totally eliminate the possibility of developing SCAR, especially in the European population.

Please review the complete therapeutic recommendations that are located here (3, 4).

Allele Nomenclature

Allele name	Other name(s)	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
HLA-B*58:01		Not applicable*	Not applicable*	Not applicable*

* For the MHC region, variations in genes such as HLA-B occur across the whole sequence of the gene, not a single locus. Therefore, the HLA-B*58:01 allele is defined by its sequence (GenBank: EU499350.1) rather than single coding or protein variants.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/
MHC, major histocompatibility complex

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Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype

Laura Dean, MD[1]

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Introduction

Amitriptyline is a tricyclic antidepressant used in the treatment of several psychiatric disorders, including major depression, obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Amitriptyline also has different off-label uses, including migraine prevention, neuropathic pain management, fibromyalgia, and enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (H1 histamine, alpha 1 α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Amitriptyline is metabolized mainly via CYP2C19 and CYP2D6 pathways. Metabolism by CYP2C19 results in active metabolites, including nortriptyline, which is also a tricyclic antidepressant. Metabolism catalyzed by CYP2D6 results in the formation of the less active 10-hydroxy metabolite. Individuals who are "CYP2D6 ultrarapid metabolizers" carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas "CYP2C19 ultrarapid metabolizers" carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 "poor metabolizers" carry two no function alleles for *CYP2D6* or *CYP2C19*, respectively.

The FDA-approved drug label for amitriptyline states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. The FDA recommendations also include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on *CYP2C19* and *CYP2D6* genotypes (2). For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., amitriptyline) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations while monitoring plasma concentrations to avoid side effects (2).

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Drug Class: Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders, including obsessive–compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for its function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).

Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry *CYP2D6* or *CYP2C19* variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

Drug: Amitriptyline

Amitriptyline is used to relieve the symptoms of depression, with endogenous depression being more likely to respond to treatment than other depressive states (e.g., reactive depression) (1). Off-label uses of amitriptyline include migraine prevention, and the treatment of neuropathic pain, fibromyalgia, and enuresis (bedwetting).

Amitriptyline blocks the uptake of both serotonin and norepinephrine, but more potently blocks the reuptake of serotonin. Amitriptyline also has strong affinities for histamine (H1), alpha-1 adrenergic, and muscarinic (M1) receptors, which account for its side effects, including sedation, weight gain, blurred vision, dry mouth, and constipation. The intensity of these side effects tends to be greater for amitriptyline compared to other tricyclics (3).

Amitriptyline is metabolized by CYP2C19 to the active metabolite, nortriptyline, which is also a tricyclic antidepressant thought to be approximately twice as potent as other TCAs. In contrast to amitriptyline, nortriptyline blocks the reuptake of norepinephrine more potently than serotonin (3).

Because both the parent drug (amitriptyline) and the CYP2C19 metabolite (nortriptyline) are pharmacologically active compounds, the plasma levels of both drugs should monitored (4). The sum of amitriptyline plus nortriptyline plasma levels may correlate with an individual's response to amitriptyline therapy (5).

The optimal therapeutic range for amitriptyline has been well-defined (6). Most individuals display an optimal response to amitriptyline when combined serum levels of amitriptyline and nortriptyline are between 80 and 200 ng/mL. Higher levels are associated with an increased risk of adverse events. At levels greater than 300 ng/ml, cardiac toxicity occurs. This is characterized by ECG changes (widening of QRS), which may lead to potentially fatal ventricular tachycardia. In some individuals, cardiac toxicity may occur at lower concentrations or even when they are within the recommended therapeutic range (7, 8).

Nortriptyline is metabolized by CYP2D6 to hydroxyl metabolites, which have been associated with cardiac toxicity. Safe levels of hydroxyl metabolites have not yet been defined (4).

Individuals who are carriers of certain *CYP2D6* and/or *CYP2C19* variants may have drug levels that are outside the therapeutic range after treated with standard doses of amitriptyline. As a result, they may have an increased risk of toxicity (if the level of amitriptyline and its active metabolites are too high) or treatment failure (if drug levels are too low).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as amitriptyline.

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Pharmacogene Variation (PharmVar) database (9).

CYP2D6 is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate *CYP2D6*.

There is substantial variation in *CYP2D6* allele frequencies among different populations (10). *CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*,**6*, **7*, **8*, and **12*) or an enzyme with decreased activity (e.g., **10*, **17*, *29, and **41*) (see Table 1) (11) . There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in the Caucasian population, **17* more common in Africans, and **10* more common in Asians (12).

Table 1: 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN* [b]

Table 1 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are intermediate or poor metabolizers carry copies of decreased or no function *CYP2D6* alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of *CYPD6* alleles are fully functional, with the reduced function **10* variant being very common (~40%, compared to ~2% in Caucasians) (13). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (14). Similarly, in Africans and African Americans, only half of *CYPD6* alleles are functional. However, a wider range of variants account for the remaining alleles (14, 15, 16).

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function **4* and **5* alleles (14). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of **1* allele) (17, 18, 19).

Individuals who are CYP2D6 poor metabolizers have higher plasma levels of amitriptyline, compared to normal metabolizers, after standard doses of amitriptyline (20). Individuals who carry at least one non-functional *CYP2D6* variant have been found to be at medium to high risk of developing side effects (21).

Because standard doses of amitriptyline may lead to an increased risk of adverse events in individuals who are CYP2D6 poor metabolizers, CPIC recommends avoiding the use of amitriptyline or other tricyclic antidepressants, and to consider using an alternative drug that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the recommended starting dose, and they strongly recommend therapeutic drug monitoring to guide dose adjustments (4).

Individuals who have more than two copies of normal function *CYP2D6* alleles are *CYP2D6* ultrarapid metabolizers. The increased rate of metabolism of amitriptyline leads to less active drug being available and a poor therapeutic response. Because of the potential lack of efficacy, CPIC recommends considering an alternative drug to amitriptyline that is not metabolized by CYP2D6. If a tricyclic is warranted, CPIC

recommends increasing the starting dose and using therapeutic drug monitoring to guide dose adjustments (4) (Table 2).

Table 2. 2016 CPIC Dosing recommendations for tricyclic antidepressants based on *CYP2D6* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2D6 ultrarapid metabolizer	Increased metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6
	Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure	If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[a]. Utilize therapeutic drug monitoring to guide dose adjustments.
CYP2D6 normal metabolizer	Normal metabolism of TCAs	Initiate therapy with recommended starting dose[b].
CYP2D6 intermediate metabolizer	Reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Consider a 25% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Higher plasma concentrations of active drug will increase the probability of side effects	
CYP2D6 poor metabolizer	Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6
	Higher plasma concentrations will increase the probability of side effects	If a TCA is warranted, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].

TCAs: Tricyclic Antidepressants
Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
The therapeutic recommendations for amitriptyline are classified as "moderate" for intermediate CYP2D6 metabolizers and "strong" for ultrarapid, normal, and poor CYP2D6 metabolizers.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

One issue with increasing the dose of amitriptyline dose for CYP2D6 metabolizers is increasing the level of hydroxyl-metabolites, which have been associated with cardiotoxicity (22, 23). Currently, the safe range of hydroxy-metabolite plasma concentrations is not known. In addition, there are few studies on how the combination of *CYP2D6* and *CYP2C19* phenotypes influences an individual's response to amitriptyline (4).

Gene: CYP2C19

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including amitriptyline.

The *CYP2C19* gene is highly polymorphic as 35 variant star (*) alleles are available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "rapid" and "ultrarapid" metabolizer phenotypes (24).

The most common no function variant is *CYP2C19*2*, which is characterized by c.681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (24, 25).

Another commonly tested no function variant is *CYP2C19*3*, which is characterized by c.636G>A in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (24, 25).

"CYP2C19 intermediate metabolizers" carry one copy of an allele that encodes decreased or no function (e.g. *1/*2), whereas "poor metabolizers" are homozygous or compound heterozygous for two no function alleles (e.g., *2/*2, *2/*3) (Table 3).

Table 3: Assignment of *CYP2C19* phenotypes by CPIC

Phenotype	Genotypes	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)[a]	An individual carrying two increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of patients)	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of patients)	An individual carrying two normal function alleles	**1/*1*
CYP2C19 Intermediate metabolizer (approximately 18–45% of patients)	An individual carrying one normal function and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 Poor metabolizer (approximately 2–15% of patients)	An individual carrying two no function alleles	**2/*2* **2/*3* **3/*3*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are CYP2C19 poor metabolizers have a reduced rate of metabolism of amitriptyline compared to normal metabolizers. As a result, standard doses of amitriptyline lead to higher plasma levels of amitriptyline, lower levels of nortriptyline, and may increase the risk of side effects (20, 26, 27, 28). Therefore, for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the recommended starting dose, and to use therapeutic drug monitoring to guide dose adjustments (4).

Individuals who are ultrarapid metabolizers may be at an increased risk of treatment failure and/or metabolites adverse effects. Being a carrier of the increased activity allele *CYP2C19*17* is not associated with an increased level of the sum of amitriptyline plus nortriptyline levels, but the ratio is altered. A higher level of nortriptyline is seen, which may be linked to increased side effects. Therefore, for ultrarapid metabolizers, CPIC have an optional recommendation of considering using an alternative drug to amitriptyline that is not metabolized by CYP2C19, or if a tricyclic is warranted, to use therapeutic drug monitoring to guide dose adjustments (4, 26) (Table 4, Table 5).

Table 4. 2016 CPIC Dosing recommendations for amitriptyline based on *CYP2C19* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer	Increased metabolism of tertiary amines as compared to normal metabolizers Greater conversion of tertiary amines to secondary amines may affect response or side effects	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
		If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments[a].
CYP2C19 normal metabolizer	Normal metabolism of tertiary amines	Initiate therapy with recommended starting dose[b].
CYP2C19 intermediate metabolizer	Reduced metabolism of tertiary amines compared to normal metabolizers	Initiate therapy with recommended starting dose[b].
CYP2C19 poor metabolizer	Greatly reduced metabolism of tertiary amines compared to normal metabolizers	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Decreased conversion of tertiary amines to secondary amines may affect response or side effects	

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as "strong" for normal and intermediate CYP2C19 metabolizers, "moderate" for poor metabolizers, and "optional" for ultrarapid metabolizers.

a Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects).

b Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.

Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Table 5. 2016 CPIC Dosing recommendations for amitriptyline based on both CYP2D6 and CYP2C19 phenotypes [a,b]

Phenotype	CYP2D6 Ultrarapid metabolizer	CYP2D6 Normal metabolizer	CYP2D6 Intermediate metabolizer	CYP2D6 Poor metabolizer
CYP2C19 ultrarapid or rapid metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19[c,e] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19 [c,e] Classification of recommendation[d]: Optional	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional
CYP2C19 normal metabolizer	Avoid amitriptyline use. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[f,g] Classification of recommendation[d]: Strong	Initiate therapy with recommended starting dose[h] Classification of recommendation[d]: Strong	Consider a 25% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Strong

Table 5. continued from previous page.

Phenotype	CYP2D6 Ultrarapid metabolizer	CYP2D6 Normal metabolizer	CYP2D6 Intermediate metabolizer	CYP2D6 Poor metabolizer
CYP2C19 intermediate metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Initiate therapy with recommended starting dose[h] Classification of recommendation[d]: Strong	Consider a 25% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Optional	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Optional
CYP2C19 poor metabolizer	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use. If amitriptyline is warranted, consider a 50% reduction of recommended starting dose[f,h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use[c] Classification of recommendation[d]: Optional

[a] Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
[b] The dosing recommendations are based on studies focusing on amitriptyline. Because tricyclic antidepressants have comparable pharmacokinetic properties, it may be reasonable to apply these guidelines to other tertiary amines including clomipramine, doxepin, imipramine and trimipramine (the classification of this recommendation is optional).
[c] If amitriptyline is warranted, utilize therapeutic drug monitoring to guide dose adjustment.
[d] The rating scheme for the recommendation classification is described in Supplementary Data (2). See CYP2D6 and CYP2C19 combined dosing recommendations for explanation of classification of recommendations for this table.
[e] TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
[f] Utilizing therapeutic drug monitoring if a tricyclic is prescribed to a patient with CYP2D6 ultrarapid, intermediate or poor metabolism in combination with CYP2C19 ultrarapid, intermediate or poor metabolism is strongly recommended.
[g] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[h] Patients may receive an initial low dose of TCAs, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Genetic Testing

Clinical genotyping tests are available for many CYP2D6 and *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides a list of test providers for "amitriptyline response," and the CYP2D6 and CYP2C19 genes.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (29). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (30).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5

- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 31)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the caucasian population (about 7 to 10% of Caucasians are so called "poor metabolizers"); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8 fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrhythmics propafenone and flecainide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent of inhibition. The extent to which SSRI-TCA interactions may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the coadministration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be coadministered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

CYP2D6 dosing recommendations.

[…]. The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as "moderate" because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate.

CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

CYP2C19 dosing recommendations.

[…]. The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

For CYP2D6 ultrarapid metabolizers:

The genetic polymorphism leads to increased metabolic capacity of CYP2D6, which may cause a decrease in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and increased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: increase the dose to 1.25 times the standard dose, monitor the plasma concentrations and be alert to potential therapy failure due to decreased amitriptyline plus nortriptyline plasma concentrations and to increased plasma concentrations of the potentially cardiotoxic, active hydroxy metabolites.

For CYP2D6 intermediate metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 60% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

For CYP2D6 poor metabolizers:

The genetic polymorphism leads to decreased metabolic capacity of CYP2D6, which may cause an increase in the plasma concentrations of amitriptyline and its active metabolite nortriptyline and decreased plasma concentrations of the active metabolites E-10-OH-amitriptyline and E-10-OH- nortriptyline.

Recommendation:

1. Choose an alternative if possible. Antidepressants that are not metabolized via CYP2D6 - or to a lesser extent - include, for example, citalopram and sertraline.
2. If an alternative is not an option: use 50% of the standard dose and monitor the plasma concentrations of amitriptyline and nortriptyline

As side effects are related to nortriptyline plasma concentrations and the efficacy to amitriptyline plus nortriptyline plasma concentrations, which are influenced to a lesser extent by CYP2D6, it is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, but the efficacy is maintained.

Please review the complete therapeutic recommendations that are located here: (32).

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947

Nomenclature for selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

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Aripiprazole Therapy and *CYP2D6* Genotype

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Introduction

Aripiprazole (brand names Abilify or Aristada) is an atypical antipsychotic used to manage schizophrenia, bipolar disorder, major depressive disorder, irritability associated with autistic disorder, and in the treatment of Tourette syndrome. (1)

The metabolism and elimination of aripiprazole is mainly mediated through 2 enzymes, CYP2D6 and CYP3A4. Approximately 8% of Caucasians, 3–8% of Black/African Americans and up to 2% of Asians cannot metabolize CYP2D6 substrates and are classified as "poor metabolizers." (2)

The FDA-approved drug label for aripiprazole states that in CYP2D6 poor metabolizers, half of the usual dose should be administered. In CYP2D6 poor metabolizers who are taking concomitant strong CYP3A4 inhibitors (for example, itraconazole, clarithromycin), a quarter of the usual dose should be used (Table 1) (1). The dosage reduction is the same regardless of the administration route (oral or long-acting injectable). (3)

The Dutch Pharmacogenetics Working group (DPWG) also recommends a reduced dosage for CYP2D6 poor metabolizers, "no more than 10 mg/day or 300 mg/month" (Table 2). No action is recommended for intermediate or ultrarapid metabolizers. While both of these metabolic variations alter the plasma concentrations of aripiprazole, there is no evidence that this increases the risk of reduced effectiveness or risk of side effects. (4)

In contrast to the recommendations by the FDA and DPWG, some recent studies have suggested CYP2D6 intermediate metabolizers may also require a dose decrease, but this was only based on aripiprazole clearance. (5, 6, 7, 8)

Table 1. The FDA Dosing Recommendations for Aripiprazole and CYP2D6 Metabolizer Status and Comedications (2020)

Factors	Dosage adjustments for aripiprazole tablets
Known CYP2D6 poor metabolizers	Administer half of usual dose
Known CYP2D6 poor metabolizers taking concomitant strong CYP3A4 inhibitors (for example, itraconazole, clarithromycin)	Administer a quarter of usual dose
Strong CYP2D6 (for example, quinidine, fluoxetine, paroxetine) or CYP3A4 inhibitors (for example, itraconazole, clarithromycin)	Administer half of usual dose
Strong CYP2D6 and CYP3A4 inhibitors	Administer a quarter of usual dose
Strong CYP3A4 inducers (for example, carbamazepine, rifampin)	Double usual dose over 1–2 weeks

This FDA table is adapted from (1).

Table 2. The DPWG Dosing Recommendations for Aripiprazole and CYP2D6 Metabolizer Status (2018)

CYP2D6 metabolizer type	Action needed	Background
Poor metabolizer	Administer no more than 10 mg/day or 300 mg/month (67–75% of the standard maximum dose of aripiprazole)[1]	The risk of side effects is increased. The genetic variation leads to an increase in the sum of the plasma concentrations of aripiprazole and the active metabolite.

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Table 2. continued from previous page.

CYP2D6 metabolizer type	Action needed	Background
Intermediate metabolizer (IM)	NO action is needed for this gene-drug interaction	The genetic variation alters the plasma concentration of the sum of aripiprazole and the active metabolite dehydroaripiprazole to a limited degree. There is no evidence that this increases the risk of reduced effectiveness for UM or risk of side effects for IM.
Ultrarapid metabolizer (UM)		

DPWG: Dutch Pharmacogenetics Working Group
1 Drug labeling within the European Union states that 15 mg/day is the starting maximum dose (9).
This DPWG table is adapted from (4) .

Drug: Aripiprazole

Aripiprazole is an atypical antipsychotic primarily used to treat schizophrenia and bipolar disorder. Aripiprazole may also be used as part of the management of major depressive disorder, irritability associated with autism, and in the treatment of Tourette syndrome. (1, 3)

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first-generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (for example, schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms.

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. However, many have serious metabolic effects. Aripiprazole is an atypical antipsychotic that is noted for having fewer metabolic side effects than other atypicals, such as clozapine, olanzapine, risperidone, and quetiapine. Other atypicals approved by the FDA include asenapine, brexpiprazole, cariprazine, lurasidone, paliperidone, and ziprasidone.

The main action of both first-generation and second-generation antipsychotics is thought to be the post-synaptic blockade of D2 dopamine receptors. All antipsychotics, with the exception of aripiprazole, are D2 antagonists.

Aripiprazole is a partial D2 agonist. Aripiprazole binds to the D2 receptor with a high affinity similar to dopamine. However, because it has low intrinsic activity, it causes much lower activation of the receptor compared with dopamine.

The combination of a high affinity for the D2 receptor and its partial agonist activity may result in aripiprazole reducing the high-frequency firing of dopamine neurons in the brain's mesolimbic system. Overactivity in this region is thought to underlie psychosis and other positive symptoms of schizophrenia. In addition, the preservation of some D2 receptor activity in other dopamine-rich pathways in the brain (mesocortical and nigrostriatal areas) may provide more protection against extrapyramidal side effects. (10, 11)

Aripiprazole also has a high affinity for the serotonin 5-HT_{2A} receptors, where it acts as an antagonist and it moderately blocks the alpha 1 adrenergic and histamine H1 receptors, which may account for the lower incidence of orthostatic hypotension and sedation compared with other antipsychotics. (12)

Adverse events with aripiprazole therapy include increased mortality in elderly individuals with psychosis caused by dementia, suicidal thoughts and behavior in children and young adults, neuroleptic malignant syndrome, tardive dyskinesia, metabolic changes including hyperglycemia, pathological gambling, and other compulsive behaviors. Additionally, orthostatic hypotension, leukopenia, neutropenia, and agranulocytosis, seizures/convulsions and potential cognitive and motor impairment have been reported. Commonly observed adverse reactions in adult schizophrenic individuals that should be reported to the FDA include akathisia.

Adverse events in pediatric individuals (13–17 years old) that should be reported are extrapyramidal disorder, somnolence, and tremor. (1)

Aripiprazole is extensively metabolized in the liver by the cytochrome P450 (CYP) superfamily of enzymes, mainly CYP2D6 and CYP3A4. Aripiprazole activity is thought to be primarily due to the parent drug, and to a lesser extent its major metabolite, dehydroaripiprazole. The mean elimination half-life is approximately 75 hours for aripiprazole, but in individuals who have no appreciable CYP2D6 activity (poor metabolizers), the mean elimination half-life for aripiprazole is around 146 hours. (1) The mean aripiprazole exposure for CYP2D6 poor and intermediate metabolizers is increased 1.5-fold compared with normal metabolizers (6).

While aripiprazole has been reported to have a minimal effect on prolactin levels (13), more recent studies have found a correlation between reduced CYP2D6 enzyme activity and increases in prolactin. (14, 15) These increases in prolactin levels were more pronounced in females and individuals with no functional CYP2D6, raising the possibility of a higher risk for hyperprolactinemia adverse reactions. Hyperprolactinemia can have significant effect in the pediatric population during growth and development and may warrant additional monitoring. (14)

Rare cardiac adverse reactions have also been observed in clinical trials with aripiprazole. (3) Two recent case reports suggest that CYP2D6 activity may play a role in predisposing an individual to atrial fibrillation or abnormal heart electrophysiology. (16, 17) One small study reported that palpitations were more commonly experienced by females versus males taking aripiprazole and more often with aripiprazole versus olanzapine treatment. (18)

The FDA-approved drug label warns that "neonates exposed to antipsychotic drugs, including aripiprazole, during the third trimester of pregnancy are at risk for extrapyramidal and/or withdrawal symptoms following delivery." (1) These exposures should be considered in light of the disease-associated maternal or embryo/fetal, or both, risks of untreated schizophrenia or bipolar I disorder. The FDA-approved label states that neonates who are exposed to aripiprazole during pregnancy should be closely monitored, as symptoms vary in severity and duration (hours to days) and may require prolonged hospitalization. The FDA encourages healthcare providers to register individuals who have aripiprazole exposure while pregnant to monitor pregnancy outcomes. For more information see (1, 3).

Limited data from scientific literature suggests a low level of aripiprazole can be present in breast milk. The literature reports the relative infant dose ranges between 0.7% and 8.3% of the maternal weight-adjusted dosage. (1) Though aripiprazole has a minimal effect on prolactin levels compared with the phenothiazines, case reports have documented both decreased lactation and hyperprolactinemia in nursing mothers taking aripiprazole, and other medications may be considered, as needed. (19)

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

CYP2D6 Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 3) (20). The combination of *CYP2D6* alleles that a person has is used to

determine their diplotype (for example, *CYP2D6*4/*4*). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (for example, CYP2D6 poor metabolizer). When duplicated alleles are detected, both copies are assigned an activity score for phenotyping. However, the activity score system is not standardized across clinical laboratories or CYP2D6 genotyping platforms. CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is defined by the sum of the allele activity scores, which is usually in the range of 0–3.0: (21)

- An ultrarapid metabolizer (UM) has an activity score greater than 2.25
- A normal metabolizer phenotype (NM) has an activity score of 1.25 to 2.25
- An intermediate metabolizer (IM) has an activity score of >0 to 1.25
- A poor metabolizer (PM) has an activity score of 0

Table 3. Activity Status of Selected *CYP2D6* Alleles

Allele type	Activity score	*CYP2D6* alleles
Normal function	1.0	**1, *2, *27, *33*
Decreased function	0.25-0.5	**10, *17, *41, *49*
No function	0	**3, *4, *5, *6, *36*

For a comprehensive list of *CYP2D6* alleles, please See PharmVar. Activity scores from (22).

The *CYP2D6*1* allele is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. In addition, the *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (23, 24, 25) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (26, 27, 28) (see Table 3). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians. (29)(30)

Allele Frequencies Vary between Populations

Among Asians and in individuals of Asian descent, only approximately 50% of *CYP2D6* alleles are normal function, and the frequency of *CYP2D6* duplications is as high as 45%, although this may have been overestimated by not accounting for tandem hybrid alleles (for example, **36+*10*) (31). Other studies of a US individual population suggested less than 50% of alleles detected within Asian-descent individuals are normal-function alleles in a single copy, with 30% of alleles arising from structural variants (duplications or deletions) (32). Common no-function variants are *CYP2D6*36* and *CYP2D6*4* (32). Both these alleles contain the variant "c.100C>T" (see Allele Nomenclature table) (29, 31, 33, 34). The *CYP2D6*36* allele is the result of a gene conversion event with the pseudogene *CYP2D7* (35). This no-function allele is most commonly found in individuals of Asian ancestry (32).

Among Africans and African Americans, only approximately 50% of *CYP2D6* alleles are normal function (23, 28, 29, 36). African Americans also have been found to have a higher frequency of no-function structural variants or decreased-function single-copy variant alleles versus Caucasian or Hispanic Americans (32).

Middle Eastern countries show a great diversity in phenotypic and allelic distribution for *CYP2D6* (37), though on average, these individuals show a lower frequency of poor metabolizer phenotypes (0.91%) and higher ultrarapid phenotypes (11.2%) than other ethnicities (Note: Oceania and Middle Eastern ethnicities combined in this study) (2).

Among European countries, there is diversity of allelic distribution (38). Gene duplications were more common in the south-eastern countries (Greece, Turkey: 6%) and less common in north-western countries (Sweden and

Denmark, <1%). Meanwhile, *CYP2D6*4* and **5* alleles were generally more common in the north and less common in the south. (38) Worldwide *CYP2D6* genotype and phenotype frequencies have been catalogued and recently published (2).

CYP2D6 Phenotype

CYP2D6 Phenotype Frequencies Vary between Populations

Normal metabolizers: Approximately 77–92% of individuals have 2 normal-function alleles (**1* or **2*), or one normal-function allele and one decreased-function allele. These individuals are "normal metabolizers" and are most likely to have a phenotypically normal response to the drug.

Intermediate metabolizers: Approximately 2–11% of individuals are intermediate metabolizers—they have either 2 decreased-function alleles or one normal- or decreased-function and one no-function allele (2). A study of a diverse US urban population of children found that roughly 8% of subjects were intermediate metabolizers (39), Within the US, it has been observed that individuals of African or Asian descent were most likely to be classified as intermediate metabolizers (20–28% of population by ethnicity) (32).

Poor metabolizers: Approximately 5–10% of individuals are poor metabolizers—they have 2 no-function alleles (40). Poor metabolizers are more commonly found in European Caucasians and their descendants. The no-function *CYP2D6*4* and **5* alleles largely account for the poor metabolizer phenotype in these populations (27, 41, 42). It should be noted that the frequency of poor metabolizers can be much lower in certain populations including East Asian, Oceania and Middle Eastern (2). Studies of US multi-ethnic populations have estimated the prevalence of poor metabolizers to be between 1.5–5.7% (32, 39).

Ultrarapid metabolizers: Individuals who are ultrarapid metabolizers have at least 3 copies of the *CYP2D6* gene. The ultrarapid metabolizer phenotype has been estimated to be present in 1–2% of individuals, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (40, 43). Ultrarapid metabolizers made up 9% of subjects in an urban multi-ethnic population with a large portion of Hispanic/Latino subjects (39). A larger study of US individuals predicted an ultrarapid metabolizer phenotype in only 2.2% of individuals, regardless of ethnicity (32).

Linking Gene Variation with Treatment Response

Genetic variations in the *CYP2D6* gene have been found to impact serum levels of aripiprazole and dehydroaripiprazole. (8)(44, 45) Because standard doses of aripiprazole lead to higher plasma levels of aripiprazole and dehydroaripiprazole, the dose of aripiprazole should be adjusted for individuals that have 2 no-function *CYP2D6* alleles causing poor metabolizer status.

The FDA recommends that individuals who are known to be CYP2D6 poor metabolizers should receive half the standard dose of aripiprazole, or a quarter of the standard dose if they are also taking medicines that strongly inhibit CYP3A4 (for example, itraconazole, clarithromycin) (see Table 1). Multiple studies substantiate the FDA recommendations by concluding that poor metabolizers should receive a reduced dose of aripiprazole (30–50% reduction). (5, 6, 8, 45, 46)

One study further suggested that individuals with increased CYP2D6 activity (ultrarapid metabolizers) may need to take an alternative antipsychotic not metabolized by CYP2D6 because of reduced drug levels. (46) Additional studies have suggested that intermediate metabolizers should also have a reduced dosage, in contrast with the current FDA-approved drug labeling. (5, 6, 7, 47) This is particularly relevant among individuals of Asian descent, where the intermediate metabolizer phenotype is highly prevalent. (7) One study reported that females and poor metabolizers are at an elevated risk for adverse events. (8)

Phenoconversion from a CYP2D6 normal metabolizer status to reduced metabolic activity has also been suggested due to drug-drug interactions in individuals with one or more wild-type *CYP2D6*1* allele. (47) The drugs observed by this study to have an effect on CYP2D6 activity were risperidone, metoprolol and propranolol. (47) Additionally, phenoconversion has been associated with a higher rate of aripiprazole discontinuation (48). Aripiprazole can lead to drug-drug interactions with other CYP2D6 substrates, particularly those with weaker affinity for the CYP2D6 enzyme—for example, the antidepressant mirtazapine—resulting in an increase in plasma concentration for the co-medication (49). The FDA drug label recommends reducing the aripiprazole dosage with concomitant use of strong CYP2D6 inhibitors (for example quinidine, fluoxetine and paroxetine) or CYP3A4 inhibitors. (1)

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are available for aripiprazole response and for the *CYP2D6* gene.

CYP2D6 is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, and pseudogenes. The complexity of genetic variation complicates making a correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories. (50)

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications are denoted by "xN" for example, *CYP2D6*1xN* with *xN* representing the number of *CYP2D6* gene copies. The functional status of the duplicated allele is also critical in interpretation of the test results, as duplication of a no-function versus a normal-function allele would result in a different total activity score and potentially different metabolizer phenotype.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6 *1/*1*, normal metabolizer", this can be confirmed by checking the diplotype and assigning an activity score to each allele (for example, 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal-function allele, Table 3). See the *CYP2D6* alleles section above for more information.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA):

Dosage adjustments are recommended in patients who are known CYP2D6 poor metabolizers and in patients taking concomitant CYP3A4 inhibitors or CYP2D6 inhibitors or strong CYP3A4 inducers (see Table 2). When the coadministered drug is withdrawn from the combination therapy, aripiprazole dosage should then be adjusted to its original level. When the coadministered CYP3A4 inducer is withdrawn, aripiprazole dosage should be reduced to the original level over 1 to 2 weeks. Patients who may be receiving a combination of strong,

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

moderate, and weak inhibitors of CYP3A4 and CYP2D6 (e.g., a strong CYP3A4 inhibitor and a moderate CYP2D6 inhibitor or a moderate CYP3A4 inhibitor with a moderate CYP2D6 inhibitor), the dosing may be reduced to one-quarter (25%) of the usual dose initially and then adjusted to achieve a favorable clinical response.

[…]

Dosage adjustment is recommended in known CYP2D6 poor metabolizers due to high aripiprazole concentrations. Approximately 8% of Caucasians and 3% to 8% of Black/African Americans cannot metabolize CYP2D6 substrates and are classified as poor metabolizers (PM).

Please review the complete therapeutic recommendations that are located here:(1).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 PM: aripiprazol [aripiprazole]

The risk of side effects is increased. The genetic variation leads to an increase in the sum of the plasma concentrations of aripiprazole and the active metabolite.

administer no more than 10 mg/day or 300 mg/month (67-75% of the standard maximum dose of aripiprazole).

CYP2D6 IM: aripiprazol [aripiprazole]

NO action is needed for this gene-drug interaction.

The genetic variation increases the plasma concentration of the sum of aripiprazole and the active metabolite dehydroaripiprazole to a limited degree. There is insufficient evidence that this increases the risk of side effects.

CYP2D6 UM: aripiprazol [aripiprazole]

NO action is needed for this gene-drug interaction.

The genetic variation decreases the plasma concentration of the sum of aripiprazole and the active metabolite dehydroaripiprazole to a limited degree. There is no evidence that this increases the risk of reduced effectiveness.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
*CYP2D6*3*	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*36*[3]	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2850C>T is also referred to as 2938C>T
[3] *CYP2D6*36* is a gene conversion with *CYP2D7*; variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (51).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Atazanavir Therapy and *UGT1A1* Genotype

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Introduction

Atazanavir is indicated for managing human immunodeficiency virus (HIV) infection as part of a multi-drug regimen (1). While it was once widely recommended as a first-line therapy, it is now primarily suggested as a second-line therapeutic option due to potential adverse effects leading to discontinuation of therapy (2, 3). Atazanavir can cause hyperbilirubinemia (not associated with liver injury) leading to jaundice, which is a common cause of drug discontinuation. Individuals with 2 decreased-function alleles for *UGT1A1* are most likely to experience jaundice leading to atazanavir discontinuation, although this can occur despite the individual having a reference *UGT1A1* genotype (4). The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that when an individual is a known UGT1A1 poor metabolizer, an alternative therapy should be considered particularly when jaundice is of concern to the individual (Table 1) (4). The US Food and Drug Administration (FDA) approved drug label states that certain comedications that depend upon UGT1A1 or the cytochrome P450 family member CYP3A are contraindications for atazanavir therapy due to the potential for elevated plasma concentrations of these comedications (1).

Table 1: The Clinical Pharmacogenetics Implementation Consortium (CPIC) Recommended Use of Boosted Atazanavir by *UGT1A1* Phenotype (2016)

Phenotype	Example *UGT1A1* genotype[a]	Implications for phenotypic measures	Dosing recommendation	Strength of recommendation
Normal metabolizer[b]	**1/*1* **1/*36* **36/*36*	Reference[c] UGT1A1 activity; very low likelihood of bilirubin-related discontinuation of atazanavir	There is no need to avoid prescribing atazanavir based on *UGT1A1* genetic test results. Inform the individual that some individuals stop atazanavir because of jaundice (yellow eyes and skin), but that this individual's genotype makes this unlikely (less than approximately a 1-in-20 chance of stopping atazanavir because of jaundice).	Strong
Intermediate metabolizer	**1/*6* **1/*27* **1/*28* **36/*37*	Somewhat decreased UGT1A1 activity; low likelihood of bilirubin-related discontinuation of atazanavir.		Strong
Poor metabolizer	**6/*6* **6/*27* **6/*28* **27/*28* **37/*37*	Markedly decreased UGT1A1 activity; high likelihood of bilirubin-related discontinuation of atazanavir	Consider an alternative agent particularly where jaundice would be of concern to the individual. If atazanavir is to be prescribed, there is a high likelihood of developing jaundice that will result in atazanavir discontinuation (at least 20% and as high as 60%).	Strong

Note: All studies correlating UGT1A1 genotype with atazanavir adverse events have involved ritonavir boosting.
[a] Example genotype (also called a diplotype) data from CPIC Diplotype-Phenotype table, as provided in (5).
[b] Original CPIC guidelines used the term of "extensive metabolizer" which has been substituted for the current, standardized term of "normal metabolizer."
[c] "Reference" function refers to the UGT1A1 allele to which other alleles are compared.
This table has been adapted from (4).

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Drug: Atazanavir

Atazanavir is an antiretroviral protease inhibitor (PI) used to treat HIV infection in adults and pediatric individuals weighing at least 15 kilograms (1, 6, 7). The current standard of care for HIV infection is combination antiretroviral therapy (cART), where multiple classes of antiretroviral medications are taken together (3). Atazanavir is most often prescribed with 2 nucleoside reverse transcriptase inhibitors (NRTIs) and a pharmacokinetic booster––either ritonavir or cobicistat––to slow the metabolism of atazanavir and ensure sufficient plasma levels for therapeutic efficacy (3). Atazanavir-containing ART is recommended by the World Health Organization (WHO) as a second-line drug regimen for individuals for whom a dolutegravir-based first-line therapy has failed (2). However, both the WHO and the U.S. Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents recommend darunavir over atazanavir due to its lower rate of drug discontinuation in response to adverse effects (2, 3). Atazanavir-containing regimens should be avoided in individuals with high viral load (HIV RNA ≥100,000 copies/mL), chronic kidney disease (creatinine clearance <60 mL/min), severe hepatic impairment (Child-Pugh Class C), or a history of clinically significant hypersensitivity (including Stevens-Johnson syndrome, erythema multiforme, or toxic skin eruptions) to components of the medication formulation (1, 3).

Primary metabolism of atazanavir occurs via the cytochrome P450, family 3, subfamily A (CYP3A) enzymes, and both isoenzymes 4 and 5 (CYP3A4/5) (1, 8). Coadministration with ritonavir or cobicistat leads to inhibition of the CYP3A4/5 enzymes and improves pharmacokinetics, decreasing the daily pill burden for the individual (8). Thus, for individuals who can tolerate ritonavir, the recommended daily dose of atazanavir is 300 mg (plus 100 mg of ritonavir); if ritonavir is not well tolerated, the daily dose of atazanavir should be 400 mg (1). Atazanavir is administered orally and should be taken with food to increase overall bioavailability; absorption is rapid, and maximum plasma concentration is reached approximately 2.5 hours after dosing (or up to 5 hours if taken with a high-fat meal) (1). In plasma, atazanavir is 86% bound to proteins, including alpha-1-acid glycoprotein and albumin (1). The WHO recommends avoiding atazanavir with rifampin, a strong CYP3A4 inducer, due to decreased plasma levels of atazanavir (2, 9). Medications that inhibit CYP3A activity can significantly increase plasma atazanavir and ritonavir levels and lead to severe, life-threatening, or fatal events and should be avoided if possible; these medications include amiodarone and indinavir (1, 9). Some medications may be used with altered dosing (see the FDA-approved drug label for full information) (1). Coadministration of ritonavir-boosted atazanavir with the antifungal voriconazole leads to altered pharmacokinetics of voriconazole, especially for individuals with a *CYP2C19* genotype associated with poor metabolism of voriconazole; experts recommend using this medication combination only when the benefits outweigh the risks (10).

Atazanavir inhibits the uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) enzyme—the only enzyme that conjugates bilirubin for excretion in bile (8, 11). Atazanavir therapy causes an elevation of unconjugated bilirubin in most individuals, though many remain asymptomatic (1). Some individuals can progress to hyperbilirubinemia and jaundice, leading to discontinuation of or non-adherence to cART (4, 12, 13). The increase in unconjugated bilirubin is not associated with other signs of hepatic injury, and any observed elevations of hepatic transaminases should be evaluated for alternative causes (1). Individuals with 2 decreased-function *UGT1A1* alleles are at highest risk of bilirubin-related atazanavir discontinuation, although bilirubin elevations can also occur in the absence of this genotype (4).

Other potential side effects associated with atazanavir (with or without ritonavir) include cardiac symptoms, severe skin reactions, liver toxicity in individuals with elevated liver transaminases, development of chronic kidney disease, diabetes mellitus and hyperglycemia, immune reconstitution syndrome, and metabolic abnormalities (1). Cardiac symptoms may include a prolonged duration of the PR interval. The PR interval is a period lasting from the beginning of the P wave (reflecting atrial depolarization) until the beginning of the QRS complex (indicating ventricular depolarization) in an electrocardiogram. The PR interval, sometimes called the PQ interval, is normally between 120 and 200 milliseconds in duration. (14) Reports of prolonged PR intervals

associated with atazanavir therapy were not acute but occurred after a few weeks of therapy, either alone or with NRTI (15). Cardiac conduction abnormalities, specifically asymptomatic first-degree atrioventricular (AV) block, were reported in 5.9% of atazanavir-treated individuals (1). In some individuals, the AV block and abnormal ECG rhythm resolved within one week following atazanavir discontinuation, but for others it persisted for one month (15). Individuals with underlying hepatitis B or C infection or preexisting renal disease may require additional monitoring or alternative medications; cases of kidney stones have been reported in individuals during atazanavir therapy and may require temporary interruption of therapy (1). Individuals with hepatic impairment should not take ritonavir to boost atazanavir pharmacokinetics. Thus, individuals with moderate (Child-Pugh class B) hepatic impairment are recommended to be given a daily dose of 300 mg atazanavir without ritonavir boost, while individuals with mild (Class A) hepatic impairment can take the standard 400 mg dose (without ritonavir) (1). Kidney disease, including nephrolithiasis and nephrotoxicity without large calculi, have also been reported in association with atazanavir therapy (16, 17). While PI therapy has been associated with diabetes mellitus and hyperglycemia (1), a pooled meta-analysis found no significant differences in insulin sensitivity during atazanavir therapy (18). Similarly, the reported association of PI therapy leading to increased risk of cardiovascular disease and dyslipidemia is less significant for atazanavir as compared with older PIs (19).

Atazanavir can be used in children, with a recommended daily dose of 200 mg (together with 100 mg ritonavir) in individuals weighing 15-35 kg, and 300 mg daily (with 100 mg ritonavir) for individuals weighing at least 35 kg. Individuals must be 13 years of age and weigh at least 40 kg for unboosted atazanavir therapy, which is dosed at 400 mg per day (1, 20). Increased age is also associated with a higher exposure to atazanavir at standard doses, which may result in a more pronounced decrease in bone mineral density in older adults when compared with darunavir cART (21).

Pregnant women may be prescribed atazanavir and should be given a form of ART to minimize the risk of maternal-fetal HIV transmission (2, 22). The FDA recommends the standard dose of atazanavir for pregnant individuals (300 mg daily with 100 mg ritonavir) (1). Despite the risk of hyperbilirubinemia and jaundice linked to atazanavir therapy, atazanavir was not associated with higher rates of neonatal jaundice (23). Pregnant individuals should not use cobicistat-boosted atazanavir (1, 3) due to reduced exposure and potential loss in efficacy during 2nd and 3rd trimesters (22). One systematic review reported an association of maternal PI therapy with an increased risk of the child being small or very small for gestational age (relative risk 1.24–1.4), though this risk was not significantly different among the multiple PIs studied, including atazanavir (24).

Individuals using hormone-based contraceptive medication may experience drug-drug interactions and altered metabolism when taking boosted-PI based cART. One study reported changes in exposure to etonogestrel (increased) and ethinyl estradiol (decreased) when used concomitantly with atazanavir (25). Similarly, a cobicistat-boosted atazanavir or darunavir regimen was found to increase exposure to drospirenone, leading to an increased risk of hyperkalemia and a subsequent recommendation to avoid atazanavir and cobicistat with drospirenone (24).

Gene: *UGT1A1*

The UGT enzymes (uridine diphosphate-glucuronosyltransferase) are a superfamily of enzymes that metabolize a wide range of lipophilic molecules, including bilirubin, steroids, toxins, and drugs. These enzymes mediate glucuronidation, a phase II metabolic pathway in which glucuronic acid is conjugated to specific targets to convert them into water-soluble metabolites that can then be eliminated from the body (11).

The UGT genes are polymorphic, and genomic processes such as variant splicing and epigenetic factors likely contribute to their diversity. As a result, the substrates catalyzed by UGT enzymes are particularly variable (26). In humans, the UGT superfamily is made up of 22 enzymes divided into 4 families, of which UGT1A is a member (27). The *UGT1A* gene locus is a cassette gene located on chromosome 2q37, where common exons 2–

5a and 5b are differentially spliced to unique first exons, resulting in the 9 functional UGT1A family members (*UGT1A1* and *UGT1A3–UGT1A10*) (28, 29). The *UGT1A1* promoter is regulated differently from other *UGT1A*s and consists of elements sensitive to various substances: xenobiotics (for example, pregnane X receptor (PXR) and constitutive androstane receptor), hydrocarbons (for example, the aryl hydrocarbon receptor), electrophilic nucleophiles and reactive oxygen species (for example, the nuclear factor 2 receptor), endobiotics, and fatty acids (such as the glucocorticoid receptor). The *UGT1A1* promoter also contains a critical Thymine-Adenine-Thymine-Adenine (TATA) box that consists of polymorphic tandem repeats, $(TA)_{58}TAA$. Several CpG islands (DNA regions rich in cytosine-guanine dinucleotide pairs) at the promoter can further alter the affinity and activity of nuclear receptors.

Whereas many UGT enzymes have overlapping glucuronidation substrates, UGT1A1 is the only enzyme that glucuronidates bilirubin, a yellow waste product produced during the catabolism of heme, a constituent of hemoglobin (30). When old or damaged red blood cells are broken down in the spleen, their hemoglobin is broken down to heme, which is then converted to bilirubin. The UGT1A1 enzyme converts this toxic, insoluble form of bilirubin (unconjugated bilirubin) to its non-toxic, soluble form (conjugated bilirubin). Since conjugated bilirubin is water-soluble, it can be dissolved in bile and eliminated with solid waste. If bilirubin is not eliminated and instead builds up to high levels (hyperbilirubinemia), it can cause a yellowish discoloration of the skin and eyes, commonly known as jaundice.

Over 150 genetic variants in the *UGT1A1* gene have been reported (11, 30, 31). Of these, the available evidence indicates that 5 polymorphic variants are of clinical importance to UGT1A1 activity (*UGT1A1*6*, *UGT1A1*27*, *UGT1A1*28*, *UGT1A1*36*, *UGT1A1*37*); 3 of these variants affect the tandem repeat of the TATA box ($(TA)_5TAA$ – *UGT1A1*36*, $(TA)_7TAA$ – *UGT1A1*28*, $(TA)_8TAA$ – *UGT1A1*37* (4)). The wild-type allele is called *UGT1A1*1,* which is associated with normal enzyme activity and the reference TATA box tandem repeat length ($(TA)_6TAA$) (Table 2).

As with all genetic variation, specific alleles or haplotypes can vary in frequency across populations based on genetic ancestry and any history of evolutionary migration or bottleneck. To characterize the range of genetic variation in different populations, studies have used a mix of ethnic, racial, and geographic descriptors to group individuals with assumed common ancestry and shared genetic traits. Those descriptors are used interchangeably below, based on the cited literature; however, the goal is to reflect a shared genetic background arising from common ancestry.

There are multiple genetic variations in the *UGT1A1* locus that reduce UGT1A1 enzyme activity and can lead to jaundice in the absence of exogenous substances, such as belinostat. The jaundice may be mild, as seen in Gilbert syndrome, or severe, as observed in Crigler-Najjar syndrome. (12)

The most common variant *UGT1A1* allele is *UGT1A1*28*, which is commonly found in individuals of African descent ("African Americans"; 0.42–0.45 allele frequency, or 17–20% frequency of homozygosity in the population), European descent ("Caucasians"; 0.26–0.31 allele frequency, or 6–9% homozygosity), and in Western and South Asian populations (0.26–0.33 allele frequency, or 6–10% homozygosity). In contrast, it is less common in East and Southeast Asian populations (0.09–0.16 allele frequency, or 0.8–2.5% homozygosity) (32, 33, 34). Within European-and African-descended American populations, the *UGT1A1*28* variant is a common cause of Gilbert syndrome (32, 35). The *UGT1A1*28* [$(TA)_7TAA$] variant contains an extra thymine-adenine (TA) repeat within the TATA box promoter region (7 TA repeats as opposed to 6 in the wild-type allele) (36). This extra TA repeat reduces the rate of transcription initiation of the *UGT1A1* gene, leading to decreased enzyme activity and bilirubin glucuronidation (37). Evidence indicates that one copy of the *UGT1A1*28* allele results in an approximately 35% decrease in transcriptional activity, and 2 copies (**28/*28*, homozygous) yield an approximate 70% decrease (38, 39).

Another variant allele, *UGT1A1*37* [$(TA)_8TAA$], has 8 TA repeats at the TATA box site, and results in reduced promoter activity to levels lower than the *UGT1A1*28* allele. In contrast, the *UGT1A1*36* [$(TA)_5TAA$] allele only has 5 repeats and is associated with increased promoter activity and a reduced risk of neonatal hyperbilirubinemia (a common and typically benign condition). The *UGT1A1*36* and *UGT1A1*37* alleles occur almost exclusively in populations of African origin, with estimated allele frequencies across African-descended populations of 0.07 for **36* (TA_5) and 0.05 for **37* (TA_8) (gnomAD browser version 3.1.2, accessed 27 April 2023) (40). By comparison, the average frequency of these alleles across all populations in gnomAD is 0.01–0.02. The *UGT1A1*80 allele* exhibits almost complete linkage disequilibrium with both *UGT1A1*28* and **37* and can be considered as a surrogate marker for these alleles (4).

Other promoter variants have been reported in the phenobarbital-responsive enhancer module of the *UGT1A* locus. A thymine (T) to guanine (G) substitution, known as *UGT1A1*60*, results in decreased transcription and is found more often in individuals with mild hyperbilirubinemia (41). However, other studies indicate no significant difference in total bilirubin concentration between individuals homozygous for *UGT1A1*60* versus wild-type homozygotes (42). The *UGT1A1*60* allele has been observed more frequently in individuals of African compared to European descent (43). It's worth noting that the *UGT1A1*28* and **60* alleles are reported to be in linkage disequilibrium in multiple ethnic groups (43, 44). This means an individual with a higher number of TA repeats in the promoter (*UGT1A1*28*) is also likely to have the T to G substitution (*UGT1A1*60*) in the phenobarbital-responsive enhancer module region. As a result, discerning the individual contribution of these variants to total enzyme activity *in vivo* can be difficult. The *UGT1A1*60* allele has a reported frequency of 0.47 in individuals of European descent and 0.85 in Americans of African descent (43).

Another variant allele, *UGT1A1*6*, is more prevalent in East Asian populations, with allele frequencies ranging from 0.10–0.30 in Taiwanese, Chinese, Korean, and Japanese populations (34, 38, 45, 46). Conversely, the *UGT1A1*6* allele is less common in Southeastern and Southern Asian populations, with frequencies ranging from 0.027–0.12 in Thai, Malay, Indonesian, Vietnamese, and Indian population studies (34). This missense variant results in a glycine to arginine amino acid change at position 71 (p.Arg71Gly), and individuals who are homozygous for this allele have reduced UGT1A1 enzyme activity, which can cause Gilbert syndrome and prolonged neonatal jaundice (47, 48, 49, 50).

The *UGT1A1*27* (p.Pro229Gln) variant is in exon 1 and has a minor allele frequency between 0.00011–0.0030 in individuals with Asian ancestry. This allele is associated with Gilbert syndrome, post-irinotecan hyperbilirubinemia, and severe or life-threatening leukopenia or diarrhea during irinotecan therapy (51, 52). The allele is also associated with a significant decrease in UGT1A1 substrate binding and catalytic activity (53).

Table 2: Relative Enzymatic Activity of UGT1A1 Variants

Allele name	Variant	Relative activity	Potential impact on drug metabolism	CPIC functional status[e]
*UGT1A1*1*	None (Promoter $[TA]_6TAA$)	100%[a]	Normal	Normal function
*UGT1A1*6*	p.Arg71Gly	70%[b]	Slower	Decreased function
*UGT1A1*27*	p.Pro229Gln	50% [c]	Slower	Decreased function
*UGT1A1*28*	Promoter $[TA]_7TAA$	65%[a]	Slower	Decreased function
*UGT1A1*36*	Promoter $[TA]_5TAA$	130%[a]	Faster	Increased function
*UGT1A1*37*	Promoter $[TA]_8TAA$	50%[a]	Slower	Decreased function

Table 2 continued from previous page.

Allele name	Variant	Relative activity	Potential impact on drug metabolism	CPIC functional status[e]
*UGT1A1*60*	c.-3279T>G	60%[d]	Slower	Normal function[f]

CPIC - Clinical Pharmacogenetics Implementation Consortium
[a] Activity level from (32)
[b] Activity level from (48)
[c] Activity level from (53)
[d] Activity level from (41)
[e] Functional status from (54)
[f] Functional status from (55)

Other genes of note:

Variation at the *NR1I2, ABCB1,* and *SLCO1B1* loci has been associated with altered atazanavir pharmacokinetics (56, 57, 58, 59). The PXR protein, encoded by *NR1I2*, controls the expression of several genes involved in drug transport and metabolism, while P-glycoprotein (also known as multi-drug resistant protein and encoded by *ABCB1*) and OATP1B1 (encoded by *SLCO1B1*) are membrane transport proteins that may facilitate the movement of atazanavir (8). Multiple studies have associated the C to T variation at rs2472677 in the *NR1I2* locus with faster atazanavir clearance (57, 58, 60). One study reported that individuals with at least 2 variants in any of these 3 loci (NG_011856.1:g.24087C>T at rs2472677 in *NR1I2*, NG_011513.1:g.208920T>C at rs1045642 in *ABCB1,* and NM_006446.5:c.521T>C at rs4149056 in *SLCO1B1)* maintained a plasma concentration of atazanavir above 150 ng/mL when the unboosted medication was administered at 200 mg twice daily, rather than 400 mg once daily (56). Variants in these drug transport proteins (or overall reduced expression of multiple transporters) can impact clearance of atazanavir, though no official guidance has been issued to suggest alternative dosing schedules based on these genotypes. Additionally, one small study reported an association of a variant in intron 1 of *SORCS2* (rs73208473, NM_001348945.2:c.3645T>G) with decreased plasma concentration and exposure to atazanavir; potentially via miRNA4798 and expression regulation of *NR1I2* mRNA (61).

Genetic variations at the *CYP3A4* and *5* loci have also been studied for their potential impact on atazanavir pharmacokinetics and clinical outcomes. Studies have reported that CYP3A5 expression is associated with altered metabolism of atazanavir (62) and decreased clearance rates (13). Individuals with 2 no-function alleles of *CYP3A5 (*such as *CYP3A5*3, *6,* or **7*) have slower oral clearance of unboosted atazanavir compared to those with at least one normal-function allele (8). Similarly, in a study of a Thai population, variation in *CYP3A5* was associated with decreased clearance of ritonavir-boosted atazanavir (63). Increased total bilirubin levels were also associated with a variant (rs4253728, NM_001393941.1:c.209-1003G>A) in the *PPARA* locus, potentially due to the role of peroxisome proliferation-activated receptor alpha as a CYP3A trans-acting factor (64).

Linking Gene Variation with Treatment Response: *UGT1A1*

Decreased UGT1A1 enzymatic activity, including decreases in activity observed in Gilbert syndrome, is associated with an increased risk of atazanavir discontinuation due to hyperbilirubinemia (65, 66, 67). Individuals who have 2 decreased-function alleles (such as *UGT1A1*28* or **37*) are at greatest risk for jaundice (4, 68, 69, 70). Significant hyperbilirubinemia (>85 micromol/L) was found in association with a haplotype involving multiple variants at the *UGT1A* locus affecting 3 members of the UGT1A family (71, 72). Some studies have reported that the correlation between *UGT1A1* decreased-function alleles and atazanavir discontinuation is stronger in individuals with lower skin melanin content (Whites), possibly due to jaundice being more noticeable in these individuals (72, 73).

Genetic Testing

The NIH Genetic Testing Registry (GTR) has tests for atazanavir response and UGT1A1 genetic variation. Variants impacting UGT1A1 enzyme activity affect both the coding sequence as well as the promoter region of the *UGT1A* locus. Genotyping for different lengths of the TA promoter requires a high degree of precision, particularly given the multiple variant alleles reported for that position. It is therefore important to consider the testing methodology when selecting a genetic test or reviewing testing results.

The *UGT1A1*28* allele has been reported to be in near complete linkage disequilibrium with the **80* allele; however, the *UGT1A1*80* variant itself is not known to influence *UGT1A1* expression (74). Instead, *UGT1A1*80* has been suggested to serve as a proxy for **28* identification in some genotyping assays (4, 74, 75).

Additionally, *UGT1A1* genotyping may reveal variants that are associated with Gilbert syndrome or Crigler-Najjar syndrome type 1 or type 2. Additional information on these conditions is available through MedGen. While more clinical data may be needed on atazanavir metabolism, studies have shown that variants associated with Gilbert syndrome or Crigler-Najjar syndrome type 2 impact the metabolism of bilirubin and multiple exogenous substances (4, 30, 53, 76).

The *UGT1A1* Gene Interactions with Medications Used for Additional Indications

Variations in *UGT1A1* and its promoter region are associated with risks of adverse reactions for a range of medications.

● Multiple oncology medications including irinotecan, belinostat, nilotinib, and sacituzumab govitecan are metabolized by UGT1A1. Decreased UGT1A1 activity may lead to increased exposure to these medications with a higher risk of adverse reactions; adjusted dosing may be necessary based on *UGT1A1* genotype.

- Additional HIV medications, including dolutegravir, are also metabolized by UGT1A1 (77)
- A medication used for acromegalia, pegvisomant, has caused liver injury in individuals positive for *UGT1A1*28* (11)

Additional information on gene-drug interactions for *UGT1A1* are available from PharmGKB, CPIC and the FDA (search for "UGT1A1").

Therapeutic Recommendations based on Genotype

This section contains excerpted [1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA)

Contraindications

Atazanavir capsules are contraindicated… when coadministered with drugs that are highly dependent on CYP3A or UGT1A1 for clearance, and for which elevated plasma concentrations of the interacting drugs are associated with serious and/or life-threatening events […]

[1] The FDA labels specific drug formulations. In this excerpt, we have substituted the generic names for any specific drug labels. The FDA may not have labeled all formulations containing the generic drug. Where necessary, certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards. We have provided the full name of abbreviations, shown in square brackets, where necessary.

Drug interactions

Atazanavir is an inhibitor of CYP3A and UGT1A1. Coadministration of atazanavir and drugs primarily metabolized by CYP3A or UGT1A1 may result in increased plasma concentration of the other drug that could increase or prolong its therapeutic and adverse effects. […]

Atazanavir is a CYP3A4 substrate; therefore, drugs that induce CYP3A4 may decrease atazanavir plasma concentrations and reduce atazanavir's therapeutic effect.

Please review the complete therapeutic recommendations that are located here: (1)

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

For individuals carrying two UGT1A1 decreased function alleles (i.e., UGT1A1*28/*28, UGT1A1*28/*37, UGT1A1*37/*37, or rs887829 T/T), the likelihood of bilirubin-related atazanavir discontinuation is substantial. Before such individuals are prescribed atazanavir (boosted with either ritonavir or cobicistat), all such patients should be advised about the substantial likelihood of developing jaundice. Prescribing atazanavir to such individuals should generally be avoided unless the patient does not consider jaundice to be a concern, or there are other compelling reasons to prescribe atazanavir.

For individuals carrying fewer than two UGT1A1 decreased function alleles (i.e., *1/*28, *1/*37, *36/*28, *36/*37, rs887829 C/C or rs887829 C/T), the likelihood of bilirubin-related atazanavir discontinuation is low. This risk is extremely low for individuals carrying no UGT1A1 decreased function alleles (i.e., UGT1A1*1/*1, UGT1A1*1/*36, UGT1A1*36/*36, or rs887829 C/C). Among patients with extensive metabolizer UGT1A1 phenotypes it may not be necessary to discuss the possibility of jaundice with atazanavir. This decision about whether to discuss possible jaundice should be based on the clinical situation and provider judgment. If advice is offered, such discussion may note that the likelihood of developing jaundice that would require discontinuation of atazanavir is very low.

Please review the complete therapeutic recommendations that are located here: (4)

Nomenclature for Selected *UGT1A1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*1*	$(TA)_6TAA$	NM_000463.2:c.-53_-52TA[7]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*6*	211G>A Gly71Arg	NM_000463.2:c.211G>A (NM_001072.4:c.862-6536G>A)	NP_000454.1:p.Gly71Arg	rs4148323
*UGT1A1*27*	Pro229Gln	NM_000463.3:c.686C>A	NP_000454.1:p.Pro229Gln	rs35350960
*UGT1A1*28*	$(TA)_7TAA$	NM_001072.4:c.862-6800AT[8]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*36*	$(TA)_5TAA$	NM_001072.4:c.862-6800AT[6]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*37*	$(TA)_8TAA$	NM_001072.4:c.862-6800AT[9]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*60*	-3263T>G -3279T>G	NM_001072.4:c.862-10021T>G	Not applicable—variant occurs in a non-coding region	rs4124874

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (78).
UGT Allele nomenclature and definitions are available from (31)
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
TATA - Thymine-Adenine-Thymine-Adenine

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Atomoxetine Therapy and *CYP2D6* Genotype

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Introduction

Atomoxetine (brand name Strattera) is a non-stimulant drug used in the treatment of attention-deficit hyperactivity disorder (ADHD). It is a selective noradrenaline reuptake inhibitor (SNRI) and is approved for children aged 6 and older, adolescents, and adults.

Atomoxetine is part of a treatment plan for ADHD that may include other measures such as psychological, educational, and social support.

Because atomoxetine is not a stimulant or a controlled substance it may be used in individuals with tics or other side effects associated with stimulants, and in individuals who have a substance abuse problem (or have a family member with a substance abuse problem).

The most common side effects associated with atomoxetine therapy include weight loss, abdominal pain, headache, dizziness, fatigue, and irritability. In addition, atomoxetine has a boxed warning on the increased risk of suicidal ideation in children and adolescents treated with atomoxetine.

The CYP2D6 enzyme is involved in the metabolism of atomoxetine (and a quarter of all prescribed drugs). Individuals who lack CYP2D6 activity ("poor metabolizers") will have increased levels of atomoxetine and an increased risk of side effects compared with CYP2D6 normal metabolizers. Approximately 7% of Caucasians are CYP2D6 poor metabolizers.

The drug label states that for known CYP2D6 poor metabolizers, the initial dose should only be increased to the usual target dose if symptoms fail to improve after 4 weeks and the initial dose is well tolerated (1). Atomoxetine dosing should be adjusted for individuals who are CYP2D6 poor metabolizers, individuals who are taking a strong CYP2D6 inhibitor (e.g., paroxetine, fluoxetine, and quinidine), and individuals with moderate or severe hepatic impairment. However, different authorities have different dosing recommendations.

The Royal Dutch Association for the Advancement of Pharmacy (KNMP) Dutch Pharmacogenetics Working Group (DPWG) provides dosing recommendations for poor, intermediate and ultrarapid metabolizers. For poor metabolizers, DPWG recommends starting with the standard initial dose and to consult a care provider if side effects occur. If the medicine is effective, but side effects occur, DPWG recommends reducing the dose and monitoring efficacy (2).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) also provide recommendations for different CYP2D6 metabolizer phenotypes. For CYP2D6 poor metabolizers, CPIC recommends that if no clinical response is observed after 2 weeks of atomoxetine therapy, then a plasma concentration exposure check be used with an individual's *CYP2D6* genotype to help clinicians guide dose selection and titration (3).

Table 1. FDA Atomoxetine Dosing in Specific Populations: CYP2D6 Poor Metabolizers (2020)

Individual	Initial daily dose	Target total daily dose	Maximum total daily dose
Child or adolescent, up to 70 kg	0.5 mg/kg	1.2 mg/kg	1.4 mg/kg

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Table 1. continued from previous page.

Individual	Initial daily dose	Target total daily dose	Maximum total daily dose
Child or adolescent, up to 70 kg, known to be CYP2D6 PM	0.5 mg/kg	Only increase to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated	Not provided
Child or adolescent over 70 kg, or adults	40 mg	80 mg	100 mg
Child or adolescent over 70 kg, or adults, known to be CYP2D6 poor metabolizer	40 mg	Only increase to the usual target dose of 80 mg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated	Not provided

This FDA table is adapted from (1).

Table 2. DPWG Atomoxetine Dosing based on *CYP2D6* Phenotype (2016)

CYP2D6 metabolizer phenotype	Dosing recommendations
Ultrarapid	Be extra alert to reduced efficacy of the treatment Advise the individual to contact their doctor in the event of inadequate effect An alternative can be selected as a precaution. Clonidine is not metabolized by CYP2D6
Intermediate	In the event of side effects occurring and/or a response later than 9 weeks: reduce the dose and check whether the effect is conserved The plasma concentration of atomoxetine is a factor of 2-3 times higher for intermediate metabolizer than for normal metabolizer at the same dose
Poor	Start with the normal initial dose, bearing in mind that an increase in this dose probably will not be required Advise the individual to seek contact if side effects occur (such as decreased appetite, vomiting, abdominal pain, constipation, insomnia, early waking, drowsiness, irritability, pupil dilation and itching) If the medicine is effective, but side effects occur reduce the dose and check whether the effect is conserved The plasma concentration of atomoxetine is a factor of 8–11 times higher for poor metabolizer than for normal metabolizer at the same dose

This DPWG table is adapted from (2).

Table 3. CPIC Dosing Recommendations for Atomoxetine based on *CYP2D6* Genotype for Children (2019)

CYP2D6 metabolizer phenotype	Activity score	Implication	Therapeutic recommendation	Classification of Recommendation[a]
Ultrarapid	>2.25	Based on very limited data available for CYP2D6 ultrarapid metabolizers taking atomoxetine, it is unlikely ultrarapid metabolizers would achieve adequate serum concentrations for the intended effect at standard dosing	Initiate with a dose of 0.5 mg/kg/day and increase to 1.2 mg/kg/day after 3 days If no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a peak plasma concentration (1 to 2 hours after dose administered) If <200 ng/ml, consider a proportional increase in dose to approach 400 ng/ml[b,c]	Moderate

Table 3. continued from previous page.

CYP2D6 metabolizer phenotype	Activity score	Implication	Therapeutic recommendation	Classification of Recommendation[a]
Normal	1.25–2.25	Normal metabolizers of atomoxetine have a lower likelihood of response as compared with poor metabolizers. This is associated with increased discontinuation due to lack of efficacy as compared with poor metabolizers	Initiate with a dose of 0.5 mg/kg and increase to 1.2 mg/kg/day after 3 days If no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a peak plasma concentration (1 to 2 hours after dose administered) If <200 ng/ml, consider a proportional [b,c] increase in dose to approach 400 ng/ml	Moderate
Intermediate	>0–<1.25	Decreased metabolism of atomoxetine and higher atomoxetine concentrations as compared with normal metabolizers. Intermediate metabolizers may be at an increased risk of discontinuation as compared with poor metabolizers	Initiate with a dose of 0.5 mg/kg/day and if no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a plasma concentration 2-4 h after dosing. If response is inadequate and concentration is <200 ng/ml, consider a proportional dose increase to achieve a concentration to approach 400 ng/ml.[b,c] If unacceptable side effects are present at any time, consider a reduction in dose	Moderate
Poor	0	Significantly decreased metabolism of atomoxetine may result in higher concentrations as compared with non-poor metabolizers. This may increase the occurrence of side effects, but also a greater improvement of ADHD symptoms as compared with non-poor metabolizers in those who tolerate treatment Poor metabolizer status is associated with lower final dose requirements as compared with non-poor metabolizers	Initiate with a dose of 0.5 mg/kg/day and if no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a plasma concentration 4 hours after dosing If response is inadequate and concentration is <200 ng/ml, consider a proportional dose increase to achieve a concentration to approach 400 ng/ml[b,c] If unacceptable side effects are present at any time, consider a reduction in dose	Strong

AS: Activity Score

[a]Rating scheme described in the Supplement.

[b]Therapeutic range of 200 to 1000 ng/ml has been proposed.

[c]Limited data are available regarding the relationship between atomoxetine plasma concentrations and clinical response. Available information suggests that clinical response is greater in poor metabolizers (PMs) compared with non-PMs and may be related to the higher plasma concentrations 1 to 1.5 hours after dosing in PMs compared with non-PMs administered a similar dose. Furthermore, modest improvement in response, defined as reduction in ADHD rating scale (ADHD-RS), is observed at peak concentrations greater than 400 ng/ml.

This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (3).

Table 4. CPIC Dosing Recommendations for Atomoxetine based on *CYP2D6* Genotype for Adults (2019)

CYP2D6 metabolizer phenotype	Activity score	Implication	Therapeutic recommendation	Classification of Recommendation[a]
Ultrarapid	>2.25	Based on very limited data available for CYP2D6 ultrarapid metabolizers taking atomoxetine, it is unlikely ultrarapid metabolizers would achieve adequate serum concentrations for the intended effect at standard dosing	Initiate with a dose of 40 mg/day and increase to 80 mg/day after 3 days If no clinical response and in the absence of adverse events after 2 weeks, consider increasing dose to 100 mg/day If no clinical response observed after 2 weeks, consider obtaining a peak plasma concentration (1 to 2 hours after dose administered) If <200 ng/ml, consider a proportional increase in dose to approach 400 ng/ml[b,c] Dosages greater than 100 mg/day may be needed to achieve target concentrations[d]	Moderate
Normal	1.25–2.25	Normal metabolizers of atomoxetine have a lower likelihood of response as compared with poor metabolizers. This is associated with increased discontinuation due to lack of efficacy as compared with poor metabolizers	Initiate with a dose of 40 mg/day and increase to 80 mg/day after 3 days If no clinical response and in the absence of adverse events after 2 weeks, consider increasing dose to 100 mg/day If no clinical response observed after 2 weeks, consider obtaining a peak plasma concentration (1 to 2 hours after dose administered) If <200 ng/ml, consider a proportional increase in dose to approach 400 ng/ml[b,c] Dosages greater than 100 mg/day may be needed to achieve target concentrations[d]	Moderate
Intermediate	>0–<1.25	Decreased metabolism of atomoxetine higher atomoxetine concentrations as compared with normal metabolizers. Intermediate metabolizers may be at an increased risk of discontinuation as compared with poor metabolizers	Initiate with a dose of 40 mg/day and if no clinical response and in the absence of adverse events after 2 weeks increase dose to 80 mg/day If response is inadequate after 2 weeks consider obtaining a plasma concentration 2–4 h after dosing If concentration is <200 ng/ml, consider a proportional dose increase to achieve a concentration to approach 400 ng/ml[b,c] If unacceptable side effects are present at any time, consider a reduction in dose	Moderate

Table 4. continued from previous page.

CYP2D6 metabolizer phenotype	Activity score	Implication	Therapeutic recommendation	Classification of Recommendation[a]
Poor	0	Significantly decreased metabolism of atomoxetine may result in higher concentrations as compared with non-poor metabolizers. This may increase the occurrence of treatment-emergent side effects, but also a greater improvement of ADHD symptoms as compared with non-poor metabolizers in those who tolerate treatment. Poor metabolizer status is associated with lower final dose requirements as compared with non-poor metabolizers	Initiate with a dose of 40 mg/day and if no clinical response and in the absence of adverse events after 2 weeks increase dose to 80 mg/day If response is inadequate after 2 weeks consider obtaining a plasma concentration 2–4 h after dosing. If concentration is <200 ng/ml, consider a proportional dose increase to achieve a concentration to approach 400 ng/ml[b,c] If unacceptable side effects are present at any time, consider a reduction in dose	Moderate

AS: Activity score.
[a] Rating scheme described in the Supplement.
[b]Therapeutic range of 200 to 1000 ng/ml has been proposed (27).
[c]Limited data are available regarding the relationship between atomoxetine plasma concentrations and clinical response. Available information suggests that clinical response is greater in poor metabolizers (PMs) compared with non-PMs and may be related to the higher plasma concentrations 1 to 1.5 hours after dosing in PMs compared with non-PMs administered a similar dose. Furthermore, modest improvement in response, defined as reduction in ADHD rating scale (ADHD-RS), is observed at peak concentrations greater than 400 ng/ml.
[d]Doses above 120 mg/day have not been evaluated.
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (3).

Drug: Atomoxetine

Atomoxetine is a selective norepinephrine reuptake inhibitor (SNRI) that is used to treat ADHD. Atomoxetine is thought to exert its therapeutic effect by increasing the concentration of synaptic norepinephrine. Unlike several other ADHD medications, atomoxetine is not a stimulant and is not a controlled substance. Therefore, atomoxetine may be used as an alternative to stimulants for individuals who have a substance abuse problem (or have a family member with a substance abuse problem), tics, or other side effects with stimulants (4).

ADHD is one of the most common neurodevelopmental childhood disorders. In the US, approximately 9.4% of children (6.1 million) have been diagnosed with ADHD, according to a 2016 parental survey (5).

The symptoms of ADHD include difficulty focusing and paying attention, difficulty controlling behavior, and hyperactivity. Symptoms may continue into adulthood. Atomoxetine may be used alone or in combination with behavioral treatment, as an adjunct to psychological, educational, social, and other remedial measures (6).

Atomoxetine is primarily metabolized via the CYP2D6 enzyme. The main metabolite, 4-hydroxyatomoxetine, is equipotent to atomoxetine as an inhibitor of the norepinephrine transporter; however, this metabolite is rapidly glucuronidated and is found at much lower concentrations in the plasma (7). In individuals who lack CYP2D6 activity, 4-hydroxyatomoxetine is formed by other CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4), but at a much slower rate (1).

CYP2C19, along with other CYP enzymes, forms the metabolite N-desmethylatomoxetine. Although this metabolite has substantially less pharmacological activity compared with atomoxetine, and is present at much lower plasma concentrations, one study found that genetic variants in the *CYP2C19* gene also influenced the pharmacokinetics of atomoxetine (7, 8).

Atomoxetine has a wide therapeutic window, but the risk of adverse effects may be increased among individuals who have variant *CYP2D6* alleles (9-11). Common adverse effects of atomoxetine therapy include a lack of response, weight loss, headache, and irritability. Psychiatric side effects may also occur; these include anxiety, depression, and the FDA-approved drug label for atomoxetine includes a boxed warning on the increased risk of suicidal ideation in children and adolescents treated with atomoxetine. Atomoxetine has not been adequately studied in pregnant women, therefore, pregnant or nursing women should not use atomoxetine unless the potential benefit justifies the potential risk to fetus or infant.

Unlike the stimulant drugs used to treat ADHD, atomoxetine has a delayed onset, and this should be taken into account during dose titration. An initial response may appear after one week, but typically, it takes 2–4 weeks for the full effect of atomoxetine on symptoms to be observed (3, 12).

Factors to be considered in determining the dose of atomoxetine include *CYP2D6* genetic variation (see below) and the child's weight. For children and adolescents who weigh less than or exactly 70 kg, the recommended starting dose is 0.5 mg/kg, which is then titrated upwards after a minimum of 3 days, to a target dose of 1.2 mg/kg. The maximum daily dose should not exceed 1.4 mg/kg or 100 mg (whichever is less). For adults, children and adolescents who weigh more than 70 kg, the recommended starting dose is 40mg, the target dose is 80mg, and the maximum daily dose is 100mg (Table 1).

The drug label states the dosage of atomoxetine should be adjusted in individuals who are receiving drugs that inhibit CYP2C26 (e.g., paroxetine, fluoxetine, quinidine); or in individuals who lack CYP2D6 activity because they have 2 non-functional copies of the *CYP2D6* gene ("CYP2D6 poor metabolizers"). For these individuals, the initial doses are the same (0.5mg/Kg or 40mg, based on body weight), but the dose should only be increased to the standard target dose if the initial dose is well tolerated and symptoms fail to improve after 4 weeks (1).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are often very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is involved in the hepatic metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. Importantly, CYP2D6 is also the main enzyme that metabolizes atomoxetine.

The *CYP2D6* gene on chromosome 22q13.2 is highly polymorphic. Over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is annotated with either normal, decreased or absent enzyme function (when functional status is known) (Table 5). The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., *CYP2D6* *4/*4), which subsequently is used to assign a phenotype (e.g., CYP2D6 poor metabolizer).

The *CYP2D6*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. Other *CYP2D6* alleles considered to have normal activity include *2, *33, and *35.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a non-functional enzyme include **3*, **4*, **5*, and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (13).

Table 5. Activity Status of selected *CYP2D6* Alleles

Effect on enzyme activity	*CYP2D6* alleles
Normal function	**1, *2, *33, *35, *45, *46*
Reduced function	**9, *10, *17, *29, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *36, *38, *40, *42*

For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

The *CYP2D6*10* variant is one of the most well studied decreased function alleles, in part due to its impact on atomoxetine therapy, and the breast cancer drug, tamoxifen. Individuals who have one or 2 copies of *CYP2D6*10* have lower than expected CYP2D6 activity and higher plasma levels of atomoxetine (and tamoxifen). This has led to CPIC making special dosing recommendations for individuals with diplotypes that contain *CYP2D6**10 for both atomoxetine (3) and tamoxifen (14). In addition, a *CYP2D6* Genotype-to-Phenotype Working Group recently revised the activity score of *CYP2D6**10 from 0.5 to 0.25 (15-18).

CYP2D6 Phenotype

Most individuals, around 70–80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41). Individuals who have more than 2 normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1 to 10% of individuals (1).

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one no function allele (e.g., *4/*41) or poor metabolizers (2 no function alleles, e.g., *4/*4). The translation of *CYP2D6* diplotype to phenotype based on the activity score system was recently reported by the CPIC and DPWG (Table 6) (18).

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalence of no function **4* and **5* alleles. Compared to Europeans, individuals of Asian descent are more likely to be intermediate metabolizers due to the prevalence of decreased function alleles, most notably *10. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are more likely to be intermediate metabolizers than Europeans because of the prevalence of a wide range of decreased function variants (13, 19-21).

Table 6. CPIC Assignment of likely *CYP2D6* Phenotype based on Diplotype (2019)

Likely CYP2D6 metabolizer phenotype[b]	Activity score	Genotype[a]	Examples of *CYP2D6* diplotype
Ultrarapid	>2.25	An individual carrying duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]
Normal	1.25 to 2.25	An individual carrying 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2, *1/*10
Intermediate	>0 to <1.25	An individual carrying one decreased function and one no function allele	*1/*4, *1/*5, *41/*41, *4/*10, *4/*41, *5/*9

Table 6. continued from previous page.

Likely CYP2D6 metabolizer phenotype[b]	Activity score	Genotype[a]	Examples of *CYP2D6* diplotype
Poor	0	An individual carrying only no functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] Assignment of allele function and citations for allele function can be found on PharmGKB: Gene Reference Materials for CYP2D6 (*CYP2D6* Allele Definition Table and *CYP2D6* Allele Functionality Table). For a complete list of *CYP2D6* diplotypes and resulting phenotypes, see the *CYP2D6* Genotype to Phenotype Table. Note that genotypes with an activity score of one are classified as normal metabolizers in the online *CYP2D6* genotype to phenotype table (22).
[b] See the *CYP2D6* Frequency Table for race-specific allele and phenotype frequencies (22) or see Gaedigk *et al* (23).
[c] Where xN represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see supplemental data for additional information on how to translate diplotype into phenotype.
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (3).

Linking *CYP2D6* Genetic Variation with the Risk of Side Effects and Treatment Response

Genetic variation in the *CYP2D6* gene has a major effect on atomoxetine pharmacokinetics. According to the FDA-approved drug label, for a given dose of atomoxetine, CYP2D6 poor metabolizers have a 10-fold higher area under the curve (AUC; a measure of exposure to a drug) and a 5-fold higher peak concentration compared with CYP2D6 normal metabolizers.

The increased exposure to atomoxetine in CYP2D6 poor metabolizers may lead to a higher rate of some adverse effects of atomoxetine therapy. The drug label cites a clinical trial where the frequency of different side effects is often higher in poor metabolizers compared with normal metabolizers (1, 10). However, some studies also report that the higher exposure to atomoxetine in CYP2D6 poor metabolizers may be linked to a greater improvement of ADHD symptoms (9, 24).

Several studies report that atomoxetine dosing based on *CYP2D6* genotype may lead to improved therapeutic outcomes (7, 25-28). Accordingly, the drug label provides dose adjustments for CYP2D6 poor metabolizers and states that laboratory tests are available to identify CYP2D6 poor metabolizers (1).

The drug label is silent on dose adjustments for individuals who have increased CYP2D6 activity ("CYP2D6 ultrarapid metabolizers"). These individuals may have a poor response to standard doses of atomoxetine because of lower plasma concentrations (1).

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are currently available for atomoxetine response and for the *CYP2D6* gene.

CYP2D6 is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, and pseudogenes. The complexity of genetic variation complicates making a correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (14).

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications are denoted by "xN" e.g., *CYP2D6**1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6* *1/*1, normal metabolizer", this may be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 6).

The *CYP2D6* phenotype is defined by the sum of the 2 activity scores, which is usually in the range of 0 to 3.0:

- An ultrarapid metabolizer has an activity score greater than 2.25
- A normal metabolizer phenotype has an activity score of 1.25 to 2.25
- An intermediate metabolizer has an activity score of >0 to 1.25
- A poor metabolizer has an activity score of 0 (14)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

2.4 Dosing in Specific Populations

Dosing adjustment for use with a strong CYP2D6 inhibitor or in patients who are known to be CYP2D6 PMs — In children and adolescents up to 70 kg body weight administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, or in patients who are known to be CYP2D6 PMs, atomoxetine capsules should be initiated at 0.5 mg/kg/day and only increased to the usual target dose of 1.2 mg/kg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated.

In children and adolescents over 70 kg body weight and adults administered strong CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine, atomoxetine capsules should be initiated at 40 mg/day and only increased to the usual target dose of 80 mg/day if symptoms fail to improve after 4 weeks and the initial dose is well tolerated.

[...]

5.12 Laboratory Tests

CYP2D6 metabolism — Poor metabolizers (PMs) of CYP2D6 have a 10 fold higher AUC and a 5 fold higher peak concentration to a given dose of atomoxetine hydrochloride compared with normal metabolizers. Approximately 7% of a Caucasian population are PMs. Laboratory tests are available to identify CYP2D6 PMs. The blood levels in PMs are similar to those attained by taking strong inhibitors of CYP2D6. The higher blood levels in PMs lead to a higher rate of some adverse effects of atomoxetine hydrochloride.

6.1 Clinical Trials Experience

[…]

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

The following adverse reactions occurred in at least 2% of child and adolescent CYP2D6 PM patients and were statistically significantly more frequent in PM patients compared with CYP2D6 normal metabolizer (EM) patients: insomnia (11% of PMs, 6% of EMs); weight decreased (7% of PMs, 4% of EMs); constipation (7% of PMs,4% of EMs); depression (7% of PMs,4% of EMs); tremor(5% of PMs,1% of EMs); excoriation

(4% of PMs, 2% of EMs); middle insomnia (3% of PMs, 1% of EMs); conjunctivitis (3% of PMs, 1% of EMs); syncope (3% of PMs, 1% of EMs); early morning awakening (2% of PMs, 1% of EMs); mydriasis (2% of PMs, 1% of EMs); sedation (4% of PMs, 2% of EMs).

7.2 Effect of CYP2D6 Inhibitors on Atomoxetine

In normal metabolizers, inhibitors of CYP2D6 (e.g., paroxetine, fluoxetine, and quinidine) increase atomoxetine steady-state plasma concentrations to exposures similar to those observed in poor metabolizers (PMs). In EM individuals treated with paroxetine or fluoxetine, the AUC of atomoxetine is approximately 6 to 8 fold and $C_{ss, max}$ is about 3 to 4 fold greater than atomoxetine alone.

In vitro studies suggest that coadministration of cytochrome P450 inhibitors to PMs will not increase the plasma concentrations of atomoxetine.

12.3 Pharmacokinetics

Atomoxetine is well-absorbed after oral administration and is minimally affected by food. It is

eliminated primarily by oxidative metabolism through the cytochrome P450 2D6 (CYP2D6) enzymatic pathway and subsequent glucuronidation. Atomoxetine has a half-life of about 5 hours. A fraction of the population (about 7% of Caucasians and 2% of African Americans) are poor metabolizers (PMs) of CYP2D6 metabolized drugs. These individuals have reduced activity in this pathway resulting in 10 fold higher AUCs, 5 fold higher peak plasma concentrations, and slower elimination (plasma half-life of about 24 hours) of atomoxetine compared with people with normal activity (normal metabolizers). Drugs that inhibit CYP2D6, such as fluoxetine, paroxetine, and quinidine, cause similar increases in exposure.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 Poor Metabolizer

The genetic variation increases the plasma concentration of atomoxetine and thereby the risk of side effects.

Recommendation:

1. start with the normal initial dose, bearing in mind that an increase in this dose probably will not be required
2. advise the patient to seek contact if side effects occur (such as decreased appetite, vomiting, abdominal pain, constipation, insomnia, early waking, drowsiness, irritability, pupil dilation and itching)
3. if the medicine is effective, but side effects occur: reduce the dose and check whether the effect is conserved

   The plasma concentration of atomoxetine is a factor of 8-11 times higher for PM than for EM at the same dose.

CYP2D6 Intermediate Metabolizer

The genetic variation increases the plasma concentration of atomoxetine and can thereby reduce the dose requirement.

Recommendation:

1 in the event of side effects occurring and/or a response later than 9 weeks: reduce the dose and check whether the effect is conserved

   The plasma concentration of atomoxetine is a factor of 2-3 times higher for IM than for EM at the same dose.

CYP2D6 Ultrarapid Metabolizer

The genetic variation results in an increased conversion of atomoxetine to the active metabolite 4-hydroxyatomoxetine, which has a much lower plasma concentration. As the plasma concentration of the active ingredients decreases as a result, this gene variation can result in reduced efficacy.

Recommendation:

1. be extra alert to reduced efficacy of the treatment
2. advise the patient to contact their doctor in the event of inadequate effect
3. an alternative can be selected as a precaution. Clonidine is not metabolised by CYP2D6.

Please review the complete therapeutic recommendations that are located here: (2).

2019 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Tables 3 and 4 summarize the therapeutic recommendations for atomoxetine based on CYP2D6 phenotype in children and adults, respectively. Although not routinely ordered, patients may benefit from a single time point atomoxetine exposure check to guide therapy. Exposure check concentrations between 200 – 1000 ng/mL are generally considered to be "therapeutic", however for individuals with comorbidities a higher exposure target may be warranted, as was done in a study evaluating children with both ADHD and Oppositional Defiant Disorder. We propose that the plasma concentration exposure check be used with an individual's CYP2D6 genotype to help clinicians guide dose selection and titration as discussed below. Based on pharmacokinetic knowledge that CYP2D6 metabolism phenotypes influence atomoxetine peak concentration and half-life, Tables 3 and 4 propose that prescribers consider measuring peak concentrations 1 to 2 hours after dosing in known CYP2D6 UMs, NMs and IMs with high activity (activity score 1.0 without a CYP2D6*10 allele), 2 to 4 hours after dosing in CYP2D6 IMs with low activity (activity score 0.5) and in individuals with AS of 1 when the CYP2D6*10 allele is present, and 4 hours after dosing in PMs.

Very limited data exist for CYP2D6 UMs taking atomoxetine, but it is unlikely these individuals would achieve adequate serum concentrations with standard atomoxetine dosing. As discussed above, CYP2D6 non-PMs have a lower likelihood of treatment response as compared to CYP2D6 PMs. Thus, for CYP2D6 UMs and NMs, recommendations are to initiate standard atomoxetine dosing (see Table 3 and 4 for pediatric and adult dosing, respectively) and if no clinical response is observed after two weeks, consider obtaining a peak plasma concentration one to two hours after dose administration. If the peak concentration is less than 200 ng/ml, consider increasing the dose proportionally to approach 400 ng/ml. It is important to note that doses above 120 mg have not been extensively evaluated, although they may be necessary to achieve target concentrations in some patients. While CYP2D6 NMs with activity scores of 1 (without the presence of the CYP2D6*10 allele) have higher atomoxetine plasma concentrations compared to NMs with an AS of 2, the clinical significance of this difference is unclear. Thus, CYP2D6 NMs with an AS of 1 (without the presence of the CYP2D6*10 allele) should be treated similarly to CYP2D6 NMs with AS of 2.

CYP2D6 PMs, IMs, and NMs with an AS of 1 in the presence of the CYP2D6*10 allele have significantly decreased metabolism of atomoxetine, which may increase the risk of side effects. However, these individuals may also have greater improvement of ADHD symptoms and lower dose requirements as compared to non-PMs. Therefore, the recommendation for these phenotype groups are to initiate with a standard starting dose (see Table 3 and 4 for pediatric and adult dosing, respectively) and if there is an inadequate trajectory of symptom improvement after 2 weeks (in the absence of side effects), consider obtaining a plasma concentration two-four hours after dosing. If response is inadequate and side effects are not present, consider adjusting the dose proportionally to approach 400 ng/ml.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major variant	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A 4180G>C	NM_000106.5:c.506-1G>A NM_000106.5:c.1457G>C	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 delT Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least 2 functional variants: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

Note: In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and the variant 988G>A occurs with 2850C>T. (Cys296Arg).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (29).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for *CYP2D6* alleles and other cytochrome P450 genes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Azathioprine Therapy and *TPMT* and *NUDT15* Genotype

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Introduction

Azathioprine (brand names Imuran, Azasan) is an immunosuppressant that belongs to the drug class of thiopurines. It is used with other drugs to prevent kidney transplant rejection and to manage autoimmune and inflammatory conditions such as systemic lupus erythematosus, inflammatory bowel disease, systemic vasculitis, and rheumatoid arthritis.

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. The active metabolites are metabolized and inactivated by the enzyme thiopurine methyltransferase (TPMT) and the enzyme nudix hydrolase 15 (NUDT15). Thus, individuals with reduced activity of either enzyme are exposed to higher levels of thioguanine and have a higher risk of toxicity side effects, including severe bone marrow suppression (myelosuppression).

The FDA-approved drug label states that testing for TPMT and NUDT15 deficiency should be considered in individuals who experience severe bone marrow toxicities or repeated episodes of myelosuppression. The FDA recommends considering an alternative therapy for individuals who are known to have homozygous TPMT or NUDT15 deficiency, or both, and to reduce dosages for individuals who have a no function allele, cautioning that a more substantial dose reduction may be required for individuals who are either TPMT or NUDT15 poor metabolizers (Table 1) (1).

Dosing recommendations for thioguanine based on *TPMT* and *NUDT15* genotype have also been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 2, Table 3) and the Dutch Pharmacogenetics Working Group (DPWG). Both the CPIC and DPWG guidelines recommend specific dose reductions for individuals who have low or deficient enzyme activity, including starting dose and more information on how and when to adjust the dose e.g., the time allowed to reach steady state after each dose adjustment (2-4).

Table 1. FDA Drug Label Dosage and Administration of Azathioprine (2020)

Enzyme	Dosage and administration
TPMT NUDT15	• Individuals with thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency may be at an increased risk of severe and life-threatening myelotoxicity if receiving conventional doses of azathioprine. • Death associated with pancytopenia has been reported in individuals with absent TPMT activity receiving azathioprine. • In individuals with severe myelosuppression, consider evaluation for TPMT and NUDT15 deficiency. • Consider alternative therapy in individuals with homozygous* TPMT or NUDT15 deficiency and reduced dosages in individuals with heterozygous deficiency.

* This also applies to compound heterozygous TPMT or NUDT15 deficiency, as multiple no function alleles exist. See Tables 4 and 5. This FDA table is adapted (1).

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Table 2. CPIC Recommended Dosing of Azathioprine by TPMT Phenotype (2018 Update)

Phenotype	Implications for azathioprine phenotypic measures	Dosing recommendations for azathioprine	Classification of recommendations[b]
TPMT normal metabolizer	Lower concentrations of TGN metabolites, higher MeTIMP, this is the "normal" pattern. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with the normal starting dose[a] (e.g., 2–3 mg/kg/day) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.	Strong
TPMT intermediate metabolizer OR TPMT possible intermediate metabolizer	Moderate to high concentrations of TGN metabolites; low concentrations of MeTIMP. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] is 2–3 mg/kg/ day (e.g., 0.6–2.4 mg/kg/day), and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after ≥ 40–60 mg/m^2/day (e.g., 20–48 mg/m^2/day) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment.	Strong
TPMT poor metabolizer	Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease; no MeTIMP metabolites. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and dose 3 times weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment.	Strong

MeTIMP, metabolites of thiopurine methyltransferase; TGN, thioguanine nucleotides; TPMT, thiopurine methyltransferase.
[a]Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, a dose reduction might not be recommended for intermediate metabolizers.
[b] Rating scheme described in Supplemental Material (2).
This CPIC table is adapted from (2).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both TPMT and NUDT15 is known. Please see (2).

Table 3. CPIC Recommended Dosing of Azathioprine by NUDT15 Phenotype (2018 Update)

Phenotype	Implications for azathioprine phenotypic measures	Dosing recommendations for azathioprine	Classification of recommendations[b]
NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with the normal starting dose[a] (e.g., 2–3 mg/kg/day) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.	Strong

Table 3. continued from previous page.

Phenotype	Implications for azathioprine phenotypic measures	Dosing recommendations for azathioprine	Classification of recommendations[b]
NUDT15 intermediate metabolizer OR NUDT15 possible intermediate metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] is 2–3 mg/kg/day (e.g., 0.6–2.4 mg/kg/day), and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment.	Strong
NUDT15 poor metabolizer	Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses[a] (reduce daily dose by 10-fold) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment.	Strong

[a] Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.
[b] Rating scheme described in Supplemental Material.
This CPIC table is adapted from (2).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both thiopurine methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15) is known. Please see (2).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine).

All 3 agents have similar effects but are typically used for different indications. Thioguanine is most commonly used to treat myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

There is increasing evidence that DNA testing for *NUDT15* and *TPMT* before initiating thiopurine therapy would be clinically useful. In Europeans and Africans, inherited TPMT deficiency is the primary genetic cause of thiopurine intolerance, whereas for Asians, risk alleles in *NUDT15* explains most thiopurine-related myelosuppression (5, 6).

Drug: Azathioprine

Azathioprine is an immunosuppressive agent used with other drugs to prevent rejection of kidney transplants. It is also used to manage autoimmune and other inflammatory conditions including active rheumatoid arthritis, (1) systemic lupus erythematosus, vasculitis, and inflammatory bowel disease (IBD).

Azathioprine is a slow-acting drug and for IBD, it typically takes at least 3 months of therapy before a therapeutic effect is observed. Therefore, azathioprine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses. Because the discontinuation of azathioprine is

associated with a high rate of relapse of IBD, azathioprine is usually continued long-term if there are no adverse effects (7-9).

The use of azathioprine or the related drug mercaptopurine has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (10, 11).

The increased risk of malignancy led to the following boxed label on the FDA-approved drug label for azathioprine:

Malignancy: Patients receiving immunosuppressants, including azathioprine, are at increased risk of developing lymphoma and other malignancies, particularly of the skin. Physicians should inform patients of the risk of malignancy with azathioprine. As usual for patients with increased risk for skin cancer, exposure to sunlight and ultraviolet light should be limited by wearing protective clothing and using a sunscreen with a high protection factor (1).

Like all thiopurines, azathioprine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Azathioprine is first metabolized to mercaptopurine (6-MP), which is then bioactivated via hypoxanthine phosphoribosyltransferase. This is followed by a series of reactions to form TGNs, which are the major active metabolites. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of 6-MP occurs via 2 major pathways: via methylation, which is catalyzed by TPMT, and via oxidation, which is catalyzed by xanthine oxidase (XO). In cells that have negligible XO levels (e.g., red blood cells), the TPMT activity is inversely correlated with TGN levels. And in individuals who take medicines that inhibit XO (e.g., allopurinol, used to manage gout), the level of azathioprine and its active metabolites may increase to a toxic level.

The NUDT15 enzyme also impacts TGN levels –– this enzyme is involved in the conversion of the active metabolites (TGNs) to inactive phosphorylated metabolites (1).

One of the most frequent adverse reactions to azathioprine is myelosuppression, which can occur in any individual and can typically be reversed by decreasing the dose of azathioprine. However, this risk increases in individuals who have reduced or absent TPMT and/or NUDT15 activity. (1).

Genetic testing to determine *TPMT* and *NUDT15* genotype does not replace the need for regular complete blood count monitoring. One study reported that in individuals with IBD receiving thiopurine therapy, *TPMT* polymorphisms were associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions such as liver damage and pancreatitis. Furthermore, *TPMT* and *NUDT15* variants do not fully explain all cases of bone marrow suppression in individuals taking azathioprine. Therefore, regular blood tests to monitor for side effects are still needed during therapy (12, 13).

Gene: *TPMT*

The *TPMT* gene encodes thiopurine S-methyltransferase, which is historically classified as a phase II metabolism enzyme. Importantly, TPMT is one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine.

The *TPMT* gene is highly polymorphic, with over 40 reported variant star (*) alleles (14-17). The *TPMT*1* allele is associated with normal enzyme activity (wild type).

The *TPMT*1* is considered the wild-type allele when no variants are detected, and is associated with normal enzyme activity and the "normal metabolizer" phenotype. Individuals who are normal metabolizers are more

likely to have a typical response to azathioprine and a low risk of myelosuppression; however, all individuals receiving azathioprine require close monitoring. (18-21).

Most individuals are TPMT normal metabolizers (~86–97%). Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (18, 19, 22):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (c.460G>A and c.719A>G in *cis*)
- *TPMT*3C* (c.719A>G)

Individuals who are TPMT poor metabolizers (~ 0.3% of individuals of European or African ancestry) have 2 non-functional *TPMT* alleles (Table 4). When treated with standard doses of azathioprine, these individuals will universally experience life-threatening bone marrow suppression because of high levels of TGNs (1).

Individuals who are TPMT intermediate metabolizers (approximately 3–14% of the general population) are heterozygous with one no function *TPMT* allele. These individuals may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs and are at an increased risk of moderate to severe bone marrow suppression. However, some of these individuals, approximately 40–70%, can tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as methylmercaptopurine nucleotides, than homozygous-deficient individuals (18, 19).

Table 4. Assignment of likely TPMT Phenotype based on Genotype (CPIC, 2018)

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*8, *3A/*7
Poor metabolizer	An individual with 2 no function alleles	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4, *2/*3C, *3A/*4
Indeterminate	An individual with 2 uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele	*6/*8 *1/*8

TPMT, thiopurine methyltransferase; NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15
[a] See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (2).

The frequency of *TPMT* variant alleles vary among different ethnic populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (14).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely (14, 23).

Gene: *NUDT15*

The *NUDT15* gene encodes an enzyme that belongs to the nudix hydrolase superfamily. Members of this superfamily catalyze the hydrolysis of nucleoside diphosphates, which are created as a result of oxidative damage (e.g., from treatment with drugs such as thiopurines).

Nudix hydrolase 15 is directly involved in the metabolism of thiopurines, as it catalyzes the conversion of active metabolites (TdGTP) to less toxic metabolites (TdGMP) and in doing so, prevents the incorporation of toxic metabolites into DNA (24).

In individuals with reduced or absent NUDT15 activity (intermediate or poor metabolizers, Table 5), the reduction in NUDT15-mediated degradation of TdGTP results in more TdGTP available for incorporation into DNA, leading to increased DNA damage and cell death. These individuals subsequently have increased sensitivity to thiopurines at standard doses, including an increased risk of severe myelosuppression (25).

Similar to *TPMT*, the *NUDT15* gene is polymorphic, as the PharmVar Consortium currently has catalogued 21 variant alleles. However, most variants are rare, and the clinical significance of many *NUDT15* star (*) alleles is currently unclear.

The first *NUDT15* variant associated with thiopurine toxicity is p.R139C (rs116855232), which is present in both the *NUDT15*2* and *NUDT15*3* haplotypes. This amino acid change results in an unstable protein with almost no enzymatic activity. (25)

The FDA drug label for thioguanine cites one study of 1028 children with acute lymphoblastic leukemia, wherein the tolerated maintenance dose of the related drug, mercaptopurine, varied greatly, depending on the degree of deficiency in TPMT, or NUDT15, or both. Individuals who were heterozygous deficient for only one gene tolerated between 50–90% of the planned dosage. However, the tolerated dosage dropped to 30–50% of the planned dosage for individuals who were heterozygous deficient for both TPMT and NUDT15. Individuals who had bi-allelic deficiency of either TPMT or NUDT15 only tolerated 5–10% of the planned mercaptopurine dosage. (24, 26)

Deficiency of NUDT15 is rare among individuals with European or African ancestry (found in less than 1%); however, NUDT15 deficiency is more common among individuals with East Asian ancestry (e.g, Korea, China, Japan, Vietnam) (~ 2%) (2, 27, 28).

Table 5. Assignment of likely *NUDT15* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*5, *3/*6
Poor metabolizer	An individual with 2 no function alleles	*2/*2, *2/*3, *3/*3
Indeterminate	An individual with 2 uncertain function alleles OR one normal function allele plus one uncertain function allele	*1/*4, *1/*5 *4/*5, *5/*6

TPMT, thiopurine methyltransferase; NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15
[a] See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (2).

Linking Gene Variation with Treatment Response

Genetic variation in the *TPMT* and *NUDT15* genes strongly influences the safety of thiopurine therapy, specifically, influencing the risk of treatment-related bone marrow suppression (29).

Thiopurine methyltransferase deficiency is the primary genetic cause of thiopurine intolerance in Europeans and Africans, and NUDT15 deficiency is a more common cause in Asians and Hispanics.

The clinical impact of variant *NUDT15* alleles was discovered more recently than for *TPMT*, and there is less evidence available to guide dose adjustments. However, there currently is one clinical trial in progress that addresses azathioprine dosing guided by the status of both *TPMT* and *NUDT15* expression, for the treatment of IBD (5, 30-32).

Currently, *TPMT* and *NUDT15* testing is not required by the FDA before starting treatment with any thiopurine (azathioprine, mercaptopurine, or thioguanine); however, both genes were listed in the recently published FDA Association tables as pharmacogenetics associations with data supporting therapeutic management recommendations (33). Consequently, routine genotyping for *TPMT* and *NUDT15* polymorphisms has not been universally adopted (34).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the azathioprine drug response, and the genes *TPMT* and *NUDT15*. The genes may be tested separately, or together, as part of a test panel that evaluates the drug response to thiopurines.

As with many commercial tests, only the most common variants are usually tested for (e.g., for *TPMT*, the **2*, **3A*, and **3C* allele, which accounts for more than 90% of known inactivating alleles). This means that rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (18, 19, 35-38).

It is important to note that for *TPMT*3A*, 2 variants, c.460G>A and c.719A>G, are in *cis*. The variant, c.460G>A by itself is *TPMT*3B* and c.719A>G by itself is *TPMT*3C*. Most clinical laboratories are unable to phase the 2 variants. In most cases, especially if the individual is of European ancestry, the laboratory will assume the 2 variants are in *cis*, though the possibility of the variants being in *trans* cannot be ruled out.

Phenotype testing is also available for *TPMT*. Phenotype tests directly measure TPMT enzyme activity in red blood cells, but accurate phenotyping is not possible in individuals who have recently received blood transfusions (20). However, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying individuals at risk of adverse reactions from thiopurine treatment, and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (21, 39-41).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

2020 Statement from the US Food and Drug Administration (FDA):

Genetic polymorphisms influence TPMT and NUDT15 activity. Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of 6-MP or azathioprine, accumulate excessive cellular concentrations of active 6-TGNs, and are at higher risk for severe myelosuppression. Because of the risk of toxicity, patients with TPMT or NUDT15 deficiency require alternative therapy or dose modification.

Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the TPMT gene and have little or no TPMT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The *TPMT*2*, *TPMT*3A*, and *TPMT*3C* alleles account for about 95% of individuals with reduced levels of TPMT activity. NUDT15 deficiency is detected in <1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the *NUDT15* gene, and approximately 21% have one loss-of-function allele. The p.R139C variant of *NUDT15* (present on the *2 and *3 alleles) is the most commonly observed, but other less common loss-of-function *NUDT15* alleles have been observed.

[…]

Patients with thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency may be at an increased risk of severe and life-threatening myelotoxicity if receiving conventional doses of azathioprine. Death associated with pancytopenia has been reported in patients with absent TPMT activity receiving azathioprine. In patients with severe myelosuppression, consider evaluation for TPMT and NUDT15 deficiency. Consider alternative therapy in patients with homozygous TPMT or NUDT15 deficiency and reduced dosages in patients with heterozygous deficiency.

[…]

TPMT and NUDT15 Testing: Consider genotyping or phenotyping patients for TPMT deficiency and genotyping for NUDT15 deficiency in patients with severe myelosuppression. TPMT and NUDT15 testing cannot substitute for complete blood count (CBC) monitoring in patients receiving azathioprine. Accurate phenotyping (red blood cell TPMT activity) results are not possible in patients who have received recent blood transfusions.

[…]

Patients with TPMT and/or NUDT15 Deficiency

Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

Homozygous deficiency in either TPMT or NUDT15 Because of the risk of increased toxicity, consider alternative therapies for patients who are known to have TPMT or NUDT15 deficiency.

Heterozygous deficiency in TPMT and/or NUDT15 Because of the risk of increased toxicity, dosage reduction is recommended in patients known to have heterozygous deficiency of TPMT or NUDT15. Patients who are heterozygous for both TPMT and NUDT15 deficiency may require more substantial dosage reductions.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

TPMT recommendation

If starting doses are already high (e.g., 75 mg/m^2 of mercaptopurine), as is true in some ALL treatment regimens, lower than normal starting doses should be considered in TPMT intermediate metabolizers and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers. This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL. Even at these markedly reduced dosages, erythrocyte TGN concentrations in TPMT poor metabolizers remain well above those tolerated and achieved by the majority of patients (who are TPMT normal metabolizers).

In some nonmalignant conditions, alternative agents may be chosen for TPMT intermediate or poor metabolizers rather than reduced doses of thiopurines; if thiopurines are used, full starting doses are recommended for TPMT normal metabolizers, reduced doses (30–80% of target dose) in TPMT intermediate metabolizers, and substantially reduced doses (or use of an alternative agent) in TPMT poor metabolizers.

Some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype, these recommendations apply regardless of the method used to assess TPMT status.

NUDT15 recommendation

Similar to TPMT, tolerated mercaptopurine dosage is also correlated with the number of nonfunctional alleles of the NUDT15 gene. In fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of TPMT vs. NUDT15 decreased function alleles, there remains a paucity of multi-ethnic studies examining both TPMT and NUDT15 variants.

Therefore, our NUDT15 recommendations parallel those for TPMT. For NUDT15 normal metabolizers (*NUDT15*1/*1*), starting doses do not need to be altered. For NUDT15 intermediate metabolizers (e.g., *NUDT15*1/*3*), reduced starting doses should be considered to minimize toxicity, particularly if the starting doses are high (e.g., 75 mg/m^2/ day for mercaptopurine). For NUDT15 poor metabolizers (e.g., *NUDT15*3/*3*), substantially reduced doses (e.g., 10 mg/m^2/ day of mercaptopurine) or the use of an alternative agent should be considered.

As for TPMT, there is substantial variability in the tolerated thiopurine dosages within NUDT15 intermediate metabolizers, with a minority of individuals who do not seem to require significant dose reduction. Therefore, genotype-guided prescribing recommendations apply primarily to starting doses; subsequent dosing adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines). In contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in NUDT15 poor metabolizers and pre-emptive dose reductions are strongly recommended.

The NUDT15 poor metabolizer phenotype is observed at a frequency of about 1 in every 50 patients of East Asian descent, which is more common than the TPMT poor metabolizer phenotype in Europeans, and, thus, genotyping *NUDT15* in the Asian populations may be of particular clinical importance. NUDT15 deficiency is also more prevalent in individuals of Hispanic ethnicity, particularly those with high levels of Native American genetic ancestry.

Please review the complete therapeutic recommendations, which include CPIC's recommended course of action if both TPMT and NUDT15 genotypes are known, located here: (2).

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

The Dutch Pharmacogenetics Working Group considers genotyping before starting azathioprine or 6-mercaptopurine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

TPMT Intermediate Metabolizer

Grade 2 leukopenia occurs in 23% of these patients with normal therapy for immunosuppression. The genetic variation increases the quantity of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

IMMUNOSUPPRESSION

- Start with 50% of the standard dose

Adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

Dose adjustment is not required for doses lower than 1.5 mg/kg per day for azathioprine or 0.75 mg/kg per day for mercaptopurine.

LEUKEMIA

- Start with 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

The initial dose should be adjusted based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also NUDT15 IM or NUDT15 PM.

TPMT Poor Metabolizer

Grade 2 leukopenia and intolerance occurred in 98% of these patients with standard therapy. The gene variation increases the quantities of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

- Choose an alternative or use 10% of the standard dose.

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

If the dose is decreased: advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

Background information:

Azathioprine is converted in the body to mercaptopurine. Mercaptopurine is an inactive pro-drug, which is converted to the active metabolites - thioguanine nucleotides - in the body.

Two catabolic routes reduce mercaptopurine bio-availability for thioguanine nucleotide formation. Thiopurine methyltransferase (TPMT) catalyses S-methylation of both mercaptopurine and the 6- mercaptopurine

ribonucleotides formed in the metabolic pathway. In addition to this, mercaptopurine is oxidised to the inactive 6-thiouric acid by the enzyme xanthine oxidase (XO), which occurs primarily in the liver and intestines.

For more information about the TPMT phenotypes: see the general background information about TPMT on the KNMP Knowledge Bank or on www.knmp.nl (search for TPMT).

NUDT15 Intermediate Metabolizer

Grade ≥ 2 leukopenia occurs in 42% of these patients with standard immunosuppression therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

IMMUNOSUPPRESSION

- start with 50% of the standard dose

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

LEUKEMIA

- start at 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also TPMT IM or TPMT PM.

NUDT15 Poor Metabolizer

Grade ≥ 2 leukopenia occurs in 96% of these patients with standard therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

- avoid azathioprine and mercaptopurine
- if it is not possible to avoid azathioprine and mercaptopurine: use 10% of the standard dose and advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and efficacy.

Background information:

NUDT15 reverses the last step in the formation of the active metabolite of mercaptopurine and its precursor azathioprine. It converts 6-thiodeoxyguanosine triphosphate (6-thio-dGTP), which is incorporated in DNA, to 6-thiodeoxyguanosine monophosphate (6-thio-dGMP). Lower metabolic activity of NUDT15 therefore leads to increased intracellular concentrations of the active metabolite 6- thio-dGTP. This increases the risk of side effects, such as myelosuppression.

For more information about TPMT and NUDT15 phenotypes: see the general background information in the KNMP Knowledge Bank or on www.knmp.nl (search for TPMT or NUDT15).

Please review the complete therapeutic recommendations that are located here: (3, 4).

Nomenclature for Selected *TPMT* and *NUDT15* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains 2 variants in cis: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345
*NUDT15*3*	p.R139C c.415C>T	NM_018283.4:c.415C>T	NP_060753.1:p.Arg139Cys	rs116855232

Note: the p.R139C variant of nudix hydrolase 15 (*NUDT15)* is present on the *NUDT15*2* and *NUDT15*3* alleles.
The TPMT Nomenclature Committee defines the nomenclature and numbering of novel thiopurine methyltransferase *(TPMT)* variants.
Nomenclature for *NUDT15* is available from the Pharmacogene Variation (PharmVar) Consortium.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS)

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Belinostat Therapy and *UGT1A1* Genotype

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Introduction

Belinostat (brand name Beleodaq) is a histone deacetylase (HDAC) inhibitor, approved for the treatment of relapsed or refractory peripheral T-cell lymphomas (PTCLs) (1). Belinostat targets 3 classes of HDACs (I, II and IV), resulting in higher levels of acetylation of both histone and non-histone proteins, thus reversing the changes in protein acetylation that are frequently disrupted during oncogenesis. Belinostat is administered as an infusion at a rate of 1000 mg/m^2 for 30 minutes on days 1–5 of a 21-day cycle (1).

Belinostat has a relatively short half-life and is primarily metabolized by uridine diphosphate (UDP)-glucuronosyltransferase 1A1 (UGT1A1)-mediated glucuronidation, with minor contributions from other UGT and cytochrome P450 (CYP) enzymes (1, 2). Genetic variation at the *UGT1A1* locus can result in decreased enzyme activity and thus increased exposure to belinostat. The US Food and Drug Administration (FDA)-approved drug label recommends a 25% decrease in dose for individuals who are known to be homozygous for the *UGT1A1*28* reduced function allele (Table 1) (1). Additional indications for dose reduction include grade 3 or 4 adverse reactions or significant decrease in neutrophil or platelet counts following belinostat administration (1). Some studies have suggested that other variant alleles may also lead to increased belinostat exposure, such as *UGT1A1*60*; however, no specific recommendations for dose reduction have been made for these alleles by either the FDA or other professional pharmacogenetic consortia. Belinostat should not be administered with other medications that can inhibit UGT1A1 function (1), such as nilotinib, ketoconazole, or ripretinib.

Table 1: The FDA (2023) Drug Label for Belinostat Dosage Recommendation based on *UGT1A1* Genotype

Genotype	Recommendation
*UGT1A1*28/*28*	Reduce the starting dose of belinostat to 750 mg/m^2

This table is adapted from (1).

Drug: Belinostat

Belinostat is a pan histone deacetylase inhibitor (HDACi) used in the treatment of relapsed or refractory PTCLs (1). Peripheral T-cell lymphomas encompass multiple subtypes that together represent 10–15% of all non-Hodgkin lymphomas. The PTCLs arise from the transformation of post-thymic T lymphocytes. (3) It is estimated that approximately 70% of individuals with PTCL either relapse or are refractory to first-line therapy (4). Classification and diagnostic criteria of the PTCL subtypes are described elsewhere (3, 5, 6, 7), but the 5-year overall survival rates vary significantly among these subtypes varying from 80–25% (4). The current indication and recommended use of belinostat is for refractory or relapsed PTCL; (1, 5) however, it has been investigated as a first-line agent for PTCL with other first-line therapies (8). Belinostat is one of several single agent options for refractory or relapsed PTCL recommended by the National Comprehensive Cancer Network (5).

Changes in gene expression are observed in most PTCL types, either owing to altered epigenetic regulation, gene fusion events, somatic genetic variation of genes involved in epigenetic regulation, or some combination of these events (9). The HDACs, key enzymes in the maintenance of protein acetylation, are also implicated in the

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pathogenesis of PTCL as well as other malignancies (10, 11). Protein acetylation has a role in epigenetics as well as modulation of many other cellular homeostatic processes (10).

Belinostat is referred to as a pan-HDACi as it can inhibit all 3 classes of HDAC isoforms in humans, impacting not only histone acetylation, but non-histone proteins as well (10). A loss of acetylation on histones is associated with condensation of the chromatin structure and reduced expression of the underlying genes (12). By inhibiting the removal of acetyl groups by HDACs, belinostat is thought to relax chromatin structure and affect the activity of non-histone nuclear proteins. This leads to expression of genes that were silenced during oncogenesis and facilitates pro-apoptotic cellular responses (10). In Phase I clinical trials in solid and hematologic malignancies, belinostat had a robust antitumor and anti-angiogenic effect, leading to phase II trials in both PTCL and cutaneous T-cell lymphoma (CTCL) (11, 13, 14).

Belinostat is administered intravenously at a dose of 1,000 mg/m^2 over 30 minutes once daily on days 1–5 of a 21-day cycle (1). It has an elimination half-life of 1.1 hours with a limited body tissue distribution; the majority of belinostat is bound to protein in plasma (1). Over 98% of belinostat is metabolized prior to excretion (15, 16). Belinostat is primarily metabolized through glucuronidation by UGT1A1 with minor contributions by UGT2B7, and the resulting metabolite is the major form detected in urine (1, 2, 15, 17). Additional metabolism of belinostat is carried out by CYP2A6, CYP2C9, and CYP3A4 to form belinostat amide and belinostat acid. However, the enzymes responsible for the formation of 2 other metabolites are currently unknown. (1, 16) The primary route of excretion of belinostat and its metabolites is through urine, with less than 10% of a single radiolabeled dose detected in the feces over 168 hours post-transfusion (15). Due to the significant role of UGT1A1 in the metabolism and elimination of belinostat, the FDA-approved drug label recommends a reduced dosage of 750 mg/m^2 for individuals with known UGT1A1 reduced function, specifically those who are homozygous for the *UGT1A1*28* allele (1). The FDA-approved drug label also cautions against concomitant use of belinostat with strong inhibitors of UGT1A1 (1).

Adverse reactions associated with belinostat include hematologic toxicity, hepatotoxicity, tumor lysis syndrome, gastrointestinal toxicity, and embryo-fetal toxicity if used during pregnancy. Hematologic toxicities include thrombocytopenia, leukopenia, anemia, or a combination of these symptoms. Hepatotoxicity and liver function should be monitored prior to initiation of therapy and at the start of each round. Nausea, vomiting, and diarrhea can also occur during belinostat therapy. (1) The most frequently reported treatment-emergent adverse effects during clinical trials include nausea, fatigue, pyrexia, constipation, vomiting, and dizziness (11, 14). Tumor lysis syndrome (TLS) is a particular concern for individuals with advanced stage disease or high tumor burden; baseline hyperuricemia and bulky disease were associated with one individual experiencing grade 4 TLS and death due to multiorgan failure during clinical trial (1).

Some observed toxicities are indications for reduced dosage or discontinuation of belinostat. For hematologic toxicity, if the individual has a nadir absolute neutrophil count less than 0.5 x10^9/L or platelet count less than 25x10^9/L, the dosage should be decreased by 25% (1). Similarly, if a non-hematologic Common Terminology Criteria for Adverse Events (CTCAE) (18) Grade 3 or 4 adverse reaction is observed, decrease the dose by 25%; a recurrence of these adverse reactions after 2 dose reductions is an indication to discontinue belinostat (1).

Due to the genotoxic effects of belinostat, there is a risk of fetal harm when administered to a pregnant woman. The FDA-approved label recommends that females use effective contraception for 6 months following the last dose of belinostat and males whose partners are females of reproductive potential should also use effective contraception for 3 months following his last dose of belinostat (1). These recommendations are based on the mechanism of action of belinostat, as there are no available data on the use of this medication in pregnant women, nor were animal reproduction studies conducted with belinostat. (1) Additionally, there are no data regarding the excretion of belinostat into breast milk, how belinostat may affect a breastfed child, nor the effect on milk production; based on the potential adverse reaction to the breastfed child, nursing mothers are advised not to breastfeed until 2 weeks after the last belinostat dose (1).

Belinostat has not been proven to be safe or effective in pediatric individuals (1). In clinical trial data, individuals aged 65 and above had a higher response rate to belinostat versus individuals under 65 (36% versus 16%) (1, 11). However, no association was found between age of the individuals and rate of adverse reactions, indicating that age alone was not a risk factor for treatment-emergent adverse events (1). Moderate to severe hepatic impairment (as indicated by total bilirubin greater than 1.5 times the upper normal limit) was an exclusionary criterion for participation in clinical trials, but less severe hepatic impairment may increase exposure to belinostat (1, 11, 14). However, the FDA-approved label does not provide any alternative dosing recommendations for individuals with liver impairment (1).

Aberrations in protein acetylation and gene dysregulation are common across many types of cancer. The efficacy and safety of belinostat in other types of malignancies is an active area of investigation. It has been tested in acute promyelocytic leukemia, CTCL, B-cell lymphoma, advanced solid tumors including neuroendocrine and lung cancers, testicular germ cell tumors, and others (14, 19, 20, 21, 22, 23). Additionally, belinostat has been investigated as a potential therapy for atopic dermatitis (24, 25). Clinical trials involving belinostat can be found at ClinicalTrials.gov.

Gene: *UGT1A1*

The UGT enzymes (uridine diphosphate-glucuronosyltransferase, or UDP-glucuronosyltransferase) are a superfamily of enzymes that metabolize a wide range of lipophilic molecules such as bilirubin, steroids, toxins, and drugs. These enzymes mediate the process of glucuronidation, which is a phase II metabolic pathway during which glucuronic acid is conjugated to specific targets to convert them to water-soluble metabolites that can then be eliminated from the body (26).

The UGT genes are polymorphic, and genomic processes, such as variant splicing and epigenetic factors, likely contribute to their diversity. As a result, the substrates that the UGT enzymes catalyze are particularly variable (27). In humans, the UGT superfamily consists of 22 enzymes divided into 4 families, of which UGT1A is a member (28). The *UGT1A* gene locus is a cassette gene located on chromosome 2q37 in which common exons 2–5a and 5b are differentially spliced to unique first exons to form the 9 functional UGT1A family members (UGT1A1 and UGT1A3–UGT1A10) (29, 30). The *UGT1A1* promoter is differentially regulated compared to other UGT1As and consists of elements sensitive to xenobiotics (for example, pregnane X receptor and constitutive androstane receptor), hydrocarbons (for example, the aryl hydrocarbon receptor (AhR)), electrophilic nucleophiles and reactive oxygen species (for example, the nuclear factor 2 receptor), endobiotics and fatty acids (such as the glucocorticoid receptor), and a critical Thymine-Adenine-Thymine-Adenine (TATA) box that consists of polymorphic tandem repeats, $(TA)_{5-8}TAA$. Several Cytosine-phosphate-Guanine islands at the promoter further alter the affinity of nuclear receptors and therefore alter receptor activity.

Whereas many UGT enzymes overlap in the substrates they glucuronidate, UGT1A1 is the only enzyme that glucuronidates bilirubin, a yellow waste product produced during the catabolism of heme, a constituent of hemoglobin (31). When old or damaged red blood cells are broken down in the spleen, their hemoglobin is broken down to heme, which is then converted into bilirubin. The UGT1A1 enzyme converts this toxic, insoluble form of bilirubin (unconjugated bilirubin) to its nontoxic form (conjugated bilirubin). Because conjugated bilirubin is water-soluble, it can be dissolved in bile and eliminated with solid waste. If bilirubin is not eliminated and instead accumulates to high levels (hyperbilirubinemia), it can cause a yellowish discoloration of the skin and eyes, a condition known as jaundice.

Over 150 genetic variants of *UGT1A1* have been reported (26, 31, 32). Of these, the available evidence indicates that 5 polymorphic variants are of clinical importance to UGT1A1 activity (*UGT1A1*6*, *UGT1A1*27*, *UGT1A1*28*, *UGT1A1*36*, *UGT1A1*37*), and 3 of these variants impact the tandem repeat of the TATA box ($[TA]_5TAA$ – *UGT1A1*36*, $[TA]_7TAA$ – *UGT1A1*28*, $[TA]_8TAA$ – *UGT1A1*37* (33)). The wild-type allele is

called *UGT1A1*1,* which is associated with normal enzyme activity and the reference TATA box tandem repeat length ($[TA]_6TAA$) (Table 2).

As with all genetic variation, specific alleles or haplotypes vary in frequency across populations based on genetic ancestry and any history of evolutionary migration or bottleneck. To characterize the range of genetic variation in different groups of people, studies have used a mix of ethnic, racial, and geographic descriptors to group individuals with presumed common ancestry and shared genetic traits. Those descriptors are used interchangeably below based on the cited literature; however, the goal is to reflect a shared genetic background arising from common ancestry.

There are multiple genetic variations in the *UGT1A1* locus that decrease UGT1A1 enzyme activity and can lead to jaundice in the absence of exogenous substances, such as belinostat. The jaundice may be mild, as seen in Gilbert syndrome, or severe, as seen in Crigler-Najjar syndrome. (34)

The most common variant *UGT1A1* allele is *UGT1A1*28*, which is commonly found in African Americans (0.42–0.45 allele frequency, or 17–20% frequency of homozygosity in the population), Caucasians (0.26–0.31 allele frequency, or 6–9% homozygosity), and in Western and South Asian populations (0.26–0.33 allele frequency, or 6–10% homozygosity); however, it is less common in East and South-East Asian populations (0.09–0.16 allele frequency, or 0.8–2.5% homozygosity) (35, 36, 37). Within Caucasian and African American populations, the *UGT1A1*28* variant is a common cause of Gilbert syndrome (35, 38). The *UGT1A1*28* ($[TA]_7TAA$) variant contains an extra TA repeat within the TATA box promoter region (7 TA repeats compared with 6 in the wild-type allele) (39). This extra TA repeat decreases the rate of transcription initiation of the *UGT1A1* gene, leading to decreased enzyme activity and decreased glucuronidation of bilirubin (40). The data suggests that one copy of the *UGT1A1*28* allele results in an approximately 35% decrease in transcriptional activity, and 2 copies (**28/*28*, homozygous) results in an approximately 70% decrease, which is the genotype that the FDA has incorporated into the belinostat dosing label (1, 41, 42).

Another variant allele, UGT1A1*37 ($[TA]_8TAA$), has 8 TA repeats at the TATA box site, and results in reduced promoter activity of the gene to levels lower than the UGT1A1*28 allele. In contrast, the UGT1A1*36 ($[TA]_5TAA$) allele only has 5 repeats and is associated with increased promoter activity and a reduced risk of neonatal hyperbilirubinemia (a common and typically benign condition). Both UGT1A1*36 and UGT1A1*37 occur almost exclusively in populations of African origin, with estimated allele frequencies across African descent populations of 0.07 for **36* (TA_5) and 0.05 for **37* (TA_8) (gnomAD browser version 3.1.2, accessed 27 April 2023) (43). By comparison, the average frequency of these alleles across all populations in gnomAD is 0.01–0.02. The allele *UGT1A1*80* demonstrates strong linkage disequilibrium with both *UGT1A1*28* and **37* and is considered a surrogate marker for these alleles (33).

Other promoter variants have been reported in the phenobarbital-responsive enhancer module of the *UGT1A* locus. A T to G substitution, referred to as *UGT1A1*60*, results in decreased transcription of the gene and was found more frequently in individuals with mild hyperbilirubinemia (44), though other studies have reported no significant difference in total bilirubin concentration in individuals homozygous for *UGT1A1*60* versus wild-type homozygotes (45). The *UGT1A1*60* allele has been observed more frequently in individuals of African descent versus European descent ('Caucasians') (46). It should be noted that the *UGT1A1*28* and **60* alleles are reported to be in linkage disequilibrium in multiple ethnic groups (46, 47), meaning an individual who has the higher number of TA repeats in the promoter (*UGT1A1*28*) is also likely to have the T to G substitution (*UGT1A1*60*) in the phenobarbital-responsive enhancer module region as well. Thus, it can be difficult to ascertain the individual contribution of these variants to total enzyme activity in vivo. The *UGT1A1*60* allele has a reported allele frequency of 0.47 in Caucasians (individuals of European descent) and 0.85 in Americans of African descent (46).

Another variant allele, *UGT1A1*6*, is more prevalent in East Asian populations, with an allele frequency of around 0.10–0.30 in Taiwanese, Chinese, Korean, and Japanese populations (37, 41, 48, 49). The *UGT1A1*6* allele is less common in South Eastern and Southern Asian populations, ranging from 0.027--0.12 in Thai, Malay, Indonesian, Vietnamese, and Indian population studies (37). This missense variant results in a glycine to arginine amino acid change at position 71 (p.Arg71Gly), and individuals who are homozygous for this allele have reduced UGT1A1 enzyme activity, which can cause Gilbert syndrome and prolonged neonatal jaundice (50, 51, 52, 53).

The *UGT1A1*27* (p.Pro229Gln) variant allele is located in exon 1 and has a minor allele frequency between 0.00011–0.0030 in those with Asian ancestry. It is associated with Gilbert's syndrome, post-irinotecan hyperbilirubinemia, and severe or life-threatening leukopenia or diarrhea during irinotecan therapy (54, 55). The allele is associated with a significant decrease in UGT1A1 substrate binding and catalytic activity (56).

Table 2: Relative Enzymatic Activity of UGT1A1 Variants

Allele name	Variant	Relative activity	Potential impact on drug metabolism	CPIC functional status[e]
*UGT1A1*1*	None (promoter $[TA]_6TAA$)	100%[a]	Normal	Normal function
*UGT1A1*6*	p.Arg71Gly	70%[b]	Slower	Decreased function
*UGT1A1*27*	p.Pro229Gln	50% [c]	Slower	Decreased function
*UGT1A1*28*	Promoter $(TA)_7TAA$	65%[a]	Slower	Decreased function
*UGT1A1*36*	Promoter $(TA)_5TAA$	130%[a]	Faster	Increased function
*UGT1A1*37*	Promoter $(TA)_8TAA$	50%[a]	Slower	Decreased function
*UGT1A1*60*	c.-3279T>G	60%[d]	Slower	Normal function[f]

CPIC – Clinical Pharmacogenetics Implementation Consortium
[a] Activity level from (35)
[b] Activity level from (51)
[c] Activity level from (56)
[d] Activity level from (44)
[e] Functional status from (57)
[f] Functional status from (58)

Phenoconversion

Mismatch between an individual's genotype-predicted phenotype and actual phenotype, or "phenoconversion," results from extrinsic factors (namely, comedications or comorbidities) altering the expression or function of enzymes. Individuals harboring an intermediate metabolizer phenotype are typically more susceptible to phenoconversion as a function of compromised drug metabolism capacity, whereas poor metabolizers (PMs) are unlikely to undergo phenoconversion but are more likely to experience toxicity from low or absent drug metabolism capacity limiting clearance of toxic drug species (59). The FDA-approved label recommends avoidance of belinostat administration with UGT1A1 inhibitors, as this may result in an increased exposure of the individual to belinostat (1). Medications that inhibit UGT1A1 include nilotinib (60), ketoconazole (61), and ripretinib along with other tyrosine kinase inhibitor drugs (62). This is highly relevant given the interest in belinostat therapy for multiple cancer types in a combination therapy approach (8, 22). The use of belinostat may, conversely, impact enzymatic activity and alter the effect of other comedications. Belinostat can interfere with rifampin-induced changes in gene expression such as the induction of CYP3A4 expression (63). Belinostat, however, did not significantly impact the metabolism of warfarin by CYP2C9 (64). Belinostat itself inhibits UGT enzymes, and thus may interfere with metabolism of other UGT substrates (65).

Linking *UGT1A1* Genetic Variation with Treatment Response

The primary mechanism of belinostat clearance is metabolism by UGT1A1 (2, 15, 66). Genetic variation in the *UGT1A1* locus can lead to a decrease in enzyme activity and thus increase an individual's exposure to belinostat, leading to a higher risk of adverse reactions. The FDA-approved drug label and initial clinical studies support a decrease in dose (750 mg/m^2) for individuals who are homozygous for the *UGT1A1*28* allele to minimize toxicities (1, 2). Given the FDA guidance to avoid belinostat and co-medication with UGT1A1 inhibitors, it is unclear why additional genetic variants with a known decrease in function are not indications for a dose decrease on the product label. Additional studies have suggested that the *UGT1A1*60* allele also results in decreased clearance of belinostat, both when it was the only detected variant but also when detected in *cis* with **28* (67, 68). Both *UGT1A1*28* and **60* have been shown to be associated with increased belinostat exposure and a higher risk of thrombocytopenia and neutropenia (67). However, it should be noted that the Clinical Pharmacogenetics Implementation Consortium (CPIC) reassessed the functional status of *UGT1A1*60* and determined it was a normal-function allele, as indicated by serum bilirubin concentrations (45, 58). The clinical function of *UGT1A1* alleles as assigned by CPIC, initially published with guidelines for atazanavir pharmacogenetics (33), are presented in Table 2.

Further clinical studies are needed to determine the role of both common and rare *UGT1A1* variants in belinostat metabolism and the risk of adverse reactions.

Genetic Testing

The NIH Genetic Testing Registry (GTR) has tests for belinostat response and UGT1A1 genetic variation. Variants impacting UGT1A1 enzyme activity affect both the coding sequence as well as the promotor region of UGT1A locus. The FDA-approved label does not recommend for or against genetic testing prior to belinostat therapy, but states that knowledge of *UGT1A1*28* genotype is sufficient indication to alter prescribing. No other clinical practice guidelines for translation of other genotypes to altered dosing are currently available. Liu and colleagues recommend *UGT1A1* genotyping before administering any medication that is metabolized by UGT1A1 (69). Indeed, germline testing for variation in *UGT1A1* along with 4 other pharmacogenes has been estimated to have impact on oncology care as significantly as somatic variant testing (70). As a result of the demonstrated impact on UGT1A1 activity and the increased risk of adverse reactions, Goey and Figg recommend *UGT1A1* genotyping for both **28* and **60* variant alleles when determining belinostat dose (68).

One pharmacogenetic study in an oncology clinical setting found that approximately 17% of individuals had a UGT1A1 PM phenotype, defined as having 2 decreased-function alleles (*UGT1A1*6* or **28*) (70). Genotyping for different lengths of the TA promoter alleles requires a high degree of precision, particularly given the multiple variant alleles reported for that position, thus it is important to consider the testing methodology when selecting a genetic test or reviewing testing results.

The *UGT1A1*28* allele has been reported to be in linkage disequilibrium with the **80* allele; however, the *UGT1A1*80* variant itself is not known to influence *UGT1A1* expression (71). Instead, *UGT1A1*80* has been suggested to serve as a proxy for **28* variant identification in some single nucleotide polymorphism-based genotyping assays (33, 70, 71).

Additionally, *UGT1A1* genotyping may reveal variants that are associated with Gilbert syndrome or Crigler-Najjar syndrome type 1 or type 2. More information and resources for these conditions are available through MedGen. While more clinical data may be needed on belinostat metabolism, studies have shown that variants associated with Gilbert syndrome or Crigler-Najjar syndrome type 2 not only impact bilirubin metabolism, but multiple exogenous substances as well (31, 33, 56, 72).

The *UGT1A1* Gene Interactions with Medications Used for Additional Indications

Variation in *UGT1A1* and its promoter region are associated with risks of adverse reactions for a range of medications.

- Multiple medications used in oncology are metabolized by UGT1A1 including irinotecan, nilotinib, and sacituzumab govitecan; decreased UGT1A1 activity may lead to increased exposure to these medications with a higher risk of adverse reactions, adjusted dosing may be necessary based on *UGT1A1* genotype.
- The human immunodeficiency virus type 1 protease inhibitor, atazanavir, inhibits UGT1A1 activity and may lead to increased indirect plasma bilirubin concentration; UGT1A1 PMs may experience jaundice leading to discontinuation of therapy (33)
- A medication used for acromegalia, pegvisomant, caused liver injury in individuals who were positive for *UGT1A1*28* (26)

Additional information on gene-drug interactions for *UGT1A1* are available from PharmGKB, CPIC and the FDA (search for "UGT1A1").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

Patients with Reduced UGT1A1 Activity

Reduce the starting dose of Beleodaq to 750 mg/m^2 in patients known to be homozygous for the UGT1A1*28 allele.

[….]

Pharmacogenomics

UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 20% of the black population, 10% of the white population, and 2% of the Asian population are homozygous for the UGT1A1*28 allele. Additional reduced function alleles may be more prevalent in specific populations.

Because belinostat is primarily (80 -90%) metabolized by UGT1A1, the clearance of belinostat could be decreased in patients with reduced UGT1A1 activity (e.g., patients with UGT1A1*28 allele). Reduce the starting dose of Beleodaq to 750 mg/m^2 in patients known to be homozygous for the UGT1A1*28 allele to minimize dose limiting toxicities.

Please review the complete therapeutic recommendations that are located here: (1)

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

Nomenclature for selected *UGT1A1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*1*	$(TA)_6TAA$	NM_000463.2:c.-53_-52TA[7]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*6*	211G>A Gly71Arg	NM_000463.2:c.211G>A (NM_001072.4:c.862-6536G>A)	NP_000454.1:p.Gly71Arg	rs4148323
*UGT1A1*27*	Pro229Gln	NM_000463.3:c.686C>A	NP_000454.1:p.Pro229Gln	rs35350960
*UGT1A1*28*	$(TA)_7TAA$	NM_001072.4:c.862-6800AT[8]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*36*	$(TA)_5TAA$	NM_001072.4:c.862-6800AT[6]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*37*	$(TA)_8TAA$	NM_001072.4:c.862-6800AT[9]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs3064744
*UGT1A1*60*	-3263T>G -3279T>G	NM_001072.4:c.862-10021T>G	Not applicable—variant occurs in a non-coding region	rs4124874
*UGT1A1*80*	c.-364C>T	NM_007120.2:c.868-7110C>T	Not applicable—variant occurs in a non-coding region	rs887829

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (73).
UGT Allele nomenclature and definitions are available from (32)
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
dbSNP - database of single nucleotide polymorphisms

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Brivaracetam Therapy and CYP2C19 Genotype

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Introduction

Brivaracetam (brand name Briviact) is an antiseizure drug used in the treatment of partial-onset (focal) epilepsy in adults. It is thought to act by binding to a synaptic vesicle glycoprotein, SV2A, and reducing the release of neurotransmitters.

Brivaracetam is primarily metabolized by hydrolysis, via amidase enzymes, to an inactive metabolite. To a lesser extent, it is also metabolized by a minor metabolic pathway via CYP2C19-dependent hydroxylation.

Individuals who have no CYP2C19 enzyme activity, "CYP2C19 poor metabolizers", will have a greater exposure to standard doses of brivaracetam. Because they are less able to metabolize the drug to its inactive form for excretion, they may have an increased risk of adverse effects. The most common adverse effects of brivaracetam therapy include sedation, fatigue, dizziness, and nausea.

The recommended starting dosage for brivaracetam monotherapy or adjunctive therapy is 50 mg twice daily (100 mg per day). Based on how the individual responds, the dose of brivaracetam may be decreased to 25 mg twice daily (50 mg per day) or increased up to 100 mg twice daily (200 mg per day) (1).

The FDA-approved drug label for brivaracetam states that patients who are CYPC19 poor metabolizers, or are taking medicines that inhibit CYP2C19, may require a dose reduction (Table 1). Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers (1).

Table 1. FDA (2017) Drug Label for Brivaracetam. Recommendations for CYP2C19 Phenotype: Pharmacokinetics.

Phenotype	Recommendations
CYP2C19 poor metabolizer	CYP2C19 poor metabolizers and patients using inhibitors of CYP2C19 may require dose reduction.

This table is adapted from (1).

Drug: Brivaracetam

Brivaracetam is an antiseizure drug that is used in the treatment of partial-onset (focal) seizures in patients aged 16 years or older. Brivaracetam can be used as monotherapy, or more commonly, is used in combination with other antiseizure drugs (1). There is also some evidence to suggest that brivaracetam may be useful in the treatment of generalized seizures (2).

Brivaracetam displays a high and selective affinity for SV2A in the brain, which is thought to contribute to the antiseizure effect (3).

In a neuron, at the synapse, vesicles store various neurotransmitters. The neurotransmitters are released and then refilled in a process regulated by voltage-dependent calcium channels. These synaptic vesicles are essential for propagating nerve impulses between neurons, and the SV2A protein is a major component of the vesicle (4).

Levetiracetam was the first antiseizure drug that was found to bind to SV2A, among other targets. Brivaracetam is an analogue of levetiracetam and was designed to selectively target SV2A with a much higher affinity (5-7).

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Over 50 million people worldwide suffer from epilepsy. Epilepsy is characterized by spontaneous recurrent epileptic seizures, which may be classified as focal or generalized. Generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase). In contrast, symptoms of focal seizures depend upon where the focus of the seizure originates in the brain; e.g., jerking of a limb indicates a focus in the contralateral motor cortex.

Most antiseizure drugs currently available target sodium channels (e.g., carbamazepine, phenytoin), calcium channels (e.g., ethosuximide), or the GABA pathway (e.g., clobazam). However, up to one-third of patients may not achieve seizure control or they may not be able to tolerate the side effects. Newer antiseizure drugs have unconventional targets, such as SV2A (8-10).

Brivaracetam was licensed in 2016, and in phase III trials and with long term follow up, brivaracetam was reported to be well tolerated with good efficacy (11, 12). Compared with the addition of placebo to a treatment regime, the addition of brivaracetam reduced the frequency of focal seizures by approximately half (13-16).

The most common side effects associated with brivaracetam therapy include dizziness, fatigue, somnolence, nausea and vomiting. Psychiatric symptoms such as irritability, insomnia and depression, and behavioral effects have also been reported, but some studies suggest these may be less likely to occur with brivaracetam compared with levetiracetam (17-20).

Brivaracetam is primarily metabolized (approximately 60%) by cytochrome P450 (CYP)-independent hydrolysis (via amidase) to inactive metabolites. Minor metabolic pathways include hydroxylation by CYP2C19.

One study found that co-administration of rifampin (a strong enzyme inducer) decreased exposure to brivaracetam by 45%, this is probably through an induction of the CYP2C19 pathway which clears brivaracetam metabolites from the blood (21). One small study (n=79) found that individuals who lacked CYP2C19 activity had increased exposure to brivaracetam (22). It is therefore possible that genetic variations associated with a loss of CYP2C19 function may reduce brivaracetam metabolism leading to increased levels of active drug levels in the plasma, and possibly increases the probability of side effects. However, the recommended therapeutic dose is 50–200 mg/day, and patients are individually titrated to optimal efficacy, safety, and tolerability.

Gene: CYP2C19

The CYP superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants (23), benzodiazepines, several proton pump inhibitors, the antifungal agent voriconazole (24), the antiplatelet agent clopidogrel (25), and antiseizure drugs such as brivaracetam, clobazam, diazepam, lacosamide, phenytoin, and phenobarbital.

The *CYP2C19* gene is highly polymorphic—35 variant star (*) alleles are cataloged at the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is the wild type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and, depending on the number of alleles present, is associated with the "rapid" (one **17* allele) and "ultrarapid" (two **17* alleles) metabolizer phenotypes.

Nonfunctional alleles include *CYP2C19*2* and **3*. The *CYP2C19* "intermediate" metabolizers carry one copy of an allele that encodes a nonfunctional allele (e.g., **1/*2*), whereas "poor" metabolizers carry 2 nonfunctional alleles (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016) *CYP2C19* Functional Status and Phenotypes

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (~2–5% of patients)[a]	An individual carrying 2 increased function alleles.	**17/*17*
CYP2C19 rapid metabolizer (~2–30% of patients)	An individual carrying one normal function allele and one increased function allele.	**1/*17*
CYP2C19 normal metabolizer (~35–50% of patients)	An individual carrying 2 normal function alleles.	**1/*1*
CYP2C19 intermediate metabolizer (~18–45% of patients)	An individual carrying one normal function allele and one no function allele, or one no function allele and one increased function allele.	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (~2–15% of patients)	An individual carrying 2 no function alleles.	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multiethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (23).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* nonfunctional allele.
This table is adapted from (23).

Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers; and up to 45% of patients are CYP2C19 intermediate metabolizers.

The most common nonfunctional variant is *CYP2C19*2*, which contains a NM_000769.1:c.681G>A variant in exon 5 that results in an aberrant splice site that produces a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians.

Another commonly tested nonfunctional variant is *CYP2C19*3*, which contains a NM_000769.1:c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other nonfunctional variants occur in less than 1% of the general population and include *CYP2C19*4-*8* (25).

Linking Gene Variation with Treatment Response

One small study (n=79) reports that *CYP2C19* allele status influences the pharmacokinetics of brivaracetam, but that this is unlikely to be clinically relevant because of the minor role of CYP-dependent hydroxylation in the metabolism of brivaracetam (22). However, the FDA does state that CYP2C19 poor metabolizers may require a reduction in the dose of brivaracetam (1).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the *CYP2C19* gene.

Usually a patient's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor).

The *CYP2C19*2* and **3* alleles are most commonly tested for, and Table 2 summarizes common CYP2C19 phenotypes. Less common nonfunctional alleles (e.g., *CYP2C19*4-*8*) may also influence drug response

similarly to *2 and *3 but they may not be tested for, and data are lacking on their effects on the brivaracetam drug response.

To facilitate *CYP2C19* genetic testing and improve genotyping concordance across laboratories, the Pharmacogenetics Working Group of the Association for Molecular Pathology Clinical Practice Committee (AMP PGx) has recommended a minimum set of *CYP2C19* alleles, referred to as "tier 1", which should be included in clinical *CYP2C19* pharmacogenomic tests. As of 2018, the tier 1 alleles are *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* (Table 3) (26).

In addition, AMP PGx have defined a list of tier 2 *CYP2C19* alleles that do not meet the criteria for inclusion in tier 1 and are thus considered optional (Table 4) (26).

Table 3. AMP PGx (2018) *CYP2C19* Tier 1 Variant Alleles.

Allele	Allele functional status[a]	Defining functional variant	Multiethnic allele frequency, %
*CYP2C19*2*	No function	rs4244285	12-54
*CYP2C19*3*	No function	rs4986893	0.3-15
*CYP2C19*17*	Increased function	rs12248560	4-21

[a] Citations for assignment of function can be found at PharmGKB Gene-specific Information for *CYP2C19*.
Note that the defining *2 variant (rs4244285) is most likely linked with the defining variant of the *35 allele (rs12769205); however, the *35 definition includes rs12769205 without rs4244285.

Table 4. AMP PGx (2018) *CYP2C19* Tier 2 Variant Alleles.

Genotype	Allele functional status*	Defining functional variant	*Multiethnic allele frequency, %
*CYP2C19*4*	No function	rs28399504	0.1-0.3
*CYP2C19*4B*	No function	rs28399504; rs12248560	0-0.2
*CYP2C19*5*	No function	rs56337013	0
*CYP2C19*6*	No function	rs72552267	0-0.1
*CYP2C19*7*	No function	rs72558186	0
*CYP2C19*8*	No function	rs41291556	0.1-0.3
*CYP2C19*9*	Decreased function	rs17884712	0.1-4.2
*CYP2C19*10*	Decreased function	rs6413438	0.1-6
*CYP2C19*35*	No function	rs12769205	0.8-3.1

* Multiethnic allele frequency from PharmVar.org (last accessed June 20, 2017.)
Both Table 3 and Table 4 are adapted from (26).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Brivaracetam is primarily metabolized by hydrolysis of the amide moiety to form the corresponding carboxylic acid metabolite, and secondarily by hydroxylation on the propyl side chain to form the hydroxy metabolite. The hydrolysis reaction is mediated by hepatic and extra-hepatic amidase. The hydroxylation pathway is mediated

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

primarily by CYP2C19. In human subjects possessing genetic variations in CYP2C19, production of the hydroxy metabolite is decreased 2-fold or 10-fold, while the blood level of brivaracetam itself is increased by 22% or 42%, respectively, in individuals with one or both mutated alleles. CYP2C19 poor metabolizers and patients using inhibitors of CYP2C19 may require dose reduction. An additional hydroxy acid metabolite is created by hydrolysis of the amide moiety on the hydroxy metabolite or hydroxylation of the propyl side chain on the carboxylic acid metabolite (mainly by CYP2C9). None of the 3 metabolites are pharmacologically active.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature of selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: the normal "wild type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (27).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Capecitabine Therapy and *DPYD* Genotype

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Introduction

Capecitabine (brand name Xeloda) is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. It is widely used in the treatment of several malignancies including colon cancer, metastatic colorectal cancer, and metastatic breast cancer. Capecitabine is a prodrug that is enzymatically converted to its active form, fluorouracil (also called 5-fluorouracil), which acts as an antimetabolite to slow tumor growth.

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Dihydropyrimidine dehydrogenase inactivates 80–90% of 5-fluorouracil (5-FU) into 5,6-dihydro-fluorouracil. Genetic variants in the *DPYD* gene can lead to enzymes with reduced or absent activity. Individuals who have at least one copy of a nonfunctional *DPYD* variant (for example, c.1905+1G>A (formerly *2A; rs3918290) or c.1679T>G (p.I560S; formerly *13; rs55886062)) will not be able to metabolize fluorouracil at normal rates. Consequently, these individuals are at risk of potentially life-threatening fluorouracil toxicity, such as bone marrow suppression, gastrointestinal toxicity and, rarely, neurotoxicity. The prevalence of DPD partial deficiency varies in different populations but is approximately 3–5%. There is an FDA-approved antidote for 5-FU overdose: uridine triacetate. Overdose can occur in individuals with partial DPD deficiency taking either capecitabine or 5-FU.

The FDA-approved drug label for capecitabine states that no capecitabine dose has been proven safe in individuals with absent DPD activity, and that there is insufficient data to recommend a specific dose in individuals with partial DPD activity as measured by any specific test (Table 1) (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have published dosing recommendations for fluoropyrimidines (capecitabine and fluorouracil) based on *DPYD* genotype (Tables 2 and 3). Both recommendations include dose reductions for intermediate metabolizers (with reduced enzyme activity), and avoiding fluorouracil and choosing an alternative agent for poor metabolizers (with absent enzyme activity) (2, 3, 4).

Table 1. The FDA Drug Label for Capecitabine: Warning DPD Deficiency (2020)

Phenotype	Capecitabine
DPD deficiency	Increased risk of severe or fatal adverse reactions in individuals with low or absent dihydropyrimidine dehydrogenase (DPD) activity. Withhold or permanently discontinue capecitabine tablets in individuals with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of (DPD) activity. No capecitabine dose has been proven safe in individuals with absent DPD activity.

Please see Therapeutic Recommendations based on Genotype for more information from FDA. This table is adapted from (1).

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Table 2. The CPIC Recommended Dosing of Fluoropyrimidines (Capecitabine or 5-Fluorouracil) by DPD Phenotype (2017, Nov 2018 Update)

Phenotype	Implications for phenotypic measures	Activity score	Dosing recommendations	Classification of recommendations[a]
DPYD normal metabolizer	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity.	2	Based on genotype, there is no indication to change dose or therapy. Use label-recommended dosage and administration.	Strong
DPYD intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs.	1-1.5	Reduce starting dose by 50%, followed by dose titration based on clinical judgement (and ideally therapeutic drug monitoring) Individuals with homozygous c.[2846A>T];[2846A>T] genotype, a >50% reduction in starting dose may be warranted.	Moderate
DPYD poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	0.5	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens. In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dose[c] with early therapeutic drug monitoring.[d]	Strong
		0	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens.	

CPIC, Clinical Pharmacogenetics Implementation Consortium
DPD, dihydropyrimidine dehydrogenase.
[a] Rating scheme is described in Supplement (2).
[b] Increase the dose in individuals experiencing no or clinically tolerable toxicity in the first 2 cycles to maintain efficacy; decrease the dose in individuals who do not tolerate the starting dose to minimize toxicities.
[c] If available, a phenotyping test (see main text for further details) should be considered to estimate the starting dose. In the absence of phenotyping data, a dose of <25% of the normal starting dose is estimated assuming additive effects of alleles on 5-FU clearance.
[d] Therapeutic drug monitoring should be done at the earliest timepoint possible (for example, minimum timepoint in steady state) in order to immediately discontinue therapy if the drug level is too high.
This table is adapted from (2). Updated information for Intermediate Metabolizers from Nov 2018 update (5).
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (6).

Table 3. The DPWG Recommendations for Capecitabine/Fluorouracil by *DPD* Gene Activity, Systemic Route of Administration (2019)

DPD gene activity score	Recommendation	Pharmacist text
Activity score 1.5	Start with 50% of the standard dose or avoid fluorouracil and capecitabine. After starting treatment, the dose should be adjusted based on toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolized by DPD.	The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose

Table 3. continued from previous page.

DPD gene activity score	Recommendation	Pharmacist text
Activity score 1.0	Start with 50% of the standard dose or choose an alternative. Adjustment of the initial dose should be guided by toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolized by DPD.	Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.
PHENO[1]	It is not possible to recommend a dose adjustment for these individuals based on the genotype only. Determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype or avoid fluorouracil and capecitabine. Tegafur is not an alternative, as this is also metabolized by DPD.	The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.
Activity score 0	Avoid fluorouracil and capecitabine Tegafur is not an alternative, as this is also metabolized by DPD. If an alternative is not possible: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly. An individual with 0.5% of the normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every 5 days). An individual with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped).	Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the standard dose is a more than 100-fold overdose.

1 Individual's genotype does not accurately predict activity level, phenotyping required.
Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (3, 4).
DPWG, Dutch Pharmacogenetics Working Group

Drug Class: Fluoropyrimidines

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. Currently, there are 3 types of fluoropyrimidines in clinical use: capecitabine (oral – pill) and 5-fluorouracil (5-FU – IV), which are licensed for use in the US, and tegafur, which is not available in the US. Capecitabine and tegafur are both active precursors of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects through several active metabolites. The main mechanism of action is thought to be the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Active metabolites can also be incorporated into RNA and DNA, ultimately leading to cell death (7). Based on their mechanism of action, fluoropyrimidines are teratogenic, as they can cause fetal harm when administered to a pregnant woman (8).

Approximately 10–40% of individuals develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (9). This toxicity typically leads to an interruption or discontinuation of potentially effective anticancer therapy and may require an emergency room visit or hospitalization in severe instances (10).

The inter-individual variation in the occurrence and severity of adverse events in individuals receiving fluoropyrimidines can be partly explained by clinical factors, such as age and gender. However, much of the variability in adverse events remains unexplained (11).

Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the *DPYD* gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive

metabolites. Individuals who have DPD deficiency have a significantly increased risk of severe fluoropyrimidine toxicity, and the stratification of individuals based on *DPYD* genotype may help prevent adverse events (12, 13, 14, 15, 16, 17).

Drug: Capecitabine

Capecitabine is a chemotherapy used as an adjunct treatment for colon cancer, and as either monotherapy or part of combination therapy for metastatic colorectal cancer, metastatic breast cancer, pancreatic cancer, esophageal cancer, head and neck cancers and neuroendocrine tumors (NETs) (1, 18, 19, 20, 21).

Capecitabine is an orally administered prodrug, which is converted to its active form, fluorouracil, by thymidine phosphorylase, an enzyme that can be found in higher concentrations in tumors compared to normal tissue and plasma. Fluorouracil (5-flourouracil, 5-FU) is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD) also catalyzes the rate-limiting step in 5-FU catabolism. Dihydropyrimidine dehydrogenase catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (22).

Once capecitabine is activated to 5-FU, further metabolism generates 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These 2 metabolites achieve cell injury by different mechanisms. The FdUMP targets thymidylate synthase (TS), inhibiting synthesis of an important DNA precursor. The FUTP is incorporated into RNA, leading to inhibition of RNA processing and protein synthesis. (1)

Uridine triacetate (brand name Vistogard) was approved December 11, 2015 as an antidote for fluorouracil and capecitabine overdose (23). Exogenous uridine competes with 5-FU for incorporation into RNA, thus diluting the toxic effects of high 5-FU levels. Uridine triacetate is 4–6-fold higher in bioavailability than equimolar doses of uridine (24).

Uridine triacetate is meant for overdose treatment of adults or children, however, it can be considered in situations of individuals with pharmacogenetic deficiency, which is technically an overdose (25, 26). The high cost of a single course of uridine triacetate therapy has been cited as a potential barrier to therapy. Nevertheless, 94% of clinical trial individuals treated with uridine triacetate survived the overdose event, a notable improvement over the historic mortality rate of 84% (24).

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs to severe neurological problems. In affected individuals, the absent or greatly reduced DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (27).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all individuals with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (28). Signs of capecitabine toxicity include severe diarrhea, severe mucositis, neutropenia, hand-foot syndrome, and neurotoxicity (1).

Capecitabine can cause fetal harm when administered to a pregnant woman; however, the limited human data are not sufficient to inform the drug-associated risk during pregnancy. There is also no information regarding the presence of capecitabine in human milk, or its effects on milk production the breast-fed infant. The FDA label advises that women should not breastfeed during treatment with capecitabine nor for 2 weeks following the final dose. (1)

Safety and efficacy of capecitabine in pediatric individuals has not been established. Additional monitoring and precautions should be employed when administering capecitabine in the elderly and individuals with mild to moderate hepatic dysfunction. Individuals with moderate and severe renal impairment have demonstrated higher exposure for capecitabine and its metabolites when compared to individuals with normal renal function. (1)

Gene: *DPYD*

The *DPYD* gene encodes the enzyme DPD, which catalyzes the first and rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. Dihydropyrimidine dehydrogenase also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. When no variant is detected (formerly known as *1), it is associated with normal enzyme activity. Individuals who have 2 copies of *DPYD* alleles with normal activity are known as "normal metabolizers" and have fully functional DPD enzyme activity (Table 4). The *DPYD* alleles c.1601G>A (*4, rs1801158), c.1627G>A (*5, rs1801159), c.2194G>A (*6, rs1801160), and c.85T>C (*9A, rs1801265) are also considered to have normal activity (29). Historically, variant haplotypes in *DPYD* have been identified by their star (*) allele names. However, the Pharmacogene Variation Database (PharmVar) now identifies these alleles by their dbSNP "rs" allele identifier or cDNA change based on the NM_000110.3 transcript, *DPYD* mRNA variant 1. All 3 of these identifiers are provided in the Nomenclature for Selected *DPYD* alleles table below.

Table 4. Activity Status of Selected *DPYD* Alleles

Allele type	Alleles	
	Strong evidence to support function	Moderate evidence to support function
Normal function	No variant detected (*1), c.1627G>A (*5, rs1801159), c.85T>C (*9A, rs1801265)	c.1601G>A (*4, rs1801158), c.2194G>A (*6, rs1801160), c.1003G>T (*11, rs72549306), c.2657G>A (*9B, rs1801267), 496A>G (rs2297595)
Decreased function	c.2846A>T (rs67376798), 1129-5923C>G and 1236G>A (HapB3)	c.557A>G (rs115232898)
No function	c.1905+1G>A (*2A, rs3918290)	c.1898delC (*3, rs72549303), c.295_298delTCAT (*7, rs72549309), c.703C>T (*8, rs1801266), c.2983G>T (*10, rs1801268), c.1156G>T (*12), c.1679T>G (*13, rs55886062)

This table is adapted from the "*DPYD* Allele Functionality Table", available from CPIC. Additional variant information from the PharmVar database. cDNA coordinates for variation are given for NM_000110.3, *DPYD* transcript variant 1.
For the nomenclature of human *DPYD* alleles, please see (30).

The nonfunctional *DPYD* variants that have been associated with absent DPD activity and an increased risk of toxicity with fluoropyrimidines include c.1905+1G>A (*2A, rs3918290) and c.1679T>G (*13, rs55886062) (22). Variants with decreased function include rs67376798 (c.2846A>T) and HapB3, which also are associated with an increased risk of fluoropyrimidine toxicity. The most well-studied variant is *DPYD* c.1905+1G>A (*2A, rs3918290), in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to exon 14 skipping, resulting in the production of a truncated protein with no enzyme activity.

Individuals who have one normal function and one decreased function or no function *DPYD* alleles are known as "intermediate metabolizers". Individuals with 2 decreased function alleles are also categorized as intermediate metabolizers, as they have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who have a combination of nonfunctional *DPYD* alleles, or decreased function *DPYD* alleles, or both are known as "poor metabolizers", as they have complete DPD deficiency and are at very high risk of capecitabine toxicity.

Activity scores may be used to distinguish between the various *DPYD* alleles and their functionality (Table 5). The use of activity scores may result in differentiated individualized dosing advice for fluoropyrimidines, which is beneficial for reducing toxic side effects while maintaining efficacy (16).

Table 5. Assignment of likely DPD Phenotype based on *DPYD* Genotype (CPIC, 2017)

Likely phenotype	Activity score[a]	Genotype[b]	Examples of genotype[c]
DPYD normal metabolizer	2	An individual with 2 normal function alleles.	c.[=]; [=] c.[85T>C]; [=] c.[1627A>G]; [=]
DPYD intermediate metabolizer (approximately 3–5% of individuals)	1 or 1.5	An individual with one normal function allele plus one no function allele or one decreased function allele, or an individual with 2 decreased function alleles.	c.[1905+1G>A]; [=] c.[1679T>G]; [=] c.[2846A>T]; [=] c.[1129–5923C>G]; [=][d] c.[1129–5923C>G]; [1129–5923C>G][d] c.[2846A>T]; [2846A>T]
DPYD poor metabolizer (approximately 0.2% of individuals)	0 or 0.5	An individual with 2 no function alleles or an individual with one no function plus one decreased function allele.	c.[1905+1G>A]; [1905+1G>A] c.[1679T>G]; [1679T>G] c.[1905+1G>A]; [2846A>T] c.[1905+1G>A]; [1129-5923C>G]

"[ ]" Square brackets are used to indicate an allele, "[=]" Indicates the allele sequence was tested and no changes were found
[a] Calculated as the sum of the 2 lowest individual variant activity scores. See (2) for further information.
[b] Allele definitions, assignment of allele function and references can be found on the CPIC website (*DPYD* Allele Functionality Table)
[c] HGVS nomenclature using the reference sequence NM_000110.3.
[d] Likely HapB3 causal variant. See *DPYD* Allele Functionality Table available or other HapB3 proxy SNPs. This table is adapted from (2).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC (6).

Overall, the prevalence of individuals who are heterozygous for nonfunctional variant *DPYD* alleles (partially DPD deficient) and at risk of severe drug reactions is estimated to be as high as 5–8%, but this varies in different populations (9, 28, 31, 32, 33, 34, 35). In Caucasians, approximately 3–5% of have partial DPD deficiency and 0.2% have complete DPD deficiency (32). Recent studies suggest that ~8% of Caucasians have at least one of the 4 best-known altered-function alleles (36).

In African-Americans, the prevalence of decreased DPD enzyme activity is 8% (35). It is notable that despite being well studied, *DPYD* c.1905+1G>A (*2A, rs3918290) is very rare in individuals of African ancestry (37). One study did note that the normal function c.85T>C (*9A, rs1801265) variant was present in 49% of African-American samples (38). The rs115232898 (c.557A>G) variant allele with reduced function was detected in 2.6% of African-heritage Brazilians (39).

Studies of Egyptian and Tunisian populations suggest the allelic frequencies for *DPYD* variants in in these 2 countries are similar to Caucasian variant allele frequencies (40, 41). The frequency of the poor-metabolizer rs67376798 (c.2846A>T) allele in Mestizo and Native Mexican populations is rare, but not significantly different than in MXL (Mexican Ancestry from Los Angeles USA) or CEU (Utah Residents (CEPH) with Northern and Western European Ancestry) populations in the 1000 genomes project (42).

Asian populations have slightly different allele frequencies as compared to African and European populations. The frequency of the c.85T>C (*9A, rs1801265) normal function variant was slightly lower in Han Chinese, Korean and Japanese populations, particularly compared to Africans, though the frequency of the c.2657G>A (*9B, rs1801267) normal function variant and c.295_298delTCAT (*7, rs72549309), c.703C>T (*8, rs1801266), and c.2983G>T (*10, rs1801268) no function alleles were similar across these groups (38). The c.1905+1G>A (*2A/*2B, rs3918290) and c.1679T>G (*13, rs55886062) no function alleles were not detected in a study of Hmong and East Asian descent individuals, underscoring the rarity of these alleles (43). An analysis of multiple

genotyping studies in South Asian populations found that the normal function rs2297595 (c.496A>G) allele was prevalent in south Asia (44).

Most individuals in the US are not screened for DPD deficiency before starting fluorouracil therapy (45). In contrast, the European Medicines Agency recommends testing for DPD deficiency before initiating treatment with any fluorouracil related chemotherapy (37).

Gene: *TYMS*

Emerging studies and reports suggest that genetic variation at another locus may also affect 5-FU efficacy and toxicity—*TYMS*. This gene encodes TS, which catalyzes the methylation of deoxyuridylate to deoxythymidylate. This reaction is a rate-limiting step in the production of an essential DNA synthesis precursor. The TS expression correlates with sensitivity to 5-FU and the TS enzyme one of the targets of 5-FU(46). While this functional link to 5-FU metabolism and tumor response has been demonstrated in multiple studies, the impact of specific genetic variants in *TYMS* is less clear (46, 47, 48, 49). The TYMS alleles have been reported in a handful of studies as being associated with increased toxicity and anti-tumor cell response with fluoropyrimidines.

The rs45445694 polymorphism is the defining variant of the TYMS "2R" allele, which has been associated with clinical response and severe toxicity events, either in homozygosity or heterozygosity (25, 50, 51, 52). This allele is in the 5'UTR and is a duplication a 28 base pair (bp) repeat. This same locus can have variable tandem repeats between 0 and 9 copies, and studies suggest that increased copy numbers of the repeat are associated with increased *TYMS* expression and TS protein levels (53).

One additional variant in *TYMS* has been found in association with adverse reactions to fluoropyrimidine therapy: a 3'UTR 9 bp-indel (rs11280056) (51, 53). There are conflicting reports as to whether this is a 6- or 9-bp-indel. One variant (rs2853542) within the TYMS enhancer region in the context of the 28bp tandem repeat triplication, called 3RG or 3RC based on the specific nucleotide present, has also been reported in association with neurotoxicity during 5-FU treatment (54). The presence of the C nucleotide at rs2853542 has been associated with decreased expression of TYMS mRNA (55).

PharmGKB has described TYMS as a Very Important Pharmacogene, though the level of evidence for TYMS and capecitabine/5-fluorouracil interaction is limited (PharmGKB "level 3") (53). CPIC also views this interaction as having limited evidence and thus provides no prescribing recommendations for these pharmacogenetic variants (56).

Linking Gene Variation with Treatment Response

Standard doses of fluorouracil increase the risk of severe toxicity in individuals who have specific *DPYD* variant alleles. No dose of capecitabine is safe in individuals with absent DPD activity (1, 57). Multiple studies have found that preemptive *DPYD* screening for individuals with cancer can significantly improve individual safety (10, 36, 58, 59, 60, 61, 62, 63, 64). Additionally, prospective genotyping decreased chemotherapy toxicities and was cost effective (10).

At least one case report indicated that the cost of administering uridine triacetate and palliative care following an adverse, overdose reaction to 5-FU was roughly $180,000 USD (25). This is significantly higher than the cost of most pre-emptive pharmacogenetic tests.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the *DPYD* gene, *TYMS* gene, and the capecitabine drug response. The *DPYD*2A* variant is the most commonly tested. Tests available for clinical practice include full gene sequencing and targeted panel-based testing of selected variants. In cases

where a targeted panel is used, only those specific variants are examined, A negative result does not mean the individual does not have DPD deficiency. Clinicians should refer to the specific testing laboratory for complete information on the test. CPIC provides a table of minor allele frequencies for *DPYD* variants per ethnic populations, which may be useful when determining what type of test or panel will be most informative for any individual (5).

Biochemical genetic tests may also be used, which assess the level of activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (for example, measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (for example, directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (65, 66, 67).

The GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme dihydropyrimidine dehydrogenase.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Based on postmarketing reports, individuals with certain homozygous or certain compound heterozygous mutations in the [*DPYD*] gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by capecitabine (e.g., mucositis, diarrhea, neutropenia, and neurotoxicity). Individuals with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by capecitabine.

Withhold or permanently discontinue capecitabine based on clinical assessment of the onset, duration and severity of the observed toxicities in individuals with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No capecitabine dose has been proven safe for individuals with complete absence of DPD activity. There is insufficient data to recommend a specific dose in individuals with partial DPD activity as measured by any specific test.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC), with November 2018 Update

[…]

Table 2 summarizes the genetics-based dosing recommendations for fluoropyrimidines using the calculated *DPYD* activity score (*DPYD*-AS). The strength of the prescribing recommendations is based on the known impact of some variants (c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129–5923C>G) on DPD activity, the demonstrated relationship between DPD activity and 5- fluorouracil clearance, and between 5-fluorouracil exposure and its toxic effects. Individuals who are heterozygous for *DPYD* decreased/no function variants demonstrate partial DPD deficiency and should receive reduced starting doses. Prospective genotyping of c.1905+1G>A followed by a 50% dose reduction in heterozygous carriers resulted in a rate of severe toxicity comparable to noncarriers[see (10)]. This study thus demonstrated that *DPYD* genetic testing can reduce the

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

occurrence of severe fluoropyrimidine-related toxicity, and that a dose reduction of 50% is suitable for heterozygous carriers of no function variants (*DPYD*-AS: 1). For decreased function variants, evidence is limited regarding the optimal degree of dose reduction. For c.2846A>T, a small retrospective study observed that the average capecitabine dose in heterozygous carriers was reduced by 25% compared to noncarriers. In a small prospective study, five individuals carrying c.1236G>A (proxy for c.1129–5923C>G) were safely treated with a 25% reduced capecitabine starting dose. This suggests that heterozygous carriers of decreased function variants (*DPYD*-AS: 1.5) may tolerate higher doses com- pared to carriers of no function variants (*DPYD*-AS: 1). In individuals with *DPYD*-AS of 1.5, the individual circumstances of a given individual should therefore be considered to determine if a more cautious approach (50% starting dose followed by dose titration), or an approach maximizing potential effectiveness with a potentially higher toxicity risk (25% dose reduction) is preferable. Of note, both studies indicating the suitability of a 25% dose reduction in decreased function variant carriers included only individuals receiving capecitabine and no data are currently available for infusional 5-fluorouracil.

Given that some individuals carrying decreased or no function variants tolerate normal doses of 5-fluorouracil, to maintain effectiveness, doses should be increased in subsequent cycles in individuals experiencing no or clinically tolerable toxicity in the first two chemotherapy cycles or with subtherapeutic plasma concentrations. Similarly, doses should be decreased in individuals who do not tolerate the starting dose.

In *DPYD* poor metabolizers (*DPYD*-AS: 0.5 or 0), it is strongly recommended to avoid use of 5-fluorouracil-containing regimens. However, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, 5-fluorouracil administration at a strongly reduced dose combined with early therapeutic drug monitoring may be considered for individuals with *DPYD*-AS of 0.5. It should be noted, however, that no reports of the successful administration of low-dose 5-fluorouracil in *DPYD* poor metabolizers are available to date. Assuming additive effects of decreased and no function alleles (*DPYD*-AS: 0.5), it is estimated that a dose reduction of at least 75% would be required (i.e., starting dose <25% of normal dose). Furthermore, in such cases a phenotyping test is advisable to estimate DPD activity and a starting dose.

The US Food and Drug Administration (FDA) and the Health Canada Santé Canada (HCSC) have added statements to the drug labels for 5-fluorouracil and capecitabine that warn against use in individuals with DPD deficiency, and prescribing recommendations for 5-fluorouracil, capecitabine, and tegafur are also available from the Dutch Pharmacogenetics Working Group.

November 2018 Update:

The current *DPYD* guideline recommends to reduce the dose of fluoropyrimidines by 25-50% (from the full standard dose) in *DPYD* Intermediate Metabolizers with an activity score of 1.5. At the time of the guideline publication, this dose range was recommended due to limited evidence for genotype-guided dosing of decreased function alleles/variants. However, a recent prospective study (PMID: 30348537) provides evidence to support a recommendation for a 50% dose reduction in heterozygous carriers of the decreased function variants c.2846A>T (rs67376798) or c.1129–5923C>G (rs75017182; HapB3 or its tagging variant c.1236G>A; rs56038477). These data suggest that all Intermediate Metabolizers with an activity score of 1.5 should receive a 50% dose reduction.

Therefore CPIC revises its recommendation such that all *DPYD* Intermediate Metabolizers should receive a 50% dose reduction from the full standard starting dose, whether the activity score is 1 or 1.5 followed by dose titration, based on clinical judgement and ideally therapeutic drug monitoring.

In addition, recent case reports from individuals who are homozygous for c.2846A>T (activity score of 1) indicate that a dose reduction of more than 50% may be required in some carriers of this genotype. Therefore, in individuals with an activity score of 1 due to a homozygous c.[2846A>T];[2846A>T] genotype, clinicians should be aware that a >50% reduction in starting dose might be warranted.

Please review the complete therapeutic recommendations that are located here: (2, 5)

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

DPD Gene Activity Score 0

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the standard dose is a more than 100-fold overdose.

- Avoid fluorouracil and capecitabine

Tegafur is not an alternative, as this is also metabolized by DPD.

- If it is not possible to avoid fluorouracil and capecitabine: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly.

An individual with 0.5% of the normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every 5 days). An individual with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped)

DPD PHENO [phenotyping indicates reduced function]

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

It is not possible to recommend a dose adjustment for this individual based on the genotype only.

- determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype, or avoid fluorouracil and capecitabine.

Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 1

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- Start with 50% of the standard dose or avoid fluorouracil and capecitabine.

Adjustment of the subsequent dose should be guided by toxicity and effectiveness. However, in one study involving 17 individuals with gene activity 1, the average dose after titration was 57% of the standard dose.

Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 1.5

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- Start with 50% of the standard dose or avoid fluorouracil and capecitabine.

After starting treatment, the dose should be adjusted based on toxicity and effectiveness. In a study involving 17 individuals with genotype *1/2846T*, the average dose after titration was 64% of the standard dose. For 51 individuals with genotype *1/1236A*, the average dose after titration was 74% of the standard dose. Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 0 (Cutaneous fluorouracil)

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- avoid fluorouracil

NOTE: If an individual has two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this individual actually has a gene activity score 1, for which no increased risk of severe, potentially fatal toxicity has been found with cutaneous use. These two situations can only be distinguished by determining the enzyme activity (phenotyping). This recommendation only applies if the individual has virtually no enzyme activity.

Background Information - Mechanism

Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of fluorouracil and its prodrug capecitabine. This leads to an increased risk of adverse events such as neutropenia, thrombopenia and hand-foot syndrome.

For more information about the phenotype gene activity score: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Please review the complete therapeutic recommendations that are located here: (3, 4).

Nomenclature for Selected *DPYD* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs3918290	*DPYD**2A, c.1905+1G>A IVS14+1G>A	NM_000110.3:c.1905+1G>A	Not applicable—deletion of exon 14 leads to the production of a truncated protein	rs3918290
rs55886062	*DPYD*13*, c.1679T>G, rs55886062.1, p.Ile560Ser	NM_000110.3:c.1679T>G	NP_000101.2:p.Ile560Ser	rs55886062
rs67376798	c.2846A>T p.Asp949Val	NM_000110.3:c.2846A>T	NP_000101.2:p.Asp949Val	rs67376798
rs75017182	c.1129–5923C>G	NM_000110.3:c.1129-5923C>G	Altered mRNA splicing introduces premature termination codon in resulting protein.	rs75017182
rs1801159	*DPYD**5, c.1627G>A	NM_000110.4:c.1627A>G	NP_000101.2:p.Ile543Val	rs1801159
rs1801265	*DPYD**9A, c.85T>C	NM_000110.4:c.85T>C	NP_000101.2:p.Cys29Arg	rs1801265
rs1801158	*DPYD**4, c.1601G>A	NM_000110.4:c.1601G>A	NP_000101.2:p.Ser534Asn	rs1801158

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs1801160	*DPYD**6, c.2194G>A	NM_000110.4:c.2194G>A	NP_000101.2:p.Val732Ile	rs1801160
rs72549306	*DPYD**11, c.1003G>T, rs72549306.1	NM_000110.4:c.1003G>T	NP_000101.2:p.Val335Leu	rs72549306
rs1801267	*DPYD**9B, c.2657G>A	NM_000110.4:c.2657G>A	NP_000101.2:p.Arg886His	rs1801267
rs72549303	*DPYD**3, c.1898delC	NM_000110.4:c.1898del	NP_000101.2:p.Pro633fs	rs72549303
rs72549309	*DPYD**7, c.295_298delTCAT	NM_000110.4:c.295_298TCAT[1]	NP_000101.2:p.Phe100fs	rs72549309
rs1801266	*DPYD**8, c.703C>T	NM_000110.4:c.703C>T	NP_000101.2:p.Arg235Trp	rs1801266
rs1801268	*DPYD**10, c.2983G>T	NM_000110.4:c.2983G>T	NP_000101.2:p.Val995Phe	rs1801268
rs78060119	*DPYD**12, c.1156G>T	NM_000110.4:c.1156G>T	NP_000101.2:p.Glu386Ter	rs78060119
rs115232898	557A>G (Y186C)	NM_000110.4:c.557A>G	NP_000101.2:p.Tyr186Cys	rs115232898
rs2297595	496A>G (M166V)	NM_000110.4:c.496A>G	NP_000101.2:p.Met166Val	rs2297595
rs75017182 rs56038477	HapB3 1129-5923C>G 1236G>A	NM_000110.4:c.1129-5923C>G NM_000110.4:c.1236G>A	Altered mRNA splicing introduces premature termination codon in resulting protein. NP_000101.2:p.Glu412=	rs75017182 rs56038477
rs45445694	2R, 3R TYMS 5'UTR	GRCh37.p13 chr 18, NC_000018.9:g.657657_657712del, NC_000018.9:g.657657_657684GGCCTGCCTCCGTCCCGCCGCGCCACTT[1]-[9]#		rs45445694
rs11280056	TYMS 3'UTR	GRCh37.p13 chr 18, NC_000018.9:g.673447_673452del, NC_000018.9:g.673447_673452dup# NM_017512.7:c.*856_*861del		rs11280056
rs2853542	TYMS 3RG, 3RC	GRCh37.p13 chr 18, NC_000018.9:g.657685G>C# NM_001071.4:c.-58=		rs2853542

This is a non-coding variant in the *TYMS* untranslated region. Coordinates given are chromosomal.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (68).
Allele information for *DPYD* can also be found at the Pharmacogene Variation Consortium (PharmVar).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Carbamazepine Therapy and *HLA* Genotype

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Introduction

Carbamazepine (brand names include Carbatrol, Epitol, Equetro, and Tegretol) is an effective antiseizure drug that is often used as a first-line agent in the treatment of epilepsy. Carbamazepine is also used to treat bipolar disorder and to relieve pain in trigeminal neuralgia.

Hypersensitivity reactions associated with carbamazepine can occur in up to 10% of patients, and typically affect the skin. Some of these reactions are mild, as in the case of maculopapular exanthema (MPE); however, conditions such as Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) are potentially life-threatening.

The risk of hypersensitivity is increased by the presence of specific human leukocyte antigen (*HLA)* alleles. The *HLA-B*15:02* allele is strongly associated with carbamazepine-induced SJS/TEN in populations where this allele is most common, such as in Southeast Asia.

According to the FDA-approved drug label for carbamazepine, testing for *HLA-B*15:02* should be done for all patients with ancestry in populations with increased frequency of *HLA-B*15:02*, prior to initiating carbamazepine therapy (Table 1). The label states that greater than 15% of the population is reported *HLA-B*15:02* positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. The label states that South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*15:02*, averaging 2 to 4%, but higher in some groups. In Japan and Korea, the *HLA- B*15:02* is present in less than 1% of the population. In individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans), the *HLA-B*15:02* allele is largely absent. These prevalence rates of *HLA-B*15:02* may be used to guide which patients should be screened. However, the FDA cautions to keep in mind the limitations of prevalence rate data when deciding which patients to screen. This is because of the wide variability in *HLA-B*15:02* rates (even within ethnic groups), the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry (1).

The FDA label also states that carbamazepine should not be used in patients who are positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks. Tested patients who are found to be negative for the allele are thought to have a low risk of SJS/TEN.

The *HLA-A*31:01* allele may also be a risk factor for SJS/TEN but is more strongly associated with other carbamazepine-induced reactions, such as DRESS and MPE. *HLA-A*31:01* is found in most populations, worldwide. *HLA-B*15:11* is another allele that has been linked with SJS/TEN. The FDA states that the risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*, but does not discuss *HLA-B*15:11* (1).

Carbamazepine dosing guidelines based on *HLA* genotype have been published by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), the Clinical Pharmacogenetics Implementation Consortium (CPIC), and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) (2-5).

DPWG recommendations include avoiding the use of carbamazepine and selecting an alternative, if possible, for individuals positive for *HLA-B*15:02*, *HLA-A*31:01* and *HLA-B*15:11* (Table 2). CPIC recommendations

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include not using carbamazepine in carbamazepine-naïve patients who are positive for *HLA-B*15:02* and any *HLA-A*31:01* genotype (or *HLA-A*31:01* genotype unknown) (Table 3). CPNDS recommends genetic testing for all carbamazepine-naïve patients before they start treatment, with a moderate level of evidence for *HLA-A*31:01* testing, and strong to optional evidence for *HLA-B*15:02* testing (based on the frequency of *HLA-B*15:02* in the population the patient originates from, and if this is known or not) (Table 4).

Table 1. FDA (2018) Drug Label for Carbamazepine. Recommendations for *HLA-B*15:02* and *HLA-A*31:01* Genotype: Warnings.

Genotype	Recommendations
SJS/TEN and *HLA-B*15:02* Allele	Prior to initiating carbamazepine therapy, testing for *HLA-B*15:02* should be performed in patients with ancestry in populations in which *HLA-B*15:02* may be present. Carbamazepine should not be used in patients positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks.
Hypersensitivity Reactions and *HLA-A*31:01* Allele	The risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

Table 2. DPWG (2017) Recommendations for Carbamazepine and *HLA* Genotype.

Genotype	Recommendations
*HLA-B*15:02* positive	Choose an alternative if possible
*HLA-A*31:01* positive	1. carefully weigh the risk of DRESS and SJS/TEN against the benefits 2. if an alternative is an option, choose an alternative
*HLA-B*15:11* positive	1. carefully weigh the risk of SJS/TEN against the benefits 2. if an alternative is an option, choose an alternative

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (2).

Table 3. CPIC (2016) Recommendations for Carbamazepine Therapy based on *HLA-B* and *HLA-A* Genotype.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
*HLA-B*15:02* negative and *HLA-A*31:01* negative	Normal risk of carbamazepine-induced SJS/TEN, DRESS, and MPE	Use carbamazepine per standard dosing guidelines.[b]	Strong	N/A
*HLA-B*15:02* negative and *HLA-A*31:01* positive	Greater risk of carbamazepine-induced SJS/TEN, DRESS, and MPE	If patient is carbamazepine-naïve and alternative agents are available, do not use carbamazepine.	Strong	Other aromatic anticonvulsants[d] have very limited evidence, if any, linking SJS/ TEN, DRESS, and/or MPE with the *HLA-A*31:01* allele, and thus no recommendation can be made with respect to choosing another aromatic anticonvulsant as an alternative agent.

Table 3. continued from previous page.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
		If patient is carbamazepine-naïve and alternative agents are not available, consider the use of carbamazepine with increased frequency of clinical monitoring. Discontinue therapy at first evidence of a cutaneous adverse reaction.	Optional	N/A
		The latency period for cutaneous adverse drug reactions is variable depending on phenotype; however, all usually occur within three months of regular dosing. Therefore, if the patient has previously used carbamazepine consistently for longer than three months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine.	Optional	Previous tolerance of carbamazepine is not indicative of tolerance to other aromatic anticonvulsants.[d]
*HLA-B*15:02* positive[c] and any *HLA-A*31:01* genotype (or *HLA-A*31:01* genotype unknown)	Greater risk of carbamazepine-induced SJS/TEN	If patient is carbamazepine-naïve, do not use carbamazepine.	Strong	Other aromatic anticonvulsants[d] have weaker evidence linking SJS/TEN with the *HLA-B*15:02* allele; however, caution should still be used in choosing an alternative agent.

Table 3. continued from previous page.

Genotype[a]	Implication	Therapeutic recommendation	Classification of recommendations	Considerations for other aromatic anticonvulsants
		The latency period for drug-induced SJS/TEN is short with continuous dosing and adherence to therapy (4-28 days), and cases usually occur within three months of dosing; therefore, if the patient has previously used carbamazepine consistently for longer than three months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine in the future.	Optional	Previous tolerance of carbamazepine is not indicative of tolerance to other aromatic anticonvulsants.[d]

DRESS, drug reaction with eosinophilia and systemic symptoms; MPE, maculopapular exanthema; N/A, not applicable; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.
[a]If only *HLA-B*15:02* was tested, assume *HLA-A**31:01 is negative and vice versa.
[b]*HLA-B*15:02* has a 100% negative predictive value for carbamazepine-induced SJS/ TEN, and its use is currently recommended to guide the use of carbamazepine and oxcarbazepine only. Because there is a much weaker association and less than 100% negative predictive value of *HLA-B*15:02* for SJS/TEN associated with other aromatic anticonvulsants, using these drugs instead of carbamazepine or oxcarbazepine in the setting of a negative *HLA-B*15:02* test in Southeast Asians will not result in prevention of anticonvulsant-associated SJS/TEN.
[c]In addition to *HLA-B*15:02*, the risk for carbamazepine-induced SJS/TEN has been reported in association with the most common B75 serotype alleles in Southeast Asia, *HLA-B**15:08, *HLA-B*15:11*, and *HLA- B*15:21*. Although not described, the possibility of carbamazepine-induced SJS/TEN in association with less frequently carried B75 serotype alleles, such as *HLA- B**15*:30* and *HLA-B*15:31*, should also be considered.
[d]Aromatic anticonvulsants include carbamazepine, oxcarbazepine, eslicarbazepine, lamotrigine, phenytoin, fosphenytoin, and phenobarbital.
This table is adapted from Phillips EJ, Sukasem C, Whirl-Carrillo M, Müller DJ, Dunnenberger HM, Chantratita W, Goldspiel B, Chen YT, Carleton BC, George ALJ, Mushiroda T, Klein T, Gammal RS, and Pirmohamed M. Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. Clinical pharmacology and therapeutics (4).

Table 4. CPNDS (2014) Recommendations for Carbamazepine and *HLA* Genotype.

Genotype	Recommendation 1.1
*HLA-B*15:02*	Genetic testing for *HLA-B*15:02* is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level A – strong in patients originating from populations where *HLA-B*15:02* is common, its frequency unknown or whose origin is unknown; Level C – optional in patients originating from populations where *HLA-B*15:02* is rare).
*HLA-A*31:01*	Genetic testing for *HLA-A*31:01* is recommended for all CBZ-naive patients before initiation of CBZ therapy (Level B – moderate in all patients; Table 7).

CBZ: Carbamazepine.
Please see Therapeutic Recommendations based on Genotype for the all the recommendations from CPNDS, and the grading scheme used for the level of evidence. Table is adapted from (5).

Drug: Carbamazepine

Carbamazepine is an antiseizure drug used in the treatment of epilepsy. Carbamazepine is also used as an analgesic in trigeminal neuralgia and may be used in the treatment of bipolar disorder (5, 7, 8).

Epilepsy is characterized by spontaneous recurrent epileptic seizures, which may be classified as focal or generalized. Carbamazepine is one of the first-line treatments for focal seizures in adults, adolescents, and children, and it may also be considered for general tonic-clonic seizures.

The symptoms of focal seizures depend upon where the focus of the seizure originates in the brain e.g., jerking of a limb indicates a focus in the contralateral motor cortex. In contrast, generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase).

Carbamazepine is a tricyclic compound that belongs to the class of antiseizure drugs that act by blocking voltage-dependent sodium channels present on neuronal cell membranes. Carbamazepine stabilizes the sodium channel in the inactivated state, leaving fewer of the channels available to open. This prolonged inactivated phase of the channel inhibits the rapid and repetitive generation of action potentials in the epileptic focus (3, 9).

Carbamazepine is metabolized in the liver by the cytochrome P-450 (CYP) system. The major metabolite is carbamazepine-epoxide, which has an anticonvulsant activity of uncertain significance. CYP3A4 is the main enzyme involved in the metabolism of carbamazepine; a lesser role is played by CYP2C8 and possibly CYP3A5. Minor metabolic pathways include multiple CYP enzymes, such as CYP2B6.

Carbamazepine stimulates transcriptional upregulation of CYP3A4 and other genes involved in its own metabolism. In addition, there are many drug-drug interactions with carbamazepine, because numerous drugs have been shown to induce or inhibit CYP3A4, or are metabolized by CYP3A4. Therefore, when carbamazepine is given with drugs that can decrease or increase carbamazepine levels, close monitoring of carbamazepine levels is indicated and dosage adjustment may be required (10, 11).

Carbamazepine-induced Adverse Drug Reactions

In general, there are two categories of adverse drug reactions. Type A reactions account for up to 85–90% of all adverse drug reactions. They are predictable, based on the known properties of the drug, and they can affect any individual if their exposure to the drug is high enough. For carbamazepine, type A adverse effects include sedation, CNS depression, and vestibular symptoms such as nystagmus and ataxia.

Type B reactions account for the remaining 10–15% of adverse drug reactions. These reactions are difficult to predict (idiosyncratic) because they can occur at any dose, and they develop through a mechanism that is unrelated to the mechanism of action of the drug. For carbamazepine, type B adverse reactions include carbamazepine-induced hypersensitivity reactions that typically involve the skin.

Approximately 5–10% of patients taking carbamazepine will experience carbamazepine-induced cutaneous reactions. Most of these are considered to be mild, such as maculopapular exanthema (MPE) and erythema multiforme. However, these cutaneous reactions can cause considerable discomfort to the patient and often lead to the discontinuation of carbamazepine therapy (5, 12, 13). In addition, treatment may be stopped because of the risk of a more severe, cutaneous drug reaction developing.

Stevens-Johnson syndrome (SJS) and the more severe form, toxic epidermal necrolysis (TEN), can be induced by carbamazepine therapy. These are life-threatening conditions that are primarily characterized by lesions of the skin (detachment of the epidermis) and mucous membranes (severe erosions) (11). SJS/TEN occurs in approximately 1–10 per 10,000 patients taking carbamazepine. Onset is delayed and may occur several weeks after the initiation of carbamazepine therapy. The mortality rate is high—up to 10% for SJS, and 50% for TEN (11, 14, 15). Pediatric patients who survive SJS/TEN usually have long-term complications, such as scarring, visual loss and chronic kidney disease (16).

Another severe and potentially life-threatening carbamazepine-induced hypersensitivity reaction is known as drug reaction with eosinophilia and systemic symptoms (DRESS, also known as drug-induced hypersensitivity syndrome, HSS).

The mechanisms underlying this hypersensitivity reaction is poorly understood, but is thought to involve the drug, or a molecule derived from the drug, interacting with the major histocompatibility complex (MHC) expressed on the surface of cells, resulting in a stimulation of the immune system, particularly T cells and eosinophils (5, 15).

Individuals who have specific *HLA* variants are known to be susceptible to carbamazepine-induced hypersensitivity reactions. In 2007, the FDA added a warning to the drug label concerning carbamazepine-induced SJS/TEN, with a recommendation for *HLA-B*15:02* screening in South-East Asian populations (17). Carbamazepine should not be used in patients who have the *HLA-B*15:02* variant (Table 1).

Screening of patients prior to carbamazepine therapy can identify those at higher risk of hypersensitivity reactions, allowing for an alternative drug to be used. Clinical practice guidelines for the treatment of epilepsy, bipolar disorder, and trigeminal neuralgia should be consulted for recommended alternative therapies to carbamazepine. However, caution should be used because of the risk of cross-reactivity between structurally similar antiseizure drugs (oxcarbazepine, lamotrigine, phenytoin, phenobarbital, primidone) (5, 18).

Up to 80% of patients who have an unexpected adverse reaction to carbamazepine will also have an adverse reaction to other antiseizure drugs, thereby restricting treatment options (19). Phenytoin and lamotrigine have both been associated with carbamazepine-induced SJS/TEN, and some evidence also links fosphenytoin, oxcarbazepine, eslicarbazepine acetate with SJS/TEN (20).

HLA gene family

The human leukocyte antigen (*HLA*) genes code for more than 200 different major histocompatibility complex (MHC*)* proteins. The MHC family has been subdivided into three subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III.

The class I region contains the proteins encoded by the HLA genes *HLA-A, HLA-B, and HLA-C*. These MHC molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting antigens. The MHC class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of MHC class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins ("self"). However, if foreign peptide fragments are presented, e.g., from a pathogen, CD8+T cells will recognize the peptides as "non-self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because MHC molecules need to present such a wide variety of "self" and "non-self" peptides, the *HLA* genes are both numerous and highly polymorphic. More than 1,500 *HLA-B* alleles have been identified (7). HLA allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a two digit number that corresponds to antigen specificity, and the assigned allele number (21). For example, the *HLA-B*15:02* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular *HLA* gene in this region)
- 15: the allele group (historically determined by serotyping, i.e., a group of alleles that share the same serotype)
- 02: the specific *HLA* allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence but differ in their genetic sequence (i.e., because of synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in the susceptibility to autoimmune disease and infections. They are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

More recently, *HLA* variants have been associated with an increasing number of drug hypersensitivity responses (Type B adverse drug reactions). The strongest HLA-associated drug responses are *HLA-B*15:02* and carbamazepine-induced SJS/TEN in Asian populations, *HLA-B*57:01* and abacavir hypersensitivity syndrome in the Caucasian population, and *HLA-B*58:01* in allopurinol hypersensitivity syndrome and SJS/TEN (22).

Gene: HLA-B, Allele HLA-B*15:02

*HLA-B*15:02* is strongly associated with carbamazepine-induced SJS/TEN in populations where the *HLA-B*15:02* is common (China, Thailand, India, Malaysia, Taiwan). In patients of Asian origin, pharmacogenetic testing for *HLA-B*15:02* is recommended before initiation of carbamazepine therapy (23). The clinical benefits of screening for *HLA-B*15:02* have been confirmed in a Taiwanese study, where genetic testing reduced the incidence of carbamazepine-induced SJS/TEN from ten expected cases to zero (24).

The association between *HLA-B*15:02* and SJS/TEN was first reported in the Han Chinese. In the initial study, every patient who had carbamazepine-induced SJS/TEN was found to have the *HLA-B*15:02* allele (44/44, 100%), whereas the allele was much less common in carbamazepine-tolerant patients (3/101, 3%) (25). The *HLA*15:02* allele has since been associated with carbamazepine-induced SJS/TEN in Taiwanese, Chinese, Indians, Malay, and Chinese-Americans, but not in Caucasians or Japanese individuals (25-32).

The prevalence of carbamazepine-induced SJS/TEN is higher in populations where the *HLA-B*15:02* allele is most common. *HLA-B*15:02* is highly prevalent in Southeast Asia, with an allele frequency of over 15% in Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines. It is slightly less prevalent (10–13%) in Taiwan and Singapore, and in North China (4%). South Asians, including Indians, have intermediate prevalence of *HLA-B*15:02* (2–4%), with higher frequencies in some sub-populations (3, 5, 33-37).

The *HLA-B*15:02* allele is rare (frequency of less than 1%) in East Asia (Japan and Korea) and in individuals who are not of Asian descent. For example, the variant is rare in Europeans, Hispanics, Africans, African Americans, and Native Americans (5, 34). The absence of this variant in these population explains the lack of association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN in Caucasians and Japanese individuals.

Current data suggest that *HLA-B*15:02* is a risk factor only for SJS/TEN because it does not appear to increase the risk of other carbamazepine-induced cutaneous reactions such as MPE and DRESS (5).

Gene: *HLA-A*, Allele *HLA-A*31:01*

The *HLA-A*31:01* allele is important for all types of carbamazepine hypersensitivity reactions. Also, in contrast to *HLA-B*15:02* which is predominantly found in Southeast Asia, the *HLA-A*31:01* is found in many populations worldwide (Table 5) (38-40).

Table 5. Comparison of *HLA-B*15:02* and *HLA-A*31:01* and Carbamazepine Therapy

	*HLA-B*15:02*	*HLA-A*31:01*
Associated phenotype	*HLA-B*15:02* is strongly associated with SJS/TEN	*HLA-A*31:01* is associated with all carbamazepine hypersensitivity phenotypes, including MPE, HSS, and (less strongly) SJS/TEN

Table 5. continued from previous page.

	*HLA-B*15:02*	*HLA-A*31:01*
Allele distribution	Predominantly concentrated in Southeast Asia, e.g., Hong Kong, Thailand, Malaysia, Vietnam, Philippines, Taiwan, Singapore.	Widely distributed across a range of populations including Europeans, Japanese, South Koreans and Han Chinese
Phenotype distribution	The strong association of *HLA-B*15:02* with carbamazepine-induced SJS/TEN is largely confined to individuals from Southeast Asian countries	*HLA-A*31:01* has been associated with carbamazepine-induced hypersensitivity reactions, particularly HSS, across different populations including European and Japanese individuals.
Pharmacogenetic screening recommendations	Screening for *HLA-B*15:02* is mandated in patients from Southeast Asia, prior to initiation of carbamazepine therapy. The FDA states that "patients with ancestry in genetically at-risk populations should be screened for the presence of *HLA-B*15:02* prior to initiating treatment with carbamazepine".	Screening is not currently mandated prior to initiation of carbamazepine therapy. The FDA states that the "risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*".
Allele frequencies (reported by the FDA (1))	Greater than 15% of the population is reported positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*15:02*, averaging 2% to 4%, but higher in some groups. *HLA-B*15:02* is present in less than 1% of the population in Japan and Korea. *HLA-B*1502* is largely absent in individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans).	*HLA-A*31:01* is expected to be positive by more than 15% of patients of Japanese, Native American, South Indian (for example, Tamil Nadu) and some Arabic ancestry; up to about 10% in patients of Han Chinese, Korean, European, Latin American, and other Indian ancestry; and up to about 5% in African- Americans and patients of Thai, Taiwanese, and Chinese (Hong Kong) ancestry.

SJS/TEN: Stevens–Johnson syndrome/ toxic epidermal necrolysis
MPE: maculopapular exanthema
HSS: drug-induced hypersensitivity syndrome

The association between *HLA-A*31:01* and DRESS and MPE has been found in Europeans, Han Chinese, Japanese, and North Americans of mixed ancestries (14, 26, 41-43). *HLA-A*31:01* is also associated with SJS/TEN, but not in Southeast Asians, where the more common HLA-B*15*:02* allele has an extremely strong association with SJS/TEN (5, 38).

The *HLA-A*31:01* variant is common globally with frequencies of at least 3% in many populations (2–5% in Northern Europeans, 2% in Han Chinese, 7–12% in Japanese populations) (5, 14, 38, 42). The highest frequencies have been reported in South American countries, such as Argentina (25%–38.6%) (38).

Gene: *HLA-B, HLA-B*15:11* and other alleles

The *HLA-B*15:11* variant has been found to be a risk factor for SJS/TEN in Japan (44, 45) and Korea (46). In Central China, *HLA-B*15:11* may be a risk factor for some patients with CBZ-induced SJS negative for *HLA-B*15:02* (47), and one study found that *HLA-A*11:01* for CBZ-induced SJS/TEN was a risk factor in the Spanish Caucasian population (39).

The *HLA-B*15:11* variant is found in frequencies above 1% in specific Asian populations only:Han Chinese, Koreans, Thai (34, 48).

Other alleles considered to be high risk, particularly in high-frequency areas such as Indonesia, Malaysia, and Thailand, include *HLA-B*15:08* and *HLA-B*15:21* (49, 50).

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are currently available for the carbamazepine response, and the *HLA-B* and *HLA-A* genes.

The FDA recommends testing for *HLA-B*15:02* prior to initiating carbamazepine therapy in patients with ancestry in populations with increased frequency of *HLA-B*15:02*. In deciding which patients to screen, the FDA states that the prevalence rates of *HLA-B*15:02* (Table 1) may offer a rough guide, keeping in mind the limitations of these figures due to wide variability in rates within ethnic groups, the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry (1).

The genotype results for an *HLA* allele such as *HLA-B*15:02* can either be "positive" or "negative" (Table 6). There are no intermediate phenotypes because the HLA genes are expressed in a codominant manner. A positive result is either "heterozygous" or "homozygous", depending upon whether the patient has one or two copies of the **15:02* allele, respectively.

For patients who are positive for *HLA-B*15:02*, the FDA states that carbamazepine should not be used unless the benefits clearly outweigh the risks. For patients who are known to be positive for *HLA-A*31:01*, the FDA states that the risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine.

A negative result indicates that the patient does not have the *HLA-B*15:02* allele. However, a negative result does not rule out the possibility of a patient developing carbamazepine hypersensitivity. Therefore, clinicians should carefully monitor all patients according to standard practices.

Table 6. CPIC (2017). Assignment of likely *HLA-B and HLA-A* genotype

Genotype	Definition	Examples of diplotypes
*HLA-B*15:02* negative	Homozygous for an allele other than *HLA-A*15:02*	$*X/*X^a$
*HLA-B*15:02* positive	Heterozygous or homozygous variant	$*15:02/*X^a$, **15:02/*15:02*
*HLA-A*31:01* negative	Homozygous for an allele other than *HLA-A*31:01*	$*Y^b/*Y^b$
*HLA-A*31:01* positive	Heterozygous or homozygous variant	$31:01/*Y^b$, **31:01/*31:01*

[a] Where *X 5 any *HLA-B* allele other than *HLA-B*15:02*.
[b] Where *Y 5 any *HLA-A* allele other than *HLA-A*31:01*.
Table is adapted from Phillips EJ, Sukasem C, Whirl-Carrillo M, Müller DJ, Dunnenberger HM, Chantratita W, Goldspiel B, Chen YT, Carleton BC, George ALJ, Mushiroda T, Klein T, Gammal RS, and Pirmohamed M. Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. Clinical pharmacology and therapeutics (4).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.*

2018 Statement from the US Food and Drug Administration (FDA)

SJS/TEN and HLA-B*1502 Allele

Retrospective case-control studies have found that in patients of Chinese ancestry there is a strong association between the risk of developing SJS/TEN with carbamazepine treatment and the presence of an inherited variant of the *HLA*-B gene, *HLA-B*15:02*. The occurrence of higher rates of these reactions in countries with higher frequencies of this allele suggests that the risk may be increased in allele-positive individuals of any ethnicity.

Across Asian populations, notable variation exists in the prevalence of *HLA-B*15:02*. Greater than 15% of the population is reported positive in Hong Kong, Thailand, Malaysia, and parts of the Philippines, compared to about 10% in Taiwan and 4% in North China. South Asians, including Indians, appear to have intermediate prevalence of *HLA-B*1502*, averaging 2 to 4%, but higher in some groups. *HLA- B*15:02* is present in <1% of the population in Japan and Korea.

*HLA- B*15:02* is largely absent in individuals not of Asian origin (e.g., Caucasians, African-Americans, Hispanics, and Native Americans).

Prior to initiating carbamazepine therapy, testing for *HLA-B*15:02* should be performed in patients with ancestry in populations in which *HLA-B*15:02* may be present. In deciding which patients to screen, the rates provided above for the prevalence of *HLA- B*15:02* may offer a rough guide, keeping in mind the limitations of these figures due to wide variability in rates even within ethnic groups, the difficulty in ascertaining ethnic ancestry, and the likelihood of mixed ancestry. Carbamazepine should not be used in patients positive for *HLA-B*15:02* unless the benefits clearly outweigh the risks. Tested patients who are found to be negative for the allele are thought to have a low risk of SJS/TEN.

Over 90% of carbamazepine treated patients who will experience SJS/TEN have this reaction within the first few months of treatment. This information may be taken into consideration in determining the need for screening of genetically at-risk patients currently on carbamazepine.

The *HLA-B*15:02* allele has not been found to predict risk of less severe adverse cutaneous reactions from carbamazepine, such as maculopapular eruption (MPE) or to predict Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS).

Limited evidence suggests that *HLA-B*15:02* may be a risk factor for the development of SJS/TEN in patients of Chinese ancestry taking other anti-epileptic drugs associated with SJS/TEN, including phenytoin. Consideration should be given to avoiding use of other drugs associated with SJS/TEN in *HLA-B*15:02* positive patients, when alternative therapies are otherwise equally acceptable.

Hypersensitivity Reactions and *HLA-A*31:01* Allele

Retrospective case-control studies in patients of European, Korean, and Japanese ancestry have found a moderate association between the risk of developing hypersensitivity reactions and the presence of *HLA-A*31:01*, an inherited allelic variant of the *HLA-A* gene, in patients using carbamazepine. These hypersensitivity reactions include SJS/TEN, maculopapular eruptions, and Drug Reaction with Eosinophilia and Systemic Symptoms.

*HLA-A*31:01* is expected to be present in the following approximate frequencies: greater than 15% in patients of Japanese and Native American ancestry; up to about 10% in patients of Han Chinese, Korean, European, and Latin American ancestry; and up to about 5% in African-Americans and patients of Indian, Thai, Taiwanese, and Chinese (Hong Kong) ancestry.

The risks and benefits of carbamazepine therapy should be weighed before considering carbamazepine in patients known to be positive for *HLA-A*31:01*.

General Information on *HLA* Genotyping and Hypersensitivity

Application of *HLA* genotyping as a screening tool has important limitations and must never substitute for appropriate clinical vigilance and patient management. Many *HLA-B*15:02*-positive and *HLA- A*31:01*-positive patients treated with carbamazepine will not develop SJS/TEN or other hypersensitivity reactions, and these reactions can still occur infrequently in *HLA-B*15:02*-negative and *HLA-A*31:01*-negative patients of any ethnicity. The role of other possible factors in the development of, and morbidity from, SJS/TEN and other hypersensitivity reactions, such as antiepileptic drug (AED) dose, compliance, concomitant medications, co-morbidities, and the level of dermatologic monitoring have not been studied.

Please review the complete therapeutic recommendations that are located here: (1).

2015 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

*HLA-B*15:02*: CARBAMAZEPINE

Patients with this genetic variation have a severely increased risk of experiencing the life-threatening cutaneous adverse event Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced SJS/TEN in these patients is 1.8-3.4%.

Recommendation:

1 choose an alternative if possible

*HLA-A*31:01*: CARBAMAZEPINE

Patients with this genetic variation have an increased risk of experiencing the life-threatening cutaneous adverse events DRESS and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced DRESS in these patients is 0.89%.

Recommendation:

1. carefully weigh the risk of DRESS and SJS/TEN against the benefits
2. if an alternative is an option, choose an alternative

*HLA-B*15:11*: CARBAMAZEPINE

Patients with this genetic variation have an increased risk of experiencing the life-threatening cutaneous adverse event Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The risk of carbamazepine-induced SJS/TEN in patients with the HLA-B*15:02 allele, which carries a 4.6-6.6 times higher risk than the HLA-B*15:11 allele, is 1.8-3.4%. This would equate to a risk of carbamazepine-induced SJS/TEN in these patients of 0.27-0.73%.

Recommendation:

1. carefully weigh the risk of SJS/TEN against the benefits
2. if an alternative is an option, choose an alternative

Please review the complete therapeutic recommendations that are located here: (2).

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

The therapeutic recommendations for *HLA-B*15:02* and carbamazepine remain unchanged from the original guideline (3) but in this update they are now also applicable to oxcarbazepine (4). These recommendations hold irrespective of the patient's region of origin or ethnic group. For patients who are *HLA-B*15:02* negative, carbamazepine or oxcarbazepine may be prescribed per standard guidelines. If a patient is carbamazepine-naïve or oxcarbazepine-naïve and *HLA-B*15:02* positive, carbamazepine and oxcarbazepine should be avoided, respectively, due to the greater risk of SJS/TEN. Other aromatic anticonvulsants, including eslicarbazepine, lamotrigine, phenytoin, fosphenytoin, and phenobarbital, have very limited evidence, if any, linking SJS/TEN with the *HLA-B*15:02* allele; however, caution should still be used when choosing an alternative agent. With regular dosing, carbamazepine- or oxcarbazepine-induced SJS/TEN usually develops within the first 4–28 days of therapy; therefore, patients who have been continuously taking carbamazepine or oxcarbazepine for longer than 3 months without developing cutaneous reactions are at extremely low risk (but not zero) of carbamazepine- or oxcarbazepine-induced adverse events in the future, regardless of *HLA-B*15:02* status.

For patients who are *HLA-A*31:01* negative, carbamazepine may be prescribed per standard guidelines (Table 3). If a carbamazepine-naïve patient also received testing for *HLA-B*15:02* and is positive for this allele, carbamazepine should be avoided regardless of the *HLA-A*31:01* genotype result. If a patient is carbamazepine-naïve and *HLA-A*31:01* positive, and if alternative agents are available, carbamazepine should be avoided due to the greater risk of SJS/TEN, DRESS, and MPE. Other aromatic anticonvulsants, including oxcarbazepine, have very limited evidence, if any, linking SJS/TEN, DRESS, and/or MPE with the *HLA-A*31:01* allele, and thus no recommendation can be made with respect to choosing another aromatic anticonvulsant as an alternative agent. If alternative agents are not available, consider the use of carbamazepine with increased frequency of clinical monitoring. Discontinue therapy at the first evidence of a cutaneous adverse reaction. As previously mentioned, since the latency period for cutaneous adverse drug reactions is known, if the patient is *HLA-A*31:01* positive and has previously used carbamazepine for longer than 3 months without incidence of a cutaneous adverse reaction, cautiously consider use of carbamazepine.

Please review the complete therapeutic recommendations that are located here: (4).

2014 Recommendations from the Canadian Pharmacogenomics Network for Drug Safety (CPNDS)

Recommendation 1.1: Genetic testing for *HLA- B*15:02* is recommended for all carbamazepine (CBZ)-naive patients before initiation of carbamazepine therapy (Level A – strong in patients originating from populations where *HLA- B*15:02* is common, its frequency unknown or whose origin is unknown; Level C – optional in patients originating from populations where *HLA-B*15:02* is rare). Genetic testing for *HLA-A*31:01* is recommended for all carbamazepine-naive patients before initiation of carbamazepine therapy (Level B – moderate in all patients; Table 6).

Recommendation 1.2: In patients who have previously taken carbamazepine for > 3 months without any adverse effects, and in whom reinitiation of carbamazepine is considered, genetic testing is NOT recommended (B). In patients who have previously taken carbamazepine for a shorter period, genetic testing should be considered (B).

Recommendation 1.3: In patients who have previously experienced a hypersensitivity reaction (HSR) potentially related to carbamazepine, genetic testing is recommended as part of the differential diagnosis and for the direction of future therapy (B).

Recommendation 1.4: In patients for whom no alternative treatment options are available, genetic testing is recommended to ensure increased alertness to hypersensitivity symptoms in positive patients (B).

Recommendation 2.1: Genetic testing for *HLA- B*15:02* is most beneficial in patients originating from a population where *HLA-B*15:02* is common (e.g., Chinese, Thai, Indian, Malay, Filipino, Indonesian; A). Nevertheless, genotyping for *HLA-B*15:02* should be considered in ALL patients, irrespective of their ancestry, as the safest option (C).

Recommendation 2.2: *HLA-A*31:01* is common in most populations studied so far. Therefore, genetic testing for this variant is recommended in patients of all ancestries (B).

Recommendation 3.1: In patients who are positive for *HLA-B*15:02* or *HLA-A*31:01*, alternative medications should be used as first-line therapy (A). Consideration in the choice of alternative medications should be given to the possibility of cross-reactivity with structurally similar antiepileptic drugs (AED) (oxcarbazepine, lamotrigine, phenytoin, phenobarbital, primidone).

Recommendation 3.2: In patients who are negative for *HLA-B*15:02* and *HLA-A*31:01*, carbamazepine can be used as first-line therapy (A). However, the occurrence of a hypersensitivity reaction cannot be excluded based on a negative genetic test result.

Table 7. Grading scheme used for clinical practice recommendations

Level	Strength	Evidence basis
A	Strong	Based on strong scientific evidence; benefits clearly outweigh risks
B	Moderate	Based on reduced confidence scientific evidence and expert opinion; benefits likely to outweigh risks
C	Optional	Based mainly on expert opinion, for use with evidence development in a research context

Table adapted from: Amstutz, U., N.H. Shear, M.J. Rieder, S. Hwang, et al., Recommendations for *HLA-B*15:02* and *HLA-A*31:01* genetic testing to reduce the risk of carbamazepine-induced hypersensitivity reactions. Epilepsia, 2014. 55(4): p. 496-506 (5).

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature

Nomenclature for selected HLA alleles

Allele name	dbSNP reference identifier for allele location	HGVS	IPD-IMGT/HLA
*HLA-B*15:02*	rs2844682 and rs3909184	NG_023187.1:c.[5T>G; 11T>C; 44C>G; 45G>A; 103T>G; 106G>A; 142T>G; 204A>G; 205G>A; 206A>T; 209A>C; 213G>C; 222G>A; 272A>C; 277G>A; 280C>A; 282G>C; 283G>A; 292G>T; 353C>T; 355C>A; 363C>G; 369C>T; 409C>T; 419A>C; 463C>A; 477C>G; 539G>T; 559G>C; 560A>T; 603C>G; 605A>C; 610G>C; 618T>G; 636C>T; 693T>C; 756T>C; 900G>A; 916G>A; 985G>A; 1008T>C; 1046G>C]	Allele Report for B*15:02:01 (HLA00165)

Nomenclature for selected continued from previous page.

Allele name	dbSNP reference identifier for allele location	HGVS	IPD-IMGT/HLA
*HLA-A*31:01*	rs1061235 and rs16333021	NM_002116.7:c.[41C>T; 97T>A; 98T>C; 238G>A; 243G>T; 282G>C; 290C>T; 363A>G; 413G>A; 448C>T; 502A>C; 524A>G; 527A>T; 555T>G; 633A>G; 642C>T; 649C>G; 651C>T; 652A>G; 691G>A; 808G>T; 829G>C; 870G>C; 899T>C; 945G>A; 952C>T; 964A>T; 967A>G; 987C>T; 992T>G; 1029T>C; 1033A>T; 1072G>A; 1077C>T]	Allele Report for A*31:01:02:01 (HLA00097)
*HLA-B*15:11*		NG_023187.1:c.[5T>G; 11T>C; 44C>G; 45G>A; 103T>G; 106G>A; 142T>G; 204A>G; 205G>A; 206A>T; 209A>C; 213G>C; 222G>A; 277G>A; 280C>A; 282G>C; 283G>A; 292G>T; 363C>G; 419A>C; 463C>A; 477C>G; 538C>T; 559G>C; 560A>T; 603C>G; 605A>C; 610G>C; 618T>G; 636C>T; 693T>C; 756T>C; 900G>A; 916G>A; 985G>A; 1008T>C; 1046G>C]	Allele Report for B*15:11:01 (HLA00174)

The IPD-IMGT/HLA Database includes the official sequences named by the WHO Nomenclature Committee for Factors of the HLA System. IPD: Immuno Polymorphism Database, IMGT: international ImMunoGeneTics project, HLA: Human Leucocyte Antigen. The sequence variation descriptions used by IMGT/HLA are in line with the HGVS recommendations for sequence variant descriptions.
Note: For the *MHC* region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*15:02* allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific *HLA* allele, the presence of these SNPs (tag SNPs) may be used for *HLA* typing (51).
Because of the extreme diversity at the HLA locus, different tag SNPs may be associated with different HLA variants in different populations. For *HLA-B*15:02*, rs2844682 and rs3909184 are the tag SNPs (51). For *HLA-A*31:01*, rs1061235 is a tag SNP in Europeans (14) and rs16333021 is a tag SNP in Japanese (41). A study involving North American children of various ancestries showed that rs1061235 is not a suitable tag SNP in non-Caucasian individuals (42).
Guidelines on nomenclature of the HLA system are available from HLA Nomenclature.

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Version History

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Carisoprodol Therapy and *CYP2C19* Genotype

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Introduction

Carisoprodol is a centrally acting muscle relaxant used to relieve acute back pain. Due to its potential for dependence and abuse, it should only be used for treatment periods of up to 2–3 weeks. Carisoprodolol is classified as a Schedule IV controlled substance, and overdose may result in central nervous system respiratory depression, seizures, or even death.

Carisoprodol is metabolized by the enzyme CYP2C19 into meprobamate, a sedative used for anxiety disorders. In individuals with low or absent CYP2C19 activity (termed "CYP2C19 poor metabolizers"), standard doses of carisoprodol can lead to a 4-fold increase in exposure to carisoprodol and a concurrent 50% decrease in meprobamate exposure compared to normal metabolizers. Approximately 3–5% of Caucasians and Africans, and 15–20% of Asians, are CYP2C19 poor metabolizers (1).

The FDA-approved drug label advises caution when prescribing carisoprodol to individuals with reduced CYP2C19 activity (Table 1) and when co-administering drugs that inhibit or induce CYP2C19 (1). The efficacy, safety, and pharmacokinetics of carisoprodol have not been established in pediatric individuals (under 16 years) or individuals over 65 years.(1). Decades of clinical use have not identified a risk of major birth defects, miscarriage, or other adverse maternal or fetal outcomes associated with carisoprodol (1).

Table 1: Clinical Management Recommendations for Carisoprodol Based on CYP2C19 Status from the US Food and Drug Administration (2024)

CYP2C19 status	Impact	Clinical action
Poor metabolizers (reduced activity)	Higher exposure (4-fold increase) to carisoprodol; 50% reduced exposure to meprobamate	Caution in administration of carisoprodol

Table adapted from (1).

Drug: Carisoprodol

Carisoprodol, also known by the brand name Soma, is a centrally acting muscle relaxant used to treat acute musculoskeletal pain, especially acute low back pain. It provides pain relief and aids in mobilization but has a high potential for abuse, classifying it as a Schedule IV controlled substance (see Alphabetical listing of Controlled Substances from (2)). Additionally, it can be toxic in overdose, which may be fatal. Carisoprodol is contraindicated in individuals with acute intermittent porphyria or hypersensitivity to carbamate medications (1).

The mechanism of action of carisoprodol is not fully understood but it acts as an indirect $GABA_A$ receptor agonist, impacting neuronal communication in the brainstem's reticular formation and spinal cord. In addition to skeletal muscle relaxation, it has weak anticholinergic, antipyretic, and analgesic properties. Adverse effects include sedation, tachycardia, shortness of breath, and dizziness (1, 3, 4).

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Carisoprodol is metabolized by CYP2C19 into meprobamate, an active metabolite used to treat anxiety. Although its exact mechanism of action remains unclear, it has barbiturate-like properties and can be toxic in overdose (5).

Individuals with reduced or absent CYP2C19 activity have higher plasma levels of carisoprodol, and an increased ratio of carisoprodol:meprobamate compared to those with normal CYP2C19 activity. Carisoprodol's narrow therapeutic index implies there may be increased risk of toxicity in CYP2C19 poor metabolizers. However, available data are limited, and small studies have not found evidence linking *CYP2C19* genotype status with increased mortality risk or adverse effects after a single dose of carisoprodol (5, 6, 7).

The FDA-approved drug label for carisoprodol recommends caution when administering carisoprodol to individuals with reduced CYP2C19 activity. Co-administration with CYP2C19 inhibitors, such as omeprazole or fluvoxamine, could result in increased carisoprodol levels and decreased meprobamate exposure. CYP2C19 inducers, such as rifampin or St. John's Wort, could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin may also induce CYP2C19. The full pharmacological impact of these potential exposure alterations on the efficacy or safety of carisoprodol is unknown (1).

Carisoprodol's safety, efficacy, and pharmacokinetics are not established in pediatric (under 16) or geriatric (over 65) individuals (1). There is no clinical evidence suggesting increased risks of major birth defects, miscarriage, or other adverse maternal or fetal outcomes (1). Carisoprodol therapy does necessarily required discontinuing breastfeeding, though other agents may be preferred for mothers nursing a newborn or preterm infant (8). Carisoprodol and meprobamate are present in breastmilk, and there is one reported case of sedation in a breastfed infant whose mother was taking the medication. Therefore, the FDA advises monitoring infants exposed through breastfeeding for sedation (1).

Gene: *CYP2C19*

The CYP2C19 enzyme contributes to the metabolism of various clinically important drugs, including several proton pump inhibitors, clopidogrel, benzodiazepines, and certain tricyclic antidepressants like imipramine.

The *CYP2C19* gene is highly polymorphic, with over 35 variant star (*) alleles catalogued by the Pharmacogene Variation Consortium (PharmVar) (9). Allele functionality is assigned by the Clinical Pharmacogenetic Implementation Consortium (CPIC) (10). The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, while the *CYP2C19*17* allele is linked to increased enzyme activity, leading to "rapid" and "ultrarapid" metabolizer phenotypes (11).

The most common loss-of-function variant is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site, producing a truncated, non-functional protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans and ~29–35% in Asians (11, 12).

Another commonly tested loss-of-function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Additional loss-of-function variants, such as *CYP2C19*4-*8,* occur in less than 1% of the general population (11, 12). Individuals classified as *CYP2C19* intermediate metabolizers have one allele that encodes reduced or absent function (for example, **1/*2*), whereas poor metabolizers are either homozygous or compound heterozygous for 2 loss-of-function alleles (for example, **2/*2*, **2/*3*) (Table 2). Estimates of the poor metabolizer phenotype frequency in the Oceanian population are notably higher than other global populations (13).

Table 2. Functional Status and Phenotypes of *CYP2C19*

Phenotype (frequency)	Genotype	Examples of diplotype
CYP2C19 ultrarapid metabolizer (0.3–4.6% of individuals)[a]	An individual with 2 increased-function alleles.	**17/*17*
CYP2C19 rapid metabolizer (2.1–27.1% of individuals)	An individual with one normal-function allele and one increased-function allele.	**1/*17*
CYP2C19 normal metabolizer (3.5–62.7% of individuals)	An individual with 2 normal-function alleles.	**1/*1*
CYP2C19 intermediate metabolizer (19–45.9% of individuals)	An individual with one normal-function allele and one no-function allele or one no-function allele and one increased-function allele.	**1/*2* **1/*3* **2/*17*
CYP2C19 poor metabolizer (1.4–57.1% of individuals)	An individual with 2 no-function alleles.	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on the range of multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (14).
Table is adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E., and Stingl J.C. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for CYP2D6 and CYP2C19 Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. 2016 Dec 20; doi: 10.1002/cpt.597. [Epub ahead of print] (15) and updated *CYP2C19* Frequency Tables allele frequency ranges.
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (16).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of genetic tests available for carisoprodol response, CYP2C19-related poor drug metabolism, and variations in the CYP2C19 gene.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2024 Statement from the US Food and Drug Administration (FDA):

Carisoprodol is metabolized in the liver by CYP2C19 to form meprobamate. Co-administration of CYP2C19 inhibitors, such as omeprazole or fluvoxamine, with carisoprodol could result in increased exposure of carisoprodol and decreased exposure of meprobamate. Co-administration of CYP2C19 inducers, such as rifampin or St. John's Wort, with carisoprodol could result in decreased exposure of carisoprodol and increased exposure of meprobamate. Low dose aspirin also showed induction effect on CYP2C19. The full pharmacological impact of these potential alterations of exposures in terms of either efficacy or safety of carisoprodol is unknown.

[...]

Patients with Reduced CYP2C19 Activity: Patients with reduced CYP2C19 activity have higher exposure to carisoprodol. Therefore, caution should be exercised in administration of carisoprodol to these patients.

[1] The FDA assigns labels to specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NG_008384.3:g.4220C>T (NM_000769.2:c.-806C>T)[a]	Not applicable—variant occurs in a non-coding region	rs12248560

[a] The *CYP2C19*17* allele has increased expression due to an upstream, non-coding variant. The legacy HGVS expression for the change relative to the coding sequence is provided, but the correct RefSeq genomic sequence is provided as well.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the PharmVar database (9) https://www.pharmvar.org/

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Version History

Version 1.0 was published on April 4, 2017.

Version 1.1 was published on October 21, 2024. This revision includes an updated citation for a more recent FDA-approved drug label, updated *CYP2C19* allele references including definitions of haplotypes, frequencies of metabolizer phentoypes, and additional citations for regarding carisoprodol use during pregnancy and lactation. Relative to version 1.0, there is no change to the prescribing recommendations from FDA or any other cited source regarding the utility of CYP2C19 phenotype and carisoprodol therapy.

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Carvedilol Therapy and *CYP2D6* Genotype

Laura Dean, MD[1]

Created: August 1, 2018.

Introduction

Carvedilol (brand name Coreg) is used to treat heart failure and high blood pressure (hypertension). It is also used in patients who developed left ventricular dysfunction after having a heart attack (myocardial infarction, MI). In patients with cardiovascular disease, carvedilol is associated with improvements in quality of life, hospitalization rates, and survival.

Carvedilol is a non-selective beta blocker (beta 1 and beta 2) and an alpha 1 blocker. It reduces the energy demands on the heart by blocking cardiac beta receptors, which decreases the heart rate and the force of heart contractions. Carvedilol lowers blood pressure by blocking alpha receptors on blood vessels, which relaxes and dilates blood vessels.

CYP2D6 is one of the primary enzymes involved in activating and metabolizing carvedilol. Approximately 8% of Caucasians and 2% of most other populations have absent CYP2D6 activity and are predicted to be "CYP2D6 poor metabolizers."

The FDA-approved drug label for carvedilol states that plasma concentrations of carvedilol may be higher in CYP2D6 poor metabolizers compared to normal metabolizers, but does not discuss altering carvedilol dosing based on a patient's *CYP2D6* genotype (1). However, the label does state the dose of carvedilol should be individualized, and the dose should be monitored as it is gradually increased (up-titrated), based on tolerability and clinical response (Table 1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) recommend that no action is needed for carvedilol and *CYP2D6* genotype. For CYP2D6 poor metabolizers, DPWG states that the plasma concentration of carvedilol can be elevated, but this does not result in an increase in side effects (Table 2) (2).

Table 1. FDA (2017) Drug Label for Carvedilol. Therapeutic recommendations based on *CYP2D6* Genotype.

Phenotype	Carvedilol
CYP2D6 poor metabolizers	Retrospective analysis of side effects in clinical trials showed that poor CYP2D6 metabolizers had a higher rate of dizziness during up-titration, presumably resulting from vasodilating effects of the higher concentrations of the α-blocking R(+) enantiomer.

Please see Therapeutic Recommendations based on Genotype for more information from FDA. This table is adapted from (1).

Table 2. DPWG (2016) Recommendations for Carvedilol and *CYP2D6*

Phenotype	Recommendations
CYP2D6 poor metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.
CYP2D6 intermediate metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

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Table 2. continued from previous page.

Phenotype	Recommendations
CYP2D6 ultrarapid metabolizers	No action is required for this gene-drug interaction. The plasma concentration of carvedilol can be reduced. This does not, however, result in a decrease in the effectiveness.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (2).

Drug: Carvedilol

Carvedilol is widely considered to be the standard of care for patients with heart failure, particularly for patients who also have hypertension. Carvedilol is used to treat mild to severe congestive heart failure, as well as hypertension, and left ventricular dysfunction in patients who recently had an MI, but are otherwise stable.

Carvedilol is a non-selective beta blocker (blocks beta 1 and beta 2 receptors) and an alpha 1 blocker. By blocking beta receptors found in the heart, carvedilol reduces the heart rate and decreases the force of heart contractions. By blocking the alpha 1 receptors found on blood vessels, carvedilol relaxes and dilates the blood vessels, which lowers blood pressure.

In the treatment of heart failure, beta blockers such as carvedilol are thought to protect the heart from increased catecholamine stimulation (catecholamines include adrenaline and noradrenaline). In the short term, adrenergic activation can help the heart maintain cardiac performance, but over time, continued activation can be detrimental. Harmful effects include a persistently increased heart rate, down-regulation and impaired functioning of the beta receptors, and myocyte hypertrophy and death—which leads to adverse re-modelling of heart tissue.

Carvedilol exerts its therapeutic effects by protecting the failing heart from harmful adrenergic stimulation. Carvedilol reduces the heart rate, improves left ventricular function, and reduces vasoconstriction. Several large trials (e.g., MOCHA, PRECISE, COPERNICUS) have reported that carvedilol reduces all-cause mortality and decreases hospitalization in patients with heart failure (3-6).

The dose of carvedilol must be individualized and monitored during up-titration. Gradual up-titration should reduce the risk of syncope (fainting) or excessive hypotension (low blood pressure). The FDA drug label recommends carvedilol to be started at 6.25 mg twice daily, which can be increased after 3 to 10 days, based on tolerability, to 12.5 mg twice daily. The dose may then be increased to the target dose of 25 mg twice daily, although patients should be maintained on a lower dose if higher doses are not tolerated. In addition, a lower starting dose may be used (3.125 mg twice daily), and the rate of up-titration may be slowed if clinically indicated (e.g., due to low blood pressure or heart rate, or fluid retention) (1).

Carvedilol is a mixture of equal amounts of left-handed *S*(-) and right-handed *R*(+) enantiomers (a "racemic mixture"). Enantiomers are molecules that are mirror images of each other, but are not superimposable on one another. The nonselective beta-adrenoreceptor blocking activity of carvedilol is present in the *S*(-) enantiomer; and the α1-adrenergic blocking activity is present in both *R*(+) and *S*(-) enantiomers at equal potency.

Even though carvedilol plasma levels are about 50% higher in the elderly compared with young subjects, no overall differences in the safety or effectiveness were observed between these two populations except for higher incidence of dizziness in hypertensive subjects (incidence 8.8% in the elderly versus 6% in younger subjects) (1).

Carvedilol is contraindicated in patients with severe hepatic (liver) impairment because patients with severe liver impairment (i.e., cirrhosis) exhibit a 4- to 7-fold increase in carvedilol plasma levels when compared with healthy subjects (1).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

CYP2D6 and CYP2C9 are the primary enzymes involved in the activation and metabolism of carvedilol. Other enzymes involved to a lesser extent include CYP3A4, CYP2C19, CYP1A2, and CYP2E1. The pharmacokinetics of carvedilol are known to be influenced by genetic variation in *CYP2D6*—data do not exist for *CYP2C9*.

Individuals who have two non-functional copies of the *CYP2D6* gene are predicted to be "CYP2D6 poor metabolizers". Plasma concentrations of *R*(+)- carvedilol are 2–3 times higher in poor metabolizers, and levels of *S*(-)-carvedilol are increased by approximately 20% to 25%, compared to normal metabolizers with normal CYP2D6 activity (1).

Retrospective analysis of side effects in clinical trials showed that individuals who are CYP2D6 poor metabolizers had a higher rate of dizziness during up-titration. This is thought to result from vasodilating effects of the 2–3 times higher concentrations of the α-blocking *R*(+) enantiomer (1).

Variation in *CYP2D6* does not appear to be associated with a change in the response to carvedilol therapy. This may be because other CYP450 enzymes can convert carvedilol to its active metabolite. One small study (n=93) reported that there were no significant differences of carvedilol dose as well as the number of adverse drug reactions among patients with different *CYP2D6* genotypes (7). Another small study (n=110) found that there were significant *CYP2D6* allele-specific differences in carvedilol pharmacokinetics, but *CYP2D6* genotype had no effect on heart rate, blood pressure or adverse effects (8).

The *CYP2D6* genotype may be associated with carvedilol dosage, however. Two small studies reported that higher maintenance doses of carvedilol were tolerated by carriers of non-functional *CYP2D6* alleles (n=65) (9), and by CYP2D6 poor metabolizers (n=93) (10).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) displays genetic tests that are currently available for carvedilol response and the *CYP2D6* gene. According to the FDA, the pharmacokinetics of carvedilol do not appear to be different in patients with decreased or absent CYP2D6 activity. Therefore, genetic testing prior to the use of carvedilol is not recommended.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

The primary P450 enzymes responsible for the metabolism of both *R*(+) and *S*(-)-carvedilol in human liver microsomes were CYP2D6 and CYP2C9 and to a lesser extent CYP3A4, 2C19, 1A2, and 2E1. CYP2D6 is

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.*

thought to be the major enzyme in the 4'- and 5'-hydroxylation of carvedilol, with a potential contribution from 3A4. CYP2C9 is thought to be of primary importance in the O-methylation pathway of *S*(-)-carvedilol.

[…]

Carvedilol is subject to the effects of genetic polymorphism with poor metabolizers of debrisoquin (a marker for cytochrome P450 2D6) exhibiting 2- to 3-fold higher plasma concentrations of *R*(+)-carvedilol compared with extensive metabolizers. In contrast, plasma levels of *S*(-)-carvedilol are increased only about 20% to 25% in poor metabolizers, indicating this enantiomer is metabolized to a lesser extent by cytochrome P450 2D6 than *R*(+)-carvedilol. The pharmacokinetics of carvedilol do not appear to be different in poor metabolizers of *S*-mephenytoin (patients deficient in cytochrome P450 2C19).

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 PM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

CYP2D6 IM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be elevated. This does not, however, result in an increase in side effects.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

CYP2D6 UM: CARVEDILOL

Pharmacist text

NO action is required for this gene-drug interaction.

The plasma concentration of carvedilol can be reduced. This does not, however, result in a decrease in the effectiveness.

Background information

Carvedilol is primarily converted by CYP2D6 to 4'-hydroxycarvedilol and 5'-hydroxycarvedilol. Data from pre-clinical studies suggest that these metabolites are active.

Carvedilol is also converted by other CYP450 enzymes to the active metabolite desmethylcarvedilol.

Please review the complete therapeutic recommendations that are located here: (2)

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Celecoxib Therapy and *CYP2C9* Genotype

Laura Dean, MD[1] and Megan Kane, PhD[2]

Created: August 18, 2016; Updated: January 25, 2021.

Introduction

Celecoxib (brand name Celebrex) is a nonsteroidal anti-inflammatory drug (NSAID) that is used to manage osteoarthritis, rheumatoid arthritis, menstrual symptoms, and acute pain.

Celecoxib is a "COX-2 inhibitor." The cyclooxygenase (COX) enzymes catalyze pathways that play a key role in the generation of the inflammatory response. Most NSAIDs inhibit both types of cyclooxygenase, COX-1 and COX-2, while celecoxib selectively inhibits COX-2.

Celecoxib is primarily metabolized by CYP2C9. Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to celecoxib, and an increased risk of side effects.

Like all non-aspirin NSAIDs, celecoxib increases the risk of serious cardiovascular events, including myocardial infarction and stroke, and serious gastrointestinal (GI) adverse events such as bleeding, ulceration, and perforation.

The FDA-approved drug label states that in individuals "who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin), initiate treatment with half the lowest recommended dose" (Table 1)(1). Recommendations from the Clinical Pharmacogenetics Implementation Consortium (CPIC) include initiating celecoxib at 25–50% of the lowest recommended starting dose. Titrate dose upward to clinical effect or 25–50% of the maximum recommended dose with caution" (Table 2)(2).

Table 1. The FDA Celecoxib Dosage in CYP2C9 Poor Metabolizers (2020)

Phenotype	Dosage
Poor Metabolizers of CYP2C9 Substrates	In individuals who are known or suspected to be poor CYP2C9 metabolizers (namely, *CYP2C9*3/*3* or **2/*3*), based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin) administer celecoxib starting with half the lowest recommended dose. Alternative management should be considered in juvenile rheumatoid arthritis individuals identified to be CYP2C9 poor metabolizers.

This FDA table is adapted from (1).

Table 2. The CPIC Therapeutic Recommendations for Celecoxib (2020)

Phenotype[a]	Implication	Therapeutic recommendation	Classification of recommendation	Other considerations
CYP2C9 normal metabolizer (NM)	Normal metabolism	Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual treatment goals.	Strong	

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Corresponding author.

Table 2. continued from previous page.

Phenotype[a]	Implication	Therapeutic recommendation	Classification of recommendation	Other considerations
CYP2C9 intermediate metabolizer (IM) AS[b] of 1.5	Mildly reduced metabolism	Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual treatment goals.	Moderate	IMs might have a higher-than-normal risk of adverse events especially in individuals with other factors affecting clearance of these drugs, such as hepatic impairment or advanced age.
CYP2C9 intermediate metabolizer AS of 1	Moderately reduced metabolism; higher plasma concentrations may increase probability of toxicities	Initiate therapy with lowest recommended starting dose. Titrate dose upward to clinical effect or maximum recommended dose with caution. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual treatment goals. Carefully monitor adverse events, such as blood pressure and kidney function during course of therapy.		
CYP2C9 poor metabolizer	Significantly reduced metabolism and prolonged half-life; higher plasma concentrations may increase probability and/ or severity of toxicities	Initiate therapy with 25–50% of the lowest recommended starting dose. Titrate dose upward to clinical effect or 25–50% of the maximum recommended dose with caution. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual treatment goals. Upward dose titration should not occur until after steady-state is reached (at least 8 days for celecoxib) Carefully monitor adverse events such as blood pressure and kidney function during course of therapy. Alternatively, consider an alternate therapy not metabolized by CYP2C9 or not significantly impacted by *CYP2C9* genetic variants *in vivo*.	Moderate	Alternative therapies not primarily metabolized by CYP2C9 include aspirin, ketorolac[c], naproxen, and sulindac. Selection of therapy will depend on individual treatment goals and risks for toxicity.
Indeterminate	N/A	No recommendation	No recommendation	N/A

AS, activity score; IMs, intermediate metabolizers; N/A, not applicable; PMs, poor metabolizers.
[a] Separate drug-specific recommendation tables are available online.
[b] Activity score of a diplotype is based on assigned activity from 0 to 1 for each allele, see Table 3.
[c] Approved for short-term use only.
This CPIC table is adapted from (2).

Drug Class: NSAIDs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation, fever, and pain. They are one of the most commonly used classes of medicine. Worldwide, it is estimated that more than 30 million people use NSAIDs daily (3).

More than 40 NSAIDs are licensed for use. Several NSAIDs (for example, aspirin, ibuprofen, and naproxen) are available over the counter, but stronger doses and other types of NSAIDs, such as celecoxib and piroxicam, are only available via prescription. It is thought that approximately 25% of the population has experienced NSAID-related side effects that require medical care (4).

The main action of NSAIDs is to inhibit cyclooxygenase (COX). Cyclooxygenase is the central enzyme in the synthesis of prostaglandins, prostacyclin, and thromboxanes from arachidonic acid. Prostaglandins can be protective (for example, protect the gastric mucosal lining and aid platelet aggregation) or inflammatory (for example, recruiting inflammatory white blood cells).

There are 2 main COX isoforms, and the safety and effectiveness of NSAIDs may be influenced by the degree they inhibit the 2 different forms. Cyclooxygenase-1 (COX-1) is a "housekeeping enzyme" that is expressed in most tissues. It protects the GI tract and induces platelet aggregation in response to injury. In contrast, COX-2 is often undetectable in tissues; however, the expression of COX-2 is increased during inflammation.

Most NSAIDs are non-selective COX inhibitors that inhibit both COX-1 and COX-2. There are exceptions, such as celecoxib, which is a selective COX-2 inhibitor that appears to be associated with fewer adverse GI events. However, GI adverse events still occur.

Approximately 25% of the exposed US population have experienced NSAID-related side effects that required medical care (4). Use of non-selective, non-aspirin NSAIDs may account for more than 30% of gastrointestinal bleeding cases in the US, resulting in 3,200 deaths per year in the 1990s (5). Overall mortality incidence rates are similar for non-selective NSAIDs and (48%) selective COX-2 inhibitors (47%) in older adults with arthritis (6). However, all non-aspirin NSAIDs have a boxed warning on the risk of serious GI and cardiovascular adverse events. For example:

> "NSAIDs cause an increased risk of serious cardiovascular thrombotic events, including myocardial infarction and stroke, which can be fatal. This risk may occur early in treatment and may increase with duration of use.
>
> NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients and patients with a prior history of peptic ulcer disease and/or GI bleeding are at greater risk for serious GI events" (1).

Drug: Celecoxib

Celecoxib is an NSAID that is used to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, painful menstruation, and acute pain. It is also used to reduce the number of colon and rectum polyps in individuals with familial adenomatous polyposis (1)

Celecoxib is a selective COX-2, but not COX-1, inhibitor that promotes the production of the gastric mucosal lining. Although celecoxib may be more gastroprotective than non-selective NSAIDs (7, 8, 9, 10), the use of celecoxib still increases the risks of gastrointestinal adverse events and the drug label has the same warning as for all NSAIDS, listing the gastric and cardiovascular risks (1).

The recommended dose in adults varies depending on the indication. For example, for osteoarthritis, the recommended dosage is 200 mg once daily or 100 mg twice daily, while for rheumatoid arthritis it is 100–200 mg twice daily. Because of the adverse events associated with any type of NSAID, the lowest effective dose of celecoxib should be used for the shortest duration consistent with the individual's treatment goals.

As for all NSAIDs, celecoxib is contraindicated in individuals with a known hypersensitivity or a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID. Celecoxib is also contraindicated to treat pain in the days following coronary heart disease surgery (NSAIDs cause an increased risk of myocardial infarction and stroke post-operatively), and celecoxib should be avoided by pregnant women starting at 30 weeks gestation (NSAID use in the third trimester causes an increased risk of premature closure of the fetal ductus arteriosus) (1).

Celecoxib is primarily metabolized by CYP2C9. Individuals with low CYP2C9 activity ("CYP2C9 poor metabolizers") have a higher exposure to celecoxib (1).

There are insufficient studies of celecoxib use in pregnant women or during labor and delivery. Animal studies suggest celecoxib use during pregnancy or labor and delivery can lead to increased rates of embryo-fetal deaths, developmental abnormalities, pre- and post-implantation loss and stillbirth (1). Celecoxib is a prostaglandin-meditated NSAID and thus may delay or prevent rupture of ovarian follicles, leading to reversible infertility in some women (1).

Celecoxib has been approved for individuals 2 years and older with signs and symptoms of Juvenile Rheumatoid Arthritis. However, safety and efficacy have not been studied beyond 6 months in the pediatric population (1). Alternative therapies should be considered for pediatric individuals who are CYP2C9 poor metabolizers (1).

Elderly individuals are at a higher risk for NSAID-associated serious cardiovascular, GI, or renal adverse reactions; if celecoxib therapy is still strongly indicated despite the elevated risk, the FDA-approved drug label recommends initiating therapy at the low end of the dosing range with close monitoring for adverse effects (1).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2C9 protein metabolizes approximately 15% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the *CYP2C9* gene can play a major role in adverse drug reactions (11, 12).

At least 16 different NSAIDS are metabolized, in part, by CYP2C9 (13). Celecoxib is extensively metabolized by CYP2C9 to form an inactive metabolite, with minor contributions from CYP3A4 (4).

The *CYP2C9* gene is highly polymorphic, with more than 60 known alleles. The *CYP2C9*1* allele is considered the wild-type allele when no variants are detected and is categorized by normal enzyme activity (14). Individuals who have 2 normal-function alleles (for example, *CYP2C9* *1/*1) are classified as "normal metabolizers".

Two common allelic variants associated with reduced enzyme activity are *CYP2C9*2* (p.Arg144Cys) and *CYP2C9*3* (p.Ile359Leu). The **2* allele is more common in Caucasian (10–20%), than Asian (1–3%) or African (0–6%) populations. The **3* allele is less common (<10% in most populations) and is rare in African populations. In African-Americans and African populations, the *CYP2C9*8* (5–8%) allele is commonly seen followed by *5 and *11 (1.9-3.8%), which are rarely seen in other populations (<1%). (15, 16, 17, 18, 19) Importantly, it is the combination of both alleles that contributes to the overall metabolizer phenotype (Table 3).

Table 3. The CPIC Assignment of Likely *CYP2C9* Phenotype based on Genotype (2020)

Likely phenotype[a,b]	Activity score	Genotype	Examples of diplotype
Normal metabolizer	2	An individual who has 2 normal function alleles	*1/*1
Intermediate metabolizer	1.5 1	An individual who has one normal function allele plus one decreased function allele; OR one normal function allele plus one no function allele OR 2 decreased function alleles	*1/*2 *1/*3 *2/*2
Poor metabolizer	0.5 0	An individual who has one no function allele plus one decreased function allele; OR 2 no function alleles	*2/*3 *3/*3
Indeterminate	n/a	An individual who has allele combinations with uncertain[c] or unknown function alleles	*1/*7 *1/*10 *7/*10 *1/*58

Note: There are no known cases of CYP2C9 ultrarapid metabolizers .
[a] Assignment of allele function and associated citations can be found at the CPIC website, also see *CYP2C9* Allele Definition Table and *CYP2C9* Allele Functionality Table in (2).
[b] See the *CYP2C9* Frequency Table for population-specific allele and phenotype frequencies. (19)
[c] Alleles with moderate to limited evidence on their effect on *CYP2C9* function.
This CPIC table has been adapted from (2).

Individuals with these variants have altered pharmacokinetics of several NSAIDs: celecoxib, flurbiprofen, ibuprofen, and tenoxicam (13, 20). This could potentially lead to dose recommendations based upon *CYP2C9* genotype and be used to identify individuals who are at increased risk of adverse events. However, pharmacogenetic testing has been limited to retrospective studies to identify the causes of an atypical response to NSAID (12).

Studies have found that *CYP2C9*3* is associated with an increased risk of bleeding associated with NSAID use (21, 22). In contrast, *CYP2C9*3* was found to be beneficial in a trial where celecoxib was given to prevent colorectal adenomas. High dose celecoxib had greater efficacy in preventing new adenomas than low-dose celecoxib, but only among individuals who had *CYP2C9*3* (23, 24).

The influence of other variant alleles, such as *CYP2C9*8* and *CYP2C9*11*, on celecoxib levels in the plasma has not yet been evaluated. However, these 2 alleles are associated with the decreased function of the enzyme and hence the CYP2C9 activity score is similar to other decreased function alleles of *CYP2C9* (for example, **2*).

Coadministration of celecoxib with other drugs that alter CYP2C9 activity may result in phenoconversion from one CYP2C9 metabolizer phenotype to another. Some drugs, such as fluconazole, are known to inhibit CYP2C9 and may increase the exposure and potential toxicity of celecoxib. Conversely, coadministration with CYP2C9 inducers like rifampin may lead to decreased efficacy of celecoxib. The FDA approved label for celecoxib advises that a dosage adjustment may be warranted when administered with CYP2C9 inhibitors or inducers. (1) Combination of NSAIDS—like celecoxib—and anticoagulants in CYP2C9 intermediate and poor metabolizers could increase the risk of gastrointestinal bleeding (25, 26).

Linking Gene Variation with Treatment Response

The FDA labeling for celecoxib recommends initiating treatment at half of the lowest recommended dose in known or suspected CYP2C9 poor metabolizers. This recommendation is supported by pharmacokinetic studies, which have shown individuals with decreased CYP2C9 activity have higher plasma levels of celecoxib (1, 27).

There is less data on how higher plasma levels of celecoxib influences the safety and efficacy of celecoxib. One study of 282 children treated with celecoxib after adenotonsillectomy found that children with a decreased function *CYP2C9* variant reported less pain than children without this variant (28).

Genetic Testing

Clinical *CYP2C9* genotyping tests are available that include a variable number of alleles. The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for the *CYP2C9* gene and celecoxib response. In addition, variant *CYP2C9* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP). (29)

Individual results are typically reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation with the predicted metabolizer phenotype (normal, intermediate, or poor). (Table 3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA): In adult patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin, phenytoin), initiate treatment with half of the lowest recommended dose.

In patients with juvenille rheumatoid arthritis (JRA) who are known or suspected to be poor CYP2C9 metabolizers, consider using alternative treatments.

[…]

Pharmacogenomics: CYP2C9 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity, such as those homozygous for the *CYP2C9**2 and *CYP2C9**3 polymorphisms. Limited data from 4 published reports that included a total of 8 subjects with the homozygous *CYP2C9**3/*3 genotype showed celecoxib systemic levels that were 3- to 7-fold higher in these subjects compared to subjects with *CYP2C9**1/*1 or *1/*3 genotypes. The pharmacokinetics of celecoxib have not been evaluated in subjects with other *CYP2C9* polymorphisms, such as *2, *5, *6, *9 and *11. It is estimated that the frequency of the homozygous *3/*3 genotype is 0.3% to 1% in various ethnic groups.

Please review the complete therapeutic recommendations that are located here: (1).

2020 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

Based on current evidence (Tables S1–S4), NMs and IMs with an AS of 1.5 are recommended to initiate therapy with the approved starting dose. Despite having mildly reduced metabolism, IMs with an AS of 1.5 do not exhibit significant increases in drug exposure relative to NMs (Figures S2–S4).

[…]

For IMs with an AS of 1, it is recommended to initiate NSAID therapy with the lowest recommended starting dose and titrate to clinical effect with close monitoring for adverse events, such as elevated blood pressure and kidney dysfunction during course of therapy.

[…]

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Individuals with a CYP2C9 PM phenotype (AS of 0) are expected to have markedly reduced metabolism and are expected to exhibit a pronounced prolongation of drug half-life and increase in plasma concentrations, which may increase the probability and/or severity of toxicities. A meta-analysis of 7 small studies showed a ~ 400% increase of celecoxib exposure (ratio of means 4.17; 95% CI 1.85–9.37 *3/*3 vs. *1/*1; P = 0.005; Figure 1), whereas insufficient data exist for formal meta-analyses of ibuprofen, flurbiprofen, and lornoxicam. In this case, therapeutic recommendations involve dose reduction or alternative therapies, coupled with careful monitoring for adverse events, which are consistent with the US Food and Drug Administration (FDA) recommendations for celecoxib and flurbiprofen. It is recommended to initiate therapy with 25–50% of the lowest recommended starting dose (i.e., 50–75% dose reduction), and careful dose titration to clinical effect. Because drug half-life is significantly prolonged in these patients, upward dose titration should not occur until after steady-state is reached, taking into consideration the PM half-life for each drug; of course, dosing may be stopped or decreased due to toxicity at any time. Treatment with an alternative therapy could also be considered. This could include NSAIDs not primarily metabolized by CYP2C9 (such as aspirin, ketorolac (approved for short-term use only), metamizole, naproxen, sulindac, etoricoxib, parecoxib, or valdecoxib), or with pharmacokinetic parameters apparently not impacted by CYP2C9 genetic variants *in vivo* despite CYP2C9 metabolism *in vitro* (diclofenac, weak level of evidence, see Table S9). Some of these alternative drugs are not available worldwide (e.g., etoricoxib, metamizole, parecoxib, and valdecoxib) because of the elevated cardiovascular risk associated with COX-2-selective NSAIDs, and some have serious adverse events that need to be considered (e.g., diclofenac and liver toxicity, metamizole and agranulocytosis). Therefore, individual NSAIDs are not always therapeutically equivalent, and the selection of an alternative agent requires careful consideration of drug properties (e.g., half-life (Table S12), potency, metabolism, COX isoenzyme selectivity, and off-target effects) that may affect efficacy and safety.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.4:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.4:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.4:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.4:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*7*	55C>A Leu 19Ile	NM_000771.4:c.55C>A	NP_000762.2:p.Leu19Ile	rs67807361
*CYP2C9*8*	449G>A Arg150His	NM_000771.4:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*9*	10535A>G His251Arg	NM_000771.4:c.752A>G	NP_000762.2:p.His251Arg	rs2256871
*CYP2C9*10*	10598A>G Glu272Gly	NM_000771.4:c.815A>G	NP_000762.2:p.Glu272Gly	rs9332130
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.4:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*58*	1009C>A Pro337Thr	NM_000771.4:c.1009C>A	NP_000762.2:p.Pro337Thr	rs1274535931

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (30).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature and consensus based functional evidence of alleles for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Version history

To view the previous version (26 August 2016), please click here.

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Cetuximab Therapy and *RAS* and *BRAF* Genotype

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Introduction

Cetuximab (brand name Erbitux) is a monoclonal antibody used in the treatment of metastatic colorectal cancer (mCRC) and cancer of the head and neck. Cetuximab is an epidermal growth factor receptor (EGFR) antagonist, which works by blocking the growth of cancer cells. It is administered as a weekly intravenous (IV) infusion, but in practice, is often given every other week to coincide with chemotherapy (for example, FOLFIRI or FOLFOX). Cetuximab has several off-label uses as well, which include non-small cell lung cancer, squamous cell carcinoma of the skin, and Menetrier's disease.

Interestingly, for colorectal cancer, the location of the primary tumor influences whether an individual with mCRC will respond to anti-EGFR therapy, and influences prognosis. Individuals with left-sided tumors are more likely to respond well to anti-EGFR therapy and have a better prognosis. Individuals with right-sided tumors have a worse prognosis and respond poorly to anti-EGFR therapy. However, currently only the mutation status of the tumor, and not the location of the tumor, is discussed in the drug label's dosing recommendations.

Resistance to cetuximab is associated with specific *RAS* mutations. The *RAS* family of oncogenes includes the *KRAS* and *NRAS* genes. When mutated, these genes have the ability to transform normal cells into cancerous cells. The *KRAS* mutations are particularly common, being detectable in 40% of metastatic colorectal tumors.

The *KRAS* mutations often lead to constitutive activation of the mitogen-activated protein kinase (MAPK) pathway and are associated with resistance to anti-EGFR drugs such as cetuximab. In addition, mutations in *NRAS* and another gene, *BRAF*, have been associated with poor response to anti-EGFR therapy; however, *BRAF* mutation does not explicitly preclude anti-EGFR therapy. Combination therapies targeting both BRAF and EGFR have shown to improve survival for individuals with wild-type *RAS* and mutant *BRAF*.

The 2018 FDA-approved drug label for cetuximab states that for mCRC, cetuximab is indicated for *K*- and *N*-*RAS* wild-type (no mutation), *EGFR*-expressing tumors. The label states that an FDA-approved test must be used to confirm the absence of a *RAS* mutation (in either *KRAS* or *NRAS*) prior to starting cetuximab (Table 1) (1). While the FDA label also states that EGFR expression should also be confirmed by an approved test prior to starting therapy for mCRC, this is largely not implemented in practice, nor is it recommended by professional oncology society guidelines.

Similarly, the 2015 Update from the American Society of Clinical Oncology (ASCO) states that anti-EGFR therapy should only be considered for the treatment of individuals whose tumor is determined to not have mutations detected after extended *RAS* testing (Table 2) (2).

The 2020 National Comprehensive Cancer Network (NCCN) guideline also strongly recommends *KRAS/NRAS* genotyping of tumor tissue in all individuals with mCRC. In addition, the guideline states the V600E mutation in the *BRAF* gene makes a response to cetuximab (and panitumumab) highly unlikely unless given a BRAF inhibitor (Table 3) (3).

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Table 1. The FDA-Approved Cetuximab Label: Dosage and Administration (2020)

Genes to be tested	Recommendations for metastatic colorectal cancer
NRAS *KRAS*	Cetuximab is not indicated for the treatment of individuals with colorectal cancer that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either *K-Ras* or *N- Ras* and hereafter is referred to as "*RAS*" or when the Ras status is unknown. Confirm the absence of a *RAS* mutation prior to initiation of treatment with cetuximab. Information on FDA-approved tests for the detection of *K-Ras* mutations in individual with metastatic colorectal cancer is available here.
EGFR	Determine EGFR expression status using FDA-approved tests prior to initiating treatment with cetuximab.

This table is created from (1). *EGFR,* epidermal growth factor receptor

Table 2. The ASCO RAS Mutational Testing of Colorectal Carcinoma Tissue (2015)

Genes to be tested	Recommendation
KRAS *NRAS*	RAS mutational testing of colorectal carcinoma tissue should be performed for all individuals who are being considered for anti-EGFR monoclonal antibody therapy (currently cetuximab and panitumumab). Before treatment with anti-EGFR antibody therapy, individuals with mCRC should have their tumor tested for mutations in: • *KRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) • *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) Anti-EGFR monoclonal antibody therapy should only be considered for treatment of individuals with mCRC carcinoma who are identified as having tumors with no mutations detected after such extended RAS mutation analysis.

This table is adapted from (2). *EGFR,* epidermal growth factor receptor; mCRC, metastatic colorectal cancer; ASCO, American Society of Clinical Oncology

Table 3. The NCCN *KRAS*, *NRAS*, and *BRAF* Mutation Testing in Metastatic Colorectal Cancer (2020)

Genes to be tested	Recommendations for colorectal cancer
KRAS *NRAS*	All individuals with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS and NRAS*) and *BRAF* mutations. individuals with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.
BRAF	*BRAF* V600E mutation makes response to cetuximab or panitumumab highly unlikely unless given with a BRAF inhibitor.

This table is created from (3). NCCN, National Comprehensive Cancer Network

Drug: Cetuximab

Cetuximab is an EGFR antagonist. It is used for the treatment of mCRC, and squamous cell carcinoma of the head and neck. Cetuximab, and the related drug panitumumab (brand name Vectibix, approved only for mCRC), are monoclonal antibodies that specifically target the extracellular domain of EGFR. They act by blocking endogenous ligand binding to the extracellular domain of EGFR, and by enhancing receptor internalization and degradation (4). Cetuximab has also been used for off-label indications that include non-small cell lung cancer (5), squamous cell carcinoma of the skin (6), and Menetrier's disease (7).

Cetuximab is a chimeric monoclonal antibody composed of regions of both mouse and human antibody, whereas panitumumab is a fully human monoclonal antibody. Both biological agents have been shown to provide a clear clinical benefit in the treatment of *RAS* wild-type mCRC (8, 9).

Colorectal cancer is the third leading cause of cancer death for men and women in the US, and the second in Europe (10). Radiation therapy is generally used for early stage rectal cancer. Surgery is the most common treatment for localized colorectal cancer that has not spread, and chemotherapy is given before (neoadjuvant) or after (adjuvant) surgery to most individuals with cancer that has penetrated the bowel wall deeply or spread to the lymph nodes (11).

Treatment regimens for advanced or metastatic colorectal carcinoma include chemotherapy such as folinic acid, fluorouracil, irinotecan, capecitabine, and oxaliplatin. Targeted biological agents may be added to such regimens, such as cetuximab, panitumumab, and bevacizumab. Bevacizumab (brand name Avastin) is a monoclonal antibody that targets vascular endothelial growth factor, VEGF. Similar FDA-approved biologics include aflibercept (a VEGF inhibitor monoclonal antibody) and regorafenib (a receptor tyrosine kinase inhibitor with activity against VEGF receptors).

Cetuximab is used in combination with FOLFIRI (FOLinic acid, Fluorouracil, IRInotecan) or FOLFOX (FOLinic acid, Fluorouracil, Oxaliplatin) for first-line treatment; or in combination with irinotecan in individuals who are refractory to irinotecan-based chemotherapy (1, 12). Cetuximab may also be used as a single agent (monotherapy) in individuals who either did not respond to oxaliplatin- and irinotecan-based chemotherapy or are intolerant to irinotecan (3).

Interestingly, the location of the primary colorectal tumor is a predictor of the prognosis for metastatic disease. Left-sided tumors derive from the embryonic hindgut (which gives rise to the splenic flexure, descending colon, sigmoid colon, rectum, and one-third of the transverse colon). Whereas right-sided tumors derive from the embryonic midgut (which gives rise to the appendix, cecum, ascending colon, hepatic flexure, and two-thirds of the transverse colon) (13). These differences in embryologic origin correlate with common genetic alterations. Right-sided tumors are more likely to have mutated *RAS* and *BRAF*, while left-sided tumors may have upregulated *EGFR* and/or *ERBB2* (*HER2*) (14). Thus, individuals with left-sided tumors benefit more from EGFR therapy than individuals with right-sided tumors (15, 16, 17).

Multiple professional guidelines suggest that cetuximab has limited benefit in first-line therapy for right-sided tumors (18, 19). Right-sided tumors may respond to bevacizumab in combination with chemotherapy, with potentially longer overall survival compared with cetuximab combination treatment (15, 16, 20). A recent review highlighted multiple retrospective studies regarding the prognostic and predictive power of right- versus left-sidedness of the primary tumor. The authors concluded that in first-line treatment, left-sided tumors were distinctly more likely to respond to anti-EGFR treatment. However, there was no clear consensus for the implications of tumor sidedness with respect to second-line (and beyond) treatment. (21)

Administration of IV anti-EGFR therapy may be associated with severe infusion reactions, including anaphylaxis (3% for cetuximab and 1% for panitumumab) and these reactions are more common in cetuximab treatment versus panitumumab (22). Other toxicities include cardiopulmonary arrest, severe skin rashes (the severity of which may predict an increased response and survival, regardless of *RAS* mutational status (23, 24)), and an increased risk of venous thrombosis and embolism (2, 11, 25). Additionally, a higher rate of cetuximab-induced infusion reactions has been reported in head and neck cancer treatment (5.4%) as compared with mCRC treatment (26). Within the United States, there appears to be a higher risk of anaphylaxis reaction in areas of the Southeast including North Carolina, Virginia, Tennessee, Florida as well as Missouri and Kansas (reviewed by (27)). Evidence suggests these infusion reactions are due to the presence of immunoglobulin E antibodies targeting a specific glycosylation moiety found on cetuximab (28). The presence of these antibodies in cetuximab-naïve individuals may be due to prior bites from the lone star tick (Amblyomma americanum) *(29).*

Cetuximab can cause fetal harm when administered during pregnancy. There are no studies in pregnant women, but in animal studies (cynomolgus monkeys) the administration of IV cetuximab in pregnancy resulted in an increased risk of fetal death. Women should be advised to use effective contraception during cetuximab therapy

and for 2 months after the last dose. Women should also be advised of the potential risks to the fetus, and to inform their healthcare provider if they know or suspect they are pregnant.

An important role in the progression of mCRC is thought to involve the impaired regulation of EGFR function, resulting in activation of the associated MAPK pathway. Cetuximab and panitumumab are important agents in metastatic disease because they can block the activation of the MAPK pathway. However, resistance to these agents can arise through constitutive activation of the MAPK pathway, which is caused by mutations in downstream signaling proteins, such as KRAS, NRAS and BRAF. Approximately 40% of cases of mCRC are found to have activating mutations in *KRAS*.

The efficacy of cetuximab in treating mCRC is confined to individuals with wild-type *KRAS* tumors. Specifically, tumors that do not harbor specific mutations in exons 2, 3, and 4 of the *KRAS* gene. The *NRAS* gene is highly similar (homologous) to *KRAS*, and mutations in the same exons—2, 3, and 4—are also associated with a lack of response to cetuximab (17, 30, 31, 32, 33).

Therefore, expanded *RAS* testing (of *KRAS* and *NRAS*) is the standard of care to determine which individuals with mCRC may benefit from anti-EGFR therapy (34, 35).

Epidermal growth factor receptor overexpression is seen commonly in squamous cell head and neck cancers and is associated with poor survival outcomes (36, 37). Trials with cetuximab in local or metastatic head and neck cancers have shown this anti-EGFR therapy to have most benefit as part of combination therapies in metastatic cancer, with limited application in local, unresectable disease (reviewed by (18)). Neither the FDA-approved drug label nor NCCN guidelines recommend RAS genetic testing before initiating therapy with cetuximab in head and neck cancers (1, 18).

Proto-oncogenes

Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene.

Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes; however, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (11). Members of the *RAS* family and the *EGFR* gene are all proto-oncogenes.

The *RAS* family contains three genes, *HRAS*, *NRAS*, and *KRAS*, and they are essential components of signaling pathways. They act as signal transducers –– coupling cell surface receptors to intracellular signaling pathways.

The RAS proteins regulate cell signal transduction by acting as a switch –– they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily by the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Mutations in *RAS* genes leads to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" –– providing a continual growth stimulus to cells. Such activating *RAS* mutations are common in colorectal cancers.

The *EGFR* gene is a member of the human epidermal growth factor receptors family (HER) along with *ERBB2* (*HER2)*, *ERBB3* (*HER3)* and *ERBB4* (*HER4)*. Overexpression of these genes has been associated with multiple cancer types. These receptors dimerize upon binding of an extracellular ligand and activate the downstream signaling pathways, including Ras/Raf/Mek/Erk proteins. In some contexts, overexpression of HER2 can promote dimerization without an extracellular signal, leading to constitutive activation. (38, 39)

Gene: *KRAS*

KRAS is the most frequently mutated *RAS* gene found in metastatic colorectal carcinoma. The most frequent individual mutations occur in *KRAS* exon 2, in codons 12 (G12D, G12V) and 13 (G13D). Collectively, these mutations account for 40% of all *RAS* mutations in mCRC (40). Individuals with mCRC that harbor *KRAS* mutations do not benefit from anti–EGFR therapy (either cetuximab or panitumumab therapy) (3, 41, 42, 43, 44, 45, 46).

Gene: *NRAS*

NRAS is highly homologous to *KRAS*, and mutations have been reported in exons 2, 3, and 4. Although *NRAS* mutations are not as frequent as *KRAS* in mCRC, occurring in approximately 2% of tumors, *NRAS* influences the response to treatment with anti-EGFR drugs (2, 47, 48, 49).

Individuals with *NRAS*-mutated tumors are less likely to respond to cetuximab or panitumumab (19, 25). Panitumumab may even have a detrimental effect in individuals with *NRAS* or *KRAS* mutations (2).

Gene: *BRAF*

RAF is a family of serine/threonine kinases that are downstream effectors of KRAS, within the MAPK signaling pathway. The RAF family has 3 members, ARAF, BRAF and CRAF (50).

BRAF mutations are detectable in approximately 5–15% of mCRCs. They tend to only occur in tumors that do not have *KRAS* exon 2 mutations (51). It is therefore unlikely that tumors with *KRAS* mutations will respond to either anti-BRAF treatment (which targets mutant BRAF) or anti-EGFR treatment (because of the presence of KRAS mutations) (52).

By far the most common *BRAF* mutation is known as V600E, which accounts for approximately 90% of *BRAF* mutations. The mutated BRAF protein is constitutively active and is a highly potent oncogene, acting downstream in the EGFR pathway, thus bypassing inhibition of EGFR by cetuximab or panitumumab (10). Constitutively active BRAF can then activate the downstream kinases MEK1 and MEK2, which ultimately activate ERK kinases at the terminus of the MAP kinase signaling pathway (53).

The *BRAF* V600E mutation is associated with a poorer prognosis for individuals with mCRC, as well as with resistance to anti-EGFR treatment. It is also possible that other *BRAF* mutations contribute to anti-EGFR resistance. In *BRAF* V600E-mutant mCRC, BRAF inhibition results in rapid feedback activation of EGFR, a likely mechanistic explanation for limited clinical utility of this monotherapy (54). Alternative treatments may include the use of drug combinations, such as the addition of a BRAF inhibitor to anti-EGFR, to overcome resistance (35, 55). Indeed, utilization of BRAF inhibitor therapy in combination with anti-EGFR (with or without additional targeting of MEK kinases) showed improved survival in the BEACON trial, with the greatest overall survival in the group targeting BRAF, EGFR and MEK simultaneously (54, 56). Guidelines from the NCCN recommend this triple therapy as one approach for *BRAF* V600E mutation-positive disease (3). The NCCN guidelines recommend additional combination therapies for *BRAF* V600E positive colorectal cancer of either vemurafenib, irinotecan and anti-EGFR monoclonal antibodies (cetuximab or panitumumab) or dabrafenib, trametinib and anti-EGFR monoclonal antibodies (3).

The NCCN Colon/Rectal Cancer Panel states that evidence increasingly suggests that the *BRAF* V600E variant makes response to panitumumab or cetuximab, as single agents or in combination with cytotoxic chemotherapy, highly unlikely unless it is also given with a BRAF inhibitor. Therefore, the panel recommends *BRAF* genotyping of tumor tissue (either primary tumor or metastasis) at diagnosis of stage IV disease (3).

Gene: *EGFR*

The HER family consists of 4 members: *ERBB2* (*HER2)*, *ERBB3* (*HER3)* and *ERBB4* (*HER4)*. All 4 members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation.

The EGFR protein is expressed in many different tissues, and is activated by the binding of a ligand, such as epithelial growth factor (EGF) or transforming growth factor α (TGFα). Binding induces receptor dimerization, either homodimers or heterodimers with other HER family members, and triggers autophosphorylation of the intracellular tyrosine kinase domain.

By activating downstream signaling pathways, EGFR has many different biological roles, including stimulating the cell cycle, cell growth, division, differentiation, as well as increased cell invasiveness, apoptosis, and angiogenesis. Therefore, overexpression of EGFR is thought to be an important step in tumor progression, making EGFR a target for anticancer drugs (57, 58, 59).

Currently, there are 2 classes of drug that target EGFR: tyrosine kinase inhibitors (for example, gefitinib and erlotinib) and anti-EGFR monoclonal antibodies (for example, cetuximab and panitumumab) (4).

The EGFR protein is overexpressed in several cancers, including squamous cell carcinoma of the head and neck, squamous cell lung cancer, and colorectal cancer. The EGFR protein is overexpressed in approximately 50–80% of colorectal tumors (2, 60). The FDA-approved drug label for cetuximab states that the drug is licensed for EGFR-expressing mCRC, and mentions in animal studies using human tumor xenografts that lacked EGFR expression, that no anti-tumor effects of cetuximab were observed (1). However, for colorectal cancer, EGFR expression has not been associated with efficacy of anti-EGFR therapy (61).

The NCCN Colon/Rectal Cancer Panel states that EGFR testing of colorectal tumor cells has no proven predictive value in determining likelihood of response to either cetuximab or panitumumab. Therefore, the panel does not recommend routine EGFR testing, and states that no individual should be considered for, or excluded from, cetuximab or panitumumab therapy based on EGFR test results (3).

Gene: *ERBB2 / HER2*

HER2 belongs to the same family of signaling kinase receptors as EGFR and is encoded by the gene *ERBB2*, also called *HER2*. Monoclonal antibodies that target HER2, such as pertuzumab and trastuzumab, are used in the treatment of breast cancer. However, HER2 is rarely expressed in colorectal tumors (approximately 3% overall), though the prevalence is higher in *RAS/BRAF* wild-type tumors (5–14%) (3). Initial evidence suggested that HER2 overexpression may be predictive of resistance to anti-EGFR therapy, yet some evidence suggested that HER2 status is not a biomarker for cetuximab response (35, 62) A recent review of HER2 retrospective studies found a consistent correlation between *ERBB2* amplification and resistance to anti-EGFR treatment (38).

The NCCN Colon/Rectal Cancer Panel recommends *ERBB2* amplification/overexpression testing for individuals with mCRC. However, if the tumor is known to have a *RAS* or *BRAF* mutation, *ERBB2* testing is not required. Based on the outcome of HER2 expression testing, the individual may be eligible for enrollment in one of the on-going clinical trials investigating targeted HER2 therapy in mCRC.(3) The NCCN guidelines emphasize that HER2 overexpression is not prognostic, but can be used to predict success of HER2-targeted therapy and resistance to anti-EGFR antibodies, including cetuximab (3). A dual tyrosine kinase inhibitor targeting HER2 and EGFR called lapatinib is also available and can be used in combination with anti-HER2 monoclonal antibodies for *ERBB2*-amplified mCRC (3).

Linking gene variation with treatment response

It has been established that specific variants in the genes *KRAS* and *NRAS* result in resistance to cetuximab therapy. In addition, the presence of the *BRAF* V600E mutation makes a beneficial response to treatment unlikely, unless given with a BRAF inhibitor (3, 56, 63). Specific point-mutation variants in *ERBB2* and *EGFR* do not appear to be associated with cetuximab resistance. However, *ERBB2* overexpression has been associated with decreased success of anti-EGFR therapies (38, 39).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the cetuximab drug response, and the genes *KRAS*, *NRAS*, *EGFR*, *BRAF* and *ERBB2*.

The 2020 NCCN Guideline for Colon Cancer (Version 4.2020) provides the following recommendations for genetic testing:

KRAS, NRAS, and BRAF Mutation and HER2 Testing

- All [individuals] with metastatic colorectal cancer should have tumor tissue genotyped for RAS (KRAS and NRAS) and BRAF mutations. [Individuals] with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab. BRAF V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor. WT RAS/BRAF tumors should also be screened for HER2 overexpression/amplification.
- No specific methodology is recommended (e.g., sequencing, hybridization) for testing KRAS, NRAS, and BRAF mutations.
- The testing can be performed on formalin-fixed paraffin-embedded tissue. The testing can be performed on the primary colorectal cancers and/or the metastasis, as literature has shown that the KRAS, NRAS, and BRAF mutations are similar in both specimen types.

Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing

- Universal MMR* or MSI* testing is recommended in all newly diagnosed [individuals] with colon cancer. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal (*IHC for MMR and DNA analysis for MSI are different assays and measure different biological effects caused by deficient MMR function)
- The presence of a BRAF V600E mutation in the setting of MLH1 absence would preclude the diagnosis of Lynch syndrome (LS) in the vast majority of cases. However, approximately 1% of cancers with BRAF V600E mutation (and loss of MLH-1) are LS. Caution should be exercised in excluding cases with a strong family history from germline screening in the case of BRAF V600E mutations
- Stage II MSI-H [individuals] may have a good prognosis […]
- Testing for MSI may be accomplished with a validated NGS panel, especially in [individuals] with metastatic disease who require genotyping of RAS and BRAF (3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

2.2 Recommended Dosage for Colorectal Cancer (CRC)

Determine EGFR-expression status using FDA-approved tests prior to initiating treatment. Also confirm the absence of a Ras mutation prior to initiation of treatment with cetuximab. Information on FDA-approved tests for the detection of K-Ras mutations in patients with metastatic CRC is available at: http://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm.

[...]

5.7 Increased Tumor Progression, Increased Mortality, or Lack of Benefit in Patients with Ras- Mutant mCRC

Cetuximab is not indicated for the treatment of patients with CRC that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either K-Ras or N- Ras and hereafter is referred to as "Ras" or when the Ras status is unknown.

Retrospective subset analyses of Ras-mutant and wild-type populations across several randomized clinical trials, including CRYSTAL, were conducted to investigate the role of Ras mutations on the clinical effects of anti-EGFR-directed monoclonal antibodies. Use of cetuximab in patients with Ras mutations resulted in no clinical benefit with treatment related toxicity. Confirm Ras mutation status in tumor specimens prior to initiating cetuximab.

Please review the complete therapeutic recommendations that are located here: (1)

2020 Clinical Practice Guidelines in Oncology: Colon Cancer, from the National Comprehensive Cancer Network (NCCN)

Version 4.2020 – Discussion update in progress.

A sizable body of literature has shown that tumors with a mutation in codon 12 or 13 of exon 2 of the *KRAS* gene are essentially insensitive to cetuximab or panitumumab therapy. More recent evidence shows mutations in *KRAS* outside of exon 2 and mutations in *NRAS* are also predictive for a lack of benefit to cetuximab and panitumumab.

The panel therefore strongly recommends *RAS* (*KRAS/NRAS*) genotyping of tumor tissue (either primary tumor or metastasis) in all patients with metastatic colorectal cancer. Patients with known *KRAS* or *NRAS* mutations should not be treated with either cetuximab or panitumumab, either alone or in combination with other anticancer agents, because they have virtually no chance of benefit and the exposure to toxicity and expense cannot be justified. ASCO released a Provisional Clinical Opinion Update on extended *RAS* testing in patients with metastatic colorectal cancer (mCRC) that is consistent with the NCCN panel's recommendations. A guideline on molecular biomarkers for CRC developed by the ASCP, CAP, AMP and ASCO also recommends resting consistent with the NCCN recommendations.

The recommendation for *RAS* testing, at this point, is not meant to indicate a preference regarding regimen selection in the first-line setting. Rather, this early establishment of *RAS* status is appropriate to plan for the treatment continuum, so that the information may be obtained in a non- time–sensitive manner and the patient and provider can discuss the implications of a *RAS* mutation, if present, while other treatment options still exist.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Note that because anti-EGFR agents have no role in the management of stage I, II, or III disease, *RAS* genotyping of colorectal cancers at these earlier stages is not recommended.

KRAS mutations are early events in colorectal cancer formation, and therefore a very tight correlation exists between mutation status in the primary tumor and the metastases. For this reason, *RAS* genotyping can be performed on archived specimens of either the primary tumor or a metastasis. Fresh biopsies should not be obtained solely for the purpose of *RAS* genotyping unless an archived specimen from either the primary tumor or a metastasis is unavailable.

The panel recommends that *KRAS*, *NRAS*, and *BRAF* gene testing be performed only in laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform highly complex molecular pathology testing. No specific testing methodology is recommended. The three genes can be tested individually or as part of an NGS panel.

[…]

HER2 is a member of the same family of signalling kinase receptors as EGFR and has been successfully targeted in breast cancer in both the advanced and adjuvant settings. HER2 is rarely amplified/overexpressed in CRC (approximately 3% overall), but the prevalence is higher in *RAF/BRAF*-wild type tumors (reported at %5-14%). Specific molecular diagnostic methods have been proposed for HER2 testing in CRC and HER2-targeted therapies are now recommended as subsequent therapy options in patients with tumors that have HER2 overexpression. Based on this, the NCCN Guidelines recommend testing for HER2 amplifications for patients with mCRC. If the tumor is already known to have a *KRAS/NRAS* or *BRAF* mutations, HER2 testing is not required. As HER2-targeted therapies are still under investigation, enrollment in a clinical trial is encouraged.

Evidence does not support a prognostic role of HER2 overexpression. In addition to its role as a predictive marker for HER2-targeted therapy, initial results indicated HER2 amplification/overexpression may be predictive of resistance to EGFR-targeting monoclonal antibodies.

Please review the complete therapeutic recommendations that are located here: (3)**.**

2015 Provisional Clinical Opinion from the American Society of Clinical Oncology (ASCO)
All patients with metastatic colorectal cancer who are candidates for anti-EGFR antibody therapy should have their tumor tested in a Clinical Laboratory Improvement Amendments–certified laboratory for mutations in both KRAS and NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146). The weight of current evidence indicates that anti-EGFR MoAb therapy should only be considered for treatment of patients whose tumor is determined to not have mutations detected after such extended RAS testing.

What's New and Different?

In addition to testing for mutations in KRAS exon 2 (codons 12 and 13) as recommended previously, before treatment with anti- EGFR antibody therapy, patients with mCRC should have their tumor tested for mutations in:

- KRAS exons 3 (codons 59 and 61) and 4 (codons 117 and 146)
- NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146)

Please review the complete therapeutic recommendations that are located here: (2)

Allele Nomenclature

Selected *KRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
G12D	p.Gly12Asp	NM_004985.4:c.35G>A	NP_004976.2:p.Gly12Asp	rs121913529
G12V	p.Gly12Val	NM_004985.4:c.35G>T	NP_004976.2:p.Gly12Val	rs121913529
G13D	p.Gly13Asp	NM_033360.3:c.38G>A	NP_004976.2:p.Gly13Asp	rs112445441

Selected *NRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
NRAS G12V	p.Gly12Val	NM_002524.4:c.35G>T	NP_002515.1:p.Gly12Val	rs121913237
NRAS G13R	p.Gly13Arg	NM_002524.4:c.37G>C	NP_002515.1:p.Gly13Arg	rs121434595
NRAS Q61R	p.Gln61Arg	NM_002524.4:c.182A>G	NP_002515.1:p.Gln61Arg	rs11554290
NRAS Q61K	p.Gln61Lys	NM_002524.4:c.181C>A	NP_002515.1:p.Gln61Lys	rs121913254

Selected *BRAF* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.4:c.1799T>C	NP_004324.2:p.Val600Glu	rs113488022

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Chloroquine Therapy and *G6PD* Genotype

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Introduction

Chloroquine is used for the treatment of uncomplicated malaria and extra-intestinal amebiasis. Malaria is caused by infection of *Plasmodium* parasites. Chloroquine is active against the erythrocytic forms of susceptible strains of *Plasmodium falciparum (P. falciparum)*, *Plasmodium malariae (P. malariae)*, *Plasmodium ovale (P. ovale)*, and *Plasmodium Vivax (P. vivax)*. Chloroquine is not active against the gametocytes and the exoerythrocytic forms including the hypnozoite stage (*P. vivax* and *P. ovale*) of the *Plasmodium* parasites. Additionally, resistance to chloroquine and hydroxychloroquine has been reported in *Plasmodium* species, thus chloroquine therapy is not indicated if the infection arose in a region with known resistance. Chloroquine is used in first-line treatment of *P. vivax* malaria with primaquine. Studies have indicated chloroquine is effective against the trophozoites of *Entamoeba histolytica (E. histolytica)*, which causes amebic dysentery, or amebiasis. (1) Chloroquine also has off-label uses for treatment of rheumatic diseases and has been investigated as a potential antiviral therapy as well as an adjuvant chemotherapy for several types of cancer. (2, 3, 4, 5)

Chloroquine accumulates in cellular acidic compartments such as the parasitic food vacuole and mammalian lysosomes, leading to alkalinization of these structures. This change in pH can impair the action of enzymes responsible for the formation of hemozoin by the parasite from ingestion of the host's hemoglobin; this reaction occurs in the parasitic vacuole (6). Thus, chloroquine targets the blood-stage of the malaria parasites but cannot eliminate dormant hypnozoites and must be administered with a drug that targets the dormant parasitic form (1). Chloroquine, developed in the 1940s, has been superseded as the first-line recommended antimalarial therapy by both the US Centers for Disease Control (CDC) and World Health Organization (WHO), with the exceptions of during the first trimester of pregnancy or for malarial prophylaxis of a pregnant individual who is also deficient for glucose-6-phosphate dehydrogenase (G6PD) (7, 8). Among antimalarial medications, chloroquine is less likely than other medicines to cause hemolysis in G6PD-deficient individuals; however, the FDA-approved drug label states there is still a risk of hemolysis (Table 1) (1). In contrast, the Clinical Pharmacogenetics Implementation Consortium (CPIC) performed a systematic review of the available clinical literature and found low-to-no risk of acute hemolytic anemia for individuals with G6PD deficiency who take hydroxychloroquine or chloroquine (9) (Table 2). It should be noted that G6PD deficiency has a range of severity; CPIC advises caution for all medications when used by an individual with a severe G6PD deficiency with chronic non-spherocytic hemolytic anemia (CNSHA).

Table 1: The FDA Drug Label for Chloroquine Phosphate (2020)

Phenotype	Precautions
G6PD deficiency	Chloroquine may cause hemolysis in G6PD deficiency. Blood monitoring may be needed as hemolytic anemia may occur in association with other drugs that cause hemolysis.

G6PD - Glucose-6-phosphate dehydrogenase. This FDA table is adapted from (1).

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Table 2: The CPIC Guidelines for Chloroquine based on G6PD Phenotype

Predicted G6PD phenotype based on genotype	Implication for phenotypic measures	Therapeutic recommendation	Classification of recommendation[a]	Evidence level[a]
Normal	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status	Strong	Weak
Deficient	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate	Weak (High)[b]
Deficient with CNSHA	High-risk of acute exacerbation of chronic hemolysis	Use all drugs cautiously in this group; if a drug is used, close monitoring for acute exacerbation of chronic hemolysis is recommended	Optional	Weak
Variable	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate	Weak
Indeterminate	Unknown risk of acute hemolytic anemia	To ascertain G6PD status, enzyme activity must be measured. Drug use should be guided per the recommendations based on the activity-based phenotype	Moderate	Weak

CNSHA - Chronic non-spherocytic hemolytic anemia; G6PD - glucose-6-phoshate dehydrogenase
[a] Rating scheme and evidence level based on clinical data from (9) Supplement.
[b] One preclinical study found no evidence of hemolysis in a humanized mouse model of G6PD deficiency.
This table is adapted from (9).

Drug: Chloroquine

Chloroquine is a 4-aminoquinoline used for the treatment of susceptible strains of malaria and extra-intestinal amebiasis caused by trophozoites of *E. histolytica* (1). It has off-label uses in rheumatic diseases, the treatment and prophylaxis of Zika virus, and it has been investigated for treatment of human immunodeficiency virus (HIV) (10, 11, 12, 13). However, in many cases hydroxychloroquine, a chloroquine derivative, is used more often for rheumatic conditions. Chloroquine has also been investigated as an adjuvant anticancer therapy (14, 15, 16). Because of its widespread use to treat and prevent malaria between the 1940s and 1980s, resistance to chloroquine has arisen in many strains of the malaria-causing parasite in several endemic areas (17). Additional antimalarial medications have since been developed and can be used to treat those resistant strains. Following recommendations by the WHO, chloroquine is no longer the first line therapy, having been replaced by artemisinin-based combination therapies (8).

Chloroquine inhibits the heme polymerase enzyme in the malarial parasite due to neutralizing the pH of the vacuole, resulting in a fatal accumulating of toxic heme within the parasite (10). Within human cells, chloroquine passively diffuses into subcellular structures important for protein synthesis and cellular waste removal: Golgi vesicles, endosomes, and lysosomes. The lysosome is the site of cellular autophagy, the mechanism whereby cells clear damaged organelles and protein masses as well as degrade foreign material. The lysosome is an acidic compartment and once inside, chloroquine becomes protonated and trapped, causing the pH of the lysosome to rise. This inhibits autophagy and is the mechanistic basis for its use as an adjuvant anticancer therapy with standard chemical and radiation oncology treatments. (14, 15, 18, 19) Chloroquine also affects innate and adaptive immunity, acting at several key points of immune regulation. Chloroquine and hydroxychloroquine both inhibit recognition of nucleic acids by the toll-like receptors, the major histocompatibility complex class II-mediated antigen presentation, inflammation induced cell proliferation and

antiphospholipid antibody activity, making them useful for the treatment of autoimmune disorders such as systemic lupus erythematous (20).

Chloroquine phosphate is not indicated for use in the treatment of complicated malaria, nor should it be used in individuals with known hypersensitivity to 4-aminoquinoline compounds. Concomitant medication with an 8-aminoquinoline is necessary to treat the hypnozoite stage of certain *Plasmodium* parasites' life cycle (see below for more details). Per the FDA-approved label, use of chloroquine phosphate for any condition other than acute malaria is contraindicated in the presence of retinal or visual changes of any etiology (1). Acute malaria therapy with chloroquine requires only 3 days to complete the full course of medication (1).

In March of 2020, hydroxychloroquine and chloroquine were granted emergency use authorization by the U.S. Food and Drug Administration for the treatment of coronavirus disease 2019 (COVID-19) caused by infection with the severe acute respiratory syndrome coronavirus 2 virus (21). This authorization was revoked on 15 June 2020 due to the risk of cardiac adverse events and other potential adverse reactions, which were determined to outweigh the potential benefit of these medications in treating COVID-19 (22).

Chronic chloroquine use may cause irreversible retinal damage. If chloroquine is to be prescribed for an extended period, the FDA-approved drug label states that a baseline visual exam must be taken within the first year of therapy to monitor for any changes in vision. The baseline exam should include "best corrected distance visual acuity, an automated threshold visual field (VF) of the central 10 degrees (with retesting if an abnormality is noted), and spectral domain optical coherence tomography (SD-OCT)." These exams should be performed annually for individuals when daily doses of chloroquine phosphate are greater than 2.3 mg/kg of actual body weight (bw), durations of use greater than 5 years, subnormal glomerular filtration, or use of some concomitant drug products such as tamoxifen citrate. Individuals without these risk factors should have their vision assessed once every 5 years. (1)

The American Academy of Ophthalmology (AAO) similarly advises that in addition to the automatic visual field exams, SD-OCT should also be performed (23) and recently updated their recommended dosage to no more than 2.3 mg/kg per day for rheumatoid condition therapy (24). The Royal College of Ophthalmologists in the United Kingdom has also published recommendations for visual screening with regular monitoring for individuals on prolonged chloroquine and hydroxychloroquine therapy (25). If ocular toxicity is suspected, discontinue use immediately, though visual changes may continue to progress after withdrawal due to the prolonged systemic half-life of chloroquine. (16)

It should be noted that in individuals of Asian descent, retinal toxicity may present first outside of the macula, thus the FDA recommends the VF screening be performed in the central 24 degrees (rather than 10) in this population. (1) The AAO also recommends SD-OCT testing should look beyond the central macula in Asian individuals (23). The specific mechanism underlying this difference in disease presentation is unknown, though genetics are suspected to play a role (23). It is hypothesized that the mechanism of retinal damage due to prolonged chloroquine therapy is due to its ability to bind to the melanin pigment in the iris, ciliary body, and retinal pigment epithelium (16, 26, 27).

Chloroquine may result in additional adverse effects on cardiac, neurological, and muscle tissues (20). Cardiac tissue toxicity can result in cardiomyopathy with conduction defects including prolonged QT interval, Torsades de pointes, and ventricular arrhythmias. Some studies have indicated that QT prolongation can present within 3 to 5 days of treatment with chloroquine or hydroxychloroquine, though arrythmias and conduction disorders are more common with chloroquine versus hydroxychloroquine (20, 28). The arrythmia risk is dose dependent and increased by co-medication with other arrhythmogenic drugs such as amiodarone or moxifloxacin. Neuromuscular toxicity is rare, but there have been reports of proximal symmetric muscle deficits and polyneuropathies (20). Chloroquine can also cause severe hypoglycemia, both with and without concomitant antidiabetic medication. Hypoglycemia is an intrinsic reaction to the drug but can also be triggered by the

nutritional status of the individual with malaria. Auditory effects have also been reported, as such the FDA advises that chloroquine should be administered with caution in individuals with pre-existing auditory damage and to immediately discontinue the medication in the event of any defects in hearing. Additional side effects or risks include acute extrapyramidal disorders, muscle weakness, increased risk of psoriatic attack or worsening of porphyria, and a potential elevated risk of convulsions in individuals with a history of epilepsy. (1) Cutaneous toxicity has also been reported; pruritus has been reported in up to half of treated individuals from several studies (29) and pigmentation changes have also been reported (20). Much of these off-target tissue toxicities are predicted to result from the alkalinization of lysosomes, modulation of immune reactions and, in some cases, off-target activation of cellular receptors (20).

There have also been reports of adverse psychiatric side effects of chloroquine use. These symptoms include insomnia, mania, paranoia and persecutory delusions and auditory and visual hallucinations (30). The incidence of these psychoses are rare, occurring once per hundreds to thousands of individuals prescribed chloroquine and mefloquine—another antimalarial medication, and is not common in the treatment of uncomplicated malaria (31). Of note, there seems to be no correlation between chloroquine-induced psychiatric symptoms and pre-existing familial or personal predisposition to mental disorders (32).

The FDA-approved label states that individuals with G6PD enzyme deficiency may be predisposed to hemolysis during chloroquine therapy (1). Therapy includes treatment for active, uncomplicated malaria that requires administration of 1.5 g base over 3 days in adults and 25 mg base/kg bw in infants and children. Prophylaxis therapy consists of a weekly dose of 300 mg base for adults or a weekly dose of 5 mg base per kg bw in children (1). The CDC advises that individuals with G6PD deficiency who may not tolerate other antimalarial medications may be prescribed a prophylactic dose of chloroquine for one year following acute malarial infection with *Plasmodium* species with hypnozoites, as most relapses from reactivation occur within this timeframe (33). As of 2023, chloroquine is not available in the Canadian market, however the last active label from Health Canada advised caution when using chloroquine in individuals with G6PD deficiency (34). In contrast, the drug regulatory agency of Switzerland (Swissmedic) states that G6PD deficiency is a contraindication for chloroquine therapy due to symptoms of hemolytic anemia and favism (35).

Chloroquine is metabolized by the cytochrome P450 family of enzymes. First, chloroquine is N-dealkylated to N-desethylchloroquine, which is an active metabolite (36). This is achieved primarily through CYP2C8 and CYP3A4 mediated metabolism, with a lesser contribution from the enzymes CYP3A5, CYP2D6, and even less by CYP1A1 (10, 36). Chloroquine and desethylchloroquine have elimination half-lives of 20–60 days, primarily via renal excretion. Much of the absorbed chloroquine is bound by albumin and P-glycoprotein and a considerable amount of chloroquine is stored in tissues, resulting in a large volume of distribution (1, 36). Multiple intrinsic factors can impact the pharmacokinetic parameters of chloroquine including age, pregnancy status, body weight, as well as *CYP* genetic variation (36, 37, 38). Although chloroquine can freely diffuse into cells, it is also a substrate of several transporter proteins. Chloroquine is a substrate, inhibitor, and inducer of the multidrug resistance-associated protein 1 (MRP1) and a substrate of organic anion transporting proteins (OATPs) (36). Various drug interactions have been documented with chloroquine, either based on absorption or metabolism. Ampicillin, cyclosporine, praziquantel, and cimetidine may all interact with chloroquine and these drugs may have altered metabolism and plasma concentrations when taken concurrently with chloroquine (1).

Chloroquine overdose is a serious risk, particularly for children with accidental ingestion, as chloroquine is rapidly absorbed. The FDA label states that even one gram of chloroquine may be fatal in children (3 years of age) (1). Toxicity symptoms include nausea, vomiting, headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions, hypokalemia, cardiac arrythmia and conduction defects, and sudden, potentially fatal respiratory and cardiac arrest; these symptoms may present within minutes of overdose. Immediate medical attention is required. Thus, it is strongly advised by the FDA-approved label to keep chloroquine phosphate out of reach of children, as these individuals are particularly sensitive to 4-aminoquinoline compounds (1).

Human studies have not shown an increase in the rate of birth defects or spontaneous abortions associated with chloroquine use by pregnant mothers, nor evidence of fetal ocular toxicity. (1, 39) The CDC states that for pregnant women with uncomplicated malaria caused by *P. malariae, P. ovale*, or chloroquine-sensitive *P. vivax* or *P. falciparum*, treatment with hydroxychloroquine or chloroquine is recommended (33). Chloroquine is also the recommended alternative therapy during pregnancy by the WHO for infection with sensitive *Plasmodium* strains (8). Furthermore, the CDC recommends continued chloroquine prophylaxis for individuals with *P. vivax* or *P. ovale* infection for the duration of pregnancy. If, upon delivery, the mother intends to breastfeed, the infant should be tested for G6PD deficiency. If neither the infant nor mother are G6PD deficient, primaquine phosphate is the recommended therapy for the mother (tafenoquine is not recommended during breastfeeding). Otherwise, women who cannot take tafenoquine or primaquine due to G6PD deficiency should continue weekly chloroquine prophylaxis for one year following acute malarial infection (33). Small amounts of chloroquine are excreted in breast milk. A once per week dosage is not sufficient to cause harm to a nursing infant, nor is it sufficient to provide protection from malaria (40). Thus, infants at risk of malaria exposure who are breastfed still require prophylaxis. One study reported a decrease in viral HIV loads in breast milk of HIV-infected women who were treated with chloroquine as compared with women who were treated with a combination of sulfadoxine and pyrimethamine (41).

Disease: Malaria

Malaria is a serious tropical disease caused by a parasite (*Plasmodium*) that spreads to humans by infected mosquitos. The only available vaccine is moderately effective and acts only against *the P. falciparum* species (42). Widely recommended antimalarial drugs such as mefloquine can be used for prevention, which is known as chemoprophylaxis. The type of chemoprophylaxis recommended depends upon the individual taking the prophylaxis (namely: age, pregnancy status, concurrent medication use, and medical comorbidities) and the nature of travel –– specifically, the countries traveled to, the length of stay, the species of *Plasmodium* that are most prevalent, and the level of drug resistance.

Despite chemoprophylaxis, travel to malaria-endemic areas is not without risk. Individuals at elevated risk for malaria complications include pregnant women (33) and adults who have had their spleen removed (43). If travel cannot be avoided, chemoprophylaxis should be combined with additional precautions to avoid mosquito bites, such as bed nets and repellents. In 2021, the WHO estimated 247 million cases of malaria occurred worldwide, and malaria was responsible for at least 619,000 deaths. (44)

Malaria is found in over 100 countries and occurs throughout most tropical regions in the world. These regions include large parts of Africa, Asia, Central and South America, and parts of the Middle East and Pacific islands (45, 46). Individuals who are heterozygous carriers for sickle cell disease or G6PD deficiency have a protective advantage against malaria, and as a result, the frequency of such genetic conditions is higher in countries where malaria is endemic (47).

Malaria is transmitted to humans by the bite of an infected *Anopheles* mosquito. Only female mosquitos spread the infection (females feed on human blood, males feed on nectar). Although malaria can also be spread by sharing contaminated needles or via a contaminated blood transfusion, these are rare means of transmission. (48)

There are several different *Plasmodium* species, but only a few species cause the most malaria cases:

- *P. falciparum*
  - The most common cause of malaria, and death from malaria
  - Predominates in sub-Saharan Africa
  - Also found in regions of Australasia (Papua New Guinea, Southeast Asia), and the Caribbean (Haiti and the Dominican Republic)

- *P. vivax*
  - A common cause of malaria outside of Africa
  - Most frequent species found in Central and South America
  - Parasite has a dormant, hypnozoite stage
  - Early gametocytes that infect mosquitos
- *P. malariae*
  - Less common
  - Found in most areas where malaria is endemic
- *P. ovale*
  - Less common
  - Parasite has a dormant, hypnozoite stage
- *P. knowlesi*
  - Less common
  - Found in some areas of Southeast Asia

The first stage of malaria infection begins when an infected mosquito bites the human host. Typically, mosquitos bite at dusk, or during the night. As the mosquito feeds, infective parasite sporozoites (the motile spore-like stage in the life cycle of this parasitic sporozoan, which is the infective agent) are inoculated into humans. The sporozoites travel to the liver, where they invade liver cells and asexually reproduce to form schizonts. The liver schizonts contain daughter merozoites. This process is asymptomatic, and because it occurs outside of the red blood cell (erythrocyte), it is known as the exoerythrocytic stage. (49)

Some species of the parasite (*P. vivax* and *P. ovale*) have an additional dormant stage in the liver. The parasite exists as hypnozoites, which can stay in the liver for weeks or months without causing any clinical symptoms.

The second stage of malaria infection is the erythrocytic stage. It begins when the liver schizonts rupture and release the daughter merozoites into the bloodstream. The merozoites invade red blood cells, digest hemoglobin, produce a toxic metabolite (hemozoin), and damage red blood cell membranes. Infected, brittle red blood cells are rapidly broken down (hemolysis) and if too many damaged red blood cells get trapped in the spleen, the spleen can rapidly enlarge (splenic sequestration). Notably, *P. vivax* preferentially infects the immature reticulocytes rather than mature erythrocytes due to expression of reticulocyte-specific receptors; G6PD levels are higher in reticulocytes than erythrocytes, which may provide more protection against oxidative stress for the *P. vivax* parasite (50, 51).

Some of the daughter merozoites differentiate into male or female gametocytes (sexual forms). When they are ingested by a mosquito, they mature, fertilize, reproduce, and develop into sporozoites. When the mosquito feeds again, the sporozoites are inoculated into another human host and the cycle of malaria transmission is complete.

The erythrocytic stage of malaria is usually associated with fever, and malaria should always be suspected in anyone with a fever who has recently returned from a malaria-endemic region, even if antimalarial chemoprophylaxis was correctly followed. Other symptoms and signs include nausea, vomiting, abdominal pain, tachycardia (fast heart rate), diaphoresis (sweating), chills, and myalgia (muscle pain). The complications of malaria infection include severe anemia, cerebral malaria, and multi-organ failure. Without correct diagnosis and prompt treatment, malaria can be fatal.

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on chromosome Xq28. As such, males are hemizygous for one *G6PD* allele, making them more susceptible to this X-linked disorder. Females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X

chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene. Males with Klinefelter syndrome have an additional X chromosome (47, XXY) and therefore 2 *G6PD* alleles. Thus, it is important to consider the number of X chromosomes for an individual when determining *G6PD* genotype or phenotype.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide, with a worldwide prevalence of approximately 5% (52). Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is or once was endemic (for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean) (53, 54, 55). In the US, G6PD deficiency is more common among African Americans, affecting approximately 12% (56).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. Red blood cells that lack G6PD also have a deficiency of NADPH. (57)

Red blood cells that are G6PD and NADPH deficient are more susceptible to oxidative stress (for example, by reactive oxygen species and hydrogen peroxide). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the antimalarial drugs primaquine and tafenoquine, the antibacterials dapsone and sulfamethoxazole, the skin cancer drug dabrafenib, and the uric acid lowering drugs pegloticase and rasburicase (9).

Most individuals with G6PD deficiency are asymptomatic, as they have a normal lifespan and may not know they have G6PD deficiency. However, at birth, they may be predisposed to neonatal jaundice, and throughout life, they will be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid exposure to oxidizing agents when possible, including antimalarial drugs such as tafenoquine and primaquine.

Symptomatic individuals with G6PD deficiency may suffer from episodes of acute hemolytic anemia or CNSHA. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions. Folic acid and iron may be given to prevent the worsening of anemia in individuals with folate deficiency and to induce formation of new red blood cells (58).

More than 200 genetic variants of the *G6PD* gene have been identified (59). Most known *G6PD* variants are missense, which can also be inherited as haplotypes that are comprised of more than one variant allele (60). Large deletions are rare, and a complete lack of G6PD activity is thought to be fatal in utero.

The normal (wildtype) copy of the *G6PD* gene is known as *G6PD* B, and is found in most individuals of European descent, individuals of Asian descent, and individuals of African descent. Common *G6PD* variants include:

- *G6PD* A (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of individuals of African descent (61)
- *G6PD* A- (p.Asn126Asp with p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (62). Additional *A-* haplotypes have also been identified, both with the A variant with a second variant (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (63)

- *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is a common pathogenic variant in individuals of European descent (64)
- *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in individuals of Asian descent (65)
- *G6PD* Viangchan (p.Val291Met) is the most common *G6PD* variant among Thais, Laotians, Cambodians, and Malaysians (based on common genetic ancestry) (66, 67)

The WHO recently updated its categorization of *G6PD* variants into 4 classes based on the median residual enzyme activity in males (expressed as a percentage of normal activity) (68). Class A variants have <20% activity and are associated with chronic hemolytic anemia. Most individuals with G6PD deficiency have variants that belong to class B (enzyme activity less than 45%). Class B variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but most of the time, affected individuals are asymptomatic. Class C variants show median G6PD activity from 60–150% and are not associated with hemolysis. In class U are the variants with any activity and unknown clinical significance. The CPIC has assigned G6PD phenotypes based on *G6PD* genotypes under the previous classification system (9); the updated WHO categories are also provided (Table 3) (9).

Table 3. Assignment of likely G6PD Phenotype based on Genotype/Diplotype (CPIC, 2022)

Likely phenotype	Definition[a]	Genotype	WHO class for G6PD variants[b]	Example of diplotype[c]
Normal	Very mild or no enzyme deficiency, no less than 60% of normal enzyme levels (60–150% of normal activity)	An X chromosome hemizygote who has a nondeficient (class IV) allele	IV (C)	B, Sao Borja
		An individual who has 2 nondeficient (class IV) alleles	IV/IV (C)	B/B, B/Sao Borja
Deficient	Less than 10–60% of normal enzyme activity (20–45% of normal activity)	An X chromosome hemizygote who has a deficient (class II–III) allele	II, III (B)	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		An individual who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III (B)	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA (<20% of normal activity)	An X chromosome hemizygote who has a class I allele	I (A)	Bangkok, Villeurbanne
		An individual who has 2 deficient (class I variants) alleles	I/I (A)	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[d]	Normal or deficient enzyme activity[c]	An individual who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III (U)	B/A–, B/Mediterranean, B/Bangkok

Table 3. continued from previous page.

Likely phenotype	Definition[a]	Genotype	WHO class for G6PD variants[b]	Example of diplotype[c]
Indeterminant	Uncertain		(U)	

CNSHA - chronic non-spherocytic hemolytic anemia; WHO - World Health Organization; G6PD - glucose-6-phosphate dehydrogenase; CPIC - Clinical Pharmacogenetics Implementation Consortium

[a] The traditional (Class I-IV) and updated (A, B, C, and U) activity levels are both provided, with the updated activity ranges provided in parentheses where relevant.

[b] WHO classifications were under revision at the time of CPIC publication, updated classification (using A, B, C and U designations) have been proposed based on enzyme activity levels and are provided in parenthesis here (68).

Class I alleles are extremely rare; the distinction between class II and III alleles is not clear. Almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.

[c] Due to the large number of G6PD variants, many other diplotypes may be possible besides those given as examples here; see Supplementary data from (9) for a more comprehensive list of alleles with their assigned WHO class. For Human Genome Variation Society terms, please see the Nomenclature table below. The alleles and diplotypes provided here are based upon the historic class I–IV definitions and may not fit the updated WHO classification.

[d] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online [G6PD heterozygotes]) (9).

This table is adapted from (9).

Additional Genes of Note

Chloroquine is metabolized by the enzymes encoded by *CYP2C8*, *CYP3A4* and *CYP2D6*, all of which are classified as "very important pharmacogenes" by PharmGKB (69, 70). Variability in the *CYP* genes can lead to reduced or, occasionally, increased enzyme function. Classification of individual phenotypes as either ultrarapid metabolizers, normal metabolizers, intermediate metabolizers, or poor metabolizers is carried out on a predictive basis from genotype/diplotype results of known alleles. Allele definitions for various genes are available from CPIC (71) and the Pharmacogene Variation Consortium (PharmVar) (72), and *CYP2D6* allele functional classifications are also available from CPIC (71) and PharmVar (72). Allele frequencies for these pharmacogenes vary across global and even regional populations (71, 73, 74, 75, 76).

Interaction of chloroquine with these CYP enzymes leads to varying degrees of enzyme inhibition (36). These interactions may decrease the enzymatic function of these CYP proteins relative to any single drug. This results in phenoconversion, a phenomenon where an individual's genetically predicted CYP metabolism is altered to a different activity level. Drugs competing for the same metabolic enzyme will often lead to phenoconversion with lower effective enzyme activity. Studies have found chloroquine administration can inhibit CYP2D6 activity in vitro and in vivo in normal metabolizers and in some cases, this led to phenoconversion to a poor-metabolizer phenotype for primaquine metabolism (77, 78, 79).

Transporter proteins encoded by solute carrier organic anion transporter (*SLCO)* genes, also known as OATPs, adenosine triphosphate (ATP)-binding cassette sub-family B member 1 *(ABCB1)* or P-glycoprotein, ATP-binding cassette sub-family C member 1 (*ABCC1)* or MRP1, and ATP-binding cassette sub-family C member 2 (*ABCC2)* or MRP2 have all been shown to have their function impacted by chloroquine (36).

Sortica and colleagues studied 164 individuals infected with *P. vivax* treated with chloroquine and primaquine and found a significant interaction between variants in *SLCO2B1* (c.935G>A) and treatment over time on the rate of parasite clearance; they further observed an effect from variants in *SLCO1A2* and *SLCO1B1* on gametocyte clearance over time. Their data suggests that individuals with *SLCO1A2*2* or **3* alleles have a slower clearance rate for gametocytes during antimalarial therapy as compared with **1* homozygous individuals, though this may be due to changes in chloroquine binding, as it is a known substrate for the encoded transporter

OATP1A2. The effect from *SLCO1B1* variants (namely the **14* allele) was significant when considered as a change in gametocyte clearance over time. (80)

The ATP-binding cassette (ABC) family of proteins and OATPs are important drug transport proteins and altered function of these enzymes can affect the efficacy of substrate medications and potential side effects. In the case of P-glycoprotein, the effect of chloroquine is weak inhibition of the transport protein and thus is not expected to significantly affect the transporter activity nor chloroquine metabolism. However other substrates may be adversely affected and co-medication with drugs that further inhibit transport may lead to significant effects (36). One study found chloroquine to be a potent inhibitor of the OATP1A2 transport protein, potentially impacting uptake of all-trans-retinol in the retinal pigment epithelium (81).

Linking Gene Variation with Treatment Response

Individuals with G6PD deficiency (less than 60% normal enzyme activity) may be at a higher risk for hemolysis when exposed to chloroquine than those individuals with normal G6PD enzyme activity (1, 63, 82). However, both the CDC and WHO recommend chloroquine prophylaxis to prevent malaria relapse for pregnant and breastfeeding women, as primaquine is contraindicated during pregnancy and in G6PD deficiency (<30% enzymatic activity) (7). The FDA-approved drug label recommends monitoring individuals on prolonged therapy for signs of hemolysis; the recommended test is complete blood counts (1).

Genetic variation in *CYP2C19*, *ABCG2,* and *UGT2B7* leading to decreased enzyme activity—when present with CYP2D6 intermediate- or poor-metabolizer status—may predispose individuals to a higher risk of *P. vivax* relapse for individuals treated with a combination primaquine and chloroquine regimen (83). Individuals with low-activity *CYP2C8* alleles have also been reported to have reduced gametocyte clearance rate as compared with those with normal function alleles (84). These pharmacogenetic effects may be due primarily to the impact on primaquine rather than chloroquine metabolism.

The CYP2D6 enzyme is critical for the metabolism of a large number of drugs and some evidence suggests that chloroquine use can further reduce enzymatic activity in individuals with already reduced CYP2D6 activity (36). Medications such as metoprolol and tamoxifen depend upon CYP2D6 metabolism and may be negatively impacted by co-medication with chloroquine (85, 86). This may lead to altered therapeutic response for all concomitant medications, and increase the risk of retinal damage in the case of tamoxifen co-medication (1).

Transport proteins for chloroquine show some genetic variation that has been associated with differences in malaria response. One study of combination therapy with chloroquine and primaquine found that variants in *SLCO2B1* (encoding OATP2B1), *SLCO1B1* (encoding OATP1B1), and *SLCO1A2* (encoding OATP1A2) were associated with differences in parasite and gametocytemia clearance rates (80).

However, there are no specific actionable guidelines from the pharmacogenetics community or the FDA (1) to alter antimalarial medication based on variation of any the genes discussed herein.

The *G6PD* Gene Interactions with Medications Used for Additional Indications

Medications that can induce oxidative stress in red blood cells can trigger hemolysis readily in individuals with G6PD enzyme deficiency. Antimalarials, such as tafenoquine and primaquine, are one class of medication that often pose a risk for G6PD deficient individuals, but many more medications require special attention to G6PD status.

- Urate-lowering medications: both refractory gout and tumor lysis syndrome can cause systemic elevation of urate levels, medications such as rasburicase and pegloticase are uricase enzymes that aid in the

breakdown of uric acid into more soluble metabolites. These reactions produce hydrogen peroxide as a byproduct, thus increasing oxidative stress in the body.
- Anti-microbial medications: nitrofurantoin, often used for urinary tract infections, was determined to be a medication of moderate risk for AHA in G6PD-deficient individuals by CPIC and may call for additional monitoring. In contrast, CPIC found sulfamethoxazole to be a medication with low-to-no risk in G6PD deficient individuals. (9)

Additional information on gene-drug interactions for *G6PD* are available from PharmGKB, CPIC and the FDA (search for "G6PD").

Genetic Testing

The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for chloroquine response and the *G6PD* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD deficiency and testing may also be used to screen females with a family history of G6PD deficiency to see if they are carriers. In routine clinical practice, G6PD deficiency is diagnosed by measuring G6PD activity in red blood cells (57, 58). Two different types of enzyme activity tests are used, and they are classified as qualitative or quantitative. For some medications, such as tafenoquine, a specific enzymatic activity threshold is used to determine the safety of the medication and as such a quantitative test may be required for individuals with intermediate levels of enzyme activity based on the qualitative test (87). False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, when necessary, screening for G6PD enzymatic activity should be performed 2–3 months after a blood transfusion or hemolytic episode (9, 57). Diagnosis using qualitative test methods is less accurate for females with intermediate G6PD activity due to heterozygous *G6PD* alleles (88).

Glucose-6-phosphate dehydrogenase deficiency occurs in homozygous and compound heterozygous individuals (who have inherited 2 copies of *G6PD* deficient alleles) and in heterozygous individuals (one normal *G6PD* allele and one deficient *G6PD* allele) with skewed X chromosome inactivation of the functional allele or in individuals who are hemizygous for a single deficient allele (54). Genetic testing alone is insufficient for heterozygous individuals with one normal function *G6PD* allele, as the expression of the 2 alleles will vary between blood cells and over time (9). Genetic testing for *G6PD* variants may reveal an incidental finding of an unexpected number of *G6PD* alleles based on the apparent gender of the tested individual; these findings may warrant additional consultation or testing.

Glucose-6-phosphate dehydrogenase deficiency is inherited in an X-linked recessive pattern and most individuals are asymptomatic throughout life. A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons. X-linked disorders affect males at a much higher rate than females because males only have one copy of the X chromosome (hemizygous, XY). Since females have 2 copies of the X chromosome (XX) they tend to be less affected. However, female carriers can present with a range of phenotypes from no symptoms through a severe deficiency. Females randomly inactivate one X chromosome in somatic cells during development, resulting in a mixed population of somatic cells expressing one *G6PD* allele or the other.

Therapeutic Recommendations based on Genotype

This section contains excerpted [1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2022 Statement from the US Food and Drug Administration (FDA):

Chloroquine may cause hemolysis in glucose-6 phosphate dehydrogenase (G-6-PD) deficiency. Blood monitoring may be needed as hemolytic anemia may occur, in particular in association with other drugs that cause hemolysis.

Please review the complete therapeutic recommendations that are located here: (1)

Nomenclature for Selected *G6PD* Alleles

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Borja	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3:c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient (A)	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient (U)	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient (B)	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3:c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient (B)	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient (B)	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient (A)	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient (B)	rs137852327

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

Table continued from previous page.

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
* WHO classifications based on (89), classification of these alleles under the updated WHO categories are taken from work described in (90) and the data deposited at (91). Please note that not all alleles have an updated classification at the time of writing.
WHO - World Health Organization; PharmGKB - Pharmacogenomics Knowledgebase; CPIC - Clinical Pharmacogenetics Implementation Consortium; CNSHA - chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phosphate dehydrogenase

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Clobazam Therapy and *CYP2C19* Genotype

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Introduction

Clobazam (brand names Onfi, Sympazan) is approved by the FDA to treat seizures associated with Lennox-Gastaut syndrome (LGS) in patients aged 2 years and older (1). The drug is widely used in the chronic treatment of focal and generalized seizures, and has application in the treatment of diverse epilepsy syndromes, including epileptic encephalopathies other than LGS, such as Dravet syndrome (2-6).

Lennox-Gastaut syndrome is characterized by different types of seizures that typically begin in early childhood and may be associated with intellectual disability. Clobazam has been shown in controlled clinical trials to reduce drop (atonic) seizures in children with LGS, but there is evidence that it is effective for other seizure types as well.

Clobazam is a 1,5-benzodiazepine that acts as a positive allosteric modulator of $GABA_A$ receptors. It is often used in combination with other drugs, including stiripentol, cannabidiol, and many others.

Clobazam is extensively metabolized in the liver by cytochrome P450 (CYP) and non-CYP transformations. The major metabolite is N-desmethylclobazam (norclobazam), which has similar activity to clobazam on $GABA_A$ receptors and is an active antiseizure agent. During chronic treatment, levels of norclobazam are 8–20 times higher than those of the parent drug so that seizure protection during chronic therapy is mainly due to this metabolite.

Norclobazam is principally metabolized by CYP2C19. Individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have higher plasma levels of norclobazam and are at an increased risk of adverse effects.

The FDA-approved drug label states that for patients known to be CYP2C19 poor metabolizers, the starting dose of clobazam should be 5 mg/day. Dose titration should proceed slowly according to weight, but to half the standard recommended doses, as tolerated. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21 (Table 1) (1).

Table 1. The FDA (2019) Drug Label for Clobazam: Recommended Total Daily Dosing by Weight Group

	Less than or equal to 30 kg body weight	Greater than 30 kg body weight	CYP2C19 poor metabolizers
Starting dose	5 mg	10 mg	In patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day and dose titration should proceed slowly according to weight, but to half the recommended total daily doses presented in this table, as tolerated. If necessary, and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21.
Starting day 7	10 mg	20 mg	
Starting day 14	20 mg	40 mg	

This FDA table is adapted from (1).

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Drug: Clobazam

Clobazam is a 1,5-benzodiazepine that is an adjunct treatment of seizures associated with LGS. Clobazam is used in patients aged 2 years and above, and is dosed according to body weight (1). Clobazam was licensed for use in the United States in 2011.

Lennox-Gastaut syndrome is characterized by severe seizures in childhood. Typically, seizures begin between 3–5 years of age, and there may be different seizure types (e.g., absent, tonic, atonic, myoclonic). In addition, there may be signs of mental retardation.

Over half of all cases of LGS are associated with another condition, such as tuberous sclerosis, meningitis, or head injuries. For approximately 40% of cases, the cause is not known, but increasingly, genetic disorders are being identified, such as de novo mutations or chromosomal syndromes.

The seizures associated with LGS are often difficult to treat. Therapy is influenced by the underlying cause of the syndrome, and certain antiseizure drugs, such as clobazam, have been found to be helpful. Previously, 2 studies reported that clobazam was effective at reducing drop seizures in children with LGS (5, 7). Other treatment options include a ketogenic diet, as well as surgical options (8).

The FDA-approved drug label for clobazam contains a boxed warning regarding the risks of the concomitant use of benzodiazepines (such as clobazam) with opioids. The warning states that this may result in profound sedation, respiratory depression, coma, and death. Other adverse effects of clobazam therapy include sedation, lethargy, drooling, severe dermatological reactions, and dependence (1).

Clobazam may cause fetal harm. There are no adequate studies in pregnant women, but in animal studies, the administration of clobazam during pregnancy resulted in fetal toxicity, including an increased incidence of fetal malformations. Therefore, clobazam should only be used during pregnancy if the potential benefit to the mother justifies the potential risk to the fetus. Infants born to mothers who have taken benzodiazepines in later stages of pregnancy can develop dependence and subsequently undergo withdrawal in the postnatal period.

Clobazam is primarily metabolized by CYP3A4, and to a lesser extent, by CYP2C19 and CYP2B6. The active metabolite, N-desmethylclobazam (norclobazam) is an antiseizure agent that is less potent than clobazam, but during chronic clobazam therapy, the circulating levels of norclobazam are 8–20 times higher than clobazam levels. Therefore, seizure protection during chronic therapy is mainly due to norclobazam.

With long-term exposure, tolerance to clobazam does occur in some patients; however, many patients exhibit continued efficacy. Therefore, the propensity for tolerance maybe less than with some other benzodiazepines (1, 9–11).

In individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers"), standard doses of clobazam lead to higher levels of norclobazam. Compared with individuals with normal CYP2C19 activity, poor metabolizers have up to 5 fold higher plasma levels of norclobazam, increasing the risk of adverse effects.

The recommended total daily doses of clobazam, by weight, are provided in the drug label (Table 1). The label states that each dose should be individualized within each body weight group based on clinical efficacy and tolerability. Any dose greater than 5 mg should be divided into a twice daily dose, and increases in doses should not be increased more rapidly than weekly, because the serum concentrations of clobazam and its active metabolite requires 5 and 9 days, respectively, to reach steady-state (1). Steady-state levels of clobazam are typically reached in under 3 weeks in normal metabolizers, but may take several months in CYP2C19 poor metabolizers (12).

Determining a patient's CYP2C19 status may be helpful in preventing an overdose when starting clobazam therapy, because levels of norclobazam will be increased in CYP2C19 poor metabolizers (13–15). The drug label

states that in patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day (1). A 2015 study recommends a lower starting dose of 2.5 mg/day (16).

In CYP2C19 poor metabolizers, the drug label states that dose titration should proceed slowly, as tolerated, and according to weight, but to half the dose presented in Table 1. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21 (1).

Gene: CYP2C19

The CYP superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, antiplatelet agents, some proton pump inhibitors, and benzodiazepines such as clobazam.

The *CYP2C19* gene is highly polymorphic as there currently are 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with "rapid" (**1/*17*) and "ultrarapid" (**17/*17*) metabolizer phenotypes. Heterozygous carriers of non-functional alleles (e.g., **2* and **3*) are classified as "intermediate metabolizers" (e.g., **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016). Assignment of *CYP2C19* Phenotypes.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual who has 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual who has one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual who has 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual who has one normal function allele and one no function allele, or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual who has 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (17).
[b] The predicted metabolizer phenotype for the **2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (17).

Approximately 2% of Caucasians, 4% of African Americans, and 15–25% in East Asians are *CYP2C19* poor metabolizers; and up to 45% of patients are CYP2C19 intermediate metabolizers (17–19).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (20).

For CYP2C19, another commonly tested no function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4–*8* (20).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the clobazam response and the *CYP2C19* gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (21).

Usually an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the individual's predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 2 summarizes common *CYP2C19* phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

2.5 Dosage Adjustments in CYP2C19 Poor Metabolizers

In CYP2C19 poor metabolizers, levels of N-desmethylclobazam, clobazam's active metabolite, will be increased. Therefore, in patients known to be CYP2C19 poor metabolizers, the starting dose should be 5 mg/day and dose titration should proceed slowly according to weight, but to half the dose presented in Table 1, as tolerated. If necessary and based upon clinical response, an additional titration to the maximum dose (20 mg/day or 40 mg/day, depending on the weight group) may be started on day 21.

[…]

8.6 CYP2C19 Poor Metabolizers

Concentrations of clobazam's active metabolite, N-desmethylclobazam, are higher in CYP2C19 poor metabolizers than in normal metabolizers. For this reason, dosage modification is recommended

[…]

12.5 Pharmacogenomics

The polymorphic CYP2C19 is the main enzyme that metabolizes the pharmacologically active N-desmethylclobazam. Compared with CYP2C19 normal metabolizers, N-desmethylclobazam AUC and Cmax are approximately 3–5 times higher in poor metabolizers (e.g., subjects with *2/*2 genotype) and 2 times higher in

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

intermediate metabolizers (e.g., subjects with *1/*2 genotype). The prevalence of CYP2C19 poor metabolism differs depending on racial/ethnic background. Dosage in patients who are known CYP2C19 poor metabolizers may need to be adjusted.

The systemic exposure of clobazam is similar for both CYP2C19 poor and normal metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable –– variant occurs in a non-coding region	rs12248560

dbSNP: The Single Nucleotide Polymorphism Database
Note: the normal "wild-type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (22).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Clopidogrel Therapy and *CYP2C19* Genotype

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Created: March 8, 2012; Updated: December 1, 2022.

Introduction

Clopidogrel (brand name Plavix) is an antiplatelet medicine that reduces the risk of myocardial infarction (MI) and stroke in individuals with acute coronary syndrome (ACS), and in individuals with atherosclerotic vascular disease (indicated by a recent MI or stroke, or established peripheral arterial disease) (1). Clopidogrel is also indicated in combination with aspirin for individuals undergoing percutaneous coronary interventions (PCI), including stent placement.

The effectiveness of clopidogrel depends on its conversion to an active metabolite, which is accomplished by the cytochrome P450 2C19 (CYP2C19) enzyme. Individuals who have 2 loss-of-function copies of the *CYP2C19* gene are classified as CYP2C19 poor metabolizers (PM). Individuals with a CYP2C19 PM phenotype have significantly reduced enzyme activity and cannot activate clopidogrel via CYP2C19, which means the drug will have a reduced antiplatelet effect. Approximately 2% of Caucasians, 4% of African Americans, 14% of Chinese, and 57% of Oceanians are CYP2C19 PMs (2). The effectiveness of clopidogrel is also reduced in individuals who are CYP2C19 intermediate metabolizers (IM). These individuals have one loss-of-function copy of *CYP2C19*, with either one normal function copy or one increased function copy. The frequency of the IM phenotype is more than 45% in individuals of East Asian descent, more than 40% in individuals of Central or South Asian descent, 36% in the Oceanian population, approximately 30% in individuals of African descent, 20–26% in individuals of American, European, or Near Eastern descent, and just under 20% in individuals of Latino descent (2).

The 2022 FDA-approved drug label for clopidogrel includes a boxed warning on the diminished antiplatelet effect of clopidogrel in CYP2C19 PMs (Table 1). The warning states that tests are available to identify individuals who are CYP2C19 PMs, and to consider the use of another platelet $P2Y_{12}$ (purinergic receptor P2Y, G-protein coupled 12) inhibitor in individuals identified as CYP2C19 PMs.

The 2022 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for clopidogrel recommends that for individuals with ACS or non-ACS indications who are undergoing PCI, being treated for peripheral arterial disease (PAD), or stable coronary artery disease following MI, an alternative antiplatelet therapy (for example, prasugrel or ticagrelor) should be considered for CYP2C19 PMs if there is no contraindication (Table 2) (3). Similarly, CPIC strongly recommends that CYP2C19 IMs should avoid clopidogrel for ACS or PCI but makes no recommendations for other cardiovascular indications (Table 2). For neurovascular indications, CPIC recommends avoidance of clopidogrel for CYP2C19 PMs and consideration of alternative medications for both IMs and PMs if not contraindicated (Table 3) (3).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) have also made antiplatelet therapy recommendations based on *CYP2C19* genotype. For individuals with ACS who undergo PCI, they recommend an alternative antiplatelet agent in PMs, and for IMs they recommend choosing an alternative antiplatelet agent or doubling the dose of clopidogrel to 150 mg daily dose, 600 mg loading dose (Table 4) (4).

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Table 1. The FDA (2022) Drug Label for Clopidogrel. Warning: Diminished Antiplatelet Effect in Individuals with 2 Loss-of-Function Alleles of the *CYP2C19* Gene.

Phenotype	Recommendations
CYP2C19 poor metabolizer	Clopidogrel bisulfate at recommended doses forms less of the active metabolite and so has a reduced effect on platelet activity in individuals who are homozygous for no-function alleles of the *CYP2C19* gene, (termed "CYP2C19 poor metabolizers"). Tests are available to identify individuals who are CYP2C19 poor metabolizers. Consider use of another platelet P2Y12 inhibitor in individuals identified as CYP2C19 poor metabolizers

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

Table 2. The CPIC (2022) Antiplatelet Therapy Recommendations based on CYP2C19 Phenotype when considering Clopidogrel for Cardiovascular Indications.

Phenotype	Examples of diplotypes	Implications for clopidogrel	Therapeutic recommendations	Classification of recommendation-ACS, or PCI[a], or both	Classification of recommendation, non-ACS, non-PCI cardiovascular indications[b]
Ultrarapid metabolizer (UM)	**17/*17*	Increased clopidogrel active metabolite formation; lower on-treatment platelet reactivity; no association with higher bleeding risk	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	No recommendation
Rapid metabolizer (RM)	**1/*17*	Normal or increased clopidogrel active metabolite formation; normal or lower on-treatment platelet reactivity; no association with higher bleeding risk			
Normal metabolizer (NM)	**1/*1* **1/*13*	Normal clopidogrel active metabolite formation; normal on-treatment platelet reactivity	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	Strong
Likely intermediate metabolizer	**1/*9* **1/*16* **1/*10*	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid standard-dose clopidogrel (75 mg/day) if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[c]	No recommendation[c]
Intermediate metabolizer (IM)	**1/*2* **1/*3* **2/*17*	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid standard-dose (75 mg/day) clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	No recommendation

Table 2. continued from previous page.

Phenotype	Examples of diplotypes	Implications for clopidogrel	Therapeutic recommendations	Classification of recommendation-ACS, or PCI[a], or both	Classification of recommendation, non-ACS, non-PCI cardiovascular indications[b]
Likely poor metabolizer	**2/*9* **3/*19* **4/*10*	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong[c]	Moderate[c]
Poor metabolizer (PM)	**2/*2* **2/*3* **3/*3*	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication	Strong	Moderate

[a] ACS or PCI, or both includes individuals undergoing PCI for an ACS or non-ACS (elective) indication.
[b] Non-ACS, non-PCI cardiovascular indications include peripheral arterial disease and stable coronary artery disease following a recent myocardial infarction outside the setting of PCI.
[c] The strength of the recommendation for "likely" phenotypes are the same as their respective confirmed phenotypes; "likely" indicates the uncertainty in phenotype assignment due to limited data for reduced-function alleles.
ACS - acute coronary syndrome; PCI - percutaneous coronary intervention; CPIC - Clinical Pharmacogenetics Implementation Consortium
Please see Therapeutic Recommendations based on Genotype for more information from CPIC. This table is adapted from (3).

Table 3. The CPIC (2022) Antiplatelet Therapy Recommendations Based on CYP2C19 Phenotype when Considering Clopidogrel for Neurovascular[a] Indications

CYP2C19 phenotype	Implications for clopidogrel	Therapeutic recommendation	Classification of recommendation[b]
Ultrarapid metabolizer (UM)	Increased clopidogrel active metabolite formation; lower on-treatment platelet reactivity; no association with higher bleeding risk	No recommendation	No recommendation
Rapid metabolizer (RM)	Normal or increased clopidogrel active metabolite formation; normal or lower on-treatment platelet reactivity; no association with higher bleeding risk		
Normal metabolizer (NM)	Normal clopidogrel active metabolite formation; normal on-treatment platelet reactivity	If considering clopidogrel, use at standard dose (75 mg/day)	Strong

Table 3. continued from previous page.

CYP2C19 phenotype	Implications for clopidogrel	Therapeutic recommendation	Classification of recommendation[b]
Likely and confirmed intermediate metabolizer (IM)	Reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Consider an alternative[c] P2Y12 inhibitor at standard dose if clinically indicated and no contraindication	Moderate
Likely and confirmed poor metabolizer (PM)	Significantly reduced clopidogrel active metabolite formation; increased on-treatment platelet reactivity; increased risk for adverse cardiac and cerebrovascular events	Avoid clopidogrel if possible. Consider an alternative[c] P2Y12 inhibitor at standard dose if clinically indicated and no contraindication	Moderate

[a] Neurovascular disease includes acute ischemic stroke or transient ischemic attack (TIA), secondary prevention of stroke, or prevention of thromboembolic events following neurointerventional procedures, such as carotid artery stenting and stent-assisted coiling of intracranial aneurysms.
[b] The strength of the recommendation for "likely" phenotypes are the same as their respective confirmed phenotypes; "likely" indicates the uncertainty in phenotype assignment due to limited data for reduced function alleles.
[c] Alternative P2Y12 inhibitors not impacted by CYP2C19 genetic variants include ticagrelor and ticlopidine. Prasugrel is contraindicated in individuals with a history of stroke or TIA. Given limited outcomes data for genotype-guided antiplatelet therapy for neurovascular indications, selection of therapy should depend on the individual's treatment goals and risks for adverse events.
Please see Therapeutic Recommendations based on Genotype for more information from CPIC. This table is adapted from (3). CPIC - Clinical Pharmacogenetics Implementation Consortium

Table 4. The DPWG (2019) Recommendations for Clopidogrel and *CYP2C19* Phenotype.

Phenotype	Recommendation
Ultrarapid metabolizer	NO action is required for this gene-drug interaction
Intermediate metabolizer	Percutaneous coronary intervention, stroke, or TIA: 1 choose an alternative or double the dose to 150 mg/day (600 mg loading dose) Prasugrel, ticagrelor, and acetylsalicylic acid/dipyridamole are not metabolized by CYP2C19 (or to a lesser extent) Other indications: 1 no action required
Poor metabolizer	Percutaneous coronary intervention, stroke, or TIA: 1 Avoid clopidogrel Prasugrel, ticagrelor, and acetylsalicylic acid/dipyridamole are not metabolized by CYP2C19 (or to a lesser extent) Other indications: 1. determine the level of inhibition of platelet aggregation by clopidogrel 2. consider an alternative in poor responders Prasugrel and ticagrelor are not metabolized by CYP2C19 (or to a lesser extent)

TIA - Transient ischemic attack; DPWG – Dutch Pharmacogenetics Working Group
Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (4).

Drug: Clopidogrel

Clopidogrel is an antiplatelet medicine used in the treatment of individuals with ACS, managed medically or with PCI. Clopidogrel is also used in the treatment of individuals with atherosclerotic vascular disease, as indicated by a recent MI, a recent ischemic stroke, or symptomatic peripheral arterial disease. Clopidogrel has been shown to reduce the rate of subsequent MI and stroke in these individuals (1, 5, 6).

Clopidogrel is a $P2Y_{12}$ inhibitor, and acts by irreversibly binding to the platelet $P2Y_{12}$ receptor and blocking adenosine diphosphate (ADP)-mediated platelet activation and aggregation. Clopidogrel belongs to the second generation of thienopyridine antiplatelet agents.

Clopidogrel is given to treat or to prevent further occurrences of arterial thrombosis, which occurs when a blood clot (thrombus) forms inside an artery. Arterial thrombosis is often triggered in response to the rupturing of the atherosclerotic plaque lining the arterial wall. If the thrombus occludes the arterial lumen, the blood flow is reduced or stopped, resulting in ischemia. In the brain, thrombosis in the cerebral arteries can cause a transient ischemic attack (TIA) or ischemic stroke. In the peripheral vessels, thrombosis can cause peripheral artery disease, and in the heart, a thrombosis in the coronary arteries is a common cause of ACS. Platelet inhibitors such as clopidogrel interrupt the formation of the thrombus, which involves the rapid recruitment and activation of platelets.

Acute coronary syndrome reflects a decreased blood flow in the coronary arteries and comprises unstable angina and MI. Unstable angina occurs suddenly, often at rest or with minimal exertion, and may be new in onset or may occur with less exertion than previously.

Among individuals with ACS, the addition of 75 mg daily clopidogrel to aspirin and other standard treatments reduces the risk of MI, stroke, and death, compared with the addition of placebo (7, 8, 9). However, despite the general efficacy of clopidogrel, resistance is common. Resistance to an antiplatelet drug occurs when there is no significant reduction in platelet function after therapy, compared with baseline platelet function. Clopidogrel treatment failure occurs when there is a thrombotic or ischemic event (for example, stent thrombosis or recurrent ACS) during clopidogrel therapy in individuals with "High on-Treatment Platelet Reactivity" (HTPR). High on-Treatment Platelet Reactivity occurs when the platelet $P2Y_{12}$ receptors are still responsive despite clopidogrel therapy. It is tested for by adding an ADP agonist to a plasma sample and measuring aggregation or intracellular markers of platelet activation. It has been estimated that between 16–50% of individuals treated with clopidogrel have HTPR (10).

Platelet function assays are used to assess platelet response by measuring 'Platelet Reactivity Units' (PRU). The PRU cut-off values vary, but the therapeutic window for clopidogrel is approximately 95–208 PRU. A PRU value higher than 208 indicates clopidogrel resistance, and a value below 95 is associated with a higher risk for major bleeding (11, 12). Many studies have reported an association between clopidogrel resistance (HTPR or high PRU) and an increased risk of thrombotic/ischemic event following PCI, such as stent thrombosis (13). Similarly, HTPR is associated with poor outcomes in stroke or TIA in the context of standard antiplatelet therapies, including clopidogrel (14).

A poor response to clopidogrel is due, in part, to genetic variants in the *CYP2C19* gene. Other genes that may influence clopidogrel response include *ABCB1*, *P2Y12*, *CES1*, *GPIIIA*, *B4GALT2,* and *PON1* (15, 16, 17, 18, 19, 20, 21, 22, 23). Clopidogrel is a prodrug, and CYP2C19 is the major enzyme involved in the conversion of clopidogrel into an active metabolite. Alternative antiplatelet drugs to clopidogrel, such as prasugrel (a third generation thienopyridine) and ticagrelor (a cyclopentyltriazolopyrimidine), are not dependent upon CYP2C19 for activation. Although both clopidogrel and prasugrel form active metabolites with similar potency, prasugrel is a more potent antiplatelet agent than clopidogrel due to the more efficient formation of the active metabolite from the prodrug (24).

The TRITON-TIMI 38 trial compared prasugrel with clopidogrel in 13,608 individuals with ACS who were undergoing PCI. Prasugrel was found to provide more potent platelet inhibition than clopidogrel; and after 15 months, individuals treated with prasugrel had a lower incidence of the combined endpoint of cardiovascular death, nonfatal MI, or nonfatal stroke as compared with individuals treated with clopidogrel (9.9% versus 12.1%) (25, 26). However, prasugrel was associated with a higher risk of bleeding, leading to the FDA warning that the use of prasugrel is contraindicated in individuals with active pathological bleeding, or a history of stroke or TIA (27, 28). In addition, prasugrel has an FDA box warning for individuals with a high probability of undergoing coronary artery bypass grafting (prasugrel should not be started, or when possible, discontinue prasugrel at least 7 days before any surgery) (29).

In an analysis from the PLATelet inhibition and patient Outcomes (PLATO) trial, ticagrelor was found to be superior to clopidogrel in a subgroup of individuals with ACS who were treated with PCI. Consistent with the overall results of the trial, ticagrelor was found to have superior efficacy and similar safety compared with clopidogrel (30, 31, 32). Other studies have similarly observed a better response to ticagrelor for antiplatelet activities but noted an increased risk of bleeding when compared with clopidogrel for various indications (stroke, unstable angina, chronic coronary syndromes after PCI and other coronary artery diseases), which may be of particular concern in elderly individuals (33, 34, 35, 36).

In addition, the latest guideline from the American College of Cardiology/American Heart Association includes a preference for alternative therapy (prasugrel or ticagrelor) over clopidogrel in individuals with ACS/PCI. This is a class IIa recommendation based on moderate quality, from the 2016 focused update on dual antiplatelet therapy (37). In full, this recommendation states in individuals with ACS who are "treated with dual antiplatelet therapy after coronary stent implantation who are not at high risk for bleeding complications and who do not have a history of stroke or TIA, it is reasonable to choose prasugrel over clopidogrel for maintenance P2Y12 inhibitor therapy." These recommendations also state that "it is reasonable to use ticagrelor in preference to clopidogrel for maintenance of P2Y12 inhibitor therapy." (37)

Although prasugrel and ticagrelor are often reported to be more effective than standard-dose clopidogrel, dual antiplatelet therapy with clopidogrel and aspirin remains the standard of care at many institutions for individuals with ACS undergoing PCI (31, 38, 39, 40, 41). This may be because clopidogrel has a lower bleeding risk and is less expensive (42). However, the availability of *CYP2C19* genetic testing can facilitate personalized antiplatelet therapy by pursuing alternative antiplatelet agents specifically for individuals with impaired CYP2C19 activity (39, 43, 44, 45, 46).

Gene: CYP2C19

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, voriconazole (47), some proton pump inhibitors, and the antiplatelet agent, clopidogrel. The variability of clopidogrel metabolism and treatment outcomes between individuals is partly determined by variant alleles of the *CYP2C19* gene. The *CYP2C19* gene is highly polymorphic with over 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild type allele when no variants are detected and is categorized as normal enzyme activity and the "normal metabolizer" phenotype. It should be noted that the *CYP2C19*1* haplotype has been determined to have a single nucleotide polymorphism in the coding region; however, the frequency of the variant nucleotide (G) is nearly 94% globally and this missense variant does not alter protein function (48, 49, 50).

The *CYP2C19*17* allele is associated with increased enzyme activity and, depending on the number of alleles present, is associated with the "rapid" (one **17* allele) and "ultrarapid" (2 **17* alleles) metabolizer phenotypes. Non-functional alleles include *CYP2C19*2* and **3*. The *CYP2C19* IMs have one copy of an allele that encodes a non-functional enzyme (for example, **1/*2*), whereas "PMs" have 2 non-functional alleles (for example, **2/*2*, **2/*3*) (Table 5).

Table 5. Activity Status of Selected *CYP2C19* Alleles

Allele type	Alleles
Increased function	*CYP2C19*17*
Normal function	*CYP2C19*1* *CYP2C19*13*
Decreased function^	*CYP2C19*9* *CYP2C19*10* *CYP2C19*16* *CYP2C19*19*
No function	*CYP2C19*2* *CYP2C19*3* *CY P2C19*4*
Uncertain function	*CYP2C19*12* *CYP2C19*23*

This table is adapted from (51).
^ Note: the evidence supporting the activity status of decreased function alleles is limited.

Approximately 2% of Caucasians, 4% of African Americans, 14% of Chinese, and 57% of Oceanians are CYP2C19 PM; and up to 45% of individuals are CYP2C19 IM (1, 2).

The most common no function variant is *CYP2C19*2*, which contains the NM_000769.1:c.681G>A variant in exon 5 that results in an aberrant splice site and produces a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are between 12–18% in individuals of European, American, or African ancestry, between 25–35% in Asians, Native Hawaiians, and Pacific Islanders, and up to 60% in Oceanian populations (2, 52). Approximately 6–12% of the observed variability in antiplatelet effect of clopidogrel is thought to be attributed to *CYP2C19* variants (53).

For *CYP2C19*, another commonly tested no functional variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other ancestral populations (52). Other non-functional variants occur in less than 1% of the general population and include *CYP2C19*4–*8* (54).

The frequency of the *CYP2C19*17* allele is approximately 22% in individuals of European ancestry, 8% for individuals from the Americas, 0.5–5.7% in Asian, Native Hawaiian, and Pacific Islander populations, 17% in African populations, and 20% in African American and Afro-Caribbean populations (2, 52).

The *CYP2C19*2, *3,* and **17* alleles are the 'Tier 1' alleles recommended by the Association for Molecular Pathology (AMP) to be included in *CYP2C19* clinical genotyping assays (55). The AMP further recommends testing laboratories consider **4A, *4B, *5, *6, *7, *8, *9, *10,* and **35* alleles as optional 'Tier 2' alleles that have all been shown to have decreased or no function but have either a low minor allele frequency, limited data characterizing the impact on enzyme function, or lack reference materials. Among the 'Tier 2' alleles, the *CYP2C19*35* allele is most common, and has a frequency of 9% in African populations (55).

Phenoconversion due to CYP2C19 Inhibitors and Inducers

Many medicines are metabolized by the CYP2C19 enzyme, and the activity level of the enzyme can be altered by administration of medications or supplements. Significant alterations in the effective enzyme activity level due to co-medication or other non-genetic factors is called phenoconversion. Increased enzymatic activity can be caused by induction of CYP2C19, such an effect can occur with medications like rifampin; this can lead to an increased bleeding risk due to increased activation of clopidogrel (1). St. John's wort and smoking may also increase CYP enzyme activity and increase the platelet inhibitory effect of clopidogrel (56). Inhibitors of CYP2C19 activity can cause reduced clopidogrel metabolism and thus trigger a blunted response to the medication. The FDA-approved drug label cautions that co-medication with proton pump inhibitors (PPIs) omeprazole or esomeprazole can decrease the antiplatelet effects of clopidogrel (1). One study found that co-medication with other CYP2C19 substrate medications was a significant risk factor for adverse drug reactions during clopidogrel treatment (57).

Linking *CYP2C19* Genetic Variation with Treatment Response

Several studies have reported an increase in adverse cardiovascular events in individuals who have one or 2 no function *CYP2C19* alleles (namely, IM or PM), compared with individuals with 2 normal copies of the *CYP2C19* gene (normal metabolizer). These studies focused on individuals with ACS undergoing PCI, with individuals who had no function alleles also being at a higher risk of stent thrombosis or major adverse cardiovascular and cerebrovascular events (58, 59, 60, 61, 62). These individuals may require much higher doses of clopidogrel (2- to 4-fold higher) or an alternative drug (63, 64, 65). A meta-analysis of 7 randomized control trials and 4 non-randomized control trials found a significant association between *CYP2C19* loss-of-function allele carriers and poorer outcomes when treated with clopidogrel as compared with an alternative $P2Y_{12}$ inhibitor (9). This analysis was limited to studies of individuals with ACS with at least 50% of participants undergoing PCI, where *CYP2C19* genotype was assessed and included in the outcomes, and clopidogrel was compared with an alternative medication. Some clinical studies did not find a significant association between *CYP2C19* and clinical outcome in individuals with ACS; however, these often included data from lower risk non-PCI individuals (66, 67, 68).

Several studies of individuals with TIA have reported that CYP2C19 status influences the risk of having an ischemic stroke or adverse clinical outcomes following a stroke when treated with clopidogrel (69, 70, 71, 72). One trial (CHANCE – Clopidogrel in High-risk Individuals with Acute Nondisabling Cerebrovascular Events; study size of 5,170 individuals) found that the use of clopidogrel plus aspirin compared with aspirin alone reduced the risk of a new stroke only in the subgroup of individuals who did not have the *CYP2C19* no function alleles (73). The CHANCE-2 trial (study size 6,412 individuals) examined the superiority of ticagrelor to clopidogrel for *CYP2C19* loss-of-function allele carriers following TIA or minor ischemic stroke and found a modest but significant improvement in the rate of strokes in the first 90 days with no significant differences in risk of severe or moderate bleeding with either $P2Y_{12}$ inhibitor (74). Additional trials examining various antiplatelet regimens following stroke or TIA have been recently reviewed, which supports the role of *CYP2C19* genotype in contributing to potential risks with clopidogrel therapy for stroke or TIA (72). At least one study of *CYP2C19* variation and clopidogrel effectiveness in neurovascular conditions found an opposite effect of *CYP2C19* loss-of-function variants on poor treatment response. The authors of this study concluded more research was needed to fully understand the impact of these variants in the context of intracranial atherosclerotic disease; however, caution should be taken when considering these findings as the study only included 188 individuals (75).

Recent studies have found that *CYP2C19*-genotype-guided antiplatelet therapy results in a higher likelihood of achieving a therapeutic level of on-treatment platelet reactivity (11, 76, 77, 78). Genotype-guided therapy may also be cost effective among ACS individuals undergoing PCI (39, 79, 80, 81). However, many authors, including the American Heart Association, find more data are needed to determine whether routine genotyping and

platelet function tests could help reduce future cardiovascular events in ACS individuals or for secondary stroke prevention (37, 82, 83, 84, 85).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of genetic tests that are available for clopidogrel response, CYP2C19-related poor drug metabolism, and the *CYP2C19* gene.

Usually, an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the individual's predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor). When a test report does not provide a predicted metabolizer phenotype, resources such as PharmVar and PharmCAT are available to assist with predicting the functional impact of identified variants.

The association between *CYP2C19*2* and **3* and clopidogrel response has been extensively studied; however, the less common no function alleles (for example, *CYP2C19*4–*8*) also likely influence clopidogrel response similar to **2* and **3,* but the body of evidence is not as extensive. Therefore, these alleles should be considered to reduce the effectiveness of clopidogrel therapy in a similar manner to the more common *CYP2C19*2* allele (54, 86). Guidance regarding inclusion of specific alleles for clinical testing is available from AMP (55). The current recommendations from CPIC advise management of likely PMs and likely IMs as if these individuals were definitively in the predicted phenotype group (3).

The *CYP2C19* Gene Interactions with Medications Used for Additional Indications

The CYP2C19 enzyme metabolizes many medications and may have impacts on other conditions. Other medications affected by *CYP2C19* genetic variation may be used to treat:

- Gastrointestinal ulcers, gastroesophageal reflux, erosive esophagitis, and *Helicobacter pylori* infection—PPIs like omeprazole, esomeprazole, and others may have reduced metabolism in CYP2C19 IMs and PMs and thus these individuals have higher exposure to these medications, which imparts risk of adverse events. Conversely, *CYP2C19*17* confers increased activity and thus a more rapid clearance of PPIs and potential for treatment failure in ultrarapid metabolizers (UMs).
- Antifungal treatment—voriconazole is metabolized by CYP2C19 and UMs may have delayed target blood concentrations due to rapid clearance; PMs may experience high exposure and have a risk of adverse events.
- Depression, anxiety disorders, obsessive-compulsive disorder, or migraine prophylaxis—both selective serotonin reuptake inhibitors (SSRIs, such as citalopram, escitalopram, and sertraline) and tricyclic antidepressants (TCAs, such as the tertiary amines amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) can be metabolized by CYP2C19. When administering SSRIs, UMs may experience treatment failure due to high clearance while PMs may require dose reduction. For the tertiary amine TCAs, UMs may experience altered responses or side effects due to rapid conversion to secondary amines while PMs are at risk of suboptimal responses and may require a lower dose. Diazepam is also partially metabolized by CYP2C19 and altered enzyme function may contribute to altered clearance of this medication.
- Epilepsy or seizures—brivaracetam, used for partial-onset (focal) epilepsy, is partially metabolized by CYP2C19 and PMs may require lower doses to avoid adverse effects due to reduced clearance of this medication. Clobazam is used to manage seizures in a variety of conditions and CYP2C19 PMs may experience higher exposure to norclobazam, putting them at higher risk for adverse effects. Lacosamide is

also metabolized by CYP2C19, though there is no indication that PMs require altered dosing or management.
- Musculoskeletal pain—carisoprodol, a centrally acting muscle relaxant, is metabolized by CYP2C19 and PMs may be at a higher risk of carisoprodol toxicity.
- Hypoactive sexual desire disorder—flibanserin is metabolized, in part, by CYP2C19 and PMs have a higher exposure to this medication, resulting in an elevated risk of hypotension, syncope, and CNS depression.

Additional information on gene-drug interactions for *CYP2C19* are available from PharmGKB, CPIC and the FDA (search for "CYP2C19").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2022 Statement from the US Food and Drug Administration (FDA)

WARNING: DIMINISHED ANTIPLATELET EFFECT IN PATIENTS WITH TWO LOSS-OF-FUNCTION ALLELES OF THE CYP2C19 GENE

The effectiveness of clopidogrel bisulfate results from its antiplatelet activity, which is dependent on its conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19 … Clopidogrel bisulfate at recommended doses forms less of the active metabolite and so has a reduced effect on platelet activity in patients who are homozygous for nonfunctional alleles of the CYP2C19 gene, (termed "CYP2C19 poor metabolizers"). Tests are available to identify patients who are CYP2C19 poor metabolizers … Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers.

[…]

Clopidogrel is a prodrug. Inhibition of platelet aggregation by clopidogrel is achieved through an active metabolite. The metabolism of clopidogrel to its active metabolite can be impaired by genetic variations in CYP2C19 … The metabolism of clopidogrel can also be impaired by drugs that inhibit CYP2C19, such as omeprazole or esomeprazole. Avoid concomitant use of clopidogrel bisulfate with omeprazole or esomeprazole because both significantly reduce the antiplatelet activity of clopidogrel bisulfate.

[…]

Rifampin strongly induces CYP2C19 resulting to both an increase level of clopidogrel active metabolite and platelet inhibition, which in particular might potentiate the risk of bleeding. As a precaution, avoid concomitant use of strong CYP2C19 inducers. […]

Clopidogrel is metabolized to its active metabolite in part by CYP2C19. Concomitant use of drugs that inhibit the activity of this enzyme results in reduced plasma concentrations of the active metabolite of clopidogrel and a reduction in platelet inhibition… Avoid concomitant use of clopidogrel bisulfate with omeprazole or esomeprazole.

[…]

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

CYP2C19 is involved in the formation of both the active metabolite and the 2-oxoclopidogrel intermediate metabolite. Clopidogrel active metabolite pharmacokinetics and antiplatelet effects, as measured by ex vivo platelet aggregation assays, differ according to CYP2C19 genotype. Patients who are homozygous for nonfunctional alleles of the CYP2C19 gene are termed "CYP2C19 poor metabolizers." Approximately 2% of White and 4% of Black patients are poor metabolizers; the prevalence of poor metabolism is higher in Asian patients (e.g., 14% of Chinese). Tests are available to identify patients who are CYP2C19 poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

2022 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

In patients with ACS and/or undergoing PCI…avoid clopidogrel in CYP2C19 Ims and PMs and use an alternative antiplatelet agent, such as prasugrel or ticagrelor, if no contraindications.

[…]

If considering clopidogrel and the patient is a CYP2C19 NM, the standard dose (75 mg/day) is recommended. Although clopidogrel-treated CYP2C19 RMs and Ums may experience lower on-treatment platelet reactivity compared with NMs, clinical data also support the use of clopidogrel at standard doses in CYP2C19 RMs and Ums due to the lack of evidence demonstrating significant differences in risk of bleeding or ischemic events compared with NMs in patients undergoing PCI.

[…]

There remain limited data regarding the potential benefit of CYP2C19-guided antiplatelet therapy on outcomes exclusively in patients undergoing PCI for a non-ACS indication. Patients undergoing elective PCI have a lower risk of cardiovascular events compared with patients with ACS, but were included in multiple studies evaluating outcomes of genotype-guided antiplatelet therapy, including the IGNITE and TAILOR-PCI studies (Table S2). Therefore, the therapeutic recommendations for patients with ACS and/or undergoing PCI may also be considered for patients undergoing elective PCI.

[…]

In patients with a cardiovascular indication for clopidogrel outside the setting of an ACS or PCI, including the treatment of patients with peripheral arterial disease or stable coronary artery disease following a recent MI, the standard dose (75 mg/day) is recommended if the patient is a CYP2C19 NM. However, there are insufficient data to make a clinical recommendation for CYP2C19 Ums, RMs, and Ims. If the patient is a CYP2C19 PM, it is recommended to avoid clopidogrel and use prasugrel or ticagrelor at standard doses if no contraindication.

[…]

If considering clopidogrel for patients with neurovascular disease, including the treatment of acute ischemic stroke or TIA, the secondary prevention of stroke, or the prevention of thromboembolic events following neurointerventional procedures, such as carotid artery stenting and endarterectomy and stent-assisted coiling of intracranial aneurysms, the standard dose (75 mg/day) is recommended in CYP2C19 NMs (Table 3). In CYP2C19 Ims and PMs, there is a “moderate” recommendation to avoid clopidogrel if possible and consider an alternative P2Y12 inhibitor at standard doses if clinically indicated and no contraindication. Alternative P2Y12 inhibitors not impacted by *CYP2C19* genetic variants with indications for patients with stroke include ticagrelor and ticlopidine. However, ticlopidine has serious hematological adverse effects that also need to be considered. Prasugrel is contraindicated in patients with a history of stroke or TIA.

Please review the complete therapeutic recommendations that are located here: (3)

2019 Summary of Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 PM: CLOPIDOGREL

The risk of serious cardiovascular and cerebrovascular events is increased in patients undergoing balloon angioplasty or stent placement (percutaneous coronary intervention) and in patients with a stroke or TIA, because the genetic variation reduces the activation of clopidogrel. No negative clinical consequences have been proved in other patients.

- PERCUTANEOUS CORONARY INTERVENTION, STROKE or TIA:
  - avoid clopidogrel
  - Prasugrel, ticagrelor and acetylsalicylic acid/dipyridamole are not metabolised by CYP2C19 (or to a lesser extent).
- OTHER INDICATIONS:
  - determine the level of inhibition of platelet aggregation by clopidogrel
  - consider an alternative in poor responders
  - Prasugrel and ticagrelor are not metabolised by CYP2C19 (or to a lesser extent).

CYP2C19 IM: clopidogrel

The risk of serious cardiovascular and cerebrovascular events is increased in patients undergoing balloon angioplasty or stent placement (percutaneous coronary intervention) and in patients with a stroke or TIA, as the genetic variation reduces the activation of clopidogrel. No negative clinical consequences have been observed in other patients.

- PERCUTANEOUS CORONARY INTERVENTION, STROKE or TIA:
  - choose an alternative or double the dose to 150 mg/day (600 mg loading dose); Prasugrel, ticagrelor and acetylsalicylic acid/dipyridamole are not metabolised by CYP2C19 (or to a lesser extent).
- OTHER INDICATIONS:
  - no action required

CYP2C19 UM: clopidogrel

NO action is required for this gene-drug interaction.

The genetic variation results in increased conversion of clopidogrel to the active metabolite. However, this can result in both positive effects (reduction in the risk of serious cardiovascular and cerebrovascular events) and negative effects (increase in the risk of bleeding).

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature of Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	12662A>G	NM_000769.4:c.332-23A>G	(Splicing defect)	rs12769205
	19154G>A	NM_000769.4:c.681G>A	NP_000760.1:p. Pro227 =	rs4244285
*CYP2C19*3*	17948G>A	NM_000769.4:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*4*	1A>G	NM_000769.4:c.1A>G	NP_000760.1:p.Met1Val	rs28399504

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*9*	12784G>A	NM_000769.4:c.431G>A	NP_000760.1:p.Arg144His	rs17884712
*CYP2C19*10*	19153C>T	NM_000769.4:c.680C>T	NP_000760.1:p.Pro227Leu	rs6413438
*CYP2C19*12*	90209A>C	NM_000769.4:c.1473A>C	NP_000760.1:p.Ter491Cys	rs55640102
*CYP2C19*13*	87290C>T	NM_000769.4:c.1228C>T	NP_000760.1:p.Arg410Cys	rs17879685
*CYP2C19*16* [a]	90060C>T	NM_000769.4:c.1324C>T	NP_000760.1:p.Arg442Cys	rs192154563
*CYP2C19*17*	-806C>T	NM_000769.4:c.-806C>T	(Variant alters mRNA expression)	rs12248560
*CYP2C19*19*	151A>G	NM_000769.4:c.151A>G	NP_000760.1:p.Ser51Gly	rs1564657013
*CYP2C19*23*	12455G>C	NM_000769.4:c.271G>C	NP_000760.1:p.Gly91Arg	rs118203756

Note: the normal "wild type" allele is *CYP2C19*1* and is reported when no variant is detected. The wild type allele is characterized by the common A>G variant at rs3758581 (NM_000769.4:c.991A>G; *CYP2C19* p.Ile331Val), which is benign and is found in many *CYP2C19* haplotypes.

[a] This allele does not have the A>G variant at rs3758581.

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (87).

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Clozapine Therapy and *CYP* Genotype

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Introduction

Clozapine is one of the most effective antipsychotics available in the treatment of schizophrenia and the only antipsychotic found to be effective in treatment-resistant schizophrenia (TRS). Clozapine is also used to reduce the risk of recurrent suicidal behavior in individuals with schizophrenia or schizoaffective disorder (1, 2).

Compared with typical antipsychotics, clozapine is far less likely to cause movement disorders, known as extrapyramidal side effects, which include dystonia, akathisia, parkinsonism, and tardive dyskinesia. However, there are significant risks associated with clozapine therapy that limits its use to only the most severely ill individuals who have not responded adequately to standard drug therapy. Most notably, because of the risk of clozapine-induced agranulocytosis, clozapine treatment requires monitoring of white blood cell counts (WBC) and absolute neutrophil counts (ANC), and in the US, the FDA requires that individuals receiving clozapine be enrolled in a computer-based registry (3). There is also a propensity for clozapine use to induce metabolic effects, resulting in substantial weight gain (1).

Clozapine is metabolized in the liver by the cytochrome P450 (CYP450) superfamily of enzymes. The CYP1A2 enzyme is the main CYP enzyme involved in clozapine metabolism, and CYP1A2 activity is a potential determinant of clozapine dose requirements (4). Other CYP enzymes involved in clozapine metabolism include CYP2D6, CYP3A4, and CYP2C19 (5).

The FDA-approved drug label states that a subset of the population (2–10%) have reduced activity of CYP2D6 ("poor metabolizers"[PMs]) and these individuals may develop higher than expected plasma concentrations of clozapine with typical standard doses. Therefore, the FDA states that a dose reduction may be necessary in individuals who are CYP2D6 PMs (Table 1) (1). However, the Dutch Pharmacogenetics Working Group (DPWG, Table 2) does not recommend dose alterations based on *CYP2D6* genotype, though the gene-drug interaction is acknowledged (6). The DPWG further states that there is not a gene-drug interaction between *CYP1A2* and clozapine due to the limited effect of known genetic variants on CYP1A2 function (6). Consequently, neither the FDA nor the DPWG recommend dose alterations based on *CYP1A2* genotype.

Additionally, clozapine clearance is affected by gender, tobacco use, and ethnicity, with further contributions from pharmacologic interactions. Females have lower CYP1A2 enzyme activity than males. Non-smokers have lower CYP1A2 activity than smokers and Asians and Amerindians have lower activity than Caucasians. Clozapine clearance can also be affected by co-medications that induce or inhibit CYP1A2 and the presence of inflammation or obesity (7, 8).

Table 1. The FDA Clozapine Dosage and CYP2D6 Poor Metabolizers (2020)

Phenotype	Dosing considerations
CYP2D6 poor metabolizers	Dose reduction may be necessary in individuals who are CYP2D6 poor metabolizers. Clozapine concentrations may be increased in these individuals, because clozapine is almost completely metabolized and then excreted.

This FDA table is adapted from (1).

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Table 2. The DPWG Recommendations for Clozapine and CYP2D6 (2016)

Phenotype	Dosing considerations
CYP2D6 poor metabolizer	No action is required for this gene-drug interaction.
CYP2D6 intermediate metabolizer	No action is required for this gene-drug interaction.
CYP2D6 ultrarapid metabolizer	No action is required for this gene-drug interaction.

This DPWG table is adapted from (6).

Drug: Clozapine

Clozapine is an antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe psychiatric disorder with a worldwide prevalence of around 1%. The specific etiology of schizophrenia is unknown; however, it is largely considered to result from a combination of complex genetic, immunologic, and environmental factors.

The symptoms of schizophrenia fall into 3 main categories: positive, negative, and cognitive. Positive symptoms include reality distortion (for example, delusions, hallucinations) and thought disorders, which both can respond well to treatment. Negative symptoms are deficits in normal emotions and behavior and may be mistaken for depression. Symptoms divide into reduced expression of emotion (for example, speaking without moving or with a monotonous voice), and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these symptoms. Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. Similarly, no treatment has major established efficacy.

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the cause), chronic psychotic disorders (for example, schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, tremors, and Parkinsonian-like symptoms. Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects such as tardive dyskinesia. These medications include aripiprazole, clozapine, olanzapine, and risperidone. Apart from aripiprazole, atypical antipsychotics can have serious metabolic side effects.

Clozapine is unique among the antipsychotics as it effectively treats positive symptoms and appears to be more effective in treating negative symptoms, and some cognitive symptoms when compared with other antipsychotics that cause negative symptoms or impair cognition (9, 10, 11). Clozapine has also been shown to reduce aggression and reduce the risk of suicide, and is the only antipsychotic found to be effective in TRS (2, 12, 13, 14). More than one third of individuals are thought to have schizophrenia that only partially responds or is resistant to standard drugs; these individuals may then be treated with clozapine (2, 14, 15).

Clozapine was introduced in 1971; however, it was withdrawn in 1975 due to safety concerns, including severe neutropenia induced by the drug (11). This severely low level of neutrophils (a type of white blood cell) places individuals at high risk of potentially lethal infections. Given that clozapine was the most effective antipsychotic used to treat TRS, in 1989 the FDA reapproved clozapine for that use (9, 11, 13). The FDA defines TRS as severe schizophrenia that does not respond adequately to standard antipsychotic treatment (1).

The main action of both first- and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. An exception is aripiprazole, which is a D2 partial agonist. Blockade of the D2 receptor in the brain's limbic system are thought to improve the "positive" symptoms of schizophrenia (16). However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway,

they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Clozapine only transiently occupies D2 receptors and then rapidly dissociates to allow normal dopamine neurotransmission. It is thought that because clozapine has a relatively low affinity for the D2 receptor and binds "loosely," extrapyramidal side effects are less likely (15, 17). In addition to binding the D2 receptor, clozapine has a high affinity for the serotonin 5-HT_{2A} receptors. Blockade of 5-HT_{2A} in the mesocortical tract may also provide some protection against extrapyramidal side effects by increasing amounts of dopamine. Clozapine and its major metabolite (N-desmethylclozapine, also called norclozapine) have been shown to indirectly activate N-methyl-D-aspartate receptors and may also modulate gamma-aminobutyric acid and cholinergic pathways. However, despite these findings, it remains unclear what gives clozapine its superior efficacy to other antipsychotics (11).

Clozapine is primarily metabolized in the liver by the CYP450 superfamily of enzymes. The primary metabolic steps are demethylation to form norclozapine and oxidation to clozapine n-oxide. The major enzymes involved in clozapine demethylation are CYP3A4 and CYP1A2, with CYP2D6 playing a minor role. Oxidation is primarily catalyzed by CYP1A2 (18). Clozapine is almost completely metabolized before excretion. Norclozapine has limited activity in some brain receptors, while the clozapine n-oxide metabolite is inactive (1, 11, 19). Norclozapine is not an effective antipsychotic and may contribute to some of the clozapine adverse drug reactions (20, 21).

The most severe side effects of clozapine therapy are included in 5 boxed warnings on the drug label: 1) severe neutropenia, 2) seizures (more likely at higher doses), 3) myocarditis (inflammation of the heart muscle induced by clozapine that can be fatal), 4) increased mortality in elderly individuals with dementia-related psychosis, and 5) an increased risk of orthostatic hypotension, bradycardia, and syncope (1). Additional side effects include weight gain and metabolic changes, QT interval prolongation, gastrointestinal hypomobility with severe complications, eosinophilia, hepatotoxicity, neuroleptic malignant syndrome and pulmonary embolism (1).

Additionally, there is a high risk that individuals with TRS taking clozapine may develop pneumonia, with a population attributable risk of pneumonia in clozapine-medicated individuals with TRS of 64%. Individuals with TRS in the absence of clozapine therapy had an estimated population attributable risk of pneumonia of 45%, which has been attributed to smoking, medication issues, and obesity prevalence in the TRS population. Clozapine use may decrease immunoglobulin levels and increase the interleukin-1 receptor antagonist, putting individuals at a higher risk for community-acquired pneumonia. Many medications used for TRS, clozapine included, can also negatively affect swallowing, increase salivation and sedation, contributing to the risk of aspiration pneumonia (22, 23, 24)

Because of the risk of neutropenia, clozapine can only be prescribed according to a schedule that monitors the patient's WBC and ANC. A severe neutropenia termed agranulocytosis is defined as an ANC of less than $500/mm^3$, is estimated to occur in around 1% of individuals, and could prove fatal if not detected early by regular monitoring (1, 25, 26). Before initiating clozapine therapy, baseline ANC must be obtained and monitored regularly during treatment. Clozapine therapy should be suspended if the ANC falls below 1500 and monitoring of ANC should be performed before reinitiating clozapine therapy (1).

Genetic risk factors for clozapine-induced neutropenia have been identified, consisting of 2 independent amino acid changes in *HLA-DQB1* (126Q) and *HLA-B* (158T). The *HLA-DQB1* gene is associated with autoimmune disease and *HLA-B* is an important component of severe drug reactions. Despite this genetic insight, a genetic test based solely on *HLA-DQB1* and *HLA-B* would not be able to adequately identify if individuals are truly at low risk of clozapine-induced neutropenia (27). More recent studies have confirmed these risk-associated loci and suggested the *SLCO1B3/SLCO1B7* locus as contributing to additional risk; however, these data do not support these loci having sufficient predictive power to recommend genetic testing (28).

A genetic variant in the *ACKR1* gene, commonly referred to as the Duffy-null genotype, causes a benign form of neutropenia usually called benign ethnic neutropenia (BEN) that can be mistaken for clozapine-induced neutropenia. This allele is common in individuals with African ancestry, but has also been reported in Middle Eastern, south west Asian and Oceania genetic backgrounds (28). For individuals known to have BEN, the FDA label indicates that a lower ANC is acceptable as compared with individuals without the Duffy-null genotype (1).

Therapeutic drug monitoring (TDM) may be employed in conjunction with the FDA-required ANC testing during treatment (29). A consensus guideline recommends a therapeutic range of 350–600 ng/ml for trough steady-state concentrations (30). The concentration to dose ratio is a useful clinical measure of clozapine clearance rates when determining appropriate dosage for individuals with altered CYP metabolic profiles, either due to genetic variation or comedication (29, 31). Moreover, the consensus guidelines strongly recommend clozapine TDM due to the narrow therapeutic range for this medication (30, 31). The importance of TDM in clozapine use is underscored by the observation that it was the third most toxic US medication between 1998-2005, when it was associated with 3277 deaths or serious non-fatal outcomes, lower than only oxycodone and fentanyl (32).

The use of clozapine in pregnancy has not been well-studied in humans, thus the FDA advises that clozapine tablets should only be used during pregnancy if clearly indicated. The increase of estrogen in the second and third trimester is associated with a decrease in the metabolism of CYP1A2 drugs. Thus, it is possible that clozapine levels may increase at the end of the pregnancy, though studies have been very limited (31). The FDA also states that clozapine is present in human breast milk and this poses a potential for serious adverse reactions in the nursing infants. A decision should be made whether to discontinue nursing or discontinue taking the drug. Additionally, safety and effectiveness in pediatric individuals has not been established (1).

The Cytochrome P450 Family

The CYP450 superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The genes that encode CYP450 proteins are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Other factors can affect CYP enzyme activity in addition to genetic variation, including concomitant medications. Inhibitors of CYP enzymes include the antibiotic ciprofloxacin (CYP1A2 inhibitor) and the antidepressant fluvoxamine (potent CYP1A2 and CYP2C19 inhibitor, moderate or weak inhibitor for CYP3A4, CYP2C9, and CYP2D6) (33). Inducers of the CYP enzymes include the antiseizure drug carbamazepine (CYP3A and CYP1A2 inducer), rifampin, phenytoin, phenobarbital, and St. John's wort (all CYP3A4 inducers) (1, 34, 35).

In addition, other agents can influence CYP enzymes—caffeine and oral contraceptives are weak or moderate CYP1A2 inhibitors (36). Tobacco smoke has polycyclic aromatic hydrocarbons that bind the aryl hydrocarbon receptor, thereby increasing the expression of CYP1A2 (37). Thus, it is a weak inducer of CYP1A2 (1). Omeprazole has similar weak inducer properties and by binding the aryl hydrocarbon receptor, omeprazole increases the expression of CYP1A2. Thus, omeprazole has clinically relevant inducer effects on non-smokers (38).

Physiologic conditions can also alter CYP enzyme expression and activity. Studies have proposed that obesity may be associated with decreased clearance of clozapine, as well as other CYP1A2 substrates (39, 40, 41). Inflammation releases cytokines that inhibit CYPs including CYP1A2 (42). One study of post-surgery inflammation suggests that inflammation decreases CYP1A2 activity by half (43).

The phenomenon by which non-genetic factors alter the enzymatic phenotype typically associated with a particular genotype is termed phenoconversion. As discussed above, medications, other ingested substances, and physiologic conditions can alter the enzymatic activity of CYP proteins. For example, an individual with a

genotype-predicted intermediate metabolizer (IM) status may have a clinical presentation more akin to a PM due to comedication with a known CYP enzyme inhibitor, such as ciprofloxacin.

Gene: *CYP1A2*

Altered function of CYP1A2 influences the clearance of the clozapine (4). Understanding the full pharmacogenomic effects of *CYP1A2* variation is still at an early stage compared with that of CYP2D6 and other CYP enzymes (44). The CYP1A2 enzyme is the main CYP enzyme involved in clozapine metabolism (4, 45).

The CYP1A2 enzyme comprises around 13% of all CYP protein in the liver, whereas CYP2D6 comprises around 2%. Approximately 25 variant *CYP1A2* alleles have been reported, some of which have been shown to alter the activity of CYP1A2 (Table 3). For example, the **1C* allele is associated with decreased enzyme activity (by altering the binding site of an unknown transcription factor in the gene promoter), and the **1F* allele is associated with increased enzyme activity (by increasing the induction of expression) (44, 46).

The frequency of *CYP1A2*1F* (g.5732C>A in intron 1, rs762551) varies across populations. Studies have reported the frequency of the *CYP1A2*1F* allele to range from 54.9% in Africans to ~60% in Chinese and 68.2% in Caucasians (47, 48). The frequency of individuals who are heterozygous or homozygous for the *CYP1A2*1F* allele ranges from 57–69% in Czech and Hungarian populations and up to 73% in Turkish populations (49). The global average allele frequency of rs762551 (agnostic to potential other variants in *cis*) is 69.2%, but this ranges from 57% in South Asian populations up to 92% in other areas of Asia (50).

Additional rare alleles associated with decreased CYP1A2 activity include *CYP1A2*7* and *CYP1A2*6,* both of which have been reported primarily in individuals of European ancestry, with the *CYP1A2*7* allele being notably absent in a study of Chinese Han PMs (51, 52, 53). Other *CYP1A2* alleles with low activity (**8*, **15*, **16*, and **11*) have been found in low numbers in East Asians (54). A study of 250 Japanese individuals found allele frequencies of 0.4% for *CYP1A2*8*, 0.2% for *CYP1A2*15*, and 0.2% for *CYP1A2*16* (55).

As previously discussed, additional factors beyond genetic variation can influence CYP1A2 activity, resulting in phenoconversion.

Table 3. Activity Status of Selected *CYP1A2* Alleles

Allele type	*CYP1A2* alleles
Normal function	**1A*
Decreased function	**1C, *1K, *3, *4, *7, *8, *11, *15, *16*
No function	**6*
Increased function (inducible)	**1F*

For a comprehensive list of *CYP1A2* alleles, please see PharmVar.

Gene: *CYP2D6*

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

CYP2D6 Alleles

The *CYP2D6* gene on chromosome 22q13.2 is highly polymorphic. Over 140 star (*) alleles have been described and catalogued at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, absent, or unknown enzyme function (Table 4) (56).

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (for example, CYP2D6 PMs). When duplicated alleles are detected, both copies are assigned an activity score for phenotyping. However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms. The CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is defined by the sum of the 2 allele activity scores, which is most commonly in the range of 0 to 3.0 (57).

- Ultrarapid metabolizer (UM) has an activity score greater than 2.25
- Normal metabolizer phenotype (NM) has an activity score of 1.25 to 2.25
- Intermediate metabolizer (IM) has an activity score of >0 to <1.25
- Poor metabolizer (PM) has an activity score of 0 (14)

Table 4. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See PharmVar.

The *CYP2D6*1* allele is considered the wild-type allele when no variants are detected, which is associated with normal enzyme activity and the "normal metabolizer" phenotype. In addition, the *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (58, 59, 60, 61) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (62, 63, 64) (see Table 4). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6,* and **41* being more common in Caucasians, **17* more common in Africans, and **10* more common in Asians (65).

Allele Frequencies Vary between Populations

Among Asians and in individuals of Asian descent, only approximately 50% of *CYPD6* alleles are normal function, and the frequency of *CYP2D6* duplications is as high as 45%, although this may have been overestimated by not accounting for tandem hybrid alleles (for example, **36+*10*) (66). Other studies of a US individual population suggested less than 50% of alleles detected within Asian-descent individuals are normal-function alleles in a single copy, with 30% of alleles arising from structural variants (duplications or deletions) (67). Common no-function variants are *CYP2D6*36* and *CYP2D6*4* (67). Both these alleles contain the variant "c.100C>T," which is the defining variant in *CYP2D6*10* (see Allele Nomenclature table) (65, 66, 68, 69). The *CYP2D6*36* allele is the result of a gene conversion event with the pseudogene *CYP2D7* (70). This no-function allele is most commonly found in individuals of Asian ancestry (67).

Among Africans and African Americans, approximately 50% of *CYP2D6* alleles are normal function (58, 64, 65, 71). African Americans also have been found to have a higher frequency of no-function structural variants or decreased-function single-copy variant alleles versus Caucasian or Hispanic-Americans (67). Intermediate and NM alleles are present in approximately 80% of the population of individuals of African descent (72).

Middle Eastern countries show a great diversity in phenotypic and allelic distribution for *CYP2D6* (73), though on average, these individuals show a lower frequency of PM phenotypes (0.91%) and higher UM phenotypes than other ethnicities (74). The highest frequencies of CYP2D6 UM reported to date are 20% in Oceanians and 9.5% Near Easterners (72, 75).

Among European countries, there is diversity of allelic distribution (76). Gene duplications were more common in the south-eastern countries (Greece, Turkey: 6%) and less common in north-western countries (Sweden and Denmark, <1%). Meanwhile, *CYP2D6*4* and **5* alleles were generally more common in the north and less common in the south (76). Worldwide *CYP2D6* genotype and phenotype frequencies have been catalogued and recently published (74).

Gene: *CYP3A4*

In contrast to *CYP2D6, CYP1A2,* and other genes that encode drug-metabolizing enzymes, *CYP3A4* shows little genetic variation with known functional consequences (Table 5). Although around 40 variant *CYP3A4* alleles have been reported, most have not been shown to alter the activity of CYP3A4 (77, 78). To date, only 3 loss-of-function *CYP3A4* alleles have been identified (*CYP3A4*6, CYP3A4*20* and *CYP3A4*26*) (79, 80).

The *CYP3A4*22* allele has decreased function and explains 12% of the variation in CYP3A4 activity (81). This variant that is present in 3.2–10.6% of the Dutch population and 5.2–8.3% of the population in America (82). The Allele Frequency Aggregator project reports this reduced-function allele to be present in approximately 5% of the global population, with the lowest prevalence in Asian and African populations (83). The 1000 Genomes Project phase 3 data release estimates global prevalence to be slightly lower (~1%); a minor allele frequency of 5% is reported for the European average (84).

The *CYP3A4*20* allele contains a premature stop codon that results in a loss-of-function of *CYP3A4*. It appears to be the most common *CYP3A4*-defective allele but is still relatively rare, with approximately 0.2% of European Americans and 0.05% African Americans who are heterozygous. However, in Spain, the *CYP3A4*20* allele is present in 1.2% of the population, and up to 3.8% in specific Spanish regions (79).

Table 5. Activity Status of Selected *CYP3A4* Alleles

Allele type	*CYP3A4* alleles
Normal function	**1A*
Decreased function	**16A, *16B, *22*
No function	**6, *20, *26*

For a comprehensive list of *CYP3A4* alleles, please see PharmVar.

Gene: *CYP2C19*

Another CYP enzyme with a minor contribution to clozapine metabolism is CYP2C19. The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, including antidepressants, antiplatelet agents, anti-fungal agents, some proton pump inhibitors, and benzodiazepines such as diazepam.

The *CYP2C19* gene is highly polymorphic, as there are over 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with 'rapid' (**1/*17*) and 'ultrarapid' (**17/*17*) metabolizer phenotypes. Individuals who have one copy of non-functional alleles (for example, **2* and **3*) are classified as 'intermediate metabolizers' (for example, **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (for example, **2/*2*, **2/*3*) (Table 6).

Table 6. The CPIC Assignment of *CYP2C19* Phenotype based on Genotype (2017)

Phenotype	Genotype	Examples of diplotype
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual with 2 increased-function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual with one normal-function allele and one increased-function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual with 2 normal-function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual with one normal-function allele and one no-function allele or one no-function allele and one increased-function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual with 2 no-function alleles	**2/*2* **2/*3* **3/*3*

CPIC: Clinical Pharmacogenetics Implementation Consortium
[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (85).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The available evidence indicates that the *CYP2C19*17* increased-function allele is unable to completely compensate for the *CYP2C19*2* no-function allele.
This CPIC table is adapted from (85).

It has been reported that approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 PMs; and up to 45% of individuals are CYP2C19 IMs (86). Other studies have found PM phenotypes to range between 10.8–16.4% in Asian populations, 3% in African descendants, and 1.6% in Middle Eastern populations (87, 88). Pacific Islanders have been reported to have higher frequencies of PMs—11.8% (88). The frequency of IMs is similarly distributed, higher in East and South Asian and Pacific Islander, lower in African or Middle Eastern populations (87).

The FDA-approved drug label for omeprazole, a CYP2C19 substrate, states that approximately 15–20% of Asians are CYP2C19 PMs, compared with 3% of Caucasians (89). The most common no-function allele is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians of European descent and Africans, and ~27–36% in Asians (87, 90).

The *CYP2C19*3* allele is another commonly identified no-function variant, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–7% in Asian populations (90), but rare in other racial groups. Other no-function variants occur in less than 1% of the general population and include *CYP2C19*4-*8* (91, 92).

The *CYP2C19*17* allele, which results in rapid and UMs, has frequencies of only 1.3–4% among Asian populations compared with approximately 20–33.7% of African, European, and Near-Eastern populations (87, 88).

Linking Gene Variation with Treatment Response

There is growing evidence to support that genetic factors, such as *CYP2D6*, play a role in determining the clinical outcome of antipsychotic treatment. However, there is no definitive evidence for clozapine treatment with regard to *CYP1A2*, *CYP2D6, CYP3A4* or *CYP2C19*, with the latter 3 playing a minor role in clozapine metabolism. Although pharmacogenetic testing is available, its use in personalizing antipsychotic treatment is minimal (93). While the FDA recommends adjusting dosage only for CYP2D6 PMs, studies investigating the potential benefit of pharmacogenomic-guided dosing have studied CYP1A2, CYP2C19, and CYP3A status (93, 94).

There is no consensus on the effect of various *CYP1A2* alleles on clozapine treatment, however, mounting evidence exists to suggest that changes in CYP1A2 activity can significantly affect clozapine plasma levels. Increased CYP1A2 activity is predicted to result in a more rapid metabolism of clozapine and lower clozapine half-life; conversely, reduced CYP1A2 activity would lead to reduced clozapine metabolism and a longer clozapine half-life (1, 18, 29). Cessation of smoking or co-medication with CYP1A2 inhibitors (for example, ciprofloxacin, fluvoxamine, or enoxacin) have led to an increase in clozapine exposure (95). Differences in gender and smoking status influence clozapine plasma levels. At least one case has been documented where high caffeine consumption was associated with an UM phenotype (40). Given that smoking is more common among individuals with schizophrenia than in the general population, the effect of tobacco use on CYP1A2 metabolism is particularly important for clozapine therapy (96). Multiple reports have found that infections, particularly pneumonia, are associated with risk of clozapine intoxication due to the release of cytokines (97, 98, 99). The decrease of clozapine clearance during infections is not specific; any inflammation with systemic manifestations, such as fever or serum C-reactive protein (CRP) elevations, can cause elevated clozapine serum concentrations. Thus, CRP elevations can help identify inflammation as a cause of clozapine concentration elevations (100). In one study, only 11% of individuals with concurrent infections during clozapine therapy were able to continue their medication without dose alteration (101).

Studies suggest that the required dose to maintain therapeutic plasma concentrations in individuals with Asian Indian and Southeast Asian ancestry is half of the dose of an individual with European ancestry, though the exact mechanism remains to be elucidated (29, 102). Several reports have documented that clozapine is used at lower doses in Asian individuals and that east Asians have a lower clozapine clearance as compared with Caucasians (53, 103, 104, 105). Similarly, studies of CYP1A2 activity have found Caucasians to have higher average enzyme activity than Asians (106). Typical clozapine doses in Asians range from 150–300 mg/day (40). Native Americans and other Amerindians may have clozapine metabolism similar to Asians and need similar lower doses as compared with Caucasians (107). A recent case of myocarditis in a Canadian of South Asian ancestry indicates that this dosing difference may be clinically relevant since the individual developed a clozapine-induced myocarditis when started with a Canadian typical titration with an initial dose of 25 mg/day and 100 mg/day was reached in the 11 day. On the other hand, a slower titration (initial dose 6.5 mg/day and final dose 81.25 mg/day) was tolerated without myocarditis (108). Furthermore, an analysis of 6 combined studies of European Caucasians established that the minimal therapeutic doses ranged from 236 (female non-smokers) to 368 mg/d (male smokers). These dosages are much lower than the ones proposed by the US clozapine package insert, which recommends targeting doses from 300-450 mg/day and then consider increases by 100 mg/day, up to 900 mg/day in rare situations (109). Thus, determination of CYP1A2 metabolizer status should account for not only genotype, but gender, obesity, inflammation, smoking or comedication, and ethnic background as potential confounding factors leading to phenoconversion (8).

Case studies have reported individuals with one or more copies of the increased-function allele *CYP1A2*1F* who responded poorly to clozapine therapy. However, evidence for a universal, clinically significant effect of *CYP1A2*1F* alleles on CYP1A2 is lacking. Out of the 7 kinetic studies, ranging in size from 58–185 individuals, only one medium-sized study (95 individuals) found a significant effect of the **1F* allele on clozapine pharmacokinetics (110, 111, 112, 113, 114, 115, 116).

The FDA drug label indicates that individuals taking strong CYP1A2 inhibitors should take a significantly reduced dose of clozapine, due to reduced clearance via CYP1A2 metabolism (1). However, experts strongly caution against co-prescribing CYP1A2 inhibitors such as fluvoxamine or ciprofloxacin with clozapine due to potentially fatal drug-drug interactions (117, 118).

While the FDA-approved drug label states that dose adjustments may be required for CYP2D6 PMs, there are no specific guidelines from any pharmacogenomic authority to pre-emptively adjust an individual's clozapine dosage based on *CYP2D6* genotype. Case studies have reported altered pharmacodynamics in CYP2D6 PMs and

UMs, however, further analysis suggests that phenoconversion due to comedication has a larger effect on CYP2D6-mediated clozapine metabolism than genotype alone (119, 120, 121, 122). Indeed, in vitro analysis suggests the CYP2D6 enzyme is responsible for approximately 6% of clozapine metabolism, further supporting a modest role for this enzyme in clinical management (5). Similarly, *CYP3A4* genotypes have not been definitively shown to be associated with a specific response or altered dosage of clozapine, likely due to the primary role of CYP1A2 in clozapine metabolism (94, 114). However, one study suggests that CYP3A4 activity may play a role in metabolic side effects from clozapine therapy (123).

Multiple studies have examined the effect of CYP2C19 genotypes on the response to antipsychotics, including clozapine, with variable conclusions being reached to determine genotype-based guidance for clozapine. Genotypes studied include *CYP2C19*2* and *CYP2C19*17* (53, 124, 125).

Genetic Testing

Genetic testing is available for common *CYP2D6*, *CYP1A2*, *CYP3A4* and *CYP2C19* alleles. Often a panel of tests is performed. These panels test for variants in multiple genes, which are involved in the metabolism of many drugs, including clozapine. For examples of the tests available for the clozapine drug response, please see the NIH Genetic Testing Registry.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (126). Gene duplications and multiplications can be denoted by "xN", for example: *CYP2D6*1xN* with *xN* representing the number of *CYP2D6* gene copies. Note: representation of duplications is also not standardized among laboratories.

In 2018, the Association for Molecular Pathology (AMP) published recommendations for *CYP2C19* genotyping allele selection. The recommendations determined varying tiers of alleles, based on the strength of evidence supporting drug response, minor allele frequencies and availability of reference materials. The AMP's tier one group represent the core alleles recommended for genotyping panels: **2, *3,* and **17* (127). These guidelines provide information for laboratories performing *CYP2C19* genotype testing and are a useful complement to CPIC prescribing recommendations.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Dosage Adjustments with Concomitant use of CYP1A2, CYP2D6, CYP3A4 Inhibitors or CYP1A2, CYP3A4 Inducers

Clozapine is a substrate for many cytochrome P450 isozymes, in particular CYP1A2, CYP3A4, and CYP2D6. Use caution when administering clozapine tablets concomitantly with drugs that are inducers or inhibitors of these enzymes.

[…]

Dose adjustments may be necessary in patients with concomitant use of:

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

- strong CYP1A2 inhibitors (e.g., fluvoxamine, ciprofloxacin, or enoxacin);
- moderate or weak CYP1A2 inhibitors (e.g., oral contraceptives, or caffeine);
- CYP2D6 or CYP3A4 inhibitors (e.g., cimetidine, escitalopram, erythromycin, paroxetine, bupropion, fluoxetine, quinidine, duloxetine, terbinafine, or sertraline);
- CYP3A4 inducers (e.g., phenytoin, carbamazepine, St. John's wort, and rifampin);
- or CYP1A2 inducers (e.g., tobacco smoking)

[…]

Concomitant use of Strong CYP1A2 Inhibitors: Reduce clozapine tablets dose to one-third when coadministered with strong CYP1A2 inhibitors (e.g., fluvoxamine, ciprofloxacin, enoxacin).

Concomitant use of Strong CYP3A4 Inducers is not recommended.

Discontinuation of CYP1A2 or CYP3A4 Inducers: Consider reducing clozapine tablets dose when CYP1A2 inducers (e.g., tobacco smoke) or CYP3A4 inducers (e.g., carbamazepine) are discontinued.

Anticholinergic drugs: Concomitant use may increase the risk for anticholinergic toxicity.

[…]

Dose reduction may be necessary in patients who are CYP2D6 poor metabolizers. Clozapine concentrations may be increased in these patients, because clozapine is almost completely metabolized and then excreted.

[…]

A subset (3%–10%) of the population has reduced activity of CYP2D6 (CYP2D6 poor metabolizers). These individuals may develop higher than expected plasma concentrations of clozapine when given usual doses.

Please review the complete therapeutic recommendations that are located here: (1).

Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) (2016, 2020)

CYP2D6 Poor, Intermediate or Ultrarapid Metabolizer-Clozapine [December 2020]

NO action is required for this gene-drug interaction.

The genetic variation results in a slightly elevated plasma concentration of clozapine, but there are no clinical consequences.

CYP1A2 [2016]

This is NOT a drug-gene interaction.

Please review the complete therapeutic recommendations that are located here: (6).

Nomenclature for selected alleles

***CYP1A2* Nomenclature**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP1A2*1C*	-3860G>A -2964G>A	Unknown	Not applicable—variant occurs in a non-coding region	rs2069514

CYP1A2 Nomenclature continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP1A2*1K*	-739T>G -729C>T -163C>A	NM_000761.5:c.-10+103T>G NM_000761.5:c.-10+113C>T NM_000761.5:c.-9-154C>A	Not applicable—variant occurs in a non-coding region	rs2069526 rs12720461 rs762551
*CYP1A2*1F*	-164C >A	NM_000761.4:c.-9-154C>A	Not applicable—variant occurs in a non-coding region	rs762551
*CYP1A2*3*	2116G>A; 5347T>C	NM_000761.5:c.1042G>A	NP_000752.2:p.Asp348Asn	rs56276455
*CYP1A2*4*	2499A>T; I386F	NM_000761.5:c.1156A>T	NP_000752.2:p.Ile386Phe	rs72547516
*CYP1A2*6*	5090C>T; R431W	NM_000761.5:c.1291C>T	NP_000752.2:p.Arg431Trp	rs28399424
*CYP1A2*7*	3533G>A	NM_000761.5:c.1253+1G>A	Splicing defect	rs56107638
*CYP1A2*8*	5347T>C; 5166G>A; R456H	NM_000761.5:c.1367G>A	NP_000752.2:p.Arg456His	rs72547517
*CYP1A2*11*	558C>A; F186L	NM_000761.5:c.558C>A	NP_000752.2:p.Phe186Leu	rs72547513

*CYP1A2*1A* is the wild-type allele and is determined to be present with no variants are detected.

CYP2D6 Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*3*	2550delA (Arg259fs)	NM_000106.6:c.775delA	NP_000097.3:p.Arg259fs	rs35742686
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T (Trp152Glyfs) CYP2D6T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.886T>C	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.1457G>C	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1319G>A	NP_000097.3:p.Glu410Lys	rs769157652

CYP2D6 Nomenclature continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.1457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.454delT	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.100C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*36*[3]	100C>T (Pro34Ser)	NM_000106.6:c.320C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts splicing).	rs28371725
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2851C>T is also referred to as 2938C>T
[3] *CYP2D6*36* is a gene conversion with *CYP2D7*; variants provided here are from the Pharmacogene Variation Consortium. *CYP2D6*1* is the wild-type allele and is presumed to be present with no variants are detected.

CYP3A4 Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A4*6*	17661_17662insA 277Frameshift	NM_017460.5:c.830_831insA	NP_059488.2:p.Asp277Glufs	rs4646438
*CYP3A4*16A*	554C>G, T185S	NM_017460.6:c.554C>G	NP_059488.2:p.Thr185Ser	rs12721627

CYP3A4 Nomenclature continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CY P3A4*16B*	554C>G, T185S	NM_017460.6:c.554C>G	NP_059488.2:p.Thr185Ser	rs12721627
	20230G>A (gDNA)	NM_017460.6:c.1026+12G>A	Not applicable—variant occurs in a non-coding region	rs2242480
*CYP3A4*20*	1461_1462insA 488Frameshift	NM_017460.5:c.1461dup	NP_059488.2:p.Pro488Thrfs	rs67666821
*CYP3A4*22*	15389C>T	NM_017460.6:c.522-191C>T	Not applicable—variant occurs in a non-coding region	rs35599367
*CYP3A4*26*	17633C>T R268Stop	NM_017460.6:c.802C>T	NP_059488.2:p.Arg268Ter	rs138105638

*CYP3A4*1A* is the wild-type allele and is determined to be present with no variants are detected.

***CYP2C19* Nomenclature**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.4:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.4:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*4*	1A>G Met1Val	NM_000769.4:c.1A>G	NP_000760.1:p.Met1Val	rs28399504
*CYP2C19*5*	90033C>T Arg433Trp	NM_000769.4:c.1297C>T	NP_000760.1:p.Arg433Trp	rs56337013
*CYP2C19*6*	12748G>A Arg132Gln	NM_000769.4:c.395G>A	NP_000760.1:p.Arg132Gln	rs72552267
*CYP2C19*7*	19294T>A	NM_000769.4:c.819+2T>A	(Splice donor variant)	rs72558186
*CYP2C19*8*	12711T>C Trp120Arg	NM_000769.4:c.358T>C	NP_000760.1:p.Trp120Arg	rs41291556
*CYP2C19*9*	12784G>A Arg144His	NM_000769.4:c.431G>A	NP_000760.1:p.Arg144His	rs17884712
*CYP2C19*17*	-806C>T	NM_000769.4:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: when no variants are detected the genotype is designated as *CYP2C19*1* and is considered the normal "wild-type" allele.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (128).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Codeine Therapy and *CYP2D6* Genotype

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Introduction

Codeine is used to relieve mild to moderately severe pain, and it belongs to the drug class of opioid analgesics. Codeine has also been prescribed to prevent coughing, though the antitussive administration is most often in liquid formulations and in conjunction with other medications. (1, 2)

The hepatic CYP2D6 enzyme metabolizes a quarter of all prescribed drugs, including codeine. The CYP2D6 enzyme converts codeine into its active metabolite, morphine, which provides its analgesic effect. Consequently, pain relief may be inadequate in individuals who have 2 inactive copies of *CYP2D6* ("poor metabolizers", PMs), because of reduced morphine levels.

In contrast, individuals who have more than 2 normal-function copies of the *CYP2D6* gene ("ultrarapid metabolizers", UMs) are able to metabolize codeine to morphine more rapidly and more completely. As a result, even with therapeutic doses of codeine, these individuals may experience the symptoms of morphine overdose, which include extreme sleepiness, confusion, and shallow breathing, which in some instances can be fatal. Nursing mothers with ultrarapid CYP2D6 metabolism may also produce breast milk containing higher than expected levels of morphine that can lead to severe adverse events in their infants. (3)

The FDA-drug label for codeine states that even at labeled dosage regimens, individuals who are UMs may have life-threatening or fatal respiratory depression or experience signs of overdose (Table 1). The label also contains a boxed warning, which states that respiratory depression and death have occurred in children who received codeine following tonsillectomy, adenoidectomy, or both, and had evidence of being CYP2D6 UMs.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that for an individual identified as a CYP2D6 UM, another analgesic should be used to avoid the risk of severe toxicity with a "normal" dose of codeine. CPIC also recommends avoiding codeine in individuals identified as CYP2D6 PMs due to the possibility of lack of effect (Table 2). (4)

The Dutch Pharmacogenetics Working Group (DPWG) have published codeine dosing recommendations based on *CYP2D6* genotype, and condition being treated (cough or pain), typical dosing, and additional risk factors, such as reduced kidney function or co-medication with CYP3A4 inhibitors. For UMs, the DPWG recommends an alternative to codeine for the treatment of pain (for example, oxycodone) (Table 3). (5)

Table 1. The FDA Recommended Dosing for Codeine based on CYP2D6 Phenotype (2019)

CYP2D6 phenotype	Codeine dose
Ultrarapid metabolizers	Individuals who are ultrarapid metabolizers should not use codeine sulfate tablets.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA.
This FDA table is adapted from (3)

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Table 2. The CPIC Codeine Therapy Recommendations based on CYP2D6 Phenotype (2020)

Phenotype[a]	Activity score[b]	Implications	Genotype	Examples of diplotypes[b]	Recommendations for codeine therapy[d]
Ultrarapid metabolizer	> 2.25	Increased enzyme activity. Increased formation of morphine leading to higher risk of toxicity.	More than 2 copies of normal-function alleles	**1/*1xN*[c] **1/*2xN*	Avoid codeine use because of potential for serious toxicity. If opioid use is warranted, consider a non-tramadol opioid.
Normal metabolizer	1.25-2.25*	Normal enzyme activity. Expected morphine formation.	2 normal-function alleles, or one normal-function allele and one decreased-function allele, or combinations of duplicated alleles that result in an activity score of 1.25 to 2.25	**1/*10* **1/*41* **1/*9* **10/*41x3* **1/*1* **1/*2* **2x2/*10*	Use codeine label-recommended age- or weight-specific dosing.
Intermediate metabolizer	0.25-1*	Intermediate enzyme activity. Reduced morphine formation.	one decreased-function allele and one no-function allele, or 2 decreased-function alleles, or one normal-function alleles and one no-function	**4/*10* **4/*41* **10/*10* **10/*41* **41/*41* **1/*5*	Use codeine label-recommended age- or weight-specific dosing. If no response, and opioid use is warranted, consider a non-tramadol opioid
Poor metabolizer	0	Absent enzyme activity. Greatly reduced morphine formation following codeine administration, leading to insufficient pain relief.	2 no-function alleles	**3/*4* **4/*4* **5/*5* **5/*6*	Avoid codeine use because of possibility of diminished analgesia. If opioid use is warranted, consider a non-tramadol option.
Indeterminant metabolizer	n/a	n/a	An individual having one or 2 uncertain-function alleles	**1/*22* **1/*25* **22/*25*	No recommendation

[a] See the CYP2D6 Frequency Table for ancestry-specific allele and phenotype frequencies from PharmGKB and CPIC.
[b] Assignment of allele function and allele activity values including citations for allele function can be found at PharmGKB (CYP2D6 Allele Definition Table and CYP2D6 Allele Functionality Table) and CPIC.
For a complete list of CYP2D6 diplotypes and resulting phenotypes, see the CYP2D6 Genotype to Phenotype Table at PharmGKB and CPIC.
[C] Where xN represents the number of CYP2D6 gene copies. For individuals with CYP2D6 duplications or multiplications, see supplemental data in (CPIC 2020) for additional information on how to translate diplotypes into phenotypes.
[d] The strength of therapeutic recommendations is "moderate" for intermediate metabolizers, and "strong" for all other metabolizers.
Table is adapted from (4)

Table 3. The DPWG Recommendations for Codeine by *CYP2D6* Genotype (2017)

CYP2D6	Recommendations for pain	Recommendations for cough
Ultrarapid	If possible, select an alternative[b] to codeine. Lower doses (<20 mg for adults, <10mg for children 12yo and older) every 6 hours (with no additional risk factors) are acceptable.	If possible, select an alternative to codeine. Noscapine* is not metabolized by CYP2D6. Lower doses (<20 mg for adults, <10mg for children 12yo and older) every 6 hours (with no additional risk factors) are acceptable.
Intermediate metabolizer[a]	1. be alert to a reduced effectiveness 2. in the case of inadequate effectiveness try a dose increase if this does not work: choose an alternative[b] 3. if no alternative is selected: advise the individual to report inadequate analgesia	No action required
Poor metabolizer[a]	1. choose an alternative[b] 2. if an alternative is not an option: advise the individual to report inadequate analgesia.	No action required

[a] It is not possible to offer adequately substantiated advice for dose adjustment based on the limited available literature for this phenotype.
[b] Do not select tramadol, as this is also metabolized by CYP2D6. Morphine is not metabolized by CYP2D6. Oxycodone is metabolized by CYP2D6 to a limited extent, but this does not result in differences in analgesia in individuals.
yo – years old
* This medication is not licensed for use within the United States, included to reflect the original source recommendations from The Netherlands.
Please see Therapeutic Recommendations based on Genotype for additional recommendations from DPWG, which take into account typical dosing, co-medication with CYP3A4 inhibitors, and risk factors such as reduced kidney function.
This table is adapted from (5)

Drug: Codeine

Codeine is an opioid analgesic. It exerts its effects via the opioid receptors found throughout the body. Target sites for the desired effects are receptors in the central nervous system, however, the gastrointestinal system is also affected due to the presence of opioid receptors, resulting in undesired, off-target effects. Codeine is a prodrug that only weakly binds the mu opioid receptor. Its analgesic properties depend upon its conversion to morphine that binds to the mu opioid receptor with 200-fold greater affinity than codeine.

Codeine is indicated for the relief of mild to moderately severe pain, where the use of an opioid analgesic is appropriate. Codeine is a Schedule II controlled substance, and there is a risk of misuse and abuse. Codeine can also be combined with acetaminophen (called Tylenol 3 or 4), which is a schedule III, controlled medication (90 milligrams or less codeine per dosage unit). Drugs and other substances that are considered controlled substances under the Controlled Substances Act (CSA) are divided into 5 schedules based on whether they have an accepted medical indication and the potential for abuse or addiction. Schedule II drugs have a high potential for abuse that may lead to severe psychological or physical dependence, and schedule III have a lower potential for abuse. Codeine is also found with some cough medications—schedule V medications that are limited to containing not more than 200 milligrams of codeine per 100 milliliters. (6) As with any opioid drug, the dosing regimen should be adjusted for each individual. When the individual no longer requires codeine, the doses should be tapered gradually to prevent withdrawal symptoms in individuals who have become physically dependent. (3)

For codeine to exert its opioid activity, it must first undergo *O*-demethylation by CYP2D6 to morphine. Only approximately 5–10% of codeine is metabolized in this pathway, with approximately 80% of an administered dose of codeine being converted to inactive metabolites and excreted. However, the percentage of codeine

converted to morphine can be much higher in individuals who have a combined enzyme "activity score" of >2.25 due to variant alleles of *CYP2D6* (*UMs; see Gene: CYP2D6 information below)*.(4) In contrast, individuals who lack active copies of *CYP2D6* (PMs) have lower levels of morphine.

Morphine is further metabolized to morphine-6-glucuronide, which also has analgesic properties. Morphine-3-glucuronide, a related morphine metabolite is presumed to not function as an analgesic but possesses neurotoxic effects. (4) Other metabolites are thought to be mostly inactive; they include codeine-6-glucuronide (~60%) and norcodeine (~5–10%), both of which share with codeine a similarly weak affinity for the mu opioid receptor. (7)

To avoid treatment complications in individuals who are either ultrarapid or PMs, opioids that are not metabolized by CYP2D6 may be used (for example, morphine, oxymorphone, buprenorphine, fentanyl, methadone, hydromorphone), alongside non-opioids, depending upon the type of pain being treated. (8, 9, 10) Tramadol is not a recommended alternative, since it is also metabolized by CYP2D6 (3, 5). Hydrocodone and oxycodone are also metabolized by CYP2D6 to more potent metabolites but the implications of CYP2D6 genotype on analgesic response and risk for toxicity is unclear (4).

The most common adverse reactions to codeine include drowsiness, lightheadedness, dizziness, sedation, shortness of breath, nausea, vomiting, and sweating. One of the main serious adverse reactions associated with codeine is respiratory depression. The FDA-drug label for codeine now includes a boxed warning that states "Warning: Death related to ultrarapid metabolism of codeine to morphine. Respiratory depression and death have occurred in children who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being ultrarapid metabolizers of codeine due to a *CYP2D6* polymorphism." (3, 11, 12)

The FDA also cautions against prolonged use of codeine during pregnancy due to the risk of neonatal opioid withdrawal and fetal harm, regardless of the mother's CYP2D6 metabolizer status. (3)

Codeine sulfate tablets are "contraindicated for all children younger than 12 years of age and in post-operative pain management in pediatric individuals younger than 18 years of age following tonsillectomy and/or adenoidectomy" (3). The FDA label also recommends avoiding codeine usage by adolescents between 12 and 18 years of age with the following risk factors: "conditions associated with hypoventilation, such as post-operative status, obstructive sleep apnea, obesity, severe pulmonary disease, neuromuscular disease, and concomitant use of other medications that cause respiratory depression." (3)

In light of the opioid-associated death of a nursing infant whose mother was found to be an UM (13), the FDA does not recommend breastfeeding during treatment with codeine (3). This recommendation against breastfeeding while taking opioids includes both codeine and tramadol, as both are pro-drug substrates of CYP2D6 (14). Additional information on the FDA's guidance regarding codeine and tramadol usage in breastfeeding women is also available from the FDA (15). However, other sources and studies—discussed below —have suggested short-term administration of codeine in the post-partum period for nursing mothers is possible with appropriate precautions and monitoring of the breastfed infant.

The American College of Obstetrics and Gynecology (ACOG) has issued a statement on the topic of pain management in the postpartum period that includes guidance for use of opioids. The ACOG recommends a stepwise approach and multimodal combination of agents in managing postpartum pain, with an emphasis on shared decision-making between the new mother and her physician regarding pain management options outside of the clinical setting. The ACOG recommends non-opioid analgesics as the first tier of medications for postpartum pain management, with low dose, mild opioids as a secondary option with acetaminophen or non-steroidal anti-inflammatory drugs. These guidelines state that oral opioids should be reserved only for breakthrough pain. Furthermore, the ACOG recommends clinicians review of the risks and benefits as well as educating the family regarding the presentation of opioid toxicity in both the woman and newborn. And the ACOG is clear to state that opiate prescriptions should be limited to the shortest reasonable course expected for acute pain. Evidence-based safety guidelines for maternal opioid use are available from the ACOG. (16)

If the clinician and new mother decide codeine is warranted, guidelines from the Lactation Database state that acute pain management for the nursing mother with established milk production can be achieved via a nonnarcotic analgesic and limiting maternal intake of low dosage oral codeine to 2–4 days. It should be noted that newborn infants seem to be particularly sensitive to the effects of narcotic analgesics and numerous professional organizations and regulatory agencies are cited as recommending other agents over codeine for pain management. (17)

A review of the literature and analysis of pharmacokinetics of codeine metabolism in nursing mothers and the rate of drug clearance in infants has indicated that longer-term (1–2 weeks) exposure via breastfeeding may be a more significant contributor to infant adverse events than maternal metabolizer status, particularly given that pregnancy-associated *CYP2D6* induction may result in higher metabolism than suggested by genotype alone. (18) Thus, even when the maternal genotype is known, the actual activity level of the CYP2D6 enzyme may be elevated and contribute to higher plasma morphine levels in the nursing mother, which may be passed into the breastmilk.

Additional factors, including further metabolism of morphine by UGT2B7, age-dependent expression levels of metabolic enzymes and clinical information not initially reported in one case of infant mortality (13) have been suggested as evidence against the likelihood that typical codeine use by a nursing mother can directly lead to infant mortality, even for UMs. (19) Codeine usage in the postnatal period with *CYP2D6* genotyping has been predicted to result in an incremental cost-effectiveness per adverse event averted (20), suggesting that when possible, determination of the mother's CYP2D6 metabolizer status is beneficial. See the section on Genetic Testing below for additional information on *CYP2D6* genotyping.

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

CYP2D6 Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and catalogued at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 4). (21)

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (for example, CYP2D6 PM). However, the activity score system is not standardized across clinical laboratories or CYP2D6 genotyping platforms. CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is defined by the sum of the 2 activity scores, which is usually in the range of 0 to 3.0: (22)

- An UM has an activity score greater than 2.25
- A normal metabolizer phenotype (NM) has an activity score of 1.25–2.25
- An intermediate metabolizer (IM) has an activity score of >0–<1.25
- A PM has an activity score of 0

Table 4. Activity Status of Selected CYP2D6 Alleles

Allele type	Activity score	*CYP2D6* alleles
Normal function	1.0	**1, *2, *27, *33*
Decreased function	0.25–0.5	**10, *17, *41, *49*
No function	0	**3, *4, *5, *6, *36*

For a comprehensive list of *CYP2D6* alleles, please See PharmVar.

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the NM phenotype. The *CYP2D6*2*, *27, and **33* alleles are also considered to have near-normal activity.

Other CYP2D6 alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (7, 23, 24, 25) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (26, 27, 28) (see Table 4). There are large inter-ethnic differences in the frequency of these alleles, with **3, *4, *5, *6*, and **41* being more common in individuals with European ancestry, **17* more common in Africans, and **10* more common in Asians. (29)

Allele Frequencies Vary between Populations

Among Asians and in individuals of Asian descent, only approximately 50% of *CYPD6* alleles are normal function, and the frequency of *CYP2D6* duplications is as high as 45%, although this may have been overestimated by not accounting for tandem hybrid alleles (for example, **36+*10*). (30) Other studies of a US population suggested less than 50% of alleles detected within Asian-descent individuals are normal-function alleles in a single copy, with 30% of alleles arising from structural variants (duplications or deletions). (31) Common no-function variants are *CYP2D6*36* and *CYP2D6*4.* (31) Both these alleles contain the variant "c.100C>T" (see Nomenclature table). (29, 30, 32, 33) The *CYP2D6*36* allele is the result of a gene conversion event with the pseudogene *CYP2D7* (34). This no-function allele is most commonly found in individuals of Asian ancestry (31).

Among Africans and African Americans, only approximately 50% of *CYP2D6* alleles are normal function. (23, 28, 29, 35) African Americans also have been found to have a higher frequency of no-function structural variants or decreased-function single-copy variant alleles versus Caucasian or Hispanic Americans. (31)

Middle Eastern countries show a great diversity in phenotypic and allelic distribution for *CYP2D6* (36), though on average, these individuals show a lower frequency of PM phenotypes (0.91%) and higher ultrarapid phenotypes (11.2%) than other ethnicities (Note: Oceania and Middle Eastern ethnicities were combined in this study). (34)

Among European countries, there is diversity of allelic distribution. Gene duplications were more common in the south-eastern countries (Greece, Turkey: 6%) and less common in north-western countries (Sweden and Denmark, <1%). Meanwhile, *CYP2D6*4* and *CYP2D6*5* alleles were more common in the north and less common in the south. (37) Worldwide *CYP2D6* genotype and phenotype frequencies have been catalogued and recently published (34).

CYP2D6 Phenotype

CYP2D6 Phenotype Frequencies Vary between Populations

Normal metabolizers: Between 43–67% of individuals have 2 normal-function alleles (**1* or **2*), or one normal-function allele and one decreased-function allele, resulting in a NM phenotype based on the CPIC/PharmGKB activity scores (38). These individuals are most likely to have a phenotypically normal response to codeine.

However, there is a large amount of variability in codeine response within individuals genotyped as NMs, and the causes of this variation, among individuals with the same diplotype, are unknown. (4)

Intermediate metabolizers: Between 10–44% of individuals are IMs—they have either 2 decreased-function alleles or one normal- or decreased-function and one no-function allele. (34, 38) These individuals may not respond as well to codeine because the metabolism of codeine to morphine is reduced. A study of a diverse US urban population of children found that roughly 8% of subjects were IMs, though this may be higher due to the broader range for IM activity scores. (39) Within the US, it has been observed that individuals of African or Asian descent were most likely to be classified as IM's (20–28% of population by ethnicity). (31) Similarly, PharmGKB reports that the highest frequency of IM activity scores are found in Sub-Saharan African and East Asian populations (38).

Poor metabolizers: Between 0.4–6.5% of individuals are PMs—they have 2 no-function alleles. (38, 40) In these individuals, codeine will provide little or no pain relief. Poor metabolizers are more commonly found in European Caucasians and their descendants. The no-function *CYP2D6*4* and **5* alleles largely account for the PM phenotype in these populations (24, 27, 41). It should be noted that the frequency of PMs can be much lower in certain populations including East Asian, Oceania and Middle Eastern (34). Studies of US multi-ethnic populations have estimated the prevalence of PMs to be between 1.5–5.7% (31, 39).

Ultrarapid metabolizers: Individuals who are UMs have an enzyme activity score greater than 2.5, often due increased copy number of the *CYP2D6* gene. The UM phenotype has been estimated to be present in 1–2% of individuals, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (3, 40). PharmGKB reports that the Oceanian population has the highest prevalence of UM phenotype (38). Ultrarapid metabolizers made up 9% of subjects in an urban multi-ethnic population with a large portion of Hispanic/Latino subjects (39). A larger study of US individuals predicted an UM phenotype in only 2.2% of individuals, regardless of ethnicity (31).

Pharmacologic Conversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and thus the metabolizer phenotype of any individual. Administration of multiple drugs, sometimes called polypharmacy or co-medication, can lead to a phenomenon called phenoconversion whereby an individual with one metabolizer genotype can have enzymatic activity of a different metabolizer group (higher or lower, depending on the medications). Enzymatic activity of CYP2D6 can be inhibited or reduced by medications including duloxetine, paroxetine, fluoxetine, bupropion, amiodarone (note: this is a weak inhibitor), and quinidine (3, 42, 43, 44). This can result in normal or IMs responding to medications as if they were PMs. Thus, co-medication with multiple CYP2D6 substrates may result in reduced metabolism of these drugs. In the case of codeine, this may present as reduced analgesic effect. In contrast, discontinuing a co-medication can increase the rate of CYP2D6 metabolism to the genotype predicted activity level.

Other Genes of Note

OPRM1

The mu opioid receptor is encoded by the *OPRM1* gene. The mu opioid receptor is a G-coupled protein receptor and is a key signal transducer for the desired analgesic effect of codeine. There are more than 200 known variant alleles of *OPRM1*, and some variants have been suggested to have a role in opioid response or predisposition to opioid use disorders (45, 46). However, CPIC's expert review found inconsistent evidence linking any of these alleles to post-operative dose requirements for some opioids and the effect on morphine dose adjustment was deemed not to be clinically actionable (4).

COMT

Catechol-o-methyltransferase (COMT) is an enzyme involved in the methylation and degradation of adrenaline, noradrenaline, and dopamine. This enzyme regulates the concentration of catecholamines and thus is a key regulator of the pain perception pathways (47). The variant rs4680 (p.Val158Met) in *COMT* has been suggested to result in decreased levels of methylation activity (4, 47). However, CPIC's review finds variable evidence associating this variant with analgesic response or opioid dose requirements and thus makes no recommendations based on *COMT* genotype (4).

Gene Family: *UGT*

Codeine and morphine are metabolized to inactive compounds by UDP-glucuronosyltransferase enzymes (UGT). The enzyme UGT2B7—along with other UGT enzymes—is involved in conversion of codeine to codeine-6-glucuronide and morphine to morphine- 3- and 6-glucuronide (48). Variation in UGT2B7 has been suggested to affect codeine and morphine metabolism, but the results have not been significantly reproducible between studies (49, 50, 51, 52, 53).

Linking Gene Variation with Treatment Response

Enzymatic activity of CYP2D6 directly correlates with the systemic dose of morphine following CYP2D6 conversion of codeine. Individuals with more than 2 copies of normal functioning alleles *(*1xN/*1*, **2xN/*1*, etc.) are at an elevated risk of codeine overdose symptoms. (3) These individuals are classified as UM, and are at elevated risk for toxicity. (54)

Each normal-function *CYP2D6* allele increases the rate of codeine metabolism, increasing the risk of an initial morphine "overdose", with more side effects. (55) Even low codeine doses can result in toxic levels of morphine in individuals with more than 2 normal-function alleles. (4) Several case reports have recorded the severe or life-threatening adverse effects that have occurred in individuals who were UMs and were treated with standard doses of codeine. (56, 57)

Multiple reports of toxic or fatal events have occurred in pediatric individuals who were later found to have UM genotypes (reviewed by (58)). Analysis of the Mayo Clinic RIGHT Protocol study suggested that UM individuals were least likely to have poor pain control but had the highest rates of adverse reactions among the various metabolizer phenotypes. (59)

In contrast, individuals with alleles that encode the no-function CYP2D6 enzyme will poorly metabolize codeine and thus are unlikely to achieve the intended therapeutic analgesic effect from codeine. Aptly classified as PMs, these individuals have a higher rate of poor pain control but lower rates of adverse reactions in the RIGHT protocol study. (59)

Additional studies of the RIGHT protocol found that both PMs and UMs experienced a higher rate of adverse effects and poor pain control from opioid prescription as compared with normal or IMs. The higher adverse effects were due to the rapid codeine metabolism in UM individuals and poor pain control due to the reduced activation of codeine to morphine in the PM population. The mechanism whereby PMs experienced higher rates of adverse events such as nausea and vomiting were not discussed. Co-medication with CYP2D6 inhibitors was also noted to affect the frequency of adverse events in all phenotypes studied. (60)

The CYP2D6 IMs may also experience reduced effectiveness in pain management of codeine due to lower rates of conversion to morphine. A dose increase can be attempted for IM, but in some cases the individual may require an alternative medication that is not primarily metabolized by CYP2D6. (5)

The CYP2D6 Gene Interactions with Medications Used for Additional Indications

The CYP family of enzymes is involved in metabolism of many substances and CYP2D6 especially has been implicated in altered pharmacologic responses for many compounds. The drugs can be categorized into many different classes:

- Antipsychotics—for example, aripiprazole, risperidone, thioridazine and—to a lesser extent—clozapine is metabolized by CYP2D6. According to the FDA, aripiprazole dosage should be reduced for PMs and thioridazine is contraindicated for individuals who are known to have reduced CYP2D6 activity due to increased risk of potentially fatal side effects. Ultrarapid metabolizers may have a decreased plasma concentration of risperidone.
- Tricyclic antidepressants—for example, amitriptyline, and imipramine may require dosage adjustments, potentially guided by therapeutic drug monitoring, to achieve the desired therapeutic range in ultrarapid or PMs. Ultimately, tricyclic antidepressants may be ineffective in CYP2D6 UMs
- Serotonin and norepinephrine reuptake inhibitors—for example atomoxetine and venlafaxine may have reduced efficacy in UMs at standard doses while PMs are at risk of elevated plasma concentrations for both medications. The DPWG advises against use of venlafaxine in CYP2D6 poor and IMs.
- Cardiovascular dysfunction—for example, carvedilol, metoprolol, and propafenone are all metabolized by CYP2D6 and PMs will have higher plasma concentrations of these medications compared with NMs resulting in potentially undesired side effects or (in the case of metoprolol) extensive slowing of the heart rate.
- Anti-cancer medications—for example, tamoxifen is activated by CYP2D6 and intermediate or PMs may have reduced benefit from tamoxifen therapy.
- Various therapies for genetic disorders—for example eliglustat used in the treatment of Gaucher disease, and deutetrabenazine used in the treatment of Huntington disease. For both medications, reduced doses are recommended for CYP2D6 PMs and UMs may not achieve adequate concentrations of eliglustat. Before initiation of eliglustat therapy, CYP2D6 genotyping is required.

It is important to note that *CYP2D6* is the most common biomarker in drug responses for FDA drug labels, the list provided here is by no means exhaustive. Additional information on gene-drug interactions for *CYP2D6* are available from PharmGKB, CPIC and the FDA (search for "CYP2D6").

Genetic Testing

Genetic testing is available for many (~30) of the variant *CYP2D6* alleles. Usually, an individual's result is reported as a diplotype, which includes one maternal and one paternal allele, for example, *CYP2D6 *1/*2*. When individuals have more than 2 copies of the *CYP2D6*, the copies of the allele are denoted by an "xN", for example, *CYP2D6*1/*2x2*. Some laboratories also use the notation of DUP to indicate an increase in copy number, but the report does not specify the number of duplications nor the allele that has been duplicated.

Genetic tests for codeine response and the *CYP2D6* gene can be found on the NIH Genetic Testing Registry. The available CYP2D6 tests include targeted single-gene tests as well as multi-gene panels and exome- or genome-wide sequencing tests.

The test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be confirmed by checking the diplotype and calculating the CYP2D6 activity score, as described in the "*CYP2D6* Alleles" section above.

Variants in other genes, such as *COMT*, *ABCB1*, *UGT2B7* and *OPRM1*, may also influence an individual's response to codeine. However, evidence is lacking on whether genetic testing for these variants will aid optimum codeine dosing. (10, 61, 62, 63)

In 2019, the US Department of Veterans Health Administration (VHA) Clinical Pharmacogenetics Subcommittee recommended that prescribers in the VHA consider *CYP2D6* genotyping before codeine use. Factors supporting this recommendation included the actionability of the genetic test result, guiding prescribers to different codeine dosing or alternate analgesic agents. (64)

Therapeutic Recommendations based on Genotype

This section contains excerpted [1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

Life-threatening respiratory depression and death have occurred in children who received codeine. Codeine is subject to variability in metabolism based upon CYP2D6 genotype (described below), which can lead to an increased exposure to the active metabolite morphine. Based upon post-marketing reports, children younger than 12 years old appear to be more susceptible to the respiratory depressant effects of codeine, particularly if there are risk factors for respiratory depression. For example, many reported cases of death occurred in the post-operative period following tonsillectomy and/or adenoidectomy, and many of the children had evidence of being ultrarapid metabolizers of codeine. Furthermore, children with obstructive sleep apnea who are treated with codeine for post-tonsillectomy and/or adenoidectomy pain may be particularly sensitive to its respiratory depressant effect.

Some individuals may be ultrarapid metabolizers because of a specific *CYP2D6* genotype (e.g., gene duplications denoted as **1/*1xN* or **1/*2xN*). The prevalence of this CYP2D6 phenotype varies widely and has been estimated at 1 to 10% for Whites (European, North American), 3 to 4% for Blacks (African Americans), 1 to 2% for East Asians (Chinese, Japanese, Korean), and may be greater than 10% in certain racial/ethnic groups (i.e., Oceanian, Northern African, Middle Eastern, Ashkenazi Jews, Puerto Rican). These individuals convert codeine into its active metabolite, morphine, more rapidly and completely than other people. This rapid conversion results in higher than expected serum morphine levels. Even at labeled dosage regimens, individuals who are ultrarapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose (such as extreme sleepiness, confusion, or shallow breathing). Therefore, individuals who are ultrarapid metabolizers should not use codeine sulfate tablets.

Please review the complete therapeutic recommendations that are located here: (3)

2020 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

For CYP2D6 normal metabolizers (i.e. CYP2D6 activity score 1.25 to 2.25), a label recommended age- or weight-specific starting dose of codeine or tramadol, as recommended in the product label, is warranted. A label recommended starting dosing is also recommended for intermediate metabolizers (i.e. activity score of 0.25 to 1); these patients should be monitored closely for less than optimal response and should be offered an alternative analgesic if warranted. For CYP2D6 poor metabolizers (i.e. activity score of 0), current evidence supports the avoidance of codeine and tramadol and the use of an alternative analgesics due to the likelihood of suboptimal

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

or lack of effect. There is insufficient evidence in the literature to recommend a higher dose of codeine or tramadol in poor metabolizers, especially considering the evidence that some adverse events do not differ between poor and normal metabolizers (19). For CYP2D6 ultrarapid metabolizers (i.e. activity score of >2.25), codeine or tramadol should not be used, in order to avoid the risk of severe toxicity with label-recommended dosing. Non-opioid analgesics and if needed, other opioids that are not affected by CYP2D6 phenotype, are potential alternatives for use in CYP2D6 poor and ultrarapid metabolizers based on the type, severity and chronicity of the pain being treated.

Please review the complete therapeutic recommendations that are located here: (4).

2017 Summary of Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 Intermediate Metabolizers

For COUGH:

1 no action required

For PAIN:

It is not possible to offer adequately substantiated advice for dose adjustment based on the limited available literature for this phenotype.

1. be alert to a reduced effectiveness
2. in the case of inadequate effectiveness:
    1. try a dose increase
    2. if this does not work: choose an alternative
        - Do not select tramadol, as this is also metabolised by CYP2D6
        - Morphine is not metabolised by CYP2D6.
        - Oxycodone is metabolised by CYP2D6 to a limited extent, but this does not result in differences in analgesia in patients.
    3. if no alternative is selected: advise the patient to report inadequate analgesia

Poor Metabolizers

For COUGH:

1 no action required

For PAIN:

It is not possible to offer adequately substantiated advice for dose adjustment based on the limited available literature for this phenotype.

1 choose an alternative

Do not select tramadol, as this is also metabolised by CYP2D6

- Morphine is not metabolised by CYP2D6.
- Oxycodone is metabolised by CYP2D6 to a limited extent, but this does not result in differences in analgesia in patients.

2 if an alternative is not an option: advise the patient to report inadequate analgesia.

Ultrarapid Metabolizers

DOSES HIGHER THAN 20 mg every 6 hours for adults and 10 mg every 6 hours for children aged 12 years or older AND/OR ADDITIONAL RISK FACTORS, such as co-medication with CYP3A4 inhibitors and/or reduced kidney function:

Codeine is contra-indicated

- if possible, select an alternative
  - For PAIN: do not select tramadol, as this is also metabolised by CYP2D6.

Morphine is not metabolised by CYP2D6. Oxycodone is metabolised by CYP2D6 to a limited extent, but this does not result in differences in side effects in patients.

- For COUGH: noscapine is not metabolised by CYP2D6.

DOSES LOWER THAN OR EQUAL TO 20 mg every 6 hours for adults and 10 mg every 6 hours for children aged 12 years or older AND NO ADDITIONAL RISK FACTORS, such as co-medication with CYP3A4 inhibitors and/or reduced kidney function:

- no action required

Please review the complete therapeutic recommendations that are located here: (5)

2013 Clinical practice Guideline from the "Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Clinical Recommendations Group: *CYP2D6* genotyping for safe and efficacious codeine therapy" are located here: https://www.ncbi.nlm.nih.gov/pubmed/24214521 (65).

Nomenclature

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
*CYP2D6*3*	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

Nomenclature of Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*36*[3]	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2851C>T is also referred to as 2938C>T
[3] *CYP2D6*36* is a gene conversion with *CYP2D7;* variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (66).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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First edition:

The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org

The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

Version History

To view an earlier version of this summary from 8 March 2016, please click here.

To view an earlier version of this summary from 18 March 2013, please click here.

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Dabrafenib Therapy and *BRAF* Genotype

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Introduction

Dabrafenib (brand name Tafinlar) is a kinase inhibitor used in the treatment of individuals with unresectable or metastatic melanoma, metastatic non-small cell lung cancer (NSCLC), locally advanced or metastatic anaplastic thyroid cancer (ATC), pediatric low-grade glioma (LGG), and other unresectable or metastatic solid tumors with specific *BRAF* variants. Dabrafenib can be used as a single agent to treat melanoma with the *BRAF* valine 600 to glutamic acid (V600E) variant or in combination with the MEK inhibitor trametinib to treat multiple tumor types with *BRAF* V600E or V600K variants. (1)

The BRAF protein is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. Functionally, BRAF regulates essential cell processes such as cell growth, division, differentiation, and apoptosis. The gene *BRAF* is also a proto-oncogene—when mutated, it transforms normal cells into cancerous cells.

Variation in the kinase domain of BRAF is associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase and causes cell proliferation in the absence of growth factors that would usually be needed. The V600E variant is detected in approximately 50% of melanomas, 25% of ATC, 2% of NSCLC, and 20% of pediatric LGGs (2, 3, 4, 5, 6, 7, 8).

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed using an FDA-approved test before starting treatment with dabrafenib. Dabrafenib is not indicated for the treatment of individuals with wild-type *BRAF* tumors, or the treatment of colorectal cancer due to intrinsic resistance to *BRAF* inhibitor monotherapy. (1)

The label also states that individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency should be monitored for signs of hemolytic anemia while taking dabrafenib (1). However, it is important to note that an independent literature review by the Clinical Pharmacogenetics Implementation Consortium found no publications to support or refute this risk and thus issued no guidance for G6PD deficiency and dabrafenib therapy (9).

Drug: Dabrafenib

Dabrafenib is a BRAF kinase inhibitor indicated for the treatment of individuals with unresectable or metastatic solid tumors bearing *BRAF* V600 mutations, including melanoma, ATC, NSCLC, and LGGs. The *BRAF* V600 mutated proteins can signal as catalytically active monomers, unlike wild-type RAFs that signal as obligatory dimers. The binding of dabrafenib to *BRAF* V600 monomers leads to decreased signaling through the MAPK pathway and reduced transcription of genes involved in various cellular responses. Resistance to dabrafenib can arise due to somatic alterations that lead to the formation of drug-resistant BRAF dimers, such as somatic variation in the *RAS* genes. Combining dabrafenib with a MEK inhibitor, such as trametinib, has been shown to extend survival (10, 11). Other medications that target MAPK signaling include vemurafenib, another BRAF kinase inhibitor, and cobimetinib, an allosteric inhibitor of the downstream kinases MEK1 and MEK2.

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Dabrafenib can be used as a single agent to treat melanoma with the *BRAF* V600E variant or in combination with trametinib to treat tumors with *BRAF* V600E or V600K variants (1). Dabrafenib, with or without trametinib, has also been investigated for treating *BRAF* V600 mutation-positive pediatric Langerhans cell histiocytosis, with phase I and II trials suggesting clinical efficacy in this population (12). Additional tumor types studied for dabrafenib plus trametinib response in the Rare Oncology Agnostic Research (ROAR) study included *BRAF* V600E positive biliary tract cancer, adenocarcinoma of the small intestine, hairy cell leukemia, and multiple myeloma (13). The ROAR results suggest that many cancer types are responsive to this comedication strategy. Notably, colorectal cancer is not an indication for dabrafenib monotherapy based on intrinsic resistance to BRAF inhibition, nor should dabrafenib be used in tumors that do not have *BRAF* mutation (1).

Members of the cytochrome P450 (CYP) family, CYP3A4 and CYP2C8, metabolize dabrafenib. Dabrafenib also induces an increase in CYP3A4 expression in a dose-dependent manner (1, 14). As a result, steady-state plasma concentration for dabrafenib is reached after 14 days of therapy (14). Metabolism by CYP3A4 and 2C8 generates 3 major metabolites, among which hydroxy-dabrafenib is thought to be an active compound, contributing to the inhibition of MAPK signaling (14). Dabrafenib is typically administered twice-daily, 150 mg per adult dose, while the pediatric dosage depends on body weight and formulation (capsule or tablet for oral suspension) (1). Taking the medication without food (either one hour before or 2 hours after a meal) significantly increases bioavailability compared to taking the medicine with a high-fat meal (1, 14).

Skin cancer is the most common of all cancers, and while melanoma only accounts for approximately 1% of cases, it is responsible for most deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.6% for Caucasian individuals, 0.6% for Hispanic individuals, and 0.1% for Black individuals (15). Most cases of malignant melanoma are diagnosed at an early stage when the tumor is localized, and surgical excision can be curative. However, the 5-year survival rate drops from more than 99% for localized disease to only 32% for individuals with metastatic (distant) disease (16). The frequency of *BRAF* V600 driver mutation in melanoma is 40–50% (17).

Anaplastic thyroid cancer has a 39% 5-year survival rate for localized tumors, but this drops to 4% for distant tumors (18). Pathogenic variation in *BRAF* occurs in 20–50% of all individuals with ATC (19). Although ATC diagnosis accounts for only 1–2% of all thyroid malignancies, it represents 15–20% of mortality (20).

Driver mutations in *BRAF* are uncommon in NSCLC, occurring in 3.4% of individuals in a cohort from the United Kingdom (5) and 1–2% of lung adenocarcinomas (21). Among lung cancer diagnoses, NSCLC accounts for 80–85% of all cases and has a 5-year relative survival rate of only 9% for distant (metastatic) disease (22, 23).

Gliomas are the most common primary brain tumor, diagnosed at a frequency of approximately 6 per 100,000 people in the US each year. However, only 5–15% of LGGs have *BRAF* V600 driver mutations (24, 25). Low-grade gliomas represent 30% of all childhood brain tumors, but *BRAF* V600E is associated with poor survival and an overall response rate (ORR) of less than 23% to conventional chemotherapy (26).

The most common adverse events associated with dabrafenib are skin lesions (benign and malignant). Cutaneous and non-cutaneous malignancies can occur during dabrafenib therapy, though the frequency is reduced when combined with trametinib. The drug label advises carrying out a dermatological evaluation before initiating dabrafenib therapy, every 2 months during therapy, and for up to 6 months following discontinuation. (1)

Skin reactions to BRAF inhibitors, such as dabrafenib, can include rashes, sarcoid-like, and granulomatous reactions (27, 28, 29). Occasionally, sarcoid-like reactions can affect other organ systems, and lesions in the pulmonary or lymphatic tissue should be assessed to determine if they are a progression of the primary cancer or a reaction to the medication (29). Dose reduction of dabrafenib/trametinib therapy or the addition of corticosteroids is often sufficient to resolve the sarcoid or granulomatous reactions when treating melanoma (28,

29). However, discontinuation of therapy for a few weeks may be necessary if intolerable grade 2 or grade 3–4 reactions occur during NSCLC treatment (30). One reported case of severe cutaneous adverse reaction to dabrafenib/trametinib dual therapy ultimately required cessation of therapy and administration of tumor-necrosis-factor-alpha blocking therapy. However, it is unclear if prior chemotherapy may have heightened the severity of the reaction. (31).

Less common but serious side effects to watch for during dabrafenib therapy include hemorrhage, cardiac toxicity, ocular effects, hyperglycemia, and hemophagocytic lymphohistiocytosis. Any grade 4 or persistent grade 3 hemorrhage event is an indication to discontinue dabrafenib therapy. (1) Cardiomyopathy characterized as a decrease in left ventricular ejection fraction of 10% or more from baseline has been observed in 6% of adult individuals during dabrafenib therapy (1). Within the context of NSCLC therapies, dabrafenib and trametinib were associated with increased odds of heart failure as compared to other targeted therapies (reporting odds ratio (ROR)) of 2–2.4) (32). Among ocular side effects, 1–2% of study participants had uveitis presenting with changes in vision, photophobia, or eye pain (1), and pharmacovigilance data from Japan reports a ROR of 6.03 for retinal disorders with dabrafenib therapy (33). Management of ocular inflammation may require the administration of topical steroids or, in severe cases, discontinuation of medication (34).

Other common side effects from dabrafenib therapy include headache, pyrexia, arthralgia, papilloma, and palmar-plantar erythrodysesthesia syndrome (1). Pyrexia is a common reaction to dabrafenib and trametinib therapy used for multiple types of *BRAF* V600 mutated cancers, with a reported frequency of approximately 50% in multiple studies (1, 35, 36), and a temporary dose interruption has been shown to be an effective management strategy (37).

Dabrafenib interacts in vitro with many proteins involved in drug metabolism, creating a possibility of drug-drug interactions. Due to its role as a CYP3A4 substrate and inducer, dabrafenib can influence the metabolism of other CYP3A4 substrates and be the victim of an interaction that significantly alters CYP3A4 activity. Increased CYP3A4 expression by a strong inducer (such as rifampin) can decrease an individual's exposure to dabrafenib, while inhibition (by ketoconazole, for example) of CYP3A4 will increase that individual's exposure to dabrafenib. Conversely, induction of CYP3A4 by dabrafenib resulted in a 65% decrease in exposure to a single dose of midazolam (a CYP3A4 substrate) (1). Dabrafenib can also inhibit CYP2C8, CYP2C9, and CYP3A5 (38). The UDP-glucuronosyltransferase (UGT) family of enzymes that contribute to drug metabolism via glucuronidation may also be inhibited by dabrafenib and lead to drug-drug interactions, particularly UGT1A1, 1A7, 1A8, and 1A9 (39). Drug-transporting enzymes also interact with dabrafenib; P-glycoprotein (encoded by the *ABCB1* gene) and breast cancer resistance protein (encoded by the *ABCG2* gene) both transport dabrafenib and can be inhibited by this interaction (1, 38).

Pregnant women should not take dabrafenib due to likely fetal harm caused by the medication. This warning is based on animal studies, as there is insufficient data on pregnant women to assess the risk of human harm (1). Similarly, there is no clinical data on the use of dabrafenib while breastfeeding. It is predicted that the amount of medication passed to breast milk would be low, given the high proportion bound to plasma proteins (1, 40). The manufacturer recommends discontinuing breastfeeding while taking the medication and for 2 weeks following the last dose (1). Both males and females of reproductive potential require counseling to use contraceptives while taking the medication and for 2 weeks after the last dose. Females should use a non-hormonal contraceptive due to the impaired efficacy of hormonal contraceptives by dabrafenib (1).

Dabrafenib has not been approved for use as a single agent in pediatric individuals but can be used for metastatic solid tumors in individuals 6 years or older or for LGG in individuals one year and older (1). Pediatric use requires weight-guided dosing that also accounts for the specific formulation of the medication; see drug labeling for more information (1). No dose adjustments are recommended for geriatric individuals, regardless of the tumor type treated with dabrafenib. However, this population may be at higher risk of specific side effects such as peripheral edema or anorexia (1). Similarly, there are no specific recommendations for dose adjustment

in the context of hepatic impairment, though moderate to severe impairment may result in increased exposure to the medication and its metabolites (1).

Gene: *BRAF*

The RAF protein family are intracellular kinases within the MAPK signaling pathway. The RAF family has 3 members: ARAF, BRAF, and CRAF (41). The *RAF* and *RAS* genes are proto-oncogenes. Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (42).

Germline variations in *BRAF*, as well as other components of the MAPK signaling pathway, are associated with congenital anomalies, such as cardiofaciocutaneous syndrome, characterized by heart defects, intellectual disabilities, and distinctive facial dysmorphology. Somatic *BRAF* mutations are also associated with several malignancies, including colorectal carcinoma, lung adenocarcinoma, mucinous adenoma, and malignant melanoma.

Variations in *BRAF* are detectable in approximately 50% of malignant melanomas and drive the progression of the disease (2, 3). The *BRAF* variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 of the coding portion of the gene and results in the substitution of valine for glutamate at codon 600 in the expressed protein; the protein-level variation (V600E) is the commonly used description for this change. The variant BRAF protein kinase is constitutively active and a highly potent oncogene, increasing kinase activity by as much as 500-fold compared with the wild-type (43). The second most common *BRAF* variant is V600K. Substitutions at other sites are rarer (44, 45). Several drugs are under development to target *BRAF* variants, and so far, 3 drugs are FDA-approved: vemurafenib, dabrafenib, and encorafenib (46).

The signaling cascade mediated by BRAF is a MAPK pathway that transmits an extracellular signal to the nucleus to influence gene expression promoting cell proliferation and survival. Mitogen ligands activate cell surface receptors that then function as a docking site for a protein complex that includes RAS and a guanine nucleotide exchange factor, leading to the activation of RAS. Activated RAS proteins can then interact with BRAF, leading to dimerization and phosphorylation of the BRAF proteins. Activated BRAF then phosphorylates MEK proteins, activating extracellular signal-regulated kinase (ERK) proteins via phosphorylation. Active ERK has several substrates, including transcription factors that promote gene expression in proliferation and survival. The V600 mutation in BRAF enables the protein to function in a constitutively active manner and as a single protein rather than as a dimer. The mutated BRAF, therefore, does not require RAS activation for its activation. Mutation of RAS that promotes dimerization in the absence of upstream activation can also lead to signaling cascade activation but rarely cooccur with *BRAF* V600 mutations in BRAF inhibitor treatment-naïve tumors. (47)

Linking *BRAF* Genetic Variation with Treatment Response

Dabrafenib increased progression-free survival (PFS), compared to cytotoxic chemotherapy (for example, dacarbazine), in individuals with advanced melanoma with the *BRAF* V600E variant (48, 49). The phase 3 COMBI-v trial for individuals with melanoma with a V600E variant found that the combination of dabrafenib plus trametinib led to a higher 3-year overall survival (OS) rate and PFS, compared to vemurafenib monotherapy (OS: 45% versus 32%, PFS: 25% versus 11%). Individuals taking the combination of dabrafenib plus trametinib experienced a decreased incidence of cutaneous squamous cell carcinoma (50). The COMBI-d trial also reported similar improved outcomes when comparing dabrafenib and trametinib to dabrafenib monotherapy (51, 52).

However, dabrafenib may not be the best first-line treatment choice for all individuals. The DREAMseq trial (EA6134, ClinicalTrials.gov identifier NCT02224781) saw a higher rate of OS in individuals with *BRAF* V600 mutated metastatic melanoma in response to treatment with nivolumab/ipilimumab as compared to dabrafenib/trametinib. As the rates of grade 3 or higher adverse reactions were similar between both treatment arms, the study authors recommend nivolumab/ipilimumab in treatment naïve metastatic melanoma with *BRAF*V600 mutation, followed by dabrafenib/trametinib if there is disease progression. (53) Dabrafenib/trametinib may be used first in symptomatic melanoma individuals who need a rapid response to therapy (54).

A clinical trial for ATC with *BRAF* V600E mutation reported an ORR of 56% when treated with dabrafenib and trametinib (55). A similar response was observed for NSCLC in clinical trials with both treatment naïve or previously treated NSCLC; subjects showed an ORR of over 60% to dabrafenib and trametinib combination therapy, leading to FDA approval in 2017 (56). The FDA-approved dabrafenib and trametinib combination therapy for pediatric LGG with *BRAF* V600E mutation in March of 2023, following a trial that showed a significant increase in ORR compared to carboplatin and vincristine dual therapy (26).

Dabrafenib and other BRAF inhibitors have also demonstrated responses in individuals with rare *BRAF* V600 variants (V600R, V600D) (57, 58, 59). As dabrafenib and other BRAF kinase inhibitors block signaling from BRAF monomers, they are selectively effective against *BRAF* V600 mutations and not effective against atypical (non-V600) variants at clinically attainable doses (for example, L597, K601) (60). In vitro experiments with BRAF inhibitors, such as dabrafenib, have been found to cause a paradoxical activation of signaling pathways and proliferation in *BRAF* wild-type cells. Therefore, clinicians should only use dabrafenib after confirming the presence of BRAF V600 variants in tumor specimens with an FDA-approved test (1). The FDA also recommends permanently discontinuing dabrafenib use in individuals who develop RAS mutation-positive non-cutaneous malignancies.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for *BRAF*.

The FDA-approved label for dabrafenib states that the presence of *BRAF* mutation in tumor specimens (V600E for dabrafenib monotherapy; V600E or V600K for dabrafenib plus trametinib) should be confirmed using an FDA-approved test before starting treatment with dabrafenib. The label also says that dabrafenib is not indicated for the treatment of individuals with wild-type *BRAF* melanoma or the treatment of colorectal cancer.

Cancer-specific guidelines on testing, treatment selection, and other best practices for clinical care are available from various clinical experts globally. Medical societies such as the European Society for Medical Oncology, the American Society of Clinical Oncology, the National Comprehensive Cancer Network, and other specialized groups have guidelines available through PubMed or the society's webpage.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

BRAF V600E Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated as a single agent for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test.

BRAF V600E or V600K Mutation-Positive Unresectable or Metastatic Melanoma: Dabrafenib is indicated, in combination with trametinib, for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test.

Adjuvant Treatment of BRAF V600E or V600K Mutation-Positive Melanoma

Dabrafenib is indicated, in combination with trametinib, for the adjuvant treatment of patients with melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test, and involvement of lymph node(s), following complete resection.

BRAF V600E Mutation-Positive Metastatic NSCLC

Dabrafenib is indicated, in combination with trametinib, for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) with BRAF V600E mutation as detected by an FDA-approved test

BRAF V600E Mutation-Positive Locally Advanced or Metastatic Anaplastic Thyroid Cancer

Dabrafenib is indicated, in combination with trametinib, for the treatment of patients with locally advanced or metastatic anaplastic thyroid cancer (ATC) with BRAF V600E mutation and with no satisfactory locoregional treatment options

BRAF V600E Mutation-Positive Unresectable or Metastatic Solid Tumors

Dabrafenib is indicated, in combination with trametinib, for the treatment of adult and pediatric patients 6 years of age and older with unresectable or metastatic solid tumors with BRAF V600E mutation who have progressed following prior treatment and have no satisfactory alternative treatment options ... This indication is approved under accelerated approval based on overall response rate (ORR) and duration of response (DoR) … Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial(s).

BRAF V600E Mutation-Positive Low-Grade Glioma

Dabrafenib is indicated, in combination with trametinib, for the treatment of pediatric patients 1 year of age and older with low-grade glioma (LGG) with a BRAF V600E mutation who require systemic therapy.

Limitation of Use: Dabrafenib is not indicated for treatment of patients with colorectal cancer because of known intrinsic resistance to BRAF inhibition … Dabrafenib is not indicated for treatment of patients with wild-type BRAF solid tumors.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with dabrafenib as a single agent. Confirm the presence of BRAF V600E or V600K mutation in tumor specimens prior to initiation of treatment with dabrafenib and trametinib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at: http://www.fda.gov/CompanionDiagnostics.

[…]

Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency

Advise patients that dabrafenib may cause hemolytic anemia in patients with G6PD deficiency. Advise patients with known G6PD deficiency to contact their healthcare provider to report signs or symptoms of anemia or hemolysis.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Selected *BRAF* Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.6:c.1799T>A	NP_004324.2:p.Val600Glu	rs113488022
V600K	p.Val600Lys	NM_004333.6:c.1798_1799delinsAA	NP_004324.2:p.Val600Lys	rs121913227
V600R	p.Val600Arg	NM_004333.6:c.1798_1799delinsAG	NP_004324.2:p.Val600Arg	rs121913227
V600D	p.Val600Asp	NM_004333.6:c.1799_1800delinsAT	NP_004324.2:p.Val600Asp	rs121913377

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Deutetrabenazine Therapy and *CYP2D6* Genotype

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Introduction

Deutetrabenazine (brand name Austedo) is used to treat chorea associated with Huntington disease (HD) and tardive dyskinesia (TD). Both HD and TD are types of involuntary movement disorders.

The recommended starting dose is 6 mg once daily for individuals with HD and 12 mg per day (6 mg twice daily) for individuals with TD. The maximum recommended daily dosage for both conditions is 48 mg (24 mg, twice daily).

The active metabolites of deutetrabenazine are reversible inhibitors of vesicular monoamine transporter 2 (VMAT2). The VMAT2 protein transports the uptake of monoamines, such as dopamine, into the nerve terminal. The inhibition of VMAT2 leads to a depletion of pre-synaptic dopamine and reduces the amount of dopamine realized when that neuron fires. This is thought to lead to fewer abnormal, involuntary movements.

The CYP2D6 enzyme converts the active metabolites of deutetrabenazine to minor, reduced activity metabolites. Individuals who have no CYP2D6 activity ("CYP2D6 poor metabolizers") are likely to have a 3- to 4-fold increased exposure to active metabolites, compared with normal metabolizers, following the recommended standard doses of deutetrabenazine.

The 2018 FDA-approved drug label for deutetrabenazine states that the daily dose of deutetrabenazine should not exceed 36 mg (maximum single dose of 18 mg) for individuals who are CYP2D6 poor metabolizers or concurrently taking a strong CYP2D6 inhibitor (e.g., quinidine, antidepressants such as paroxetine, fluoxetine, and bupropion) (Table 1).

In addition, the drug label cautions that tetrabenazine, a closely related VMAT2 inhibitor, causes QT prolongation. Therefore, a clinically relevant QT prolongation may occur in some individuals treated with deutetrabenazine who are CYP2D6 poor metabolizers or are co-administered a strong CYP2D6 inhibitor (1).

Table 1. The FDA (2017) Deutetrabenazine Dosage and Administration.

Disorder	Maximum dose of deutetrabenazine	
	Standard recommendation	Recommendation for CYP2D6 poor metabolizers
Chorea association with Huntington disease	48 mg (24 mg twice daily)	36 mg per day (18 mg twice daily)
Tardive dyskinesia	48 mg (24 mg twice daily)	36 mg per day (18 mg twice daily)

Please see Therapeutic Recommendations based on Genotype for more information from the FDA.
This FDA table is adapted from (1).

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Drug: Deutetrabenazine

Deutetrabenazine (brand name Austedo) is used in the management of involuntary movement disorders: chorea associated with HD, and TD in adults. The use of deutetrabenazine is also being investigated for the management of tics associated with Tourette syndrome (2, 3).

Deutetrabenazine belongs to the drug class of VMAT2 inhibitors. These agents act centrally by depleting dopamine storage in presynaptic vesicles in the central nervous system. This reduces the amount of dopamine released when neurons fire, which may result in fewer abnormal, involuntary movements. Other drugs in this class include valbenazine (brand name Ingrezza) and tetrabenazine (brand name Xenozine).

The recommended initial dose of deutetrabenazine is 6 mg/day for chorea associated with HD, and 12 mg/day for TD. The dose should be titrated up by weekly increments of 6 mg/day, based on tolerability and the reduction of chorea or TD, until the individual optimal and tolerated dose is established in both conditions. The standard maximum dose is 48 mg/day (24 mg, twice daily) (1). However, in the Aim to Reduce Movements in Tardive Dyskinesia study, the maximum allowable dose was 72 mg/day (4).

Tetrabenazine was the first drug to be licensed for the treatment of chorea associated with HD, but its use was limited by frequent dosing (at least 3 times daily) and dose-related adverse events (e.g., somnolence, anxiety, and depression).

Deutetrabenazine is a modified (deuterated) form of tetrabenazine. In deuterated drugs, key hydrogen atoms have been replaced with the heavier hydrogen isotope, deuterium, while preserving pharmacological activity. Because deuterium-carbon bonds are stronger than hydrogen-carbon bonds, these drugs tend to be more resistant than non-deuterated drugs to metabolizing enzymes (e.g., CYP2D6), resulting in a longer half-life that allows less frequent dosing. In addition, peak drug concentrations are reduced, potentially reducing any side effects that are associated with peak concentrations. The maximum daily dose of tetrabenazine is 100 mg, compared with 48 mg for deutetrabenazine; and deutetrabenazine is dosed less frequently (twice daily, compared with at least 3 times daily for tetrabenazine) (5-11).

The exact mechanism of action of deutetrabenazine is unknown, but it is thought to involve the reversible depletion of monoamines (such as dopamine, serotonin, norepinephrine, and histamine) from nerve terminals. Deutetrabenazine's active alpha and beta metabolites (α-HTBZ and β-HTBZ) are reversible inhibitors of VMAT2, a transporter protein that is localized in the presynaptic neurons in the central nervous system.

Dopamine and other monoamine transmitters are transported by VMAT2 from the neuronal cell cytoplasm into the neuronal synaptic vesicle. Dopamine that is not taken up into the presynaptic vesicle as a result of VMAT2 blockade by deutetrabenazine is rapidly degraded by monoamine oxidase, resulting in presynaptic depletion of dopamine (7, 11-14).

Huntington Disease

Huntington disease is primarily an adult-onset hereditary autosomal dominant progressive neurodegenerative disorder, which is characterized by involuntary movements ("chorea"), psychiatric symptoms, and cognitive dysfunction that can lead to dementia. More than 35,000 people in the US have HD. There is a juvenile form of HD that is characterized by onset of signs and symptoms before 20 years of age.

The prevalence of HD varies across regions of the world. For individuals of European ancestry, the prevalence of HD is estimated to be 3–10 per 100,000. Individuals from the US, Europe, and Australia generally fall within this range. Huntington disease is less common in Japan, China, Korea, Finland, Africa, and South Africa, with estimated prevalence values ranging from 0.1–2 per 100,000 (15, 16).

In the US, more than 35,000 people have HD, and, in Caucasians, the prevalence of HD is estimated to be 4.8 per 100,000. Interestingly, the prevalence of HD is higher for Black Americans (6.4 per 100,000). This suggests that HD is far more common in Blacks living in the US compared with Blacks living in Africa. For example, for Blacks living in South Africa the prevalence of HD is 0.02 per 100,000, and for those living in Zimbabwe it is 1.00 per 100,000 (15).

Huntington disease is caused by an unstable expanded repeat of the cytosine-adenine-guanine (CAG) trinucleotide coding for polyglutamine in the *huntingtin* (*HTT*) gene on chromosome 4. A repeat of 39 or more repeats invariably causes HD. The pathogenesis of HD is not understood, but the mutated huntingtin protein is thought to become toxic, which is accompanied by selective loss of neurons in the caudate and putamen (striatum).

Electron microscopy reveals aggregates of mutated huntingtin protein, which may form because the mutated protein is less soluble, or because it is likelier to form bonds with other proteins, or both of these mechanisms may contribute. The MRI scans of the brain reveal early progressive atrophy of the striatum, with the caudate often more severely affected than the putamen.

Chorea is a defining motor symptom, occurring in approximately 90% of individuals with HD, and is characterized by sudden, random, jerky, involuntary movements that can affect any part of the body. Initially, these movements may be mild and misinterpreted as restlessness, but as HD progresses, the movements increasingly interfere with daily functioning, causing social isolation, and increasing the risk of injury from instability and falls. Chorea tends to stabilize and dissipate during the later stages of HD when other movement impairments such as rigidity and dystonia (involuntary twisting movements) become more prominent (11, 13, 17).

Currently, the mainstay of treatment for HD is symptomatic and supportive care –– no drugs are available to stop or prevent the progression of HD. Deutetrabenazine therapy has been shown to effectively control chorea symptoms compared with placebo and is generally well tolerated. However, comparison data with other VMAT2 inhibitors is limited due to a lack of reported head-to-head trials (6, 9-11, 13, 14, 17, 18).

Tardive Dyskinesia

Tardive dyskinesia (TD) is a medication-induced movement disorder. These movements are involuntary and repetitive, and most commonly affect the tongue, mouth, jaw, and face, but can also affect limbs and trunk. Severe cases are associated with difficulty speaking and swallowing. The condition can be disfiguring and stigmatizing, severely negatively impacting the individual's quality of life (19).

Tardive dyskinesia is caused by medicines that block dopamine receptors –– these include antipsychotic medications (e.g., aripiprazole, clozapine, risperidone, thioridazine) and antiemetic drugs used to treat nausea and vomiting (e.g., metoclopramide and prochlorperazine). Tardive dyskinesia is irreversible and life long, persisting after the causative medicine has been stopped.

Approximately one-third of individuals with schizophrenia treated with antipsychotics have TD (20). Initially, it was thought that the prevalence of TD would decrease as newer antipsychotics were developed that are less likely to cause TD; however, TD remains prevalent. This is partly because the newer antipsychotics are indicated to treat conditions other than schizophrenia, such as depression, bipolar disorder, personality disorder, irritability in autism spectrum disorder, as well as off-label uses including insomnia and anxiety. Therefore, the population exposed to the risk of TD has increased (21-24).

Because TD is irreversible, prevention is crucial –– requiring both the limited use of drugs that cause TD and early diagnosis (25, 26). While not a cure, deutetrabenazine has been shown to reduce the abnormal movements associated with TD and is generally well tolerated. However, there are no head-to-head comparisons between

VMAT2 inhibitors, and TD returns approximately 4 weeks after treatment is discontinued (4, 12, 20, 22, 25, 27-29).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse superfamily of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are often very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. Importantly, CYP2D6 is also the main enzyme that metabolizes the active metabolites of deutetrabenazine (1).

CYP2D6 Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 2).

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., CYP2D6 *4/*4). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (e.g., CYP2D6 poor metabolizer). However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms.

Table 2. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42*

For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

*CYP2D6*1* is assigned when no variant is detected and is assumed to have normal enzyme activity (CYP2D6 normal metabolizer phenotype). The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have near-normal activity.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a nonfunctional enzyme include **3*, **4*, **5*, and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (30).

Additional variant alleles and their multi-ethnic population frequencies have previously been reported (31). Moreover, given the structural variability of the *CYP2D6* region at chromosome 22q13.2, full gene deletion and duplication alleles, as well as complex tandem alleles with *CYP2D6*'s pseudogene, *CYP2D7*, also occur in some individuals, and populations (32).

CYP2D6 Phenotypes

In the US and globally, most individuals, around 70-80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41).

Individuals who have one normal function and one no function allele (e.g., *1/*4) or 2 decreased function alleles (e.g., *41/*41) are also categorized as "normal metabolizers" by recent nomenclature guidelines (33), but have also been categorized as "intermediate metabolizers" (34).

Individuals who have more than 2 normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1–10% of Caucasian individuals. For individuals of North African, Ethiopian and Saudi ancestry, the frequency is 16–28% (Table 3) (35).

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one no function allele, e.g., *4/*41) or poor metabolizers (2 no function alleles, e.g., *4/*4).

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent nonfunctional **4* and **5* alleles. Compared with Europeans, individuals of Asian descent are likelier to be intermediate metabolizers because of increased prevalence of decreased function alleles, such as *10. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are likelier to be intermediate metabolizers than Europeans because of the prevalence of a wide range of decreased function variants (30, 36-38).

Table 3. CPIC (2017). Assignment of likely CYP2D6 Phenotype based on Genotype

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 ultrarapid metabolizer	>2.0	An individual with duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]
CYP2D6 normal metabolizer	1.5–2.0	An individual with 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0	An individual with 2 decreased function alleles or one normal function and one no function allele	*1/*4, *1/*5, *41/*41
CYP2D6 intermediate metabolizer	0.5	An individual with one decreased function and one no function allele	*4/*10, *4/*41, *5/*9
CYP2D6 poor metabolizer	0	An individual with only no functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] See the *CYP2D6* frequency table in (35) for race-specific allele and phenotype frequencies.
[b] For a complete list of *CYP2D6* diplotypes and resulting phenotypes, see the *CYP2D6* genotype to phenotype table in (35). Note that genotypes with an activity score of 1 are classified as normal metabolizers in the *CYP2D6* genotype to phenotype table on the CPIC website (35).
[c] Where xN represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see supplemental data for additional information on how to translate diplotypes into phenotypes.
[d] Individuals with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories. A group of *CYP2D6* experts are currently working to standardize the *CYP2D6* genotype to phenotype translation system.
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (35)

Linking Gene Variation with Treatment Response

The CYP2D6 enzyme is responsible for converting deutetrabenazine active metabolites to minor, reduced activity metabolites. Individuals who are taking a strong CYP2D6 inhibitor (e.g., quinidine, antidepressants such as paroxetine, fluoxetine, and bupropion) have an approximately 3–4 fold higher exposure to active deutetrabenazine metabolites after standard dosing. Therefore, it is likely that individuals who are CYP2D6 poor metabolizers will have a similarly increased exposure to deutetrabenazine.

The FDA-approved drug label for deutetrabenazine cautions that a clinically relevant QT prolongation may occur in CYP2D6 poor metabolizers or individuals who are taking a strong CYP2D6 inhibitor. The drug label also states that a closely related VMAT2 inhibitor, tetrabenazine, has been shown to prolong the QT interval (the time taken for the heart ventricles to depolarize and repolarize). Other drugs with this potential have been associated with life-threatening ventricular tachycardia.

The FDA states that the total daily dosage of deutetrabenazine should be reduced in CYP2D6 poor metabolizers or individuals who are taking a strong CYP2D6 inhibitor. The total daily dose of deutetrabenazine should not exceed 36 mg, with a maximum single dose of 18 mg taken twice daily (the standard recommended total daily dose is 48 mg) (1).

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are currently available for the *CYP2D6* gene.

The *CYP2D6* gene is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, and pseudogenes. The complexity of genetic variation complicates the correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (of the more than 100 alleles that have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary between laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (35).

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications are denoted by "xN"; e.g., *CYP2D6*1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "CYP2D6 *1/*1, normal metabolizer", this can be confirmed by checking the diplotype and assigning an activity score assigned to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 3).

The *CYP2D6* phenotype is defined by the sum of the 2 activity scores, which is usually in the range of 0–3.0:

- An ultrarapid metabolizer has an activity score greater than 2
- A normal metabolizer phenotype has an activity score of 1.5–2.0
- A normal metabolizer or intermediate metabolizer has a score of 1.0
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0 (35)

A standardized *CYP2D6* genotype to phenotype assignment logic is currently being developed by an international working group of *CYP2D6* experts and both the CPIC and the Dutch Pharmacogenetics Working Group (DPWG).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants

2017 Statement from the US Food and Drug Administration (FDA)

2.4 Dosage Adjustment in Poor CYP2D6 Metabolizers

In patients who are poor CYP2D6 metabolizers, the total daily dosage of deutetrabenazine should not exceed 36 mg (maximum single dose of 18 mg).

[...]

5.3 QTc Prolongation

Tetrabenazine, a closely related VMAT2 inhibitor, causes an increase (about 8 msec) in the corrected QT (QTc) interval. A clinically relevant QT prolongation may occur in some patients treated with deutetrabenazine who are CYP2D6 poor metabolizers or are co-administered a strong CYP2D6 inhibitor.

For patients who are CYP2D6 poor metabolizers or are taking a strong CYP2D6 inhibitor, dose reduction may be necessary. The use of deutetrabenazine in combination with other drugs that are known to prolong QTc may result in clinically significant QT prolongations.

[...]

8.7 Poor CYP2D6 Metabolizers

Although the pharmacokinetics of deutetrabenazine and its metabolites have not been systematically evaluated in patients who do not express the drug metabolizing enzyme, it is likely that the exposure to α-HTBZ and β-HTBZ would be increased similarly to taking a strong CYP2D6 inhibitor (approximately 3-fold).

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947

may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

SNP= Single Nucleotide Polymorphism
Note: In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and the variant 988G>A occurs with 2850C>T (Cys296Arg).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (39).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Diazepam Therapy and *CYP2C19* Genotype

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Introduction

Diazepam is a benzodiazepine with several clinical uses, including managing anxiety, insomnia, muscle spasms, seizures, and alcohol withdrawal (1). Brand names include Valium, Diastat Acudial, Diastat, and Diazepam Intensol.

Diazepam is primarily metabolized by CYP3A4 and CYP2C19 to the major active metabolite, desmethyldiazepam. Approximately 2% of Europeans, 13% of East Asians, and as much as 57% of Oceanians have reduced or absent CYP2C19 enzyme activity ("poor metabolizers") (2).

The FDA-approved drug label for diazepam gel states that "the marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19"(3). However, the most recent drug label for oral formulations (tablet and liquid) only briefly discusses CYP2C19 in the context of potential drug interactions (Table 1)(1). Diazepam is available in multiple formulations including tablets (1), rectal gel (3), injectable solution (4), and oral solutions (5). The injectable solution does not discuss potential pharmacogenetic interactions with CYP2C19.

Table 1. The FDA Diazepam Statements on Metabolism and Drug Interactions (2020, 2017)

Drug (formulation)	Metabolism and pharmacogenetic interactions
Diazepam (tablet, oral solution)[a]	Diazepam is N-demethylated by CYP3A4 and 2C19 to the active metabolite N-desmethyldiazepam, and is hydroxylated by CYP3A4 to the active metabolite temazepam. […] There is a potentially relevant interaction between diazepam and compounds that inhibit certain hepatic enzymes (particularly CYP3A and CYP2C19). Data indicate that these compounds influence the pharmacokinetics of diazepam and may lead to increased and prolonged sedation. At present, this reaction is known to occur with cimetidine, ketoconazole, fluvoxamine, fluoxetine, and omeprazole.
Diazepam (gel)[b]	The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of *CYP2C19* (which is known to exhibit genetic polymorphism; approximately 3–5% of Caucasians have little or no activity and are "poor metabolizers") and *CYP3A4*. […] … Potential interactions may occur when diazepam is given concurrently with agents that affect CYP2C19 and CYP3A4 activity. Potential inhibitors of CYP2C19 and CYP3A4 could decrease the rate of diazepam elimination, while inducers of CYP2C19 and CYP3A4 could increase the rate of elimination of diazepam. Effect of diazepam on the metabolism of other drugs: There are no reports as to which isozymes could be inhibited or induced by diazepam. But, based on the fact that diazepam is a substrate for CYP2C19 and CYP3A4, it is possible that diazepam may interfere with the metabolism of drugs that are substrates for CYP2C19, (for example, omeprazole, propranolol, and imipramine) and CYP3A4 (for example, cyclosporine, paclitaxel, terfenadine, theophylline, and warfarin) leading to a potential drug-drug interaction.

[a] Information adapted from (1, 5).
[b] Information adapted from (3).

Drug: Diazepam

Diazepam is used to manage anxiety disorders or for the short-term relief of the symptoms of anxiety. In acute alcohol withdrawal, diazepam may provide symptomatic relief from agitation, tremor, delirium tremens, and

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hallucinations. Diazepam is also useful as an adjunct treatment for the relief of acute skeletal muscle spasms, as well as spasticity caused by upper motor neuron disorders (1, 6).

There are 16 benzodiazepines licensed by the FDA. Diazepam was the second benzodiazepine to be used clinically (after chlordiazepoxide), after being approved for use in 1963. It remains a commonly used drug today, and is included in the World Health Organization's core list of essential medicines needed for a basic healthcare system (7).

The use of benzodiazepines has replaced the use of barbiturates. Although these drug classes share similar therapeutic effects, barbiturates have a narrower therapeutic index, they are more sedative at therapeutic doses, and a barbiturate overdose is more likely to be fatal (8).

Like all benzodiazepines, diazepam is a controlled substance. Chronic use, either at standard therapeutic doses or through recreational abuse, can lead to tolerance and physical dependence. If diazepam treatment is abruptly discontinued, withdrawal symptoms can arise that can be severe and include seizures. Therefore, a gradual tapering of dose is recommended after chronic therapy.

Diazepam has several therapeutic effects—it is a sedative, anxiolytic, anticonvulsant muscle relaxant, and has amnesic effects. Diazepam is thought to exert these effects through an interaction with gamma-aminobutyric acid (GABA) A-type receptors ($GABA_A$), and GABA is the major inhibitory neurotransmitter in the central nervous system. When GABA binds to the $GABA_A$ receptor, the receptor opens, allowing the influx of chloride ions into neurons. This reduces the ability of neurons to depolarize and produce action potentials (excessive action potentials are implicated in seizures). It is thought that diazepam enhances the effects of GABA by increasing the affinity between GABA and its receptor, causing GABA to bind more tightly to the $GABA_A$ receptor (1, 3).

Diazepam is primarily metabolized via CYP2C19 and CYP3A4 to the major active metabolite (desmethyldiazepam), which is found in the plasma at concentrations equivalent to diazepam. Two minor active metabolites include temazepam and oxazaepam, which are usually not detectable. Other cytochrome P450 (CYP) enzymes involved in diazepam metabolism include CYP2C9, CYP2B6, and CYP3A5 (9).

Safe and effective use of oral diazepam in pediatric individuals below the age of 6 months has not been established. Pharmacokinetics indicate that the mean half-life of diazepam in children aged 3–8 years old is less than 18 hours, with elimination half-lives in full-term infants closer to 30 hours. Diazepam rectal gel has been studied and shown to be effective in children 2 years or older; safety and efficacy has not been established in children under 2 (3). Diazepam can be transmitted in breast milk to nursing infants, thus breastfeeding is not recommended for individuals receiving oral diazepam (1). Diazepam and desmethyldiazepam have long half-lives, thus there is little to no benefit to timing of breastfeeding with respect to the dose of diazepam (10). Breastfeeding after acute use of diazepam gel is possible, though "an appropriate period of time" should pass between dosage and nursing (3).

The FDA-approved label for diazepam tablets states that there is a suggested increased risk of congenital malformations and other developmental abnormalities associated with benzodiazepine drug use during pregnancy. Neonatal flaccidity, respiratory and feeding difficulties, and hypothermia have been reported in children born to mothers who have received benzodiazapines late in pregnancy or during labor and delivery. (1)

Both gel and tablet formulations of diazepam have reduced clearance and increased elimination half-lives in geriatric populations. This can alter the peak and trough concentrations for oral diazepam as well as the time to achieve steady state concentrations (1) and leads to a higher risk of ataxia or overdose in the gel formulation (3). Diazepam gel should be used with caution in individuals with compromised respiratory function (3). Similarly, individuals with renal or hepatic impairment should be administered oral or gel formulations of diazepam with caution, as these individuals have reduced clearance of diazepam and their metabolites (1, 3).

Gene: *CYP2C19*

The CYP superfamily is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, including antidepressants, antiplatelet agents, antifungal agents, some proton pump inhibitors, and benzodiazepines such as diazepam.

The *CYP2C19* gene is highly polymorphic, as there are over 35 variant star (*) alleles cataloged by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with 'rapid' (**1/*17*) and 'ultrarapid' (**17/*17*) metabolizer phenotypes. Heterozygous carriers of non-functional alleles (for example, **2* and **3*) are classified as 'intermediate metabolizers' (for example, **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (for example, **2/*2*, **2/*3*) (Table 2).

Table 2. The CPIC Assignment of CYP2C19 Phenotype based on Genotype (2017)

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual with 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual with one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual with 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual with one normal function allele and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual with 2 no function alleles	**2/*2* **2/*3* **3/*3*

CPIC: Clinical Pharmacogenetics Implementation Consortium
[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (11).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (11).

Linking Gene Variation with Treatment Response

It is well documented that wide inter-individual variation in the metabolism of benzodiazepines occurs, which includes diazepam metabolism. This can result in marked differences in drug levels when standard dosing is used and may potentially influence both therapeutic and adverse effects. It is thought that the variability in clearance of many benzodiazepines, including diazepam, is due to the variability in *CYP2C19* and *CYP3A4* genotypes (1, 9, 12-14).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are available for the diazepam response and the CYP2C19 gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (15).

Usually an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor). Table 2 summarizes common CYP2C19 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA): [Diazepam tablets, oral solution] Diazepam is N-demethylated by CYP3A4 and 2C19 to the active metabolite N-desmethyldiazepam, and is hydroxylated by CYP3A4 to the active metabolite temazepam.

[…]

There is a potentially relevant interaction between diazepam and compounds which inhibit certain hepatic enzymes (particularly CYP3A and CYP2C19). Data indicate that these compounds influence the pharmacokinetics of diazepam and may lead to increased and prolonged sedation. At present, this reaction is known to occur with cimetidine, ketoconazole, fluvoxamine, fluoxetine, and omeprazole.

Please review the complete therapeutic recommendations that are located here: (1 , 5).

2017 Statement from the US Food and Drug Administration (FDA): [Diazepam gel] The metabolism of diazepam is primarily hepatic and involves demethylation (involving primarily CYP2C19 and CYP3A4) and 3-hydroxylation (involving primarily CYP3A4), followed by glucuronidation. The marked inter-individual variability in the clearance of diazepam reported in the literature is probably attributable to variability of CYP2C19 (which is known to exhibit genetic polymorphism; about 3-5% of Caucasians have little or no activity and are "poor metabolizers") and CYP3A4.

[…]

Effect of Other Drugs on Diazepam Metabolism: *In vitro* studies using human liver preparations suggest that CYP2C19 and CYP3A4 are the principal isozymes involved in the initial oxidative metabolism of diazepam. Therefore, potential interactions may occur when diazepam is given concurrently with agents that affect CYP2C19 and CYP3A4 activity. Potential inhibitors of CYP2C19 (for example, cimetidine, quinidine, and tranylcypromine) and CYP3A4 (for example, ketoconazole, troleandomycin, and clotrimazole) could decrease the rate of diazepam elimination, while inducers of CYP2C19 (for example, rifampin) and CYP3A4 (for example, carbamazepine, phenytoin, dexamethasone and phenobarbital) could increase the rate of elimination of diazepam.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Effect of Diazepam on the Metabolism of Other Drugs: There are no reports as to which isozymes could be inhibited or induced by diazepam. But, based on the fact that diazepam is a substrate for CYP2C19 and CYP3A4, it is possible that diazepam may interfere with the metabolism of drugs which are substrates for CYP2C19, (for example omeprazole, propranolol, and imipramine) and CYP3A4 (for example cyclosporine, paclitaxel, terfenadine, theophylline, and warfarin) leading to a potential drug-drug interaction.

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

dbSNP: The Single Nucleotide Polymorphism Database
Note: the normal "wild-type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (16).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.
Please note that the *CYP2C19*2* defining variant (rs4244285) has recently been reported to be in high linkage disequilibrium with an intronic variant implicated in aberrant slicing (rs12769205) (17).

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Version history

To view the 2016 version of this summary (created on 25 August 2016) please click here.

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Dronabinol Therapy and *CYP2C9* Genotype

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Introduction

Dronabinol (brand names Marinol, Syndros) is the main psychoactive component in marijuana. Dronabinol is used in the treatment of chemotherapy-induced nausea and vomiting (CINV) among individuals who have not responded to conventional antiemetic therapy, and to treat anorexia associated with weight loss in individuals with acquired immunodeficiency syndrome (AIDS).

Dronabinol is primarily metabolized by CYP2C9, which is responsible for the formation of the major active metabolite (11-hydroxy-delta-9-THC). Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to dronabinol and an increased risk of side effects. Adverse events associated with dronabinol therapy include sedation, physical weakness, facial flushing, and palpitations.

The FDA-approved drug label for dronabinol recommends monitoring for the increased adverse reactions that could potentially occur in individuals who are known to have genetic variants associated with diminished CYP2C9 function (Table 1). The label states that published data indicates these individuals may have a 2- to 3-fold higher exposure to dronabinol (1).

Table 1. The FDA Drug Label for Dronabinol. Effect of *CYP2C9* Polymorphism (2020)

Phenotype	Recommendations
CYP2C9 poor metabolizer	Published data suggest that systemic clearance of dronabinol may be reduced and concentrations may be increased in the presence of *CYP2C9* genetic polymorphism. Monitoring for potentially increased adverse reactions is recommended in individuals known to have genetic variants associated with diminished CYP2C9 function.

This table is adapted from (1).

Drug: Dronabinol

Dronabinol is used to stimulate appetite in individuals with AIDS-related anorexia associated with weight loss, and to treat CINV in individuals who have failed to respond adequately to conventional antiemetic treatments (1).

Dronabinol is taken orally, as capsules or a solution, and the recommended dose varies depending on the indication. Dronabinol is a cannabinoid and is also known as delta-9-tetrahydrocannabinol (delta-9-THC, or "THC").

Cannabinoids are lipids derived from the cannabis (marijuana) plant. The plant contains more than 100 cannabinoids and dronabinol is a synthetic form of the primary psychoactive cannabinoid THC (2). Psychoactive agents affect brain function, and can cause a change in perception, mood, behavior, and level of consciousness.

Dronabinol is a Schedule III controlled substance. This means it is a drug that has a potential for abuse, but this is less of a risk than for Schedule I or II drugs. Abuse of dronabinol (taking high doses) will increase the risk of adverse psychiatric reactions such as psychosis, hallucinations, mood alteration, and paranoia. Continued abuse

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can lead to addiction (1).Two drugs that contain dronabinol as their active ingredient are Marinol, also Schedule III, and Syndros, which is a Schedule II substance (3).

In individuals with AIDS, to stimulate appetite, the recommended starting dose of dronabinol is 2.5 mg orally, twice daily, one hour before lunch and dinner (1). Weight loss associated with human immunodeficiency virus (HIV) and AIDS is common. Despite treatment with antiretroviral therapy, it is thought that the prevalence of HIV wasting syndrome is between 14 38%. Dronabinol appears to be well tolerated, improves appetite and may stop weight loss, but has variable results in terms of weight gain (4, 5, 6, 7).

In individuals undergoing chemotherapy, to treat CINV, the typical dose of dronabinol is 5 mg/m^2, 3–4 times a day. According to the drug label, the first dose of 5 mg/m^2 should be taken 1–3 hours before chemotherapy and at least 30 minutes before eating. This dose can be repeated every 2–4 hours after chemotherapy (without regard to meals) for a total of 4–6 doses per day (1).

Nausea and vomiting are common symptoms of chemotherapy and can have a significant detrimental impact on an individual's quality of life. Several types of antiemetics are commonly used to prevent and treat CINV before cannabinoids such as dronabinol are considered. These include corticosteroids (for example, dexamethasone), serotonin receptor antagonists (for example, ondansetron), and neurokinin receptor antagonists (for example, aprepitant).

There are many other medicines that can be used for CINV, but they tend to have more adverse effects. These medicines include antihistamines (for example, cyclizine), dopamine antagonists (for example, metoclopramide, olanzapine), benzodiazepines (for example, lorazepam), and anticonvulsants (for example, levetiracetam) (8).

Data on the efficacy of dronabinol is conflicting and limited to small trials (9). As an antiemetic, dronabinol has been reported to be superior to placebo in one trial, and inferior to metoclopramide in another (10, 11, 12). Dronabinol is only indicated for CINV in adults, although some data suggests adjunct dronabinol may be helpful in children (13). One review found no evidence to suggest that cannabinoids such as dronabinol were of value for anorexia or cachexia (extreme weight loss and wasting due to illness) in individuals with cancer or HIV (14). Because of the potential risk to the fetus, dronabinol should not be taken during pregnancy (1).

The mechanism of action of dronabinol is not clear –– it has complex effects on the central nervous system, including impairment of higher order processing, short term memory loss, and enhanced sensation. Central sympathomimetic activity can cause tachycardia (experienced as palpitations) and redness of the eyes (conjunctival injection).

Two cannabinoid receptors have been identified so far, CB1 –– which is located in the brain and some peripheral tissues also and may account for behavioral effects of cannabinoids; and CB2 –– mainly found peripherally and may affect immune function (15). Appetite stimulation is thought to be mediated at the lateral hypothalamus, antiemetic actions may be mediated by the vomiting center in the medulla, and the area subpostrema of the nucleus tractus solitarii may be involved in both appetite stimulation and antiemesis.

Outside of the US, synthetic cannabinoids such as dronabinol have been licensed to treat spasticity associated with multiple sclerosis (MS) and licensed as an adjunctive treatment of moderate to severe cancer pain. In the US, state medical marijuana programs may authorize eligible individuals to legally use marijuana for these conditions. However, there are many medical and legal differences between dronabinol and medical marijuana. Marijuana contains over 400 compounds, it does not have FDA approval, it is a schedule I controlled substance, and is illegal at the federal level (16).

The importance of dronabinol in the management of chronic pain is unclear –– studies give a mixed picture, but dronabinol as an adjunct treatment may be more beneficial in neuropathic pain (for example, MS) than cancer pain (17, 18).

CYP2C9 is the main enzyme involved in the metabolism of dronabinol to its major active metabolite, 11-hydroxy-delta-9-THC. Individuals who have low CYP2C9 activity ("CYP2C9 poor metabolizers") have a higher exposure to dronabinol (19). Data suggest that the route of THC administration affects the rate of metabolism, with oral administration—as in the case of dronabinol—showing significant inter-individual variation not only due to CYP2C9 activity, but also fed versus fasted states (1, 20, 21). Total exposure to dronabinol was increased in the fed state, particularly following a high-fat content, high calorie meal (1, 20). Of note, CYP2C9 activity can be altered by co-medication, infection, and other disease processes, resulting in phenoconversion from normal metabolizer to poor metabolizer phenotypes (22, 23, 24).

While the FDA-approved label for dronabinol states that both CYP2C9 and CYP3A4 contribute to the metabolism of dronabinol, it is important to note that CYP3A4 is not involved in the formation of the primary active metabolite (11-hydroxy-delta-9-THC) (1). As such, adverse reactions due to coadministration with CYP2C9 inhibitors may differ compared with reactions due to CYP3A4 inhibitors.

The FDA states that "Dronabinol, a synthetic cannabinoid, may cause fetal harm. Avoid use of dronabinol capsules in pregnant women. Although there is little published data on the use of synthetic cannabinoids during pregnancy, use of cannabis (for example, marijuana) during pregnancy has been associated with adverse fetal/neonatal outcomes" (1). Similarly, dronabinol is not advised for nursing mothers, either because of HIV positive status or due to possible adverse effects on the nursing infant in individuals with CINV (1).

Gene: CYP2C9

The cytochrome P450 (CYP450) superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are polymorphic and can result in reduced, absent, or increased enzyme activity.

The *CYP2C9* gene is highly polymorphic, with more than 50 known star (*) alleles, which are catalogued by the Pharmacogene Variation (PharmVar) consortium (25). The *CYP2C9*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype (26). Individuals who have 2 normal function alleles (for example, *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. The CPIC Assignment of likely *CYP2C9* Phenotype based on Genotype (2020)

Likely phenotype[a,b]	Activity score	Genotype	Examples of diplotype
Normal metabolizer	2	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	1.5 1	An individual with one normal function allele plus one decreased function allele; OR one normal function allele plus one no function allele OR 2 decreased function alleles	*1/*2 *1/*3 *2/*2
Poor metabolizer	0.5 0	An individual with one no function allele plus one decreased function allele; OR 2 no function alleles	*2/*3 *3/*3
Indeterminate	n/a	An individual with allele combinations with uncertain or unknown function alleles	*1/*7 *1/*10 *7/*10 *1/*58

This CPIC table has been adapted from (27). Additional information on allele function is available from PharmVar and CPIC.

Multiple allelic variants associated with reduced enzyme activity (with definitive or moderate evidence), these include *CYP2C9*2, *5, *8, *11, *14* and others (28, 29). The **2* allele is more common in Caucasian (10–20%)

than Asian (1–3%) or African (0–6%) populations. Alleles assigned an activity score of zero include *CYP2C9*3* and **13*. The **3* allele has a frequency of <10% in most populations and is extremely rare in African populations. Of note, among African-Americans, the *CYP2C9*5*, **6*, **8* and **11* variants are more common (30, 31, 32).

Linking Gene Variation with Treatment Response

One small study (43 healthy volunteers) reported that while orally administered THC pharmacokinetics did not differ by *CYP2C9*2* allele status, the *CYP2C9*3* allele did influence pharmacokinetics, therapeutics, and adverse effects. On average, individuals who were *CYP2C9*3/*3* homozygotes had a greater exposure to THC (the median area under the curve of THC was 3-fold higher than in than in *CYP2C9*1/*1* homozygotes), and these individuals showed a trend toward increased sedation following administration of THC (19). However, it is not possible to draw conclusions for the influence of the *CYP2C9*3/*3* genotype on dronabinol response, due to the small sample size (n=43) and nonblinded study design (19). Another study examined the pharmacokinetics of intravenously- and orally-administered THC and observed there was a significant contribution from the *CYP2C9*3* allele to individual dose exposure following oral ingestion, contributing to adverse effects in these individuals (21).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are available for the *CYP2C9* gene and the dronabinol response. In addition, variant *CYP2C9* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP) (33).

The *CYP2C9* variants are usually reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the individual's predicted metabolizer phenotype (normal, intermediate, or poor) (Table 2).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Dronabinol is primarily metabolized by CYP2C9 and CYP3A4 enzymes based on published in vitro studies. Inhibitors of these enzymes may increase, while inducers may decrease, the systemic exposure of dronabinol and/or its active metabolite resulting in an increase in dronabinol-related adverse reactions or loss of efficacy of dronabinol capsules.

Monitor for potentially increased dronabinol-related adverse reactions when dronabinol capsules is coadministered with inhibitors of CYP2C9 (e.g., amiodarone, fluconazole) and inhibitors of CYP3A4 enzymes (e.g., ketoconazole, itraconazole, clarithromycin, ritonavir, erythromycin, grapefruit juice).

[…]

Published data suggest that systemic clearance of dronabinol may be reduced and concentrations may be increased in the presence of CYP2C9 genetic polymorphism. Monitoring for potentially increased adverse reactions is recommended in patients known to carry genetic variants associated with diminished CYP2C9 function.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[…]

Published data indicate a potentially 2-to 3-fold higher dronabinol exposure in individuals carrying genetic variants associated with diminished CYP2C9 function.

Please review the complete therapeutic recommendations that are located here: (1)

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.4:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.4:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.4:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.4:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*7*	55C>A Leu19Ile	NM_000771.4:c.55C>A	NP_000762.2:p.Leu19Ile	rs67807361
*CYP2C9*8*	449G>A Arg150His	NM_000771.4:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*10*	10598A>G Glu272Gly	NM_000771.4:c.815A>G	NP_000762.2:p.Glu272Gly	rs9332130
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.4:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685
*CYP2C9*13*	3276T>C Leu90Pro	NM_000771.4:c.269T>C	NP_000762.2:p.Leu90Pro	rs72558187
*CYP2C9*14*	3552G>A Arg125His	NM_000771.4:c.374G>A	NP_000762.2:p.Arg125His	rs72558189
*CYP2C9*58*	1009C>A Pro337Thr	NM_000771.4:c.1009C>A	NP_000762.2:p.Pro337Thr	rs1274535931

Note: the normal "wild type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (34).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Eliglustat Therapy and *CYP2D6* Genotype

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Introduction

Eliglustat (brand name CERDELGA) is a glucosylceramide synthase inhibitor used in the treatment of Gaucher disease (GD). Eliglustat is indicated for the long-term treatment of adult individuals with Gaucher disease type 1 (GD1) who are CYP2D6 normal metabolizers, intermediate metabolizers, or poor metabolizers as detected by an FDA-cleared test (1).

Gaucher disease is an autosomal recessive metabolic disorder characterized by accumulation of glucosylceramide (a sphingolipid also known as glucocerebroside) within lysosomes. This is caused by a malfunction of the enzyme acid beta-glucosidase, encoded by the gene GBA. Type 1 GD may present in childhood or adulthood with symptoms including bone disease, hepatosplenomegaly, thrombocytopenia, anemia and lung disease and –– unlike Gaucher types 2 and 3 –– does not directly affect the central nervous system primarily (2). Eliglustat, a ceramide mimic, inhibits the enzyme that synthesizes glucosylceramides (UDP-Glucose Ceramide Glucosyltransferase), thereby reducing the accumulation of these lipids in the lysosome (3).

Eliglustat is broken down to inactive metabolites by CYP2D6 and, to a lesser extent, CYP3A (3). The dosage of eliglustat is based on the individual's CYP2D6 metabolizer status. Individuals with normal CYP2D6 activity are termed normal metabolizers (NM), those with reduced activity are termed intermediate metabolizers (IM), and if activity is absent, poor metabolizers (PM).

The FDA-approved drug label for eliglustat provides specific dosage guidelines based on their CYP2D6 status and concomitant usage of CYP2D6 or CYP3A inhibitors, and states that hepatic and renal function should also be considered when determining the appropriate dosage (Table 1). The label also states that CYP2D6 ultrarapid metabolizers (UM) may not achieve adequate concentrations of eliglustat for a therapeutic effect, and that for individuals for whom a CYP2D6 genotype cannot be determined, a specific dosage cannot be recommended (1).

Dosing recommendations for eliglustat have also been published by the Dutch Pharmacogenetics Working Group (DPWG) based on CYP2D6 metabolizer type (Table 2) and include dose adjustments for dosing eliglustat with medications that alter CYP2D6 and or CYP3A function (Table 3).

Table 1. The FDA Recommended Eliglustat Dosage Regimen by CYP2D6 Metabolizer Status (2020)

CYP2D6 metabolizer status	Eliglustat dosage
Ultrarapid metabolizer	May not achieve adequate concentrations
Normal metabolizer	84 mg twice daily
Intermediate metabolizer	84 mg twice daily
Poor metabolizer	84 mg once daily
Indeterminate metabolizer	Specific dosage cannot be recommended

For dose alterations based on decreased hepatic function, see Therapeutic Recommendations based on Genotype This FDA table is adapted from (1).

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Table 2. The DPWG Recommended Dosing of Eliglustat based on *CYP2D6* Phenotype (2018)

Phenotype	Implications for eliglustat therapy	Recommendation
CYP2D6 intermediate metabolizer	This gene variation reduces the conversion of eliglustat to inactive metabolites. This increases the risk of side effects, such as a (small, dose-dependent) elongation of the QT interval. The CYP3A inhibitors increase this risk even further.	No co-medication –– use the standard dose of 84 mg twice daily. Co-medication with a moderate or strong CYP2D6 or CYP3A inhibitor or strong CYP3A inducer: see Table 3.
CYP2D6 poor metabolizer		No co-medication –– use a dose of 84 mg once daily. Co-medication with a CYP3A inhibitor or strong CYP3A inducer: see Table 3.
CYP2D6 ultrarapid metabolizer	This gene variation increases the conversion of eliglustat to inactive metabolites. As a result, a normal dose is not effective. There is not enough scientific substantiation to suggest an effective dose for all ultrarapid metabolizers	Eliglustat is contraindicated. Choose an alternative if possible.

For dosage recommendations that incorporate co-medications and altered hepatic function, please see Therapeutic Recommendations based on Genotype
This DPWG table is adapted from (4) DPWG: Dutch Pharmacogenetics Working Group

Table 3. The DPWG Adjusted Daily Dosage for Eliglustat 84 mg based on Co-medications and Altered Hepatic Function (2018)

Co-medication(s)	Relative strength of CYP inhibitor/inducer	IM	PM
CYP2D6 inhibitor	Strong[1]	Once daily	--
	Moderate[2]	Once daily[#]	--
CYP3A inhibitor	Strong[3]	Once daily[#]	CI
	Moderate[4]	Once daily[#]	CI
	Weak[5]	--	Once daily[#]
CYP3A inducer	Strong[6]	CI	CI
CYP2D6 inhibitor with CYP3A inhibitor	Strong[1], strong[3]	CI	--
	Strong[1], moderate[4]	CI	--
	Moderate[2], strong[3]	CI	--
	Moderate[2], moderate[4]	CI	--

CI: Contraindicated, IM: Intermediate Metabolizer, PM: Poor metabolizer
[1] Strong CYP2D6 inhibitor: for example, paroxetine, fluoxetine, quinidine, bupropion.
[2] Moderate CYP2D6 inhibitor: for example, duloxetine, terbinafine, moclobemide, mirabegron, cinacalcet, dronedarone.
[3] Strong CYP3A inhibitor: for example, ketoconazole, clarithromycin, itraconazole, cobicistat, indinavir, lopinavir, ritonavir, saquinavir, telaprevir, tipranavir, posaconazole, voriconazole, telithromycin, conivaptan, boceprevir.
[4] Moderate CYP3A inhibitor: for example, erythromycin, ciprofloxacin, fluconazole, diltiazem, verapamil, aprepitant, atazanavir, darunavir, fosamprenavir, imatinib, cimetidine.
[5] Weak CYP3A inhibitor: amlodipine, cilostazol, fluvoxamine, goldenseal, isoniazid, ranitidine, ranolazine
[6] Strong CYP3A inducer: rifampicin, carbamazepine, phenobarbital, phenytoin, rifabutin, hypericum
[#] Individual requires additional monitoring for side effects.
DPWG guidelines available here (4) DPWG: Dutch Pharmacogenetics Working Group

Drug: Eliglustat

Eliglustat (brand name Cerdelga) is an oral substrate reduction therapy, indicated for the long-term treatment of adults with GD1 who are CYP2D6 NMs, IMs or PMs as detected by an FDA-approved test. Eliglustat is a

selective substrate inhibitor of glucosylceramide synthase (3, 5). Eliglustat is an oral therapy alternative to injection perfusion enzyme replacement therapy (ERT) for the long-term treatment of GD1.

Gaucher disease is an inborn error of metabolism and lysosomal storage disorder. It is a rare monogenic, autosomal recessive disorder due to biallelic variant of the *GBA* gene, which encodes the lysosomal enzyme acid beta-glucosidase. Loss of acid beta-glucosidase function results in accumulation of glucosylceramide within the lysosome. Gaucher disease is divided into 3 major clinical types (types 1, 2 and 3) and 2 subtypes (perinatal-lethal form—a subtype of GD type 2 [GD2], and cardiovascular form—a subtype of GD type 3[GD3]). Gaucher disease type 1 is characterized by bone disease, hepatosplenomegaly, thrombocytopenia, anemia, lung disease and a distinct lack of primary CNS disease (in contrast with GD2 and GD3, which present with primary CNS involvement). Gaucher disease type 1 presents in childhood with bone disease occurring in 70–100% of individuals; this bone disease ranges from asymptomatic osteopenia to focal lytic or sclerotic lesions and osteonecrosis. However, bone disease may be the most debilitating aspect of GD1. Liver enlargement and cytopenia are also both very common, nearly universal (2, 6). Biochemical testing of GBA1 enzyme activity in peripheral blood is the gold standard to confirm GD diagnosis; molecular sequencing approaches are limited due to significant sequence identity between *GBA* and a pseudogene, *GBAP* (2, 7).

Eliglustat is a ceramide analog that specifically inhibits UDP-glucose ceramide glucosyltransferase, which catalyzes the first glycosylation step in glycosphingolipid biosynthesis (transfer of glucose to ceramide) (3, 7). Inhibition of the ceramide glycosyltransferase reduces the burden of glucosylceramides, which have been shown to accumulate in the lysosomes of GD individuals.

Eliglustat is metabolized primarily by CYP2D6 and, to a lesser extent, CYP3A. No active metabolites are known (3). Metabolized eliglustat is excreted through the urinary and gastrointestinal tracts. The CYP2D6 metabolizer status must be considered when determining the appropriate dosage of eliglustat; NMs, IMs, and PMs with normal hepatic and renal function can take the recommended dosage. Ultrarapid metabolizers "may not achieve adequate concentrations of eliglustat to achieve therapeutic effect." (1) Dosage levels cannot be recommended for individuals of undetermined CYP2D6 metabolizer status. Individuals who are CYP2D6 NMs concomitantly taking CYP2D6 inhibitors, with moderate or severe hepatic impairment, or mild hepatic impairments and CYP2D6 inhibitor use should not take eliglustat. Both IMs and PMs taking CYP2D6 or CYP3A inhibitors, or both, demonstrating hepatic impairment are also contraindicated from eliglustat use.

Potential risk of cardiac arrhythmias should be considered in individuals taking eliglustat with CYP2D6 or CYP3A inhibitors, with certain metabolizer status and with varying degrees of hepatic impairment. More moderate adverse reactions in individuals during clinical trials included abdominal pain, diarrhea, flatulence, and back/extremity pain and were reported at least once in over 80% of individuals (8). Headache, arthralgia, fatigue and nausea have also been reported at least once in >60% of individuals, but one study found no correlation between CYP2D6 metabolizer status and frequency of these adverse events (8). Real world evidence for use of eliglustat shows most individuals tolerate long-term treatment without adverse effects (9).

Use of eliglustat during pregnancy has not been sufficiently studied to assess drug-associated risks of major birth defects, spontaneous abortion, or other adverse maternal/fetal outcomes. "Women with Gaucher disease type 1 have an increased risk of spontaneous abortion, especially if disease symptoms are not treated and controlled pre-conception and during a pregnancy. Pregnancy may exacerbate existing Gaucher disease type 1 symptoms or result in new disease manifestations. Gaucher disease type 1 manifestations may lead to adverse pregnancy outcomes including, hepatosplenomegaly which can interfere with the normal growth of a pregnancy and thrombocytopenia which can lead to increased bleeding and possible hemorrhage" (1). There are no human data available on the presence of eliglustat in human milk, effects on the breastfed infant, or effects on milk production. Based on animal studies, it is likely that eliglustat would be present in human milk (1).

Eliglustat has not been sufficiently studied for safety and effectiveness in pediatric individuals or subjects over 65. Individuals with renal impairment should be dosed based on their CYP2D6 metabolizer status. Individuals with hepatic impairment should similarly be dosed in light of CYP2D6 metabolizer status and concomitant use of CYP2D6 or CYP3A inhibitors (1).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity. The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

CYP2D6 Alleles

The *CYP2D6* gene on chromosome 22q13.2 is highly polymorphic. Over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 4) (10). In addition to the wildtype *CYP2D6*1* allele, variant *CYP2D6* alleles (or haplotypes) can harbor single nucleotide polymorphisms (SNPs), insertions or deletions, gene conversions, as well as copy number variations.

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (for example, CYP2D6 poor metabolizer). However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms. The CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The *CYP2D6* phenotype is defined by the sum of the 2 allele activity scores, which is most commonly in the range of 0 to 3.0 (11):

- An UM has an activity score greater than 2.25
- A NM phenotype has an activity score of 1.25–2.25
- An IM has an activity score of >0–1.25
- A PM has an activity score of 0 (14)

Table 4. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles
Normal function	*1, *2, *27, *33
Decreased function	*10, *17, *41, *49
No function	*3, *4, *5, *6, *36

For a comprehensive list of *CYP2D6* alleles, please See PharmVar.

The *CYP2D6*1* allele is considered the wildtype allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. In addition, the *CYP2D6*2*, *27*, and *33 alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, *3, *4, *5, and *6) (12, 13, 14, 15) or an enzyme with decreased activity (for example, *10, *17, and *41) (16, 17, 18) (see Table 4). There are large inter-ethnic differences in the frequency of these alleles, with *3, *4, *5, *6, and *41 being more common in Caucasians, *17 more common in Africans, and *10 more common in Asians (19).

Allele Frequencies Vary between Populations

Among Asians and in individuals of Asian descent, only approximately 50% of *CYPD6* alleles are normal function, and the frequency of *CYP2D6* duplications is as high as 45%, although this may have been overestimated by not accounting for tandem hybrid alleles (for example, **36+*10*) (20). Other studies of a US individual population suggested less than 50% of alleles detected within Asian-descent individuals are normal function alleles in a single copy, with 30% of alleles arising from structural variants (duplications or deletions) (21). Common no-function variants are *CYP2D6*36* and *CYP2D6*4* (21). Both these alleles contain the variant "c.100C>T" (see Allele Nomenclature table) (19, 20, 22, 23). The *CYP2D6*36* allele is the result of a gene conversion event with the pseudogene *CYP2D7* (24). This no-function allele is most commonly found in individuals of Asian ancestry (21).

Among Africans and African-Americans, only approximately 50% of *CYP2D6* alleles are normal function (12, 18, 19, 25). African-Americans also have been found to have a higher frequency of no-function structural variants or decreased function single-copy variant alleles versus Caucasian or Hispanic-Americans (21).

Middle Eastern countries show a great diversity in phenotypic and allelic distribution for *CYP2D6* (26), though on average, these individuals show a lower frequency of PM phenotypes (0.91%) and higher UM phenotypes (11.2%) than other ethnicities (Note: Oceania and Middle Eastern ethnicities combined in this study) (27).

Among European countries, there is diversity of allelic distribution (28). Gene duplications were more common in the south-eastern countries (Greece, Turkey: 6%) and less common in north-western countries (Sweden and Denmark, <1%). Meanwhile, *CYP2D6*4* and **5* alleles were generally more common in the north and less common in the south. (28) Worldwide *CYP2D6* genotype and phenotype frequencies have been catalogued and recently published (27).

CYP2D6 Phenotype

CYP2D6 Phenotype Frequencies Vary between Populations

Normal metabolizers: Approximately 77–92% of individuals have 2 normal function alleles (**1* or **2*), or one normal function allele and one decreased function allele. These individuals are "normal metabolizers" and are most likely to have a phenotypically normal response to the drug.

Intermediate metabolizers: Approximately 2–11% of individuals are IMs—they have either 2 decreased function alleles or one normal or decreased function and one no allele (27). A study of a diverse US urban population of children found that roughly 8% of subjects were IMs (29), Within the US, it has been observed that individuals of African or Asian descent were most likely to be classified as IMs (20–28% of population by ethnicity) (21).

Poor metabolizers: Approximately 5–10% of individuals are PMs—they have 2 no alleles (30). The PMs are more commonly found in European Caucasians and their descendants. The no *CYP2D6*4* and **5* alleles largely account for the PM phenotype in these populations (14, 17, 31). It should be noted that the frequency of PMs can be much lower in certain populations including East Asian, Oceania and Middle Eastern (27). Studies of US multi-ethnic populations have estimated the prevalence of PM to be between 1.5–5.7% (21, 29).

Ultrarapid metabolizers: Individuals who are UMs have at least 3 copies of the *CYP2D6* gene. The UM phenotype has been estimated to be present in 1–2% of individuals, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African-Americans, and up to 1% in Hispanics, Chinese, and Japanese (30, 32). The UMs made up 9% of subjects in an urban multi-ethnic population with a large portion of Hispanic/Latino subjects (29). A larger study of US individuals predicted a UM phenotype in only 2.2% of individuals, regardless of ethnicity (21).

Linking Gene Variation with Treatment Response

Genetic variation in the *CYP2D6* gene can influence whether an individual achieves adequate concentrations of eliglustat and therapeutic benefit (namely, CYP2D6 ultrarapid metabolizers) or is at an increased risk of adverse events (namely, CYP2D6 poor metabolizers). Eliglustat dose, metabolizer status/genotype and concomitant CYP inhibitor medication use should all be considered in assessing potential risk of cardiac arrhythmias. Conditions that decrease the clearance of eliglustat put individuals in this elevated risk category (namely, hepatic impairment, CYP2D6 IMs or PMs taking CYP inhibitors), for complete information please see (1).

Six GD individuals in the International Collaborative Gaucher Group Gaucher Registry who were known UMs voluntarily reported their 1–3 year outcomes after taking eliglustat. This included individuals who had been on ERT and switched to eliglustat as well as some who were treatment naive. For these individuals, the reported dosages were 84 mg 3 times daily (4 individuals) or twice daily (2 individuals). Four of the 6 individuals maintained hemoglobin concentration and platelet counts within the therapeutic goal range after 2 years; however, one individual developed anemia and another developed moderate thrombocytopenia. One individual reported discontinuation of eliglustat (rationale unclear). Adverse events and reasons for discontinuation of therapies are not recorded within the Registry; however, very few individuals of any metabolizer status discontinued treatment with eliglustat over the course of this study (22/231, 9%). (9)

Genetic Testing

The NIH Genetic Testing Registry provides examples of the genetic tests that are available for eliglustat response and for the *CYP2D6* gene.

The *CYP2D6* is a particularly complex gene that is difficult to genotype due to highly homologous neighboring pseudogenes, as well as the large number of variants and the presence of gene deletions, duplications, and multiplications. The complexity of genetic variation complicates making a correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (33).

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications can be denoted by "xN", for example: *CYP2D6*1xN* with *xN* representing the number of *CYP2D6* gene copies. Note representation of duplications is also not standardized among laboratories.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6 *1/*1*, normal metabolizer", this may be confirmed by checking the diplotype and assigning an activity score to each allele (for example, 0 for no, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 4). See the *CYP2D6* alleles section above for more information.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants

2018 Statement from the US Food and Drug Administration (FDA):

The recommended dosage of Eliglustat in adults is based on the patient's CYP2D6 metabolizer status.

[…]

Reduce dosage frequency of Eliglustat 84 mg to once daily in CYP2D6 NMs and IMs with or without hepatic impairment taking CYP2D6 or CYP3A inhibitors.

Table [5] : Recommended Dosage of Eliglustat: 84 mg Once Daily based on CYP2D6 Metabolizer, Hepatic Impairment Status, and Concomitant CYP Inhibitors

CYP2D6 Metabolizer Status	Hepatic Impairment Status	Concomitant CYP Inhibitor
NMs	Without Hepatic Impairment	Taking a strong or moderate CYP2D6 inhibitor Taking a strong or moderate CYP3A inhibitor
	Mild (Child-Pugh Class A) Hepatic Impairment	Taking a weak CYP2D6 inhibitor Taking a strong, moderate, or weak CYP3A inhibitor
IMs	Without hepatic involvement	Taking a strong or moderate CYP2D6 inhibitor

4 CONTRAINDICATIONS

Eliglustat is contraindicated in the following patients based on CYP2D6 metabolizer status due to the risk of cardiac arrhythmias from prolongation of the PR, QTc, and/or QRS cardiac intervals.

NMs

- Taking a strong or moderate CYP2D6 inhibitor concomitantly with a strong or moderate CYP3A inhibitor
- Moderate or severe hepatic impairment
- Mild hepatic impairment and taking a strong or moderate CYP2D6 inhibitor

IMs

- Taking a strong or moderate CYP2D6 inhibitor concomitantly with a strong or moderate CYP3A inhibitor
- Taking a strong CYP3A inhibitor Any degree of hepatic impairment

PMs

- Taking a strong CYP3A inhibitor
- Any degree of hepatic impairment

7 DRUG INTERACTIONS

7.1 Effect of other drugs on Eliglustat

Coadministration of Eliglustat with:

- CYP2D6 or CYP3A inhibitors may increase eliglustat concentrations which may increase the risk of cardiac arrhythmias from prolongation of the PR, QTc, and/or QRS cardiac interval.
- strong CYP3A inducers decreases eliglustat concentrations which may reduce efficacy.

Table [6] : Prevention and Management Strategies of Drug Interactions Affecting eliglustat based on CYP2D6 Metabolizer status and Concomitant Interacting drug

Concomitant Drug(s)	CYP2D6 Metabolizer Status		
	NMs	IMs	PMs
CYP2D6 Inhibitor			
Strong	Reduce frequency of eliglustat 84mg to once daily		Continue eliglustat 84mg once daily*
Moderate			
Weak	Continue eliglustat 84mg twice daily		
CYP3A Inhibitor			
Strong	Reduce frequency of eliglustat 84 mg to once daily	Contraindicated	
Moderate		Avoid coadministration.	
Weak	Continue eliglustat 84mg twice daily		Avoid coadministration.
CYP2D6 Inhibitor Concomitantly with a strong CYP3A Inhibitor			
Strong	Contraindicated		
Moderate			
CYP2D6 Inhibitor Concomitantly with a moderate CYP3A Inhibitor			
Strong	Contraindicated		Avoid coadministration
Moderate			
CYP3A Inducer			
Strong	Avoid coadministration		

* No effect of CYP2D6 inhibitor due to little or no CYP2D6 activity in CYP2D6 PMs.

8 Use In Specific Populations

8.6 Renal Impairment

Use eliglustat in patients with renal impairment based on the patient's CYP2D6 metabolizer status

NMs

- Avoid eliglustat in patients with end-stage renal disease (ESRD) (estimated creatinine clearance (eCLcr) less than 15 mL/min not on dialysis or requiring dialysis).
- No dosage adjustment is recommended in patients with mild, moderate, or severe renal impairment (eCLcr at least 15 mL/min).

IMs and PMs

Avoid eliglustat in patients with any degree of renal impairment.

8.7 Hepatic Impairment

Use eliglustat in patients with hepatic impairment based on CYP2D6 metabolizer status and concomitant use of CYP2D6 or CYP3A inhibitors

NMs

Eliglustat is contraindicated in patients with [see Contraindications]:

- severe (Child-Pugh Class C) hepatic impairment
- moderate (Child-Pugh Class B) hepatic impairment
- mild (Child-Pugh Class A) hepatic impairment taking a strong or moderate CYP2D6 inhibitor

Reduce dosage frequency of eliglustat 84 mg to once daily [see Dosage and Administration] in patients with mild hepatic impairment taking:

- a weak CYP2D6 inhibitor
- a strong, moderate, or weak CYP3A inhibitor

No dosage adjustment is recommended in patients with mild hepatic impairment, unless otherwise specified above.

IMs and PMs

Eliglustat is contraindicated in patients with any degree of hepatic impairment [see Contraindications].

Please review the complete therapeutic recommendations that are located here: (1).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 UM:

Eliglustat is contra-indicated.

1 choose an alternative if possible

CYP2D6 IM:

Recommendation:

- Co-medication with BOTH a MODERATE to STRONG CYP2D6 INHIBITOR AND a MODERATE to STRONG CYP3A INHIBITOR: Eliglustat is contra-indicated.
  1 choose an alternative if possible

Strong CYP2D6 inhibitor: for example paroxetine, fluoxetine, quinidine, bupropione. Moderate CYP2D6 inhibitor: for example duloxetine, terbinafine, moclobemide, mirabegron, cinacalcet, dronedarone. Strong CYP3A inhibitor: for example ketoconazole, clarithromycin, itraconazole, cobicistat, indinavir, lopinavir, ritonavir, saquinavir, telaprevir, tipranavir, posaconazole, voriconazole, telithromycin, conivaptan, boceprevir. Moderate CYP3A inhibitor: for example erythromycin, ciprofloxacin, fluconazole, diltiazem, verapamil, aprepitant, atazanavir, darunavir, fosamprenavir, imatinib, cimetidine.

- Co-medication with a STRONG CYP2D6 INHIBITOR (e.g. paroxetine, fluoxetine, quinidine, bupropione):
  1 use a dose of 84 mg eliglustat 1x daily
- Co-medication with a MODERATE CYP2D6 INHIBITOR (for example duloxetine, terbinafine, moclobemide, mirabegron, cinacalcet, dronedarone):
  1. consider a dose of 84 mg eliglustat 1x daily
  2. be alert to side effects
- Co-medication with a STRONG CYP3A INHIBITOR (for example ketoconazole, clarithromycin, itraconazole, cobicistat, indinavir, lopinavir, ritonavir, saquinavir, telaprevir, tipranavir, posaconazole, voriconazole, telithromycin, conivaptan, boceprevir):

-1. choose an alternative if possible

- if an alternative is not an option:
- consider a dose of 84 mg eliglustat 1x daily

- be alert to side effects
    - Co-medication with a MODERATE CYP3A INHIBITOR (for example erythromycin, ciprofloxacin, fluconazole, diltiazem, verapamil, aprepitant, atazanavir, darunavir, fosamprenavir, imatinib, cimetidine):
        1. choose an alternative
        2. if an alternative is not an option:
            1. consider a dose of 84 mg eliglustat 1x daily
            2. be alert to side effects
    - Co-medication with a STRONG CYP3A INDUCER (for example rifampicin, carbamazepine, phenobarbital, phenytoin, rifabutine, hypericum): Eliglustat is not recommended. The plasma concentration may decrease so sharply that a therapeutic effect cannot be achieved.
        1 choose an alternative if possible
    - NO co-medication with a moderate or strong CYP2D6 or CYP3A inhibitor or strong CYP3A inducer:
        1 use the standard dose of 84 mg 2x daily

CYP2D6 PM:

Recommendation:

- Co-medication with a STRONG CYP3A INHIBITOR (for example ketoconazole, clarithromycin, itraconazole, cobicistat, indinavir, lopinavir, ritonavir, saquinavir, telaprevir, tipranavir, posaconazole, voriconazole, telithromycin, conivaptan, boceprevir):

Eliglustat is contra-indicated.

1 choose an alternative if possible
    - Co-medication with a MODERATE CYP3A INHIBITOR (for example erythromycin, ciprofloxacin, fluconazole, diltiazem, verapamil, aprepitant, atazanavir, darunavir, fosamprenavir, imatinib, cimetidine):

Eliglustat is not recommended.

1 choose an alternative if possible
    - Co-medication with a WEAK CYP3A INHIBITOR (for example amlopidine [amlodipine], cilostazole [cilostazol], fluvoxamine, goldenseal, isoniazide [isoniazid], ranitidine, ranolazine):
        1. choose an alternative for the weak CYP3A inhibitor if possible
        2. if an alternative is not an option:
            1. use a dose of 84 mg eliglustat 1x daily
            2. be alert to side effects
    - Co-medication with a STRONG CYP3A INDUCER (for example rifampicin, carbamazepine, phenobarbital, phenytoin, rifabutine, hypericum):

Eliglustat is not recommended. The plasma concentration may decrease so sharply that a therapeutic effect cannot be achieved.

1 choose an alternative if possible
    - NO co-medication with a CYP3A inhibitor or strong CYP3A inducer:
        1 use a dose of 84 mg 1x daily

Please review the complete therapeutic recommendations that are located here:(4).

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*3*	2550delA (Arg259fs)	NM_000106.6:c.775delA	NP_000097.3:p.Arg259fs	rs35742686
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T (Trp152Glyfs) CYP2D6T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.886T>C	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.1457G>C	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1319G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.1457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.454delT	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.100C>T	NP_000097.3:p.Ser486Thr	rs1135840

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*36*[3]	100C>T (Pro34Ser)	NM_000106.6:c.320C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts splicing).	rs28371725
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2851C>T is also referred to as 2938C>T
[3] *CYP2D6*36* is a gene conversion with *CYP2D7;* variants provided here are from the Pharmacogene Variation Consortium. Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (34). Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS). Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Esomeprazole Therapy and *CYP2C19* Genotype

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Introduction

Esomeprazole (brand name Nexium) is a proton pump inhibitor (PPI) used to treat gastroesophageal reflux disease (GERD) and to reduce the risk of gastric ulcers associated with nonsteroidal anti-inflammatory drug NSAID use. Esomeprazole is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and in combination with antibiotics to eradicate *Helicobacter pylori (H. pylori)* infection.

Esomeprazole reduces the acidity (raises the pH) in the stomach by inhibiting the secretion of gastric acid. The level of esomeprazole an individual is exposed to is influenced by several factors, such as the dose used and how quickly the drug is metabolized and inactivated.

Esomeprazole is primarily metabolized by the CYP2C19 enzyme. Individuals with increased CYP2C19 enzyme activity ("CYP2C19 ultrarapid metabolizers") may have an insufficient response to standard doses of esomeprazole, because the drug is inactivated at a faster rate. In contrast, individuals who have reduced or absent CYP2C19 enzyme activity (i.e., CYP2C19 intermediate and poor metabolizers) have a greater exposure to esomeprazole.

The 2018 FDA-approved drug label for esomeprazole states that 3% of Caucasians, and 15–20% of Asians are CYP2C19 poor metabolizers, and that poor metabolizers have approximately twice the level of exposure to esomeprazole, compared with CYP2C19 normal metabolizers. However, the drug label does not include dosing recommendations for CYP2C19 poor metabolizers (1).

Esomeprazole recommendations have been published by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP), which indicates that no change in dosing is recommended for CYP2C19 poor, intermediate, or ultrarapid metabolizers. The DPWG states that although genetic variation in *CYP2C19* influences the plasma concentration of esomeprazole, there is insufficient evidence to support an effect on treatment outcomes or side effects (2).

Table 1. The FDA (2018) Drug Label for Esomeprazole: *CYP2C19*

Phenotype	Esomeprazole
CYP2C19 poor metabolizer	The CYP2C19 isoenzyme exhibits polymorphism in the metabolism of esomeprazole, since some 3% of Caucasians and 15–20% of Asians lack CYP2C19 and are termed poor metabolizers. At steady state, the ratio of AUC in poor metabolizers to AUC in the rest of the population (normal metabolizers) is approximately 2.

AUC: Area Under the plasma drug concentration-time Curve. AUC reflects the body's exposure to the drug being administered.
Note: "normal metabolizers" were previously termed "extensive metabolizers".
Please see Therapeutic Recommendations based on Genotype for more information from FDA. This FDA table is adapted from (1).

Table 2. The DPWG (2018) Recommendations for Esomeprazole and *CYP2C19* Genotype

Phenotype	Action	Pharmacist text
CYP2C19 poor metabolizer	No action is required for this gene-drug interaction.	Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.
CYP2C19 intermediate metabolizer	No action is required for this gene-drug interaction.	

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Table 2. continued from previous page.

Phenotype	Action	Pharmacist text
CYP2C19 ultrarapid metabolizer	No action is required for this gene-drug interaction.	Although the genetic variation may lead to faster inactivation of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This Dutch Pharmacogenetics Working Group (DPWG) table is adapted from (2).

Drug class: Proton Pump Inhibitors

Proton pump inhibitors block the secretion of gastric acid. They are among the most commonly prescribed drugs in the United States and globally, and some PPI formulations are available without a prescription.

Proton pump inhibitors can be used to treat a number of conditions in adults:

- Active duodenal ulcers
- Active gastric (peptic) ulcers
- *Helicobacter pylori* infection eradication (in combination with antibiotics, to reduce the risk of duodenal ulcer recurrence)
- Hypersecretory conditions (e.g., Zollinger-Ellison syndrome)

Proton pump inhibitors are also used in infants, children, and adults to treat:

- Symptomatic GERD
- Erosive esophagitis (EE) due to acid-mediated GERD
- Maintenance of healing of EE due to acid-mediated GERD

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid into the stomach (gastric lumen). Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B. Gastric acid also helps maintain a sterile environment by suppressing the growth of bacteria (3).

Hydrogen ions (H+) are actively secreted into the gastric lumen in exchange for potassium ions (K+) via an H^+/K^+-ATPase, which is also known as a "proton pump". Located on the luminal surface of gastric parietal cells, the proton pump controls the last step in acid secretion. Proton pump inhibitors potently suppress gastric acid secretion by covalently binding to and irreversibly inactivating this proton pump.

Six PPIs are currently FDA-approved for clinical use: esomeprazole (brand name Nexium), dexlansoprazole (Dexilant, Kapidex), lansoprazole (Prevacid), omeprazole (Prilosec), pantoprazole (Protonix), and rabeprazole (Aciphex). All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (4, 5).

There are a few differences between the indications of different PPIs. For example, for the treatment of GERD in young children, only esomeprazole is indicated for infants from one month old (lansoprazole is indicated from one year of age, omeprazole and dexlansoprazole from 2 years of age, and rabeprazole from age 12) (6).

All 6 PPIs, to varying degrees, are metabolized and inactivated by CYP2C19 (and to a lesser extent by CYP3A4). Additionally, given that PPIs are also inhibitors of CYP2C19 and that CYP2C19 is involved in the metabolism of many drugs, PPI administration can lead to clinically significant drug interactions. For example, the concomitant use of a PPI and clopidogrel, which requires CYP2C19 for bioactivation, has been associated with reduced antiplatelet activity, and thus, the concurrent administration of omeprazole with clopidogrel must balance overall risks and benefits, considering both cardiovascular and gastrointestinal complications (7-11).

Genetic variation in the *CYP2C19* gene influences the clearance of PPIs, which may in turn influence treatment outcomes. Second-generation PPIs are being developed that are not primarily metabolized by CYP2C19, and therefore less likely to be influenced by *CYP2C19* genotype (12-14).

Drug: Esomeprazole

Esomeprazole is a PPI that is available via prescription medication or over-the-counter. It is closely related to omeprazole, which was the first PPI to be licensed in the United States. Esomeprazole is the S-isomer of omeprazole (mirror image of the same chemical structure) whereas omeprazole is a racemic mixture (50:50 mix) of R- and S-isomers.

In adults, esomeprazole is used to reduce the risk of NSAID-associated gastric ulcers and to reduce the risk of recurrence of duodenal ulcers by eradicating *H. pylori* infection. Esomeprazole is also used to treat pathological hypersecretory conditions, including Zollinger-Ellison syndrome.

Esomeprazole is used to treat GERD and to support healing of EE in adults, children, and infants from one month of age.

Esomeprazole is metabolized and inactivated in the liver by the cytochrome P450 system. CYP2C19 is the principal enzyme involved, although other enzymes such as CYP3A4 also contribute to a lesser degree.

The long term use of PPIs has been associated with several adverse effects. Daily treatment with any PPI for longer than 3 years may lead to malabsorption of vitamin B12, caused by hypochlorhydria. Given that prolonged hypochlorhydria also increases the risk of *Clostridium difficile* infection and may increase the risk for osteoporosis-related fractures, the FDA recommends that individuals should use the lowest dose and shortest duration of PPI therapy appropriate for the condition being treated (1).

Studies have not adequately assessed the safety of esomeprazole therapy during pregnancy. For omeprazole use during pregnancy, epidemiology studies failed to find an increased risk of major congenital malformations or other adverse pregnancy outcomes.

Studies have reported that genetic variations in the *CYP2C19* gene influence the plasma concentration of esomeprazole. However, there is insufficient evidence to support that *CYP2C19* genotype influences the efficacy or safety of esomeprazole therapy (15-21). For other PPIs, such as omeprazole, alterations in dose have been recommended by the FDA and the DPWG (1, 2, 16).

Incidental Findings

Genetic variation in the *CYP2C19* gene influences the metabolism of other medications used for the treatment of several conditions:

- Acute coronary syndrome – individuals who are CYP2C19 poor metabolizers and undergoing percutaneous coronary intervention have an increased risk of cardiovascular events if they are treated with the antiplatelet drug clopidogrel (a prodrug that is activated via CYP2C19 metabolism)
- Depression – *CYP2C19* influences the metabolism of tricyclic antidepressants e.g., amitriptyline, imipramine; and selective serotonin reuptake inhibitors (SSRIs) e.g., citalopram. Individuals who are CYP2C19 poor metabolizers may have an increased risk of side effects, whereas there may be an increased risk of treatment failure in ultrarapid metabolizers.

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the esomeprazole response and the

CYP2C19 gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (22).

Individual results are typically reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation with the predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Metabolism

Esomeprazole is extensively metabolized in the liver by the cytochrome P450 (CYP) enzyme system. The metabolites of esomeprazole lack antisecretory activity. The major part of esomeprazole's metabolism is dependent upon the CYP2C19 isoenzyme, which forms the hydroxy and desmethyl metabolites. The remaining amount is dependent on CYP3A4, which forms the sulphone metabolite. CYP2C19 isoenzyme exhibits polymorphism in the metabolism of esomeprazole, since some 3% of Caucasians and 15 to 20% of Asians lack CYP2C19 and are termed Poor Metabolizers. At steady state, the ratio of AUC in Poor Metabolizers to AUC in the rest of the population (Normal Metabolizers) is approximately 2.

[...]

Interaction with Clopidogrel

Avoid concomitant use of esomeprazole magnesium with clopidogrel. Clopidogrel is a prodrug. Inhibition of platelet aggregation by clopidogrel is entirely due to an active metabolite. The metabolism of clopidogrel to its active metabolite can be impaired by use with concomitant medications, such as esomeprazole, that inhibit CYP2C19 activity. Concomitant use of clopidogrel with 40 mg esomeprazole reduces the pharmacological activity of clopidogrel. When using esomeprazole magnesium consider alternative anti-platelet therapy.

Please review the complete therapeutic recommendations located here: (1).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 Poor Metabolizer (PM)

No action is needed for this gene-drug interaction.

Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

CYP2C19 Intermediate Metabolizer (IM)

No action is needed for this gene-drug interaction.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Although the genetic variation leads to a higher plasma concentration of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

CYP2C19 Ultrarapid Metabolizer (UM)

No action is required for this gene-drug interaction.

Although the genetic variation may lead to faster inactivation of esomeprazole, there is insufficient evidence to support an effect on the therapeutic effectiveness and side effects.

Background information

For more information about the PM, IM, and UM phenotypes: see the general background information about *CYP2C19* on the KNMP Knowledge Bank or on www.knmp.nl (search for *CYP2C19*). Access requires KNMP membership.

Please review the complete therapeutic recommendations that are located here: (2).

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Flibanserin Therapy and *CYP2C19* Genotype

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Introduction

Flibanserin (brand name Addyi) is indicated for the treatment of "hypoactive sexual desire disorder" (HSDD) in premenopausal women. It is the first drug to be approved by the FDA for female sexual dysfunction. Flibanserin acts on central serotonin receptors and was initially developed to be an antidepressant. Although it was not effective for depression, flibanserin did appear to increase sex drive.

The use of flibanserin is limited by modest efficacy and the risk of severe hypotension and syncope (fainting). This risk is increased by alcohol, and by medications that inhibit CYP3A4 (the primary enzyme that metabolizes flibanserin). Consequently, alcohol use is contraindicated during flibanserin therapy, and flibanserin is contraindicated in individuals taking moderate or strong CYP3A4 inhibitors, which include several antibiotics, antiviral agents, cardiac drugs, and grapefruit juice.

The CYP2C19 enzyme also contributes to the metabolism of flibanserin, and individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have a higher exposure to flibanserin than normal metabolizers.

The risk of hypotension, syncope, and CNS depression may be increased in individuals who are CYP2C19 poor metabolizers, according to the FDA-approved drug label, which also states that approximately 2–5% of Caucasians and Africans and 2–15% of Asians are CYP2C19 poor metabolizers. However, the drug label does not provide alternative dosing for poor metabolizers (Table 1). The standard recommended dosage of flibanserin is 100 mg once per day, taken at bedtime (1).

Table 1. The FDA (2015) Drug Label for Flibanserin. Recommendations for CYP2C19 Poor Metabolizers.

Phenotype	Recommendations
CYP2C19 poor metabolizer	Increase monitoring for adverse reactions (e.g., hypotension) in individuals who are CYP2C19 poor metabolizers.

This FDA table is adapted from (1).

Drug: Flibanserin

Flibanserin is the first drug to be approved by the FDA to treat premenopausal women with "acquired, generalized hypoactive sexual desire disorder (HSDD), as characterized by low sexual desire that causes marked distress or interpersonal difficulty". The drug label states that flibanserin should not be used when problems with sexual desire are due to a coexisting medical or psychiatric condition, problems within the relationship, or the effects of medicine or other drugs. In addition, flibanserin should not be used to treat postmenopausal women or men, and it is not indicated to enhance sexual performance (1, 2).

Approximately 10% of adult women in the US are thought to have HSDD, which can significantly affect quality of life. The symptoms of HSDD vary, but may include a lack of sexual desire, impaired arousal, an inability to achieve orgasm, or a general decrease in sexual satisfaction, with accompanying distress (3, 4).

Note: in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM5), disorders of desire and arousal were joined into one classification titled female sexual interest/excitement and arousal disorder (FSIAD). However, HSDD is still referred to in the literature, and remains a central element of FSAID (5).

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A common reason for sexual problems is insufficient or inadequate sexual stimulation, and the mainstay of treatment is counselling (sex therapy, couples therapy, psychotherapy). Sex therapy includes education on the differences in male and female genital anatomy; e.g., in women, the clitoris is the structure for sexual pleasure, whereas the vagina is a birth canal and not in itself a source of pleasure - therefore, sexual intercourse is unpleasurable and even painful when the woman is insufficiently aroused. Sex therapy provides a safe and respectful space for sexual feelings to emerge and can identify issues such as anxiety and sexual trauma.

In addition to counselling, the management of female sexual dysfunction may also include lifestyle changes (e.g., relaxation techniques, increasing quality time with partner), and physical therapy; e.g., for problems related to pelvic floor hypertonus such as dyspareunia (painful intercourse) and vaginismus (inability for the penis to enter the vagina despite a woman's wish to do so). Medications may include hormone therapy and phosphodiesterase inhibitors (although the latter is not licensed for use in women, and studies report inconsistent results) (6).

Flibanserin is thought to work by targeting central serotonin receptors – it is a postsynaptic 5-HT-1A agonist and 5-HT-2A antagonist. It also has a weak antagonist effect on HT2B, 5-HT2C and dopamine D4 receptors (7-9).

The FDA approval of flibanserin in 2015 was controversial, primarily because of modest efficacy and safety concerns. A daily dose of 100 mg of flibanserin, taken at bedtime, has been associated with a modest increase in sexual desire, and a modest increase of sexually satisfying events – an additional "one half" of an event, per month, on average (9-15). Although only indicated for premenopausal women, one study reported that flibanserin was generally well tolerated and may have efficacy in post-menopausal women (16, 17).

The safety concerns of flibanserin therapy include the risk of severe hypotension, syncope, and CNS depression (e.g., daytime sleepiness). These risks are further increased if flibanserin is taken during the day (it should be taken at bedtime), is taken with alcohol, or taken with CYP3A4 inhibitors (flibanserin is primarily metabolized by CYP3A4). Both alcohol use and the use of strong or moderate CYP3A4 inhibitors are contraindicated with flibanserin use (18, 19). There have been no studies of flibanserin in pregnant women, and it is unknown whether flibanserin causes fetal harm.

Inhibitors of CYP3A4 include antibiotics (e.g., clarithromycin, ciprofloxacin, telithromycin), antifungals (e.g., ketoconazole, itraconazole, posaconazole, fluconazole), HIV drugs – antiretrovirals (e.g., ritonavir, saquinavir, nelfinavir, indinavir, atazanavir) and protease inhibitors – (e.g., amprenavir, fosamprenavir); hepatitis C virus protease inhibitors (e.g., boceprevir, telaprevir), calcium channel blockers (e.g., diltiazem, verapamil), the diuretic conivaptan, the antidepressant nefazodone, and grapefruit juice.

In addition, several drugs can induce CYP3A4 and although concomitant use of these drugs with flibanserin therapy is not contraindicated, it is not recommended. This is because exposure to flibanserin will be decreased, potentially to subtherapeutic levels. The antibiotic rifampin, which is a strong CYP3A4 inducer, decreased concentrations of flibanserin by 95% (20).

The CYP2C19 enzyme has a less prominent role in the metabolism of flibanserin. However, strong CYP2C19 inhibitors may increase flibanserin exposure, and individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") may have higher drug levels of flibanserin compared with normal metabolizers (20).

Gene: CYP2C19

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as benzodiazepines, antiplatelet agents, some proton pump inhibitors, antidepressants, and flibanserin –– flibanserin was originally developed to be an antidepressant.

The *CYP2C19* gene is highly polymorphic, as currently there are 35 variant star (*) alleles catalogued by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with "rapid" (**1/*17*) and "ultrarapid" (**17/*17*) metabolizer phenotypes. Heterozygous carriers of nonfunctional alleles (e.g., **2* and **3*) are classified as "intermediate metabolizers" (e.g. **1/*2*), and individuals who have 2 nonfunctional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 2).

Table 2. CPIC (2016). Assignment of CYP2C19 Phenotype based on Genotype.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual who has 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual who has one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual who has 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual who has one normal function allele and one no function allele, or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual who has 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (21).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This table is adapted from (21).

Approximately 2% of Caucasians, 4% of African Americans, and 15–25% of East Asians are CYP2C19 poor metabolizers, and up to 45% of individuals are CYP2C19 intermediate metabolizers (2, 22-24).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and nonfunctioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (25).

For CYP2C19, another commonly tested no function variant is *CYP2C19*3*, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (25).

Linking Gene Variation with Treatment Response

Currently, data are lacking on the influence of the CYP2C19 genotype on the efficacy and toxicity of flibanserin.

The drug label for flibanserin cites one study that compared 100 mg daily flibanserin in CYP2C19 poor metabolizers and normal metabolizers. In nine women who were poor metabolizers, the maximum serum concentration of flibanserin was 1.5 times higher, compared with normal metabolizers. In addition, exposure to

flibanserin was 1.3 times higher, and the drug's half-life increased by over 2 hours (from 11.1 hours in normal metabolizers to 13.5 hours in poor metabolizers).

Because CYP2C19 poor metabolizers have increased exposure to flibanserin, the FDA recommends increasing monitoring for adverse reactions (e.g., hypotension) in individuals who are CYP2C19 poor metabolizers.

In contrast to CYP3A4, the concurrent use of strong CYP2C19 inhibitors is not contraindicated with flibanserin therapy. However, the drug label does caution that the concomitant use of strong CYP2C19 inhibitors may increase flibanserin exposure, which in turn increases the risk of hypotension, syncope, and CNS depression. The label recommends discussing the use of a strong CYP2C19 inhibitor with the patient when prescribing flibanserin.

Drugs that are CYP2C19 inhibitors include selective serotonin reuptake inhibitors and other types of antidepressants (e.g., fluoxetine, fluvoxamine, moclobemide), antibiotics (e.g., chloramphenicol, isoniazid), antifungals (e.g., fluconazole, voriconazole), proton pump inhibitors and histamine antagonists (e.g., cimetidine, esomeprazole, omeprazole), HIV drugs (e.g., delavirdine, efavirenz), benzodiazepines, and other types of anti-seizure drugs (e.g., oxcarbazepine, felbamate, topiramate), and antiplatelet agents (e.g., clopidogrel, ticlopidine) (1, 20).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the *CYP2C19* gene and flibanserin response. In addition, variant CYP2C19 alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP) (27).

Clinical *CYP2C19* genotyping results are reported as a diplotype, such as *CYP2C19 *1/*1*, that typically also include an interpretation of the individual's predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 2 summarizes common CYP2C19 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): CYP2C19 poor metabolizers had increased flibanserin exposures compared to CYP2C19 extensive metabolizers. Additionally, syncope occurred in a subject who was a CYP2C19 poor metabolizer. Therefore, increase monitoring for adverse reactions (e.g., hypotension) in patients who are CYP2C19 poor metabolizers. The frequencies of poor CYP2C19 metabolizers are approximately 2–5% among Caucasians and Africans and approximately 2– 15% among Asians.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: the normal "wild type" allele is *CYP2C19*1*.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium database: https://www.pharmvar.org/

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Fluorouracil Therapy and *DPYD* Genotype

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Introduction

Fluorouracil, or 5-flourouracil (5-FU), is a chemotherapy agent that belongs to the drug class of fluoropyrimidines. When given as an intravenous (IV) solution, 5-FU is used in the palliative management of carcinoma of many solid tumors including (but not limited to) colon, rectum, breast, esophagus, cholangiocarcinoma (bile duct cancers), stomach, and pancreas. When prescribed as a cream or solution for topical use, fluorouracil (brand names Carac, Efudex, Fluoroplex, Tolak) is used to treat multiple actinic or solar keratoses of the face and scalp. Capecitabine (brand name Xeloda or CAPE) is the oral pill form of 5-FU chemotherapy, which is used interchangeably with 5-FU IV chemotherapy. Although it is the same drug, the oral pill version has certain side effects that are more pronounced (for example, diarrhea or skin related side effects – 'hand-foot' syndrome). Given the common usage of 5-FU for a variety of malignancies and potentially fatal overdoses, an antidote has been developed—uridine triacetate—which may be useful for pharmacogenetic-related overdoses, as well.

The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyzes the rate-limiting step in fluorouracil metabolism. Genetic variations in the *DPYD* gene can lead to enzymes with reduced or absent activity. Individuals who have at least one copy of a non-functional *DPYD* variant [for example, *DPYD*2A* (c.1905+1G>A) or *DPYD*13* (c.1679T>G)] will not be able to metabolize fluorouracil at normal rates. Consequently, they are at risk of potentially life-threatening fluorouracil toxicity, such as bone marrow suppression, diarrhea, and neurotoxicity. The prevalence of DPD partial deficiency varies in different populations but is approximately 35%. Complete absence of DPD function, which is often fatal with exposure to 5-FU chemotherapy, occurs in <1% (~0.2%) of the general population.

The FDA-approved drug label for fluorouracil states that no dose of fluorouracil has been proven safe in individuals with absent DPD activity (Table 1). Fluorouracil is contraindicated in individuals who are known to have complete DPD deficiency, or when complete deficiency is suspected because of early-onset or unusually severe fluorouracil toxicity (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have published dosing recommendations for fluoropyrimidines (fluorouracil and capecitabine) based on *DPYD* genotype (Tables 2 and 3). Both recommendations include dose reductions for intermediate metabolizers (with reduced enzyme activity) and avoiding fluorouracil or capecitabine and choosing an alternative agent for poor metabolizers (with absent enzyme activity).

Table 1. The FDA Drug Label for Fluorouracil: Warning DPD Deficiency (2020)

Phenotype	Fluorouracil
DPD deficiency	Increased risk of serious or fatal adverse reactions in individuals with low or absent dihydropyrimidine dehydrogenase (DPD) activity. Withhold or permanently discontinue fluorouracil or its oral pill version capecitabine in individuals with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No fluorouracil dose has been proven safe in individuals with absent DPD activity.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

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Table 2. The CPIC Recommended Dosing of Fluoropyrimidines (5-fluorouracil or capecitabine) by DPD Phenotype (2017, Nov 2018 Update)

Phenotype	Implications for phenotypic measures	Activity score	Dosing recommendations	Classification of recommendations[a]
DPYD normal metabolizer	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity.	2	Based on genotype, there is no indication to change dose or therapy. Use label-recommended dosage and administration.	Strong
DPYD intermediate metabolizer	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs.	1–1.5	Reduce starting dose by 50%, followed by dose titration based on clinical judgement (and ideally therapeutic drug monitoring) Individuals with homozygous c.[2846A>T];[2846A>T] genotype, a >50% reduction in starting dose may be warranted.	Moderate
DPYD poor metabolizer	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	0.5	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens. In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dose[c] with early therapeutic drug monitoring.[d]	Strong
		0	Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens.	

CPIC, Clinical Pharmacogenetics Implementation Consortium
DPD, dihydropyrimidine dehydrogenase.
[a] Rating scheme is described in Supplement (2).
[b] Increase the dose in individuals experiencing no or clinically tolerable toxicity in the first 2 cycles to maintain efficacy; decrease the dose in individuals who do not tolerate the starting dose to minimize toxicities.
[c] If available, a phenotyping test (see main text for further details) should be considered to estimate the starting dose. In the absence of phenotyping data, a dose of <25% of the normal starting dose is estimated assuming additive effects of alleles on 5-flourouracil clearance. [d]Therapeutic drug monitoring should be done at the earliest timepoint possible (for example, minimum timepoint in steady state) in order to immediately discontinue therapy if the drug level is too high.
This table is adapted from (2, 3) .
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC in a 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" (4).

Table 3. The DPWG Recommendations for Capecitabine/Fluorouracil by *DPYD* Gene Activity, Systemic Route of Administration (2019)

DPYD gene activity score	Recommendation	Pharmacist text
Activity score 1.5	Start with 50% of the standard dose or avoid fluorouracil and capecitabine. After starting treatment, the dose should be adjusted based on toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolized by DPD.	The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose

Table 3. continued from previous page.

DPYD gene activity score	Recommendation	Pharmacist text
Activity score 1.0	Start with 50% of the standard dose or choose an alternative. Adjustment of the initial dose should be guided by toxicity and effectiveness. Tegafur is not an alternative, as this is also metabolized by DPD.	Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.
PHENO[1]	It is not possible to recommend a dose adjustment for these individuals based on the genotype only. Determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype or avoid fluorouracil and capecitabine. Tegafur is not an alternative, as this is also metabolized by DPD.	The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.
Activity score 0	Avoid fluorouracil and capecitabine Tegafur is not an alternative, as this is also metabolized by DPD. If an alternative is not possible: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly. An individual with 0.5% of the normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every 5 days). An individual with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped).	Genetic variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the standard dose is a more than 100-fold overdose.

1 Individual's genotype does not accurately predict activity level, phenotyping required.
Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (5, 6).
DPWG - Dutch Pharmacogenetics Working Group

Drug Class: Fluoropyrimidines

Fluoropyrimidines are a class of antimetabolite drugs that are widely used in the treatment of cancer. There are 3 types of fluoropyrimidines in clinical use: capecitabine (oral - pill) and 5-fluorouracil (5-FU – IV), which are licensed for use in the US, and tegafur, which is not available in the US. Capecitabine and tegafur are both active precursors of fluorouracil.

Fluoropyrimidines are thought to exert their chemotherapeutic effects in multiple ways, through several active metabolites. The main mechanism of action is thought to be the inhibition of thymidylate synthase, which plays an important part in the folate-homocysteine cycle, and purine and pyrimidine synthesis pathways. Also, active metabolites can be incorporated into RNA and DNA, ultimately leading to cell death (7). Based on their mechanism of action, fluoropyrimidines can cause fetal harm when administered to a pregnant woman (1).

Approximately 10–40% of individuals develop severe and potentially life-threatening toxicity early during treatment with fluoropyrimidines (8). This toxicity typically leads to an interruption or discontinuation of potentially effective anticancer therapy, and may require an emergency room visit or hospitalization in severe instances (9).

The interindividual variation in the occurrence and severity of adverse events in individuals receiving fluoropyrimidines can be partly explained by clinical factors, such as age and gender. However, much of the variability in adverse events remains unexplained (10).

Of the genetic factors thought to contribute to fluoropyrimidine intolerance, the *DPYD* gene has been the most studied. This gene encodes the primary enzyme involved in breaking down fluoropyrimidines to inactive metabolites. Individuals who have a deficiency of the DPD enzyme have a significantly increased risk of suffering

from severe fluoropyrimidine toxicity, and the stratification of individuals on the basis of the *DPYD* genotype may help to prevent such adverse events (11, 12, 13, 14, 15, 16).

Drug: Fluorouracil

Fluorouracil is a form of chemotherapy that is given as an IV solution, and is used to manage many cancers, including carcinoma of the colon, rectum, breast, stomach, and pancreas. Fluorouracil may also be used topically as a cream or a solution, for the treatment of multiple actinic or solar keratoses of the face and anterior scalp.

Fluorouracil is structurally similar to pyrimidines, and the enzyme that catalyzes the rate-limiting step in the breakdown of pyrimidines (DPD) also catalyzes the rate-limiting step in 5-FU catabolism. The DPD enzyme catalyzes the conversion of fluorouracil to the non-cytotoxic dihydrofluorouracil (DHFU) (17).

The FDA states that fluorouracil therapy should be discontinued promptly whenever one of the following signs of toxicity appears:

- Cardiotoxicity including angina, myocardial infarction/ischemia, arrhythmia, and heart failure
- Hyperammonemic encephalopathy
- Neurologic toxicity, including acute cerebellar syndrome
- Mucositis, stomatitis or esophagopharyngitis, which may lead to mucosal sloughing or ulceration
- Myelosuppression, which may include neutropenia, thrombocytopenia, and anemia
- Diarrhea (grade 3 or 4), frequent bowel movements, or watery stools
- Palmar-plantar erythrodysesthesia (hand-foot syndrome), grade 2 or 3

The FDA label also indicates that uridine triacetate should be administered within 96 hours following the end of fluorouracil infusion for management of fluorouracil overdose. (1) Uridine triacetate (brand name Vistogard) was approved December 11, 2015 (18). Exogenous uridine competes with 5-FU for incorporation into RNA, thus diluting the toxic effects of high 5-FU levels. Uridine triacetate is 4–6-fold higher in bioavailability than equimolar doses of uridine (19).

Uridine triacetate is meant for overdose treatment of adults or children; however, can be considered in situations of individuals with pharmacogenetic deficiency, which is technically an overdose (20, 21). This compound can also be used in the context of capecitabine overdose (19). The high cost of a single course of uridine triacetate therapy has been cited as a potential barrier to therapy. Nevertheless, 94% of clinical trial participants treated with uridine triacetate survived the overdose event, a notable improvement over the historic mortality rate of 84% (19).

Symptomatic DPD deficiency is a rare autosomal recessive disorder with a wide range of symptoms, ranging from no symptoms or signs, to severe neurological problems. In affected individuals, the absent or greatly decreased DPD activity results in uracil and thymine accumulating in the blood, urine, and cerebrospinal fluid. Neurological symptoms typically manifest in early childhood and include seizures, small head size, and delayed cognitive and motor development (22).

Symptomatic DPD deficiency is typically caused by homozygous inactivation of *DPYD*; whereas individuals who are heterozygotes tend to be asymptomatic. However, all individuals with less than 70% DPD activity are considered at risk for the development of severe drug toxicity when treated with fluoropyrimidines (23). Signs of fluorouracil toxicity include severe diarrhea, severe mucositis, neutropenia, neurotoxicity, and hand-foot syndrome (redness, swelling, and blisters on the palms of the hands an soles of the feet) (1).

Gene: *DPYD*

The *DPYD* gene encodes the enzyme DPD, which catalyzes the first and rate-limiting step in the breakdown of the pyrimidine nucleotides thymine and uracil. The DPD enzyme also catalyzes the rate-limiting step in the breakdown of fluoropyrimidines.

Many *DPYD* variants have been described, although only a few have been demonstrated to influence DPD enzyme activity. When no variant is detected (formerly known as the **1* allele), it is associated with normal enzyme activity. Individuals who have 2 copies of normal activity *DPYD* alleles are known as "normal metabolizers" and have fully functional DPD enzyme activity (Table 4). The *DPYD* alleles c.1601G>A (*4, rs1801158), c.1627G>A (*5, rs1801159), c.2194G>A (*6, rs1801160), and c.85T>C (*9A, rs1801265) are also considered to have normal activity (24). Historically, variant haplotypes in *DPYD* have been identified by their star (*) allele names. However, the Pharmacogene Variation Database (PharmVar) now identifies these alleles by their dbSNP "rs" allele identifier or cDNA change based on the NM_000110.3 transcript, *DPYD* mRNA variant 1. All 3 of these identifiers are provided in the Nomenclature for Selected *DPYD* alleles table below.

Table 4. Activity Status of Selected *DPYD* Alleles

Allele type	Alleles	
	Strong evidence to support function	Moderate evidence to support function
Normal function	No variant detected (*1), c.1627G>A (*5, rs1801159), c.85T>C (*9A, rs1801265)	c.1601G>A (*4, rs1801158), c.2194G>A (*6, rs1801160), c.1003G>T (*11, rs72549306), c.2657G>A (*9B, rs1801267), 496A>G (rs2297595)
Decreased function	c.2846A>T (rs67376798), 1129-5923C>G and 1236G>A (HapB3)	c.557A>G (rs115232898)
No function	c.1905+1G>A (*2A, rs3918290)	c.1898delC (*3, rs72549303), c.295_298delTCAT (*7, rs72549309), c.703C>T (*8, rs1801266), c.2983G>T (*10, rs1801268), c.1156G>T (*12), c.1679T>G (*13, rs55886062)

This table is adapted from the "*DPYD* Allele Functionality Table", available from CPIC. Additional variant information from the PharmVar database. The cDNA coordinates for variation are given for NM_000110.3, *DPYD* transcript variant 1.
For the nomenclature of human *DPYD* alleles, please see (25).
CPIC, Clinical Pharmacogenetics Implementation Consortium

The non-functional *DPYD* variants that have been associated with absent DPD activity and an increased risk of toxicity with fluoropyrimidines include c.1905+1G>A (*2A, rs3918290) and c.1679T>G (*13, rs55886062) (26). Variants with decreased function include rs67376798 (c.2846A>T) and HapB3, which also are associated with an increased risk of fluoropyrimidine toxicity. The most well studied variant is *DPYD* c.1905+1G>A (*2A, rs3918290), in which a single nucleotide substitution at the invariant splice donor site of intron 14 leads to translation skipping exon 14, resulting in the production of a truncated protein with virtually no enzyme activity.

Individuals who have combinations of one normal function and one decreased function or no function *DPYD* alleles are known as "intermediate metabolizers". Individuals with 2 decreased function alleles are also categorized as intermediate metabolizers. They have partial DPD deficiency and are at increased risk of capecitabine toxicity. And individuals who have a combination of non-functional *DPYD* alleles, or decreased function *DPYD* alleles, or both, are known as "poor metabolizers". They have complete DPD deficiency and are at an even higher risk of capecitabine toxicity.

Activity scores may be used to distinguish between the various *DPYD* alleles and their functionality (Table 5). The use of activity scores may result in differentiated individualized dosing advice for fluoropyrimidines, which is beneficial for reducing toxic side effects while maintaining efficacy (15).

Table 5. Assignment of likely DPD Phenotype based on *DPYD* Genotype (CPIC, 2017)

Likely phenotype	Activity score[a]	Genotype[b]	Examples of genotype[c]
DPYD normal metabolizer	2	An individual with 2 normal function alleles.	c.[=]; [=] c.[85T>C]; [=] c.[1627A>G]; [=]
DPYD intermediate metabolizer (approximately 3–5% of individuals)	1 or 1.5	An individual with one normal function allele plus one no function allele or one decreased function allele, or an individual with 2 decreased function alleles.	c.[1905+1G>A]; [=] c.[1679T>G]; [=] c.[2846A>T]; [=] c.[1129–5923C>G]; [=][d] c.[1129–5923C>G]; [1129–5923C>G][d] c.[2846A>T]; [2846A>T]
DPYD poor metabolizer (approximately 0.2% of individuals)	0 or 0.5	An individual with 2 no function alleles or an individual with one no function plus one decreased function allele.	c.[1905+1G>A]; [1905+1G>A] c.[1679T>G]; [1679T>G] c.[1905+1G>A]; [2846A>T] c.[1905+1G>A]; [1129-5923C>G]

"[ ]" Square brackets are used to indicate an allele, "[=]" Indicates the allele sequence was tested and no changes were found
[a] Calculated as the sum of the 2 lowest individual variant activity scores. See (2) for further information.
[b] Allele definitions, assignment of allele function and references can be found on the CPIC website (*DPYD* Allele Functionality Table)
[c] HGVS nomenclature using the reference sequence NM_000110.3. Note: Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
[d] Likely HapB3 causal variant. See *DPYD* Allele Functionality Table available or other HapB3 proxy SNPs.
This table is adapted from (2).
Note: The nomenclature used in this table reflects the standardized nomenclature for pharmacogenetic terms proposed by CPIC (4).
CPIC, Clinical Pharmacogenetics Implementation Consortium

Overall, the prevalence of individuals who are heterozygous for non-functional *DPYD* alleles (partially DPD deficient) and at risk of severe drug reactions is estimated to be as high as 5–8%, but this varies in different populations (8, 23, 27, 28, 29, 30, 31, 32). In Caucasians, approximately 3–5% of have partial DPD deficiency and 0.2% have complete DPD deficiency (29). Recent studies suggest that ~8% of Caucasians have at least one of the 4 well characterized altered-function alleles (33).

In African-Americans, the prevalence of decreased DPD enzyme activity is 8% (32). It is notable that despite being well studied, *DPYD* c.1905+1G>A (*2A, rs3918290) is incredibly rare in individuals of African ancestry (34). One study reported that the normal function c.85T>C (*9A, rs1801265) allele was present in 49% of African-American samples (35). The rs115232898 (c.557A>G) variant allele with reduced function was detected in 2.6% of African-heritage Brazilians (36).

Studies of Egyptian and Tunisian populations suggest the allelic frequencies for *DPYD* variants in these 2 countries are similar to Caucasian variant allele frequencies (37, 38). The frequency of the poor-metabolizer rs6376798 (c.2846A>T) allele in Mestizo and native Mexican populations is rare, but not significantly different than in MXL (Mexican Ancestry from Los Angeles USA) or CEU (Utah Residents (CEPH) with Northern and Western European Ancestry) populations in the 1000 Genomes Project (39).

Asian populations have slightly different allele frequencies as compared with African and European populations. The frequency of the c.85T>C (*9A, rs1801265) normal function variant was slightly lower in Han Chinese, Korean and Japanese populations, particularly compared with Africans, though the frequency of the c.2657G>A (*9B, rs1801267) normal function variant and c.295_298delTCAT (*7, rs72549309), c.703C>T (*8, rs1801266), and c.2983G>T (*10, rs1801268) no function alleles were similar across these groups (35). The c.1905+1G>A (*2A/*2B, rs3918290) and c.1679T>G (*13, rs55886062) no function alleles were not detected in a study of Hmong and East Asian descent individuals, underscoring the rarity of these alleles (40). An analysis of multiple

genotyping studies in South Asian populations found that the rs2297595 (c.496A>G) allele was prevalent in south Asia (41).

Most individuals in the U.S. are not screened for DPD deficiency before starting fluorouracil therapy (42) and the FDA-approved label does not specifically recommend DPD testing (1). In contrast, the European Medicines Agency recommends testing for DPD deficiency before initiating treatment with any fluorouracil related substance via infusion or injection (43).

Gene: *TYMS*

Emerging studies and reports suggest genetic variation at another locus may also affect 5-FU efficacy and toxicity—*TYMS*. This gene encodes thymidylate synthase (TS), which catalyzes the methylation of deoxyuridylate to deoxythymidylate. This reaction is a rate-limiting step in production of an essential DNA synthesis precursor. The TS protein expression correlates positively with sensitivity to 5-FU, and the TS enzyme is one of the targets of 5-FU (44). While this functional link to 5-FU metabolism and tumor response has been demonstrated in multiple studies, the impact of specific genetic variants in *TYMS* is less clear (44, 45, 46, 47). Some *TYMS* alleles have been reported in a handful of studies as being associated with increased toxicity and anti-tumor cell response with fluoropyrimidines.

The rs45445694 variant is the basis of the *TYMS* "2R" allele, which has been associated with clinical response and severe toxicity events, either in homozygosity or heterozygosity (20, 48, 49, 50). This 2R variant occurs in the 5'UTR and is duplication of a 28 base pair (bp) repeat. This same locus can have variable tandem repeats between 0 and 9 copies, and studies suggest that the increased copy number of the repeat is associated with increased *TYMS* expression and TS protein levels (51).

One additional variant in *TYMS* has been found in association with adverse reactions to fluoropyrimidine therapy: a 3'UTR 9bp-indel (rs11280056) (49, 51). There are conflicting reports as to whether this is a 6- or 9-bp indel. One variant (rs2853542) within the *TYMS* enhancer region in the context of the 28bp tandem repeat triplication, called 3RG or 3RC based on the specific nucleotide present, has also been reported in association with neurotoxicity during 5-FU treatment (52). The presence of the C nucleotide at rs2853542 has been associated with decreased expression of *TYMS* mRNA (53).

PharmGKB has described *TYMS* as a 'Very Important Pharmacogene', though the level of evidence for *TYMS* and capecitabine/5-fluorouracil interaction is limited (PharmGKB "level 3") (51). The CPIC also views this interaction as having limited evidence and thus provides no prescribing recommendations for these pharmacogenetic variants (54).

Linking Gene Variation with Treatment Response

Standard doses of fluorouracil increase the risk of severe toxicity in individuals who are carriers of specific *DPYD* variant alleles, and no dose of fluorouracil is considered safe among individuals with absent DPD activity (1, 55). Multiple studies have found that preemptive *DPYD* screening for individuals with cancer can significantly improve individual safety (56, 57, 58, 59, 60, 61). Of note, at least one case report indicated that the cost of administering uridine triacetate and palliative care following an adverse, overdose reaction to 5-FU was roughly $180,000 USD (20).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the *DPYD* gene and the fluorouracil drug response. The *DPYD* c.1905+1G>A (*2A, rs3918290) variant is the most commonly tested;

however, newly discovered and rare variants may also lead to enzyme inactivation and toxicity to fluoropyrimidine-based chemotherapy (36, 62, 63, 64, 65).

Biochemical genetic tests may also be used, which assess the activity of the DPD enzyme. These tests include biochemical assays such as analyte testing (for example, measuring the amount of thymine and uracil in the urine or blood) or an enzyme assay (for example, directly measuring the activity of DPD using RNA extracted from blood cells and measuring the DPD mRNA copy number) (66, 67, 68).

The GTR provides a list of biochemical tests that assess the levels of thymine and uracil analytes, and the activity of the enzyme DPD.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

WARNINGS AND PRECAUTIONS: Increased Risk of Serious or Fatal Adverse Reactions in Patients with Low or Absent [Dihydropyrimidine] Dehydrogenase (DPD) Activity

Based on postmarketing reports, patients with certain homozygous or certain compound heterozygous mutations in the DPD[2] gene that result in complete or near complete absence of DPD activity are at increased risk for acute early-onset of toxicity and severe, life-threatening, or fatal adverse reactions caused by fluorouracil (e.g., mucositis, diarrhea, neutropenia, and neurotoxicity). Patients with partial DPD activity may also have increased risk of severe, life-threatening, or fatal adverse reactions caused by fluorouracil.

Withhold or permanently discontinue fluorouracil based on clinical assessment of the onset, duration and severity of the observed toxicities in patients with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No fluorouracil dose has been proven safe for patients with complete absence of DPD activity. There is insufficient data to recommend a specific dose in patients with partial DPD activity as measured by any specific test.

[…]

PATIENT COUNSELING INFORMATION

Advise patients to notify their healthcare provider if they have a known DPD deficiency.

Advise patients if they have complete or near complete absence of DPD activity, they are at an increased risk of severe and life-threatening mucositis, diarrhea, neutropenia and neurotoxicity.

[…]

OVERDOSAGE

Administer uridine triacetate within 96 hours following the end of fluorouracil infusion for management of fluorouracil overdose.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

[2] Note: the official gene symbol is *DYPD*, *DPD* is an alternate gene symbol.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC), with November 2018 Update

[…]

Table 2 summarizes the genetics-based dosing recommendations for fluoropyrimidines using the calculated DPYD activity score (DPYD-AS). The strength of the prescribing recommendations is based on the known impact of some variants (c.1905+1G>A, c.1679T>G, c.2846A>T, c.1129–5923C>G) on DPD activity, the demonstrated relationship between DPD activity and 5- fluorouracil clearance, and between 5-fluorouracil exposure and its toxic effects. Patients who are heterozygous for DPYD decreased/no function variants demonstrate partial DPD deficiency and should receive reduced starting doses. Prospective genotyping of c.1905+1G>A followed by a 50% dose reduction in heterozygous carriers resulted in a rate of severe toxicity comparable to noncarriers[see (9)]. This study thus demonstrated that DPYD genetic testing can reduce the occurrence of severe fluoropyrimidine-related toxicity, and that a dose reduction of 50% is suitable for heterozygous carriers of no function variants (DPYD-AS: 1). For decreased function variants, evidence is limited regarding the optimal degree of dose reduction. For c.2846A>T, a small retrospective study observed that the average capecitabine dose in heterozygous carriers was reduced by 25% compared to noncarriers. In a small prospective study, five patients carrying c.1236G>A (proxy for c.1129–5923C>G) were safely treated with a 25% reduced capecitabine starting dose. This suggests that heterozygous carriers of decreased function variants (DPYD-AS: 1.5) may tolerate higher doses com- pared to carriers of no function variants (DPYD-AS: 1). In patients with DPYD-AS of 1.5, the individual circumstances of a given patient should therefore be considered to determine if a more cautious approach (50% starting dose followed by dose titration), or an approach maximizing potential effectiveness with a potentially higher toxicity risk (25% dose reduction) is preferable. Of note, both studies indicating the suitability of a 25% dose reduction in decreased function variant carriers included only patients receiving capecitabine and no data are currently available for infusional 5-fluorouracil.

Given that some patients carrying decreased or no function variants tolerate normal doses of 5-fluorouracil, to maintain effectiveness, doses should be increased in subsequent cycles in patients experiencing no or clinically tolerable toxicity in the first two chemotherapy cycles or with subtherapeutic plasma concentrations. Similarly, doses should be decreased in patients who do not tolerate the starting dose.

In DPYD poor metabolizers (DPYD-AS: 0.5 or 0), it is strongly recommended to avoid use of 5-fluorouracil-containing regimens. However, if no fluoropyrimidine-free regimens are considered a suitable therapeutic option, 5-fluorouracil administration at a strongly reduced dose combined with early therapeutic drug monitoring may be considered for patients with DPYD-AS of 0.5. It should be noted, however, that no reports of the successful administration of low-dose 5-fluorouracil in DPYD poor metabolizers are available to date. Assuming additive effects of decreased and no function alleles (DPYD-AS: 0.5), it is estimated that a dose reduction of at least 75% would be required (i.e., starting dose <25% of normal dose). Furthermore, in such cases a phenotyping test is advisable to estimate DPD activity and a starting dose.

The US Food and Drug Administration (FDA) and the Health Canada Santé Canada (HCSC) have added statements to the drug labels for 5-fluorouracil and capecitabine that warn against use in patients with DPD deficiency, and prescribing recommendations for 5-fluorouracil, capecitabine, and tegafur are also available from the Dutch Pharmacogenetics Working Group.

November 2018 Update:

The current DPYD guideline recommends to reduce the dose of fluoropyrimidines by 25-50% (from the full standard dose) in DPYD Intermediate Metabolizers with an activity score of 1.5. At the time of the guideline publication, this dose range was recommended due to limited evidence for genotype-guided dosing of decreased

function alleles/variants. However, a recent prospective study (PMID: 30348537) provides evidence to support a recommendation for a 50% dose reduction in heterozygous carriers of the decreased function variants c.2846A>T (rs67376798) or c.1129–5923C>G (rs75017182; HapB3 or its tagging SNP c.1236G>A; rs56038477). These data suggest that all Intermediate Metabolizers with an activity score of 1.5 should receive a 50% dose reduction.

Therefore CPIC revises its recommendation such that all DPYD Intermediate Metabolizers should receive a 50% dose reduction from the full standard starting dose, whether the activity score is 1 or 1.5 followed by dose titration, based on clinical judgement and ideally therapeutic drug monitoring.

In addition, recent case reports from patients who are homozygous for c.2846A>T (activity score of 1) indicate that a dose reduction of more than 50% may be required in some carriers of this genotype. Therefore, in patients with an activity score of 1 due to a homozygous c.[2846A>T];[2846A>T] genotype, clinicians should be aware that a >50% reduction in starting dose might be warranted.

Please review the complete therapeutic recommendations that are located here: (2, 3)

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

DPD Gene Activity Score 0

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the standard dose is a more than 100-fold overdose.

- Avoid fluorouracil and capecitabine

Tegafur is not an alternative, as this is also metabolized by DPD.

- If it is not possible to avoid fluorouracil and capecitabine: determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose accordingly.

A patient with 0.5% of the normal DPD activity tolerated 0.8% of the standard dose (150 mg capecitabine every 5 days). A patient with undetectable DPD activity tolerated 0.43% of the standard dose (150 mg capecitabine every 5 days with every third dose skipped)

DPD [PHENO] [phenotyping indicates reduced function]

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

It is not possible to recommend a dose adjustment for this patient based on the genotype only.

- determine the residual DPD activity in mononuclear cells from peripheral blood and adjust the initial dose based on phenotype and genotype, or avoid fluorouracil and capecitabine.

Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 1

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- Start with 50% of the standard dose or avoid fluorouracil and capecitabine.

Adjustment of the subsequent dose should be guided by toxicity and effectiveness. However, in one study involving 17 patients with gene activity 1, the average dose after titration was 57% of the standard dose.

Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 1.5

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- Start with 50% of the standard dose or avoid fluorouracil and capecitabine.

After starting treatment, the dose should be adjusted based on toxicity and effectiveness. In a study involving 17 patients with genotype *1/2846T*, the average dose after titration was 64% of the standard dose. For 51 patients with genotype *1/1236A*, the average dose after titration was 74% of the standard dose. Tegafur is not an alternative, as this is also metabolized by DPD.

DPD Gene Activity Score 0 (Cutaneous fluorouracil)

The gene variation increases the risk of severe, potentially fatal toxicity. A reduced conversion of fluorouracil/capecitabine to inactive metabolites means that the normal dose is an overdose.

- avoid fluorouracil

NOTE: If a patient has two different genetic variations that lead to a non-functional DPD enzyme (e.g. *2A and *13), this recommendation only applies if the variations are on a different allele. If both variations are on the same allele, this patient actually has a gene activity score 1, for which no increased risk of severe, potentially fatal toxicity has been found with cutaneous use. These two situations can only be distinguished by determining the enzyme activity (phenotyping). This recommendation only applies if the patient has virtually no enzyme activity.

Background Information - Mechanism

Fluorouracil is mainly (> 80%) converted by dihydropyrimidine dehydrogenase (DPD) to inactive metabolites. Lower metabolic activity of DPD leads to increased intracellular concentrations of fluorodeoxyuridine monophosphate, the active metabolite of fluorouracil and its prodrug capecitabine. This leads to an increased risk of adverse events such as neutropenia, thrombopenia and hand-foot syndrome.

For more information about the phenotype gene activity score: see the general background information about DPD on the KNMP Knowledge Bank or on www.knmp.nl (search for DPD).

Please review the complete therapeutic recommendations that are located here: (5) .

Nomenclature for Selected Alleles

***DPYD* Alleles**

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs3918290	*DPYD*2A*, c.1905+1G>A	NM_000110.4:c.1905+1G>A	Not applicable—deletion of exon 14 leads to the production of a truncated protein	rs3918290
	IVS14+1G>A			
rs55886062	*DPYD*13*, c.1679T>G, rs55886062.1, p.Ile560Ser	NM_000110.4:c.1679T>G	NP_000101.2:p.Ile560Ser	rs55886062

DPYD Alleles continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs67376798	c.2846A>T p.Asp949Val	NM_000110.4:c.2846A>T	NP_000101.2:p.Asp949Val	rs67376798
rs75017182	c.1129–5923C>G	NM_000110.4:c.1129-5923C>G		rs75017182
rs1801159	*DPYD*5*, c.1627G>A	NM_000110.4:c.1627A>G	NP_000101.2:p.Ile543Val	rs1801159
rs1801265	*DPYD*9A*, c.85T>C	NM_000110.4:c.85T>C	NP_000101.2:p.Cys29Arg	rs1801265
rs1801158	*DPYD*4*, c.1601G>A	NM_000110.4:c.1601G>A	NP_000101.2:p.Ser534Asn	rs1801158
rs1801160	*DPYD*6*, c.2194G>A	NM_000110.4:c.2194G>A	NP_000101.2:p.Val732Ile	rs1801160
rs72549306	*DPYD*11*, c.1003G>T, rs72549306.1	NM_000110.4:c.1003G>T	NP_000101.2:p.Val335Leu	rs72549306
rs1801267	*DPYD*9B*, c.2657G>A	NM_000110.4:c.2657G>A	NP_000101.2:p.Arg886His	rs1801267
rs72549303	*DPYD*3*, c.1898delC	NM_000110.4:c.1898del	NP_000101.2:p.Pro633fs	rs72549303
rs72549309	*DPYD*7*, c.295_298delTCAT	NM_000110.4:c.295_298TCAT[1]	NP_000101.2:p.Phe100fs	rs72549309
rs1801266	*DPYD*8*, c.703C>T	NM_000110.4:c.703C>T	NP_000101.2:p.Arg235Trp	rs1801266
rs1801268	*DPYD*10*, c.2983G>T	NM_000110.4:c.2983G>T	NP_000101.2:p.Val995Phe	rs1801268
rs78060119	*DPYD*12*, c.1156G>T	NM_000110.4:c.1156G>T	NP_000101.2:p.Glu386Ter	rs78060119
rs115232898	557A>G (Y186C)	NM_000110.4:c.557A>G	NP_000101.2:p.Tyr186Cys	rs115232898
rs2297595	496A>G (M166V)	NM_000110.4:c.496A>G	NP_000101.2:p.Met166Val	rs2297595
rs75017182 rs56038477	HapB3 1129-5923C>G 1236G>A	NM_000110.4:c.1129-5923C>G NM_000110.4:c.1236G>A	Altered mRNA splicing due to cryptic splice donor site leads to retention of intronic sequence, introduces premature termination codon in resulting protein.	rs75017182 rs56038477
			NP_000101.2:p.Glu412=	

TYMS Alleles

Common allele name	Alternative names	HGVS reference sequence	dbSNP reference identifier for allele location
rs45445694	2R, 3R *TYMS* 5'UTR	GRCh37.p13 chr 18, NC_000018.9:g.657657_657712del, NC_000018.9:g.657657_657684GGCCTGCCTCCGTCCCGCCGCGCCACTT[1]-[9] #	rs45445694
rs11280056	*TYMS* 3'UTR	GRCh37.p13 chr 18, NC_000018.9:g.673447_673452del, NC_000018.9:g.673447_673452dup# NM_017512.7:c.*856_*861del	rs11280056

TYMS Alleles continued from previous page.

rs2853542	*TYMS* 3RG, 3RC	GRCh37.p13 chr 18, NC_000018.9:g.657685G>C[#] NM_001071.4:c.-58=	rs2853542

This is a non-coding variant in the *TYMS* untranslated region. Coordinates given are chromosomal.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (69).
Allele information for *DPYD* can also be found at the Pharmacogene Variation Consortium (PharmVar).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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To view the 2016 version of this summary (created 3 November 2016), please click here.

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Flurbiprofen Therapy and *CYP2C9* Genotype

Laura Dean, MD[1]

Created: February 11, 2019.

Introduction

Flurbiprofen (brand name Ansaid) is a nonsteroidal anti-inflammatory drug (NSAID). Tablets and skin patches are used in the management of osteoarthritis and rheumatoid arthritis. Flurbiprofen provides pain relief and reduces inflammation. Flurbiprofen eye drops (brand name Ocufen) may also be used to prevent miosis (excessive constriction of the pupil) during eye operations; e.g., cataract surgery.

Flurbiprofen is primarily metabolized by CYP2C9. Individuals who lack CYP2C9 activity (CYP2C9 poor metabolizers) have an increased exposure to flurbiprofen, and an increased risk of side effects.

Like all NSAIDs, flurbiprofen increases the risk of serious cardiovascular events, including myocardial infarction and stroke, and serious gastrointestinal (GI) adverse events such as bleeding, ulceration, and perforation, which may be fatal.

The recommended starting dose of flurbiprofen tablets in adults is 200–300 mg per day, divided for administration 2, 3, or 4 times a day. But for all patients, the lowest effective dose of flurbiprofen should be used for the shortest length of time, consistent with the treatment goals of each individual.

The FDA-approved drug label for flurbiprofen states that the dose of flurbiprofen should be reduced in "patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin)" (Table 1). This dose reduction is to avoid the abnormally high plasma levels of flurbiprofen in these patients caused by reduced metabolic clearance. However, specific dose reductions based on *CYP2C9* phenotype are not provided (1).

As for all NSAIDs, flurbiprofen is contraindicated in patients with a known hypersensitivity; a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID; and for coronary artery bypass graft (CABG) surgery. Flurbiprofen should also be avoided by pregnant women starting at 30 weeks gestation (1).

Table 1. The FDA (2017) Drug Label for Flurbiprofen. Poor Metabolizers of CYP2C9 Substrates.

Phenotype	Recommendations
CYP2C9 Poor metabolizers	In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin), reduce the dose of flurbiprofen to avoid abnormally high plasma levels due to reduced metabolic clearance.

This table is adapted from (1).

Drug Class: NSAIDs

NSAIDs are widely used to treat inflammation, fever, and pain. They are one of the most commonly used class of drugs. Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2).

Currently, more than 20 NSAIDs are licensed for use. Several NSAIDs (e.g., aspirin, ibuprofen, and naproxen) are available over-the-counter, but higher doses and other types of NSAIDs, such as celecoxib, piroxicam, and flurbiprofen, are only available via prescription.

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The main action of NSAIDs is to inhibit cyclooxygenase (COX). Cyclooxygenase is the central enzyme in the synthesis of prostaglandins, prostacyclin, and thromboxanes from arachidonic acid. Prostaglandins can be protective (e.g., protect the gastric mucosal lining and aid platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

There are 2 main isoforms of COX, and the safety and effectiveness of NSAIDs may be influenced by the degree they inhibit the 2 different forms. COX-1 is a "housekeeping enzyme" that is expressed in most tissues. It protects the GI tract and induces platelet aggregation in response to injury. In contrast, COX-2 is often undetectable in tissues. However, the expression of COX-2 is increased during inflammation.

Most NSAIDs are non-selective COX inhibitors that inhibit both COX-1 and COX-2. There are exceptions, such as celecoxib, which is a selective COX-2 inhibitor that appears to be associated with fewer adverse GI events. However, GI adverse events still occur.

Approximately 25% of the exposed population in the US has experienced NSAID-related side effects that required medical care (3). All NSAIDs carry a boxed warning regarding the risk of serious GI and cardiovascular adverse events; e.g.,

"*NSAIDs cause an increased risk of serious cardiovascular thrombotic events, including myocardial infarction and stroke, which can be fatal. This risk may occur early in treatment and may increase with duration of use.*

NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients and patients with a prior history of peptic ulcer disease and/or GI bleeding are at greater risk for serious GI events" (1).

Drug: Flurbiprofen

Flurbiprofen is an NSAID used for the relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis. It may also be used for soft tissue injuries, such as bursitis and tendinitis.

The recommended starting dose of flurbiprofen tablets in adults is 200–300 mg per day, divided into doses to be taken 2, 3, or 4 times a day. The largest recommended single dose in a multiple-dose daily regimen is 100 mg (1).

Because of the adverse events associated with any type of NSAID, the lowest effective dose of flurbiprofen should be used, for the shortest duration. And, as for all NSAIDs, flurbiprofen is contraindicated in patients with a known hypersensitivity, or a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID. Flurbiprofen is also contraindicated to treat pain in the days following CABG surgery –– this is because NSAIDs increase the risk of myocardial infarction and stroke after surgery. Flurbiprofen should be avoided by pregnant women starting at 30 weeks gestation –– this is because NSAID use in the third trimester causes an increased risk of premature closure of the fetal ductus arteriosus. There are no well-controlled studies of flurbiprofen in pregnant women, but in animal studies, flurbiprofen was lethal to the embryos of pregnant rats and rabbits. Flurbiprofens' safety, efficacy, and pharmacokinetics have not established for pediatric patients.

Flurbiprofen can be taken orally (tablets) or topically (via a skin patch or cream) for the treatment of osteoarthritis and rheumatoid arthritis. Flurbiprofen is also available as an ophthalmic solution (eye drops) –– it is used before eye surgery to prevent miosis (excessive constriction of the pupil), which can occur in surgical procedures such as cataract surgery.

CYP2C9 is the main enzyme involved in the metabolism of flurbiprofen to its inactive metabolite: 4'-hydroxyflurbiprofen. Both flurbiprofen and its metabolite are eliminated as acyl glucuronides. Individuals who have decreased CYP2C9 activity, such as CYP2C9 intermediate and poor metabolizers, have a higher exposure to flurbiprofen (1, 4, 5).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (6).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected and is categorized as having normal enzyme activity (7). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual carrying one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual carrying 2 decreased function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers.
[a] Global frequencies are approximate. Because haplotype frequencies vary considerably among populations, please see (7) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (7) for activity ranges.
This table is adapted from (7). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (8).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10–20%) than Asian (1–3%) or African (0–6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (9-11).

Linking Gene Variation with Treatment Response

Studies have shown that CYP2C9 intermediate or poor metabolizers have increased drug exposure when taking standard doses of flurbiprofen.

Although data are lacking that link CYP2C9 intermediate or poor metabolizers with an increased risk of the adverse effects associated with NSAID therapy, the dose, and duration of NSAID therapy do influence the risk of adverse effects, such as severe GI bleeding.

Therefore, the FDA drug label for flurbiprofen recommends reducing the dose of flurbiprofen in CYP2C9 poor metabolizers. The FDA label does not however recommend a dose reduction in CYP2C9 intermediate metabolizers, despite the observed high levels of the drug in this genotype group in other studies (1, 4, 5, 12).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH's Genetic Testing Registry (GTR) displays genetic tests that are currently available for flurbiprofen response and for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin), reduce the dose of flurbiprofen to avoid abnormally high plasma levels due to reduced metabolic clearance.

Please review the complete therapeutic recommendations that are located here:(1)

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (13).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Gentamicin Therapy and *MT-RNR1* Genotype

Laura Dean, MD[1] and Megan Kane, PhD[2]

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Introduction

Gentamicin (brand names Garamycin, Cidomycin, and Septopal) is an aminoglycoside antibiotic that is used to treat sepsis. Gentamicin is administered by injection to treat serious infections caused by gram-negative bacteria (for example, *Pseudomonas aeruginosa*, *Proteus* species, *Escherichia coli*, *Klebsiella-Enterobacter-Serratia* species, and *Citrobacter* species). Additionally, gentamicin is used as an adjuvant treatment for infections caused by gram-positive bacteria (such as *Staphylococcus* species) (1). Gentamicin may also be used topically to treat ophthalmic and dermatological infections.

In most individuals, prolonged exposure to high gentamicin levels will cause ototoxicity (damage to the inner ear). However, among individuals who have specific variants in the mitochondrial gene *MT-RNR1*, a single dose of gentamicin can result in hearing loss (cochleotoxicity). This toxicity occurs in genetically susceptible individuals, despite serum drug concentrations within the normal therapeutic range (2). This hearing loss can be triggered not only by gentamicin, but by other aminoglycoside antibiotics and is referred to as aminoglycoside-induced hearing loss (AIHL).

Substantial literature has reported that a high proportion of individuals with the *MT-RNR1* m.1555A>G variant (NC_012920.1:m.1555A>G) develop hearing loss after receiving aminoglycoside therapy. The onset of hearing loss among these individuals varies, but once it occurs, it is usually moderate to profound, bilateral, and irreversible (3). Additional *MT-RNR1* genotypes associated with increased risk of AIHL include m.1095T>C and m.1494C>T (4).

The FDA-approved drug label for gentamicin does not include a statement regarding *MT-RNR1* (5); however, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has published guidelines for administration of aminoglycosides, including gentamicin, with respect to variants in the *MT-RNR1* gene (4). These guidelines (Table 1) recommend avoiding the use of aminoglycoside antibiotics, such as gentamicin unless there are no satisfactory alternatives, by individuals who have a *MT-RNR1* genotype that puts them at high risk for AIHL. The CPIC guideline further advises that individuals with normal-risk alleles or uncertain-risk alleles at the *MT-RNR1* locus should all use aminoglycosides at standard doses for the shortest feasible course, with regular evaluation for hearing loss. This extends the 2014 American College of Medical Genetics and Genomics guideline with the following recommendation: "Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA variants associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics" (6, 7).

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Table 1: The CPIC Recommendations for Aminoglycoside Use and *MT-RNR1* Phenotype

Phenotype	Example genotype(s)*	Recommendation (strength)	Implications	Considerations
MT-RNR1 increased risk of aminoglycoside-induced hearing loss	m.1095T>C m.1494C>T m.1555A>G	Avoid aminoglycoside antibiotics unless the high risk of permanent hearing loss is outweighed by the severity of infection and lack of safe or effective alternative therapies. (Strong)	Very high risk of developing hearing loss if administered an aminoglycoside antibiotic	If no effective alternative to an aminoglycoside antibiotic is available, evaluate for hearing loss frequently during therapy and ensure that all appropriate precautions are utilized (namely, lowest possible dose and duration, utilization of therapeutic drug monitoring, hydration, renal function monitoring).
MT-RNR1 normal risk of aminoglycoside-induced hearing loss	m.827A>G	Use aminoglycoside antibiotics at standard doses for the shortest feasible course with therapeutic dose monitoring. Evaluate regularly for hearing loss in line with local guidance. (Strong)	Normal risk of developing hearing loss if administered an aminoglycoside antibiotic.	Individuals without *MT-RNR1* aminoglycoside-induced hearing loss increased risk variants are still at risk of aminoglycoside-associated hearing loss, especially with high drug levels or prolonged courses.
MT-RNR1 uncertain risk of aminoglycoside-induced hearing loss	m.663A>G m.961T>G m.961T>del+Cn m.1189T>C m.1243T>C m.1520T>C	Use aminoglycoside antibiotics at standard doses for the shortest feasible course with therapeutic drug monitoring. Evaluate regularly for hearing loss in line with local guidance. (Optional)	Weak or no evidence for an increased risk of *MT-RNR1*-associated hearing loss if administered an aminoglycoside antibiotic.	Individuals without *MT-RNR1* aminoglycoside-induced hearing loss increased risk variants are still at risk of aminoglycoside-associated hearing loss, especially with high drug levels or prolonged courses.

This table is adapted from (4). *Example genotypes are based on mitochondrial reference sequence NC_012920.1.

Drug: Gentamicin

Aminoglycosides such as gentamicin are among the earliest formulations of antibiotics (8). They are effective against most aerobic bacteria, both gram positive and gram negative. However, because they are inactive against anaerobes, they are often used with another antibiotic, such as a beta-lactam antibiotic or a cephalosporin, to increase coverage (9).

Aminoglycoside drugs approved for use by the FDA include amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, and tobramycin. The ending of these drug names, -mycin or -micin, reflects from which genus of bacteria the aminoglycoside was derived, either *Streptomyces* or *Micromonospora* (10).

Aminoglycosides exert antibacterial effects by binding to bacterial ribosomes and inhibiting bacterial protein synthesis. They bind to the 30S ribosomal subunit, which interferes with the decoding site, which is where the ribosome facilitates the accurate match of the tRNA in accordance with the appropriate mRNA codon. Errors here lead to inappropriate translation of the mRNA codons, so that incorrect amino acids are inserted into the polypeptide chain, which can disrupt elongation of the peptide chain (11, 12).

Like all aminoglycosides, gentamicin is poorly absorbed from the gut, so it is not taken orally. It is either given by intravenous (IV) or, less commonly, intramuscular (IM) injection with regular blood tests to monitor drug levels, or given topically in the form of drops, cream, or ointment to treat infections of the eye or skin. Gentamicin is also used for prevention and treatment of recurrent urinary tract infections (UTIs) via intravesical

delivery, also called bladder irrigation (13, 14). Systemic exposure following topical gentamicin cream has been reported to be low, though this may be higher among individuals being treated for burns (15, 16). The FDA-approved labels for topical gentamicin formulations do not discuss absorption kinetics, nor a need for therapeutic drug monitoring (17, 18).

The scope of this document is limited to gentamicin therapy, though much of the guidance and literature related to therapy-induced ototoxicity is inclusive of multiple aminoglycoside antibiotics. Readers are encouraged to review regulatory labeling and the CPIC guideline for aminoglycosides regarding those related medications (4).

The toxicity of aminoglycosides, along with the discovery of equally potent but less toxic antibiotics, has meant that the use of aminoglycoside injections is reserved for serious infections that are proven, or strongly suspected to be, caused by susceptible bacteria. Aminoglycosides are most commonly used in the treatment of neonatal septicemia, especially in premature or low-birth-weight babies, with estimates of 61–90% receiving some kind of aminoglycoside therapy during the first weeks of life (19, 20, 21). Aminoglycosides are also used with other antibiotics as surgical prophylaxis among individuals who are allergic to penicillin, and for febrile neutropenia, septic shock, and drug-resistant tuberculosis (8). Individuals with cystic fibrosis frequently require aminoglycoside antibiotic therapy to manage recurrent lung infections, though tobramycin and amikacin are the recommended medications (22, 23). Aminoglycoside antibiotics are also recommended for management of peritoneal dialysis-associated peritonitis with gram-negative bacterial infection, and empiric antibiotic selection that covers both gram-positive and -negative bacterial species administered promptly is associated with better outcomes (24).

Neonatal sepsis is often treated with aminoglycosides such as gentamicin with ampicillin. The World Health Organization (WHO) recommends one of 2 treatment courses for children in the first 60 days of life who present with clinical severe infection in a setting without access to hospitalization. The first course is IM gentamicin injection (when hospitalization and IV administration are not feasible) (5–7.5 mg/kg or 3–4 mg/kg for low-birth-weight infants) once daily for 7 days with twice daily oral amoxicillin (50 mg/kg per dose) for 7 days. The second option for managing a neonate with clinical severe infection is IM injection of gentamicin (5–7.5 mg/kg or 3–4 mg/kg for low-birth-weight infants) once daily for 2 days and twice daily oral amoxicillin (50 mg/kg per dose) for 7 days. If the second option is selected, a close evaluation of the child must be made on day 4 to verify improvement. (25) Recently, simplified dosing regimens for neonates have been proposed (26). The American Academy of Pediatrics recommends empirical use of use of either ceftazidime or gentamicin (IM or IV, 4 mg/kg per dose) along with ampicillin for the treatment of well-appearing febrile infants (aged 8–21 days old) with either an unknown source of infection or known UTI (27).

The main toxicities associated with aminoglycoside therapy are kidney damage (nephrotoxicity) and damage to the inner ear (ototoxicity) (28). Nephrotoxicity primarily involves the proximal tubules and is often, but not always, reversible (29). A Fanconi-like syndrome, with aminoaciduria and metabolic acidosis, has occurred in some adults and infants receiving gentamicin injection (5). Studies for medications that can be co-administered to reduce gentamicin nephrotoxicity have shown promising results (30, 31). In contrast, aminoglycoside-induced ototoxicity is typically irreversible. Ototoxicity from gentamicin may damage the cochlea (cochleotoxicity)—resulting in sensorineural hearing loss—or the vestibular system (vestibulotoxicity)—causing problems with balance, vertigo, ataxia, nausea, and vomiting. Increased risk of vestibulotoxicity has not been reported to be associated with *MT-RNR1* variation. Gentamicin is more toxic to the vestibular system so is used for vestibular ablation to treat Ménière's disease via intratympanic dosing (32). However, the risk of hearing loss is greater with gentamicin therapy as compared with other pharmacologic methods, despite improved outcomes with respect to vertigo (32). Amikacin and neomycin are examples of aminoglycosides that are more toxic to the cochlea (28, 33). Monitoring for signs of ototoxicity or co-administration of aminoglycosides with N-acetylcysteine have been recommended for individuals with an elevated infection risk that require aminoglycoside therapy, such as those with cystic fibrosis or individuals undergoing peritoneal dialysis (23, 24). The proposed mechanism

underlying the otoprotective effects of N-acetylcysteine is its ability to act as an antioxidant, thereby reducing levels of reactive oxygen species that may be damaging the inner ear (34). The frequency of hearing loss or other ototoxicity in the pediatric population following aminoglycoside use has been reported to range from 0–57% (35), and among adults the incidence of hearing loss following aminoglycoside therapy ranges from 20–63% (36).

Rarely, neuromuscular blockade can occur after aminoglycoside therapy. The boxed warning on the FDA-approved drug label recommends that aminoglycosides "be used with caution in individuals with neuromuscular disorders, such as myasthenia gravis or parkinsonism, because they may aggravate muscle weakness (9)"; whereas in 2014, the British National Formulary stated that aminoglycosides should not be given to individuals with myasthenia gravis (37). The European Medicines Agency cautions that acute renal failure and Fanconi-like syndrome are 2 very rare adverse reactions to gentamicin medication and notes that irreversible hearing loss or deafness is also a risk of unknown frequency (38).

Elderly individuals who may have reduced renal function may experience elevated exposure to aminoglycoside antibiotics, increasing the risk of toxicity. The FDA-approved drug label for injectable gentamicin recommends that creatinine clearance is a more useful indication of renal function as compared with other tests.

The use of gentamicin or other aminoglycoside antibiotics during pregnancy can result in fetal exposure to the medication, as these compounds can cross the placenta. There are documented reports of total irreversible congenital deafness in children whose mothers were administered streptomycin during pregnancy, though serious effects on either the mother, fetus, or newborn have not been observed for other aminoglycosides. The FDA states that pregnant individuals should be advised of the risk to the fetus if gentamicin is used during pregnancy (5). Gentamicin is poorly excreted into breastmilk following systemic administration and maternal use of ear or eye drop medications that include gentamicin present little to no risk for a nursing infant (39). While infant gastrointestinal absorption of gentamicin from breastmilk can occur, the resulting serum concentrations in newborns and older infants were well below the therapeutic levels and are reportedly unlikely to have systematic effects (39).

Gene: *MT-RNR1*

Mitochondria are the main source of energy in most cells, as they use oxygen, sugars, and fats to create energy in the form of adenosine triphosphate. This process is known as oxidative phosphorylation. Any genetic variation that disrupts normal mitochondrial function can have severe effects on health.

Mitochondria have their own genome, which is small, circular, and resembles the bacterial prokaryotes from which they evolved. The mitochondrial genome (mtDNA) is passed down from mother to child (maternal inheritance) and has 37 genes, one of which is the *MT-RNR1* gene ("mitochondrially encoded 12S RNA"). The ribosomal RNA (rRNA) encoded by *MT-RNR1* is essential in the synthesis of the proteins that perform oxidative phosphorylation. (3) Each mitochondrion holds multiple copies of its genome, and the number of mitochondria can vary between cells and tissues. It is possible for these multiple copies of mtDNA to vary in genotype at specific loci within an individual. This is called heteroplasmy and the relative frequencies of genotypes can differ from cell to cell and tissue to tissue. Homoplasmy is the term for all mitochondria having the same genotype at the locus of interest.

Consistent with their bacterial origin, mitochondrial rRNA more closely resembles bacterial rRNA than human rRNA. However, at a highly conserved decoding region in the *MT-RNR1* gene, the sequence in humans is distinct from the sequence in bacteria. This difference means that aminoglycosides, which target the decoding region in bacteria, normally do not bind to this region in humans (11).

However, genetic variation in the ribosomal decoding region can result in mitochondrial rRNA appearing more like bacterial rRNA, thereby facilitating the binding of aminoglycosides. This results in inhibition of protein

synthesis, as in the bacterial ribosome, leading to cellular toxicities. The mechanism of cellular toxicity is unclear, but aminoglycosides preferentially damage the sensory hair cells in the cochlea that mediate hearing (40, 41, 42). Hearing loss is a common symptom in many mitochondrial disorders, pointing to a critical role for mitochondria in the auditory system.

The most common *MT-RNR1* variant is a single nucleotide substitution of a guanine nucleotide at position 1555 in place of an adenine nucleotide (m.1555A>G). Individuals with this variant are exquisitely sensitive to AIHL, which is moderate to profound, bilateral, irreversible, and may have a rapid onset. Even a single dose of aminoglycoside can be sufficient to cause ototoxicity (2, 43).

Genetically susceptible individuals who are not exposed to aminoglycosides may nonetheless develop hearing loss, referred to as "non-syndromic mitochondrial hearing loss," though more data are needed to understand this relationship. The course of hearing loss may be affected by the presence of additional genetic factors as well as environmental factors, such as exposure to loud noise. However, preliminary findings suggest that normal hearing may be preserved until at least 44 years of age (2).

The prevalence of the m.1555A>G variant varies among different populations. In the US, the UK, and Finland, the prevalence is estimated to be 0.2% (7, 21, 44, 45). Data summarized from CPIC reports the overall frequency of the m.1555A>G variant to be 0.11% for both central/south Asia and Europe, 1.81% for East Asian ancestry, 0.14% for near Eastern populations, and 0.3% for sub-Saharan Africa (46). Individuals with either m.1555A>G or m.1494C>T (described below) were observed at a frequency of 0.227% in one study from Beijing, China (47). Among hearing-impaired populations, the prevalence is much greater; however, the estimates vary widely based on study differences, such as the age of onset of hearing loss and whether there has been exposure to aminoglycosides. Estimates include a prevalence of 3.5% among the hearing-impaired population in Japan (48), 5% among deaf individuals in Indonesia (49), and 6% of individuals with postlingual hearing loss from the UK and Southern Italy (50). Additionally, a prevalence of 15% has been reported in "ethnically diverse patients in the United States with hearing loss after aminoglycoside exposure" (51), and in 15-–20% of individuals from Spain with hearing loss (52).

The m.1555A>G variant is the best studied *MT-RNR1* variant with regards to aminoglycoside ototoxicity, but other mitochondrial variants are also associated with hearing loss. In 10 small studies, all individuals with the m.1494C>T (NC_012920.1:m.1494C>T, rs267606619) variant developed hearing loss after receiving an aminoglycoside antibiotic. The CPIC guidelines state that both the m.1555A>G and m.1494C>T variants are risk alleles for AIHL, with high levels of evidence. Another variant, m.1095T>C (NC_012920.1:m.1095T>C, rs267606618), is similarly associated with a risk of AIHL by CPIC, with a moderate level of evidence (3, 4). In addition, the m.827A>G (NC_012920.1:m.827A>G) variant, and variants at position 961, have also been associated with nonsyndromic hearing loss, both with and without the use of aminoglycosides (3); however, due to the high frequency of m.827A>G in certain populations, CPIC determined that this allele is associated with normal risk of AIHL. (4)

Several studies have highlighted the complex issues raised by screening for pathogenic *MT-RNR1* variants. The aim of screening is to prevent avoidable hearing loss in genetically susceptible individuals by administering an alternative antibiotic whenever possible. Issues include the costs of universal screening, for example, as part of the newborn screening program—given that the prevalence of m.1555A>G is thought to be one in 385 Caucasians (2, 53, 54) —versus limiting genetic testing to a case-by-case basis (that is, individuals with tuberculosis, children with leukemia, individuals with cystic fibrosis, and individuals requiring surgery who are allergic to beta-lactam antibiotics) (7, 55). A family history of hearing loss may be useful in identifying candidates for genetic testing, but not necessarily indicative of AIHL risk due to *MT-RNR1* variation, as multiple genes can contribute to inherited hearing loss in syndromic or nonsyndromic forms (6). A report from the WHO's Essential Medicines and Pharmaceutical Policies comments that "pre-treatment screening is an

important consideration to prevent aminoglycoside related hearing loss but given cost and access issues, asking about a maternal family history of deafness may be more practical" (56).

In the US, aminoglycosides are most commonly used in the neonatal intensive care unit, where acute, life-threatening sepsis means that aminoglycoside therapy cannot be delayed to wait for the results of genetic testing (57). However, recent advances in screening have allowed for rapid, accurate, and inexpensive testing (58, 59, 60). A recent study from the UK described the development and implementation of a rapid point-of-care test for septic infants requiring intensive care, which was successfully integrated into routine care and returned results in less than 30 minutes (61).

Linking Genetic Variation to Treatment Response

Individuals who have one of the *MT-RNR1* at-risk alleles will not always develop hearing loss upon exposure to aminoglycosides. The highest risk allele, based on a review of over 40 studies, is the m.1555A>G variant. As reviewed by Barbarino and colleagues, over 96% of individuals with this genotype developed some degree of AIHL (466 individuals with hearing loss as compared with 16 without hearing loss out of 482 individuals in the reviewed articles) (3). One study of a Spanish family with heteroplasmy for this variant reported that individuals with <20% variant burden at the m.1555 locus did not develop hearing loss, or the loss was mild (62). Other recent studies that show a lack of AIHL among individuals with m.1555A>G were primarily performed in the neonatal setting, following use of gentamicin in septic newborns. The proportion of infants with m.1555A>G who failed the newborn screening test after aminoglycoside treatment was 4 out of 15, leading one group to conclude that this genotype is a risk factor for a failed newborn hearing test (63, 64, 65). However, the degree and timing of the onset of AIHL attributed to at-risk genotypes is still unclear. While some reports suggest the degree of mitochondrial heteroplasmy may affect the penetrance of the hearing-loss phenotype (62, 66), CPIC's recommendations to avoid aminoglycosides (including gentamicin) apply to individuals who are heteroplasmic or homoplasmic for the at-risk genotype(s) (4).

The variants at m.1555 and m.1494 have been shown to affect the structure of the eukaryotic rRNA, increasing the relative affinity of aminoglycosides for the ribosome (3). The m.1095T>C variant is associated with increased cellular apoptosis in the presence of aminoglycosides, presumably due to a similar increased affinity between the ribosome and medication (67).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are available for the *MT-RNR1* gene and Gentamicin response. Targeted mutation panels vary among testing laboratories, but most laboratories that interrogate the *MT-RNR1* gene routinely test for m.1555A>G.

The *MT-RNR1* variants are associated with 2 conditions: aminoglycoside hypersensitivity resulting in post-exposure deafness, and nonsyndromic mitochondrial hearing loss that tends to develop gradually over time. While the presence of an *MT-RNR1* variant indicates a high risk of aminoglycoside ototoxicity, the test results do not predict the age of onset or severity of nonsyndromic mitochondrial hearing loss (43).

Mitochondria are exclusively maternally inherited. Therefore, identification of an individual with an *MT-RNR1* risk allele may be relevant to any maternal relatives, or children of a female identified to have the variant, or both (4).

Therapeutic Recommendations based on Genotype

Excerpt from the CPIC guidelines for Aminoglycosides and MT-RNR1 variants.

The critical pharmacogenetics recommendation for a person with an *MT-RNR1* variant which predisposes to AIHL is that aminoglycoside antibiotics are relatively contraindicated, meaning that aminoglycosides should be avoided unless the increased risk of hearing loss is outweighed by the severity of infection and lack of safe or effective alternative therapies. There is insufficient evidence to suggest that the adverse drug reaction may be more profound with some members of the aminoglycoside class than others. As such, this guidance covers all aminoglycoside antibiotics irrespective of class. We provide a strong recommendation that carriers of *MT-RNR1* variants that predispose to AIHL should avoid aminoglycosides unless the increased risk of permanent hearing loss is outweighed by the risk of infection without safe or effective alternative therapies… If no effective alternative to an aminoglycoside is thought to be available, we advise use for the shortest possible time, consultation with an infectious disease expert for alternative approaches, therapeutic drug monitoring and frequent assessment for hearing loss, both during and after therapy, in consultation with an audiovestibular physician.

An individual with no detectable *MT-RNR1* variant or carrying *MT-RNR1* variants not considered to be predisposing to AIHL (normal risk), including the m.827A>G variant, should still be considered at risk of AIHL. In addition to *MT-RNR1*, AIHL is often associated with other risk factors such as prematurity, renal impairment, severe inflammatory response syndrome, prolonged therapy regimens, and supratherapeutic plasma concentrations. As such, irrespective of the presence of an *MT-RNR1* variant which predisposes to AIHL, precautions such as renal monitoring, therapeutic drug monitoring, and utilizing the lowest effective dose should be applied. Finally, if an individual with an actionable *MT-RNR1* variant has previously received aminoglycosides and not developed AIHL, this does not preclude them from developing AIHL with subsequent doses.

Considerations for aminoglycoside use in patients at increased risk of AIHL. For the purposes of this guideline, appropriateness for use of aminoglycoside antibiotics can be considered for three scenarios: First, an equally or more effective agent is available for the condition; second, there is reason to believe that an aminoglycoside might lead to superior outcomes, but evidence is poor, the effect-size is small, or the outcome is not clinically meaningful; and third, there is good evidence for significantly superior efficacy of an aminoglycoside-containing treatment regimen for a clinically meaningful outcome.

[….]

In all cases, an aminoglycoside used in patients at increased risk of AIHL due to the presence of an *MT-RNR1* variant should be administered for the shortest possible period, under expert supervision, with therapeutic drug and ototoxicity monitoring, and with clinical audiovestibular assessment performed during and after treatment. Irrespective of whether an individual carries a pathogenic *MT-RNR1* variant, all patients who receive aminoglycoside antibiotics, especially those prescribed prolonged courses, should be monitored for ototoxicity in line with existing local and international guidelines.

[….]

Based on the available literature, at present there is not sufficient evidence to define a level of heteroplasmy where aminoglycoside administration becomes safe, especially as the mutational load may differ from tissue to tissue and be dependent upon the genotyping technique utilized. As such, we have not tailored this guideline based on the level of heteroplasmy. Rather, we recommend that if a relevant *MT-RNR1* variant is detected, the guidance should be followed as set out for a homoplasmic variant.

Please review the complete CPIC therapeutic recommendations that are located here: (4).

Excerpt from the American College of Medical Genetics and Genomics (ACMG) Guideline for the Clinical Evaluation and Etiologic Diagnosis of Hearing Loss:

For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, a tiered diagnostic approach should be implemented.

Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing should be ordered.

Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics.

Please review the complete ACMG therapeutic recommendations that are located here: (6).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence			dbSNP reference identifier for allele location
		Genomic	Coding*	Protein#	
m.1555A>G	A1555G	NC_012920.1:m.1555A>G	N/A	N/A	rs267606617
m.1494C>T	C1494T	NC_012920.1:m.1494C>T	N/A	N/A	rs267606619
m.1095T>C	T1095C	NC_012920.1:m.1095T>C	N/A	N/A	rs267606618
m. 827A>G	A827G	NC_012920.1:m.827A>G	N/A	N/A	rs28358569
m.663A>G	A663G	NC_012920.1:m.663A>G	N/A	N/A	rs56489998
m.961T>G	T961G	NC_012920.1:m.961T>G	N/A	N/A	rs3888511
m.961T>del+Cn	T961del+Cn	NC_012920.1:m.961delTinsC(2_7)	N/A	N/A	rs1556422499
m.1189T>C	T1189C	NC_012920.1:m.1189T>C	N/A	N/A	rs28358571
m.1243T>C	T1243C	NC_012920.1:m.1243T>C	N/A	N/A	rs28358572

* RNA coordinates not available.
# MT-RNR1 encodes an RNA gene product, there is no translated protein.

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Hydroxychloroquine Therapy and *G6PD* Genotype

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Introduction

Hydroxychloroquine, which is closely related to chloroquine, can be used for the prevention and treatment of some forms of malaria and rheumatic conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. Malaria is an infection caused by the *Plasmodium* parasite, transmitted via mosquito bites. Hydroxychloroquine sulfate is indicated for the prevention and treatment of uncomplicated malaria due to sensitive strains of *Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium malariae (P. malariae), Plasmodium ovale (P. ovale),* and *Plasmodium knowlesi* (*P. knowlesi)* by both the US Centers for Disease Control (CDC) and World Health Organization (WHO) (1, 2). Resistance to chloroquine and hydroxychloroquine has been reported in *Plasmodium* species, thus hydroxychloroquine therapy is not recommended if the infection arose in a region with known resistance. Most *P. falciparum* infections are resistant to the 4-aminoquinolines (chloroquine and hydroxychloroquine), and as such these drugs are no longer used widely for these infections. Hydroxychloroquine must be co-administered with an 8-aminoquinoline compound for the radical cure of *P. vivax* or *P. ovale* infection to eliminate the hypnozoite forms of these parasites. (3) Additionally, hydroxychloroquine is indicated for the treatment of many rheumatoid conditions in adults, including chronic discoid lupus erythematosus, systemic lupus erythematosus, as well as acute and chronic rheumatoid arthritis. Hydroxychloroquine has also been used in an off-label capacity for the management of Sjögren syndrome (4).

Hydroxychloroquine accumulates in cellular acidic compartments such as the parasitic food vacuole and mammalian lysosomes, leading to alkalinization of these structures. Among antimalarial medications, hydroxychloroquine is less likely than other medicines to cause hemolysis in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals; however, the U.S. FDA-approved drug label states there is still a risk of acute hemolytic anemia (AHA) (Table 1) (3). In contrast, the Clinical Pharmacogenetics Implementation Consortium (CPIC) performed a systematic review of the available clinical literature and found low-to-no risk of AHA for individuals with G6PD deficiency who take hydroxychloroquine (5). It should be noted that G6PD deficiency has a range of severity; CPIC advises caution for all medications when used by an individual with a severe G6PD deficiency with chronic non-spherocytic hemolytic anemia (CNSHA) (Table 2) (5). Regardless of G6PD phenotype, chronic use of hydroxychloroquine can cause irreversible retinal damage and regular visual exams are recommended by the FDA (3).

hydroxychloroquine therapy, hydroxychloroquine response, G6PD, lupus, malaria, rheumatoid arthritis

Table 1: The FDA Drug Label for Hydroxychloroquine Sulfate (2022)

Phenotype	Warnings and precautions
G6PD deficiency	Hydroxychloroquine should be administered with caution in individuals that have G6PD deficiency.

G6PD - Glucose-6-phosphate dehydrogenase. This FDA table is adapted from (3).

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Table 2: The CPIC Guidelines for Hydroxychloroquine based on G6PD Phenotype

Predicted G6PD phenotype based on genotype	Implication for phenotypic measures	Therapeutic recommendation	Classification of recommendation[a]
Normal	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status	Strong
Deficient	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate
Deficient with CNSHA	High risk of acute exacerbation of chronic hemolysis	Use all drugs cautiously in this group; if a drug is used, close monitoring for acute exacerbation of chronic hemolysis is recommended	Optional
Variable	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate
Indeterminant	Unknown risk of acute hemolytic anemia	To ascertain G6PD status, enzyme activity must be measured. Drug use should be guided per the recommendations based on the activity-based phenotype	Moderate

CNSHA - Chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phoshate dehydrogenase, CPIC - Clinical Pharmacogenetics Implementation Consortium
[a] Rating scheme from (5) Supplement.

Drug: Hydroxychloroquine

Hydroxychloroquine is a 4-aminoquinoline used for the treatment of specific forms of malaria, rheumatoid arthritis, SLE, and Sjögren syndrome, with potential utility in additional rheumatoid and autoimmune disorders (3, 6, 7, 8). Hydroxychloroquine is a chloroquine derivative and both medications have significant overlap in clinical use, pharmacodynamics, and metabolism. However, hydroxychloroquine is more frequently prescribed for rheumatoid conditions. Hydroxychloroquine and chloroquine have additionally been investigated as an adjuvant anticancer therapy (8, 9, 10, 11). Because of widespread use to treat and prevent malaria between the 1940s and 1980s, resistance to chloroquine and hydroxychloroquine has arisen in many strains of the malaria-causing parasite (12). Additional antimalarial medications have since been developed and can be used to treat those resistant strains; however, in many countries chloroquine and hydroxychloroquine remain first-line treatments.

In March of 2020, hydroxychloroquine and chloroquine were granted emergency use authorization by the FDA for the treatment of 2019 coronavirus disease (COVID-19) caused by infection with the severe acute respiratory syndrome coronavirus 2 (13). This authorization was revoked on 15 June 2020 due to risk of cardiac adverse events and other potential serious side-effects, which were determined to outweigh the potential benefit of these medications in treating COVID-19 (14). Experimental data suggested hydroxychloroquine and chloroquine may inhibit viral entry to the cell by suppressing angiotensin-converting enzyme 2 protein glycosylation and reduce viral particle release within cells due to neutralization of endosomal pH (15). However, the potential risk of cardiac adverse events and other serious side-effects were determined to outweigh the potential benefit of these medications in treating COVID-19 (14). For up-to-date information please see the latest information from the FDA, CDC, and NIH.

Hydroxychloroquine inhibits the heme polymerase enzyme in the malarial parasite, resulting in a fatal accumulation of toxic heme as well as accumulation in the lysosomes raising the pH. Within human cells, hydroxychloroquine also accumulates in lysosomes. The lysosome is the site of cellular autophagy, the mechanism whereby cells clear damaged organelles and protein masses as well as degrade foreign material. Inhibition of autophagy and lysosomal enzymes occurs once hydroxychloroquine or chloroquine raise the

internal pH of the lysosomes. This results in altered antigen processing and presentation, and prevents major histocompatibility complex components from dimerizing, thus reducing the inflammatory response (15). Chloroquine and hydroxychloroquine both inhibit recognition of nucleic acids by the toll-like receptors, the major histocompatibility complex class II-mediated antigen presentation, inflammation-induced cell proliferation, and antiphospholipid antibody activity, making them useful for the treatment of autoimmune disorders such as SLE (16). Chloroquine may be of more potential benefit as an oncology adjuvant therapy compared with hydroxychloroquine (4, 9, 10, 17, 18).

Hydroxychloroquine sulfate is not indicated for use in the treatment of complicated malaria nor should it be used in individuals with known hypersensitivity to 4-aminoquinoline compounds (3). Concomitant medication with an 8-aminoquinoline is necessary to treat the hypnozoite life cycle stage of certain *Plasmodium* parasites (see below for more details). Additionally, malaria that is resistant to chloroquine will also not respond to hydroxychloroquine.

Chronic hydroxychloroquine sulfate use may cause irreversible retinal damage. If the medication is to be prescribed for an extended period, the FDA-approved drug label states that a baseline visual exam must be taken within the first year of therapy to monitor for any changes in vision. The baseline exam should include "best corrected distance visual acuity, an automated threshold visual field (VF) of the central 10 degrees (with retesting if an abnormality is noted), and spectral domain optical coherence tomography (SD-OCT)" (3). These exams should be performed annually in individuals taking daily doses of hydroxychloroquine sulfate greater than 5 mg/kg of actual body weight, a duration of use greater than 5 years, experiencing subnormal glomerular filtration, or using concomitant drug products such as tamoxifen citrate (3).

The American Academy of Ophthalmology (AAO) also advises that in addition to the automatic VF exams, SD-OCT should be performed (19). The American College of Rheumatology, American Academy of Dermatology, Rheumatologic Dermatology Society and AAO recently issued a joint statement regarding hydroxychloroquine and retinal toxicity(20). In this joint recommendation, it is stated that the initial baseline examination should be completed within the first few months of hydroxychloroquine use and further clarifies that in the absence of risk factors (such as daily dose above 5 mg/kg body weight or tamoxifen co-medication), the next retinal exam can be deferred for 5 years, but afterwards should be performed annually. They further advise that hydroxychloroquine therapy should not be stopped prematurely based on borderline findings from visual testing, rather there should be a team-based approach and clear communication between the eye care providers and prescribing care provider with the treated individual regarding the risks, screening importance, and treatment options (20).The Royal College of Ophthalmologists in the United Kingdom has also published recommendations for visual screening with regular monitoring for individuals on prolonged chloroquine and hydroxychloroquine therapy (21). If ocular toxicity is suspected, immediate discontinuation of use is recommended, although visual changes may continue to progress after withdrawal due to the prolonged systemic half-life of chloroquine (11).

It should be noted that in individuals of Asian descent, retinal toxicity may present first outside of the macula, thus the FDA recommends the VF screening be performed in the central 24 degrees (rather than 10) in this population (3). The AAO also recommends SD-OCT testing should look beyond the central macula in Asian individuals (19). The specific mechanism underlying this difference in disease presentation is unknown, though genetics are suspected to play a role (19).

Hydroxychloroquine use may be associated with rare adverse effects on cardiac, neurological and muscle tissues (4, 16). Cardiac tissue toxicity can present as cardiomyopathy with conduction defects including prolonged QT interval, torsades de pointes, or ventricular arrhythmias. Some studies have indicated that QT prolongation can present within 3 to 5 days of treatment with hydroxychloroquine, though arrythmias and conduction disorders are more common with chloroquine versus hydroxychloroquine (16, 22). Acute cardiotoxicity is dose-dependent and often associated with overdose, while chronic cardiomyopathy is primarily a concern for individuals after

chronic, high-level dosing. The arrythmia risk is increased by co-medication with other arrhythmogenic drugs such as moxifloxacin. However, other studies have reported limited to no increased risk for arrythmia associated with hydroxychloroquine therapy alone (23, 24). One study found that after an acute course of hydroxychloroquine, drug levels remained at or above therapeutic goal levels for an average of 16 days following cessation of treatment, which may delay the timeline to safely administer other arrhythmogenic medications (25). Overall, the literature suggests standard dosing protocols (which vary between indications) for chloroquine and hydroxychloroquine have minimal risk of inducing either chronic or acute cardiotoxicity, with reported cardiotoxicity occurring in <1 out of 100 individuals (26, 27).

Additional side effects or risks include muscle weakness, increased risk of psoriatic attack or worsening of porphyria, and a potential elevated risk of convulsions among individuals with a history of epilepsy (3, 28). Rare instances of acute intermittent porphyria have been reported in some individuals with SLE on long-term hydroxychloroquine therapy (28). Cutaneous toxicity has also been reported, including pruritus, alopecia, and pigmentation changes (3, 7, 16). Much of these off-target tissue toxicities are predicted to result from the alkalinization of lysosomes, modulation of immune reactions and, in some cases, off-target activation of cellular receptors (16). Neuromuscular toxicity is rare, but there have been reports of proximal symmetric muscle deficits and polyneuropathies (16). Hydroxychloroquine can also cause severe hypoglycemia, both with and without concomitant antidiabetic medication. (3)

There have been reports of psychiatric adverse reactions with hydroxychloroquine use. Rare instances of suicidal behavior have been associated with hydroxychloroquine sulfate (3). However, in one study of long-term use for rheumatoid arthritis, hydroxychloroquine showed no increased risk of psychiatric side effects compared with sulfasalazine (29). When they do occur, psychiatric side effects seem to predominate in the context of short-term use (30) and may be the result of multiple predisposing risk-factors, with few studies of these risks in an elderly population (31).

Individuals with G6PD enzyme deficiency may experience hemolysis following treatment with hydroxychloroquine (3). However, other medications, including the antimalarials primaquine and tafenoquine, have a much higher risk of AHA in G6PD-deficient individuals (5, 32). The CDC advises that individuals with G6PD deficiency who may not tolerate other antimalarial medications may be prescribed a prophylactic dose of chloroquine for one year following acute malarial infection with *Plasmodium* species with hypnozoites, as most relapses from reactivation occur within this timeframe; hydroxychloroquine is not specifically recommended (33). The drug regulatory agency of Switzerland (Swissmedic) states that G6PD deficiency is a contraindication for hydroxychloroquine therapy (34).

Hydroxychloroquine is metabolized by the cytochrome P450 family of enzymes. First, hydroxychloroquine is N-dealkylated to N-desethylhydroxychloroquine. This is achieved primarily through the action of CYP3A4, with additional contributions from the enzymes CYP3A5, CYP2D6, CYP2C8, and CYP1A1 (15, 35). Hydroxychloroquine and desethylhydroxychloroquine have elimination half-lives of 40 to 50 days, primarily via renal excretion. This long half-life is due to extensive tissue uptake and storage rather than slow excretion (3). It is hypothesized that the mechanism of retinal damage with prolonged hydroxychloroquine therapy is due to its ability to bind to the melanin pigment in the iris, ciliary body, and retinal pigment epithelium. Notably, some animal research data suggests that chloroquine is more toxic than hydroxychloroquine. (11, 36, 37, 38)

Although hydroxychloroquine can freely diffuse into cells, it is also a substrate of several transporter proteins. Hydroxychloroquine is derived from chloroquine and shares many of the same enzyme interactions. Chloroquine is a substrate, inhibitor, and inducer of the multidrug resistance-associated protein 1 (MRP1) and a substrate of organic anion transporting proteins (OATPs) (35). Hydroxychloroquine has been shown to inhibit OATP1A2, which in turn can affect all-*trans*-retinol uptake in retinal cells (35). Various drug interactions have been documented with hydroxychloroquine due to their metabolism by cytochrome P450 enzymes described above. Altered plasma level of digoxin and cyclosporin have been correlated with hydroxychloroquine co-

medication. Based on studies in chloroquine, it is possible that ampicillin, praziquantel, and cimetidine could have similar drug-drug interactions with hydroxychloroquine (3).

The FDA-approved drug label states that hydroxychloroquine has not been determined to be safe or efficacious for pediatric individuals with SLE or juvenile idiopathic arthritis (3). However, the use of hydroxychloroquine is recommended for managing both pediatric and adult SLE (39, 40), for selected cases of juvenile idiopathic arthritis (41), for managing lupus nephritis (42), and has been reported to manage pediatric Sjögren disease (43). It should be noted that overdose is a serious risk, particularly for children with accidental ingestion, as these medications are rapidly and completely absorbed. The FDA label states that even one gram of chloroquine may be fatal in children (44); it should be noted that both chloroquine and hydroxychloroquine are dosed based on the body mass of the individual and the severity of poisoning would depend upon the size of the child. Toxicity symptoms include nausea, vomiting, headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions, hypokalemia, cardiac arrythmia and conduction defects and sudden potentially fatal respiratory and cardiac arrest; these symptoms may present within minutes of overdose. Immediate medical attention is required. Thus, it is strongly advised to keep hydroxychloroquine and chloroquine phosphate out of reach of children, as these individuals are particularly sensitive to 4-aminoquinoline compounds. (3)

There are also limited data regarding use in individuals over 65 years of age, though this is an active area of clinical research (45). As kidney function may be decreased in these individuals and thus slow hydroxychloroquine clearance, there is a risk of toxic accumulation in geriatric individuals. The FDA-approved label suggests monitoring of renal function and that care should be taken during dose selection for these individuals (3).

Human studies have not shown an increase in the rate of birth defects associated with hydroxychloroquine use by pregnant mothers, nor evidence of fetal ocular toxicity (3, 46). The CDC states that pregnant women with uncomplicated malaria caused by *P. malariae, P. ovale*, or chloroquine-sensitive *P. vivax* or *P. falciparum* should be treated with hydroxychloroquine or chloroquine (1). The WHO recommends chloroquine as an alternative therapy during pregnancy for infection with sensitive *Plasmodium* strains (2). Furthermore, the CDC advises continued chloroquine prophylaxis for individuals with *P. vivax* or *P. ovale* infection for the duration of pregnancy. If, upon delivery, the mother intends to breastfeed, the infant should be tested for G6PD deficiency. If neither the infant nor mother are G6PD deficient, primaquine phosphate is the recommended therapy for the mother (tafenoquine is not recommended during breastfeeding). Otherwise, women who cannot take tafenoquine or primaquine should continue weekly chloroquine prophylaxis for one year following acute malarial infection (33). Small amounts of hydroxychloroquine are excreted in breast milk (3). However, studies have found no adverse effects on growth, vision, or hearing, leading international experts to state that hydroxychloroquine is acceptable during breastfeeding (47).

Disease: Malaria

Malaria is a serious tropical disease caused by a parasite (*Plasmodium*) that spreads to humans by infected mosquitos. The only available vaccine is moderately effective and acts only against the *P. falciparum* species (48). Widely recommended antimalarial drugs such as mefloquine or atovaquone-proguanil can be used for prevention, which is known as chemoprophylaxis. The type of chemoprophylaxis recommended depends upon the individual taking the prophylaxis (namely, age, pregnancy status, and medical and psychiatric comorbidities) and the nature of travel –– specifically, the countries travelled to, the length of stay, the species of *Plasmodium* that are most prevalent, and the level of drug resistance. For individuals residing in malaria-endemic regions, the WHO recommends a variety of preventative chemotherapies that can be used in infants, children, during pregnancy or collectively for the population of endemic areas (49).

Despite chemoprophylaxis, travel to malaria-endemic areas is not without risk. Individuals at elevated risk for malaria complications include pregnant women (33) and adults who have had their spleen removed (50). If

travel cannot be avoided, chemoprophylaxis should be combined with additional precautions to avoid mosquito bites, such as bed nets and repellents. In 2021, the WHO estimated 247 million cases of malaria occurred worldwide, and malaria was responsible for at least 619,000 deaths. (51)

Malaria is found in over 100 countries and occurs throughout most tropical regions in the world. These regions include large parts of Africa, Asia, Central and South America, and parts of the Middle East and Pacific islands (51, 52). Individuals who are heterozygous carriers for sickle cell disease or G6PD deficiency have a protective advantage against malaria, and as a result, the frequency of such genetic conditions is higher in countries where malaria is endemic (53).

Malaria is transmitted to humans by the bite of an infected *Anopheles* mosquito. Only female mosquitos spread the infection (females feed on blood, males feed on nectar). Although malaria can also be spread by sharing contaminated needles or via a contaminated blood transfusion, these are rare means of transmission.

There are several different *Plasmodium* species, but only a few species cause most human malaria cases:

● *P. falciparum*

o The most common cause of malaria, and death from malaria

o Predominates in sub-Saharan Africa

o Also found in regions of Australasia (Papua New Guinea, Southeast Asia), and the Caribbean (Haiti and the Dominican Republic)

● *P. vivax*

o A common cause of malaria outside of Africa

o Most frequent species found in Central and South America, and South-East Asia

o Parasite has a dormant, hypnozoite stage in the liver

o Early gametocytes that infect mosquitos

● *P. malariae*

o Less common

o Found in most areas where malaria is endemic

● *P. ovale*

o Less common

o Parasite has a dormant, hypnozoite stage in the liver

● *P. knowlesi*

o Less common

o Found in some areas of Southeast Asia

The first stage of malaria infection begins when an infected mosquito bites the human host. Typically, mosquitos bite at dusk, or during the night. As the mosquito feeds, infective parasite sporozoites (the motile spore-like stage in the life cycle of this parasitic sporozoan, which is the infective agent) are inoculated into humans. The sporozoites travel to the liver, where they invade liver cells and asexually reproduce to form schizonts. The liver

schizonts contain daughter merozoites. This process is asymptomatic, and because it occurs outside of the red blood cell (erythrocyte), it is known as the exoerythrocytic stage.

Some species of the parasite (*P. vivax* and *P. ovale*) have an additional dormant stage in the liver–– the hypnozoite. These parasites can stay in the liver for weeks or months without causing any clinical symptoms.

The second stage of malaria infection is the erythrocytic stage. It begins when the liver schizonts rupture and release the daughter merozoites into the bloodstream. The merozoites invade red blood cells, digest hemoglobin, produce a toxic metabolite (hemozoin), and damage red blood cell membranes. Infected, brittle red blood cells are rapidly broken down (hemolysis) and if too many damaged red blood cells get trapped in the spleen, the spleen can rapidly enlarge (splenic sequestration).

Some of the daughter merozoites differentiate into male or female gametocytes (sexual forms). When they are ingested by a mosquito, they mature, fertilize, and reproduce, and develop into sporozoites. When the mosquito feeds again, the sporozoites are inoculated into another human host and the cycle of malaria transmission is complete.

The erythrocytic stage of malaria is usually associated with fever, and malaria should always be suspected in anyone with a fever who has recently returned from a malaria-endemic region, even if antimalarial chemoprophylaxis was correctly followed. Other symptoms and signs include nausea, vomiting, abdominal pain, tachycardia (fast heart rate), diaphoresis (sweating), chills, and myalgia (muscle pain). The complications of malaria infection include severe anemia, cerebral malaria, and multi-organ failure. Without correct diagnosis and prompt treatment, malaria can be fatal.

Disease Class: Rheumatic and Autoimmune Disorders

Several rheumatic and autoimmune disorders are treated with long-term hydroxychloroquine administration. These disorders include acute and chronic rheumatic arthritis, SLE, chronic discoid lupus erythematosus, and Sjögren syndrome, with potential use in many more conditions (4). In these conditions, there is a common theme of altered immune reactions leading to the immune system attacking endogenous tissues and cells. This causes cellular death and breakdown of the affected tissues, with potentially widespread systemic symptoms. The specific etiology of auto-antigen production may vary by specific conditions and individuals, but activation of toll-like receptors, release of interferon, or other inflammatory cytokines can all drive inflammation and activate innate and adaptive immunity. This can lead to auto-antigen presentation in lupus, (7) aberrant B-cell maturation in primary Sjögren syndrome, (54) and contribute to pathogenesis of rheumatoid arthritis (55). Antimalarial drugs, including the 4-aminoquinoline compounds like hydroxychloroquine have been utilized to manage these wide-ranging inflammatory processes (4, 16).

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on the long arm of the X chromosome (Xq28). Variants in the *G6PD* gene that eliminate enzymatic activity are not viable; variants observed in living humans impact the stability of the enzyme. As such, males can only be hemizygous (have one *G6PD* allele) while females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene. Males with Klinefelter syndrome have an additional X chromosome (47, XXY) and thus 2 *G6PD* alleles. Thus, it is important to consider the number of X chromosomes for an individual when determining *G6PD* genotype or phenotype.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide, with a worldwide prevalence of approximately 5% (56). Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once was, endemic; for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean (57, 58, 59). In the US, G6PD deficiency is more common among individuals of African descent, affecting approximately 12% (60).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. (61)

Red blood cells that are G6PD deficient have a normal function but are more susceptible to increased oxidative stress (for example, by reactive oxygen species and hydrogen peroxide). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the antimalarial drugs primaquine and tafenoquine, the antibacterials dapsone and sulfamethoxazole, the skin cancer drug dabrafenib, and the uric acid lowering drugs pegloticase and rasburicase.

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. At birth, they are at a higher risk of developing neonatal jaundice, and throughout their lives will be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid exposure to oxidizing agents when possible, including drugs such as tafenoquine.

Symptomatic individuals with G6PD deficiency may suffer from episodes of AHA or, the more severe condition, CNSHA. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions.

More than 200 genetic variants of the *G6PD* gene have been identified so far (62). Most known *G6PD* variants are missense, and variants that are in cis as a haplotype have also been described, including the A- variant that is most common in individuals of African and South-American genetic ancestry (63). Large deletions are rare, and a complete lack of G6PD activity is fatal in utero.

The normal (wild type, referred to as B) copy of the *G6PD* gene is found in most individuals of European descent, individuals of Asian descent, and individuals of African descent. Common *G6PD* variants include:

● *G6PD* A (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of individuals of African descent and approximately 1.5% of Latinos (64, 65)

• *G6PD* A- (p.Asn126Asp with p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (66). Additional *A-* haplotypes have also been identified, both with the A+ variant with a second single nucleotide polymorphism (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (67)

• *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is a common pathogenic variant in individuals of European descent (68)

• *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in individuals of Asian descent (69)

● *G6PD* Viangchan (p.Val291Met) is the most common *G6PD* variant among Thais, Laotians, Cambodians, and Malaysians (based on common genetic ancestry) (70, 71)

The WHO recently updated its categorization of *G6PD* variants into 4 classes based on the median residual enzyme activity in males (expressed as a percentage of normal activity) (72). Class A variants have <20% activity and are associated with chronic hemolytic anemia. Most individuals with G6PD deficiency have variants that belong to class B (enzyme activity less than 45%). Class B variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but most of the time, affected individuals are asymptomatic. Class C variants show median G6PD activity from 60–150% and are not associated with hemolysis. In class U are the variants with any activity and unknown clinical significance. The CPIC has assigned G6PD phenotypes based on *G6PD* genotypes under the previous classification system; the updated WHO categories are also provided (Table 3) (5).

Table 3. Assignment of likely G6PD Phenotype based on Genotype/Diplotype (CPIC, 2022)

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Normal	Very mild or no enzyme deficiency no less than 60% of normal enzyme levels (60–150% of normal activity)	An X chromosome hemizygote who has a nondeficient (class IV) allele	IV (C)	B, Sao Borja
		An individual who has 2 nondeficient (class IV) alleles	IV/IV (C)	B/B, B/Sao Borja
Deficient	Less than 10–60% of normal enzyme activity (20–45% of normal activity)	An X chromosome hemizygote who has a deficient (class II–III) allele	II, III (B)	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		An individual who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III (B)	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA (<20% of normal activity)	An X chromosome hemizygote who has a class I allele	I (A)	Bangkok, Villeurbanne
		An individual who has 2 deficient (class I variants) alleles	I/I (A)	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[d]	Normal or deficient enzyme activity[c]	An individual who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III (U)	B/A–, B/Mediterranean, B/Bangkok

Table 3. continued from previous page.

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Indeterminant	Uncertain		(U)	

CNSHA - chronic non-spherocytic hemolytic anemia, WHO - World Health Organization, G6PD - glucose-6-phosphate dehydrogenase

[a] The traditional (Class I-IV) and updated (A, B, C, and U) activity levels are both provided, with the updated activity ranges provided in parentheses where relevant.

[b] WHO classifications were under revision at the time of CPIC publication, updated classification (using A, B, C and U designations) have been proposed based on enzyme activity levels and are provided in parenthesis here (72).

Class I alleles are extremely rare; the distinction between class II and III alleles is not clear. Almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.

[c] Due to the large number of *G6PD* variants, many other diplotypes may be possible besides those given as examples here; see Supplementary data from (5) for a more comprehensive list of alleles with their assigned WHO class. For Human Genome Variation Society terms, please see the Nomenclature table below. The alleles and diplotypes provided here are based upon the historic class I-IV definitions and may not fit the updated WHO classification.

[d] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online (G6PD heterozygotes)) (5).

This table is adapted from (5).

Additional Genes of Note

Hydroxychloroquine is metabolized by the enzymes encoded by *CYP2C8*, *CYP3A4,* and *CYP2D6*, all of which are classified as "very important pharmacogenes" by PharmGKB (73, 74). Variability in the *CYP* genes can lead to reduced or increased enzyme function. Classification of individual phenotype as either ultrarapid metabolizers, normal metabolizers, intermediate metabolizers, or poor metabolizers is carried out on a predictive basis from genotype results of known alleles. Allele classifications for various *CYP* genes and other pharmacogenes are available from CPIC (75) and the Pharmacogene Variation Consortium (76). Allele frequencies for these pharmacogenes vary across global and even regional populations, where populations are defined as groups with shared genetic variants due to ancestry (75, 77, 78, 79, 80).

Interaction of hydroxychloroquine with these CYP enzymes (for example, CYP2D6) leads to varying degrees of enzyme inhibition depending on the method of testing used. In vivo measurement of CYP2D6 inhibitory activity ranges from moderate to no inhibition. Thus, drug-drug interactions may occur due to co-medication of hydroxychloroquine with other CYP substrates(35). This results in phenoconversion, a phenomenon where an individual's genetically predicted CYP metabolism is altered to a different activity level. Drugs competing for the same metabolic enzyme will often lead to phenoconversion with lower effective enzyme activity.

Transporter proteins encoded by solute carrier organic anion transporter (*SLCO)* genes, also known as OATPs, adenosine triphosphate (ATP)-binding cassette sub-family B member 1 (*ABCB1)* or P-glycoprotein, ATP-binding cassette sub-family C member 1 (*ABCC1)* or MRP1, and ATP-binding cassette sub-family C member 2 (*ABCC2) or* MRP2 have all been shown to have their function impacted by chloroquine, which is likely to extend to hydroxychloroquine as well (35). Both chloroquine and hydroxychloroquine exerted potent inhibition of OATP1A2, but only moderate inhibition of breast cancer resistance protein 1 (BCRP1), human serotonin transporter (hSERT), and OATP2B1, with no inhibition of the bile salt export pump (BSEP) transporter (35).The ATP ABC family of proteins and OATPs are important drug transport proteins and altered function of these enzymes can affect the efficacy of substrate medications and potential side effects. One study found hydroxychloroquine to be a potent inhibitor of the OATP1A2 transport protein, potentially impacting uptake of all-*trans*-retinol in the retinal pigment epithelium (81).

Linking Gene Variation with Treatment Response

Some guidelines report that individuals with G6PD deficiency may be at a higher risk for hemolysis during medication with antimalarials such as hydroxychloroquine and chloroquine than those individuals with normal G6PD enzyme activity (3, 67, 82). However, both the CDC and WHO recommend chloroquine prophylaxis to prevent malaria relapse for pregnant and breastfeeding women, as primaquine is contraindicated during pregnancy and in G6PD deficiency (<30% enzymatic activity) (1). The FDA-approved drug label recommends monitoring individuals on prolonged therapy for signs of hemolysis; the recommended test is complete blood counts (3). Several studies have reported there is no increased risk of hemolysis for G6PD-deficient individuals taking chloroquine or hydroxychloroquine (83, 84, 85, 86, 87) Similarly, multiple studies regarding the safety and efficacy of hydroxychloroquine for a variety of rheumatic conditions did not report significant rates of hemolysis in their cohorts (88, 89, 90). However, despite extensive use of chloroquine in areas with high a high proportion of G6PD deficiency and no evidence of hemolysis, there are case reports documenting hemolysis in individuals with G6PD deficiency who were undergoing experimental treatment for COVID-19 with hydroxychloroquine (91, 92, 93, 94, 95). The specific cause of hemolysis in this context is a matter of scientific debate. Many authors suggest that the systemic response to SARS-CoV2 infection or co-medications or both may be the actual triggers for hemolysis in these cases, with the underlying G6PD deficiency and hydroxychloroquine medication playing minor roles (96, 97, 98).

Studies have reported differences in levels of hydroxychloroquine and its metabolites in individuals with variant *CYP2D6* alleles, however a clear clinical correlation in efficacy or dose requirement has not been demonstrated (99, 100). Medications such as metoprolol and tamoxifen depend upon CYP2D6 metabolism and may be negatively impacted by co-medication with chloroquine or hydroxychloroquine (101, 102). This may lead to altered therapeutic response for all concomitant medications and increase the risk of retinal damage in the case of tamoxifen co-medication (3). There have been reports of different variants in *ABCA4* associating with either increased risk for or protection from hydroxychloroquine toxicity (19, 103, 104).

However, there are no specific actionable guidelines from the pharmacogenetics community or the FDA (3) to alter hydroxychloroquine dosage based on variation of any the genes discussed herein.

The *G6PD* Gene Interactions with Medications Used for Additional Indications

Medications that can induce oxidative stress in red blood cells can trigger hemolysis readily in individuals with G6PD enzyme deficiency. Many of these medications are antimalarial (tafenoquine, for example) but many more medications pose a hazard for G6PD deficient individuals.

- Urate-lowering medications: both refractory gout and tumor lysis syndrome can cause systemic elevation of urate levels, medications such as rasburicase and pegloticase are uricase enzymes that aid in the breakdown of uric acid into more soluble metabolites. These reactions produce hydrogen peroxide as a byproduct, thus increasing oxidative stress in the body.

- Kinase inhibitors: anti-cancer medications such as dabrafenib may also increase oxidative stress.

- Anti-microbial medications: nitrofurantoin, often used for urinary tract infections, was determined to be a medication of moderate risk for AHA in G6PD deficient individuals by CPIC and may call for additional monitoring. In contrast, CPIC found sulfamethoxazole to be a medication with low-to-no risk in G6PD deficient individuals. (5)

Additional information on gene-drug interactions for *G6PD* are available from PharmGKB, CPIC and the FDA (search for "G6PD").

Genetic Testing

Glucose-6-phosphate dehydrogenase deficiency is inherited in an X-linked pattern and most individuals are asymptomatic throughout life. A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons. X-linked disorders affect males at a much higher rate than females because their frequency is the same as the allelic frequency (males only have one copy of the X chromosome, XY). Since females have 2 copies of the X chromosome (XX) and the deficient phenotype is mostly expressed in the homozygous/compound heterozygous mutated genotype, the frequency of the phenotypic disorder is the square root of the allelic frequency. However, female heterozygotes with one mutated allele can present with a range of phenotypes from no symptoms through a severe deficiency; frequency of "intermediate" phenotypes (not deficient but not normal either) in females can be as high as the frequency of deficiency in males (105).

The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for the *G6PD* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD deficiency in males and testing may also be used to screen females with a family history of G6PD to see if they are carriers. In females, G6PD deficiency occurs mostly in homozygous and compound heterozygous individuals (who have inherited 2 copies of *G6PD* deficiency alleles); in heterozygous individuals (one normal *G6PD* allele and one deficiency *G6PD* allele) skewed X chromosome inactivation of the functional allele (58) can result in deficient phenotypes. Therefore, genetic testing alone is insufficient to assess risk of hemolysis in heterozygous individuals.

In routine clinical practice, G6PD deficiency is diagnosed by measuring G6PD activity in red blood cells (61, 106). Two different types of enzyme activity tests are used, and they are classified as qualitative or quantitative. For some medications, such as tafenoquine, a specific enzymatic activity threshold is used to determine the safety of the medication and as such a quantitative test may be required for individuals with intermediate levels of enzyme activity based on the qualitative test (107). False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, when necessary, screening for G6PD enzymatic activity should be performed 2–3 months after a blood transfusion or hemolytic episode (5, 61). Diagnosis using qualitative test methods is less accurate for females with intermediate G6PD activity due to heterozygous *G6PD* alleles (108).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2022 Statement from the US Food and Drug Administration (FDA):

Hemolysis has been reported in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor for hemolytic anemia as this can occur, particularly in association with other drugs that cause hemolysis.

Please review the complete therapeutic recommendations that are located here: (3)

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

Nomenclature for Selected *G6PD* Alleles

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Borja	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3:c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient (A)	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient (U)	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient (B)	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3:c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient (B)	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient (B)	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient (A)	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient (B)	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society.
* WHO classifications based on (109), classification of these alleles under the updated WHO categories are taken from work described in (110) and the data deposited at (111). Please note that not all alleles have an updated classification at the time of writing.
WHO - World Health Organization, PharmGKB - Pharmacogenomics Knowledgebase, CPIC - Clinical Pharmacogenetics Implementation Consortium, CNSHA - chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phosphate dehydrogenase

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A minor revision was published (version 1.1) on August 22, 2024 to provide current links to NIH and CDC guidance regarding coronavirus treatment and to remove an inactive link to retired NIH guidelines. There is no change in recommendations regarding the use of hydroxychloroquine in COVID-19 or other disease treatment relative to the previous version of this chapter.

Version 1.2 was published on October DD, 2024 to provide correct links to updated CDC pages. There is no change in recommendations in this revision relative to version 1.0.

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Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype

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Introduction

Imipramine is a tricyclic antidepressant used in the treatment of several psychiatric disorders including major depression, obsessive-compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder, and bulimia. Imipramine may also be useful as an adjunctive treatment in the management of panic attacks, neuropathic pain, attention-deficit disorder, and childhood enuresis (bedwetting) (1).

Tricyclic antidepressants (TCAs) primarily mediate their therapeutic effect by inhibiting the reuptake of both serotonin and norepinephrine, leaving more neurotransmitter in the synaptic cleft stimulating the neuron. Because tricyclics can also block different receptors (histamine H1, α1-adrenergic, and muscarinic receptors), side effects are common. As such, more specific selective serotonin reuptake inhibitors (SSRIs) have largely replaced the use of them. However, TCAs still have an important use in specific types of depression and other conditions.

Imipramine is primarily metabolized via CYP2C19 to active metabolites, including desipramine, also a tricyclic antidepressant. Further metabolism is catalyzed by CYP2D6. Individuals who are "CYP2D6 ultrarapid metabolizers" carry more than two normal function alleles (i.e., multiple copies) (Table 1, 2), whereas individuals who are "CYP2C19 ultrarapid metabolizers" carry two increased function alleles (Table 3, 4). Individuals who are CYP2D6 or CYP2C19 "poor metabolizers" carry two no function alleles for *CYP2D6* or *CYP2C19*, respectively.

The FDA-approved drug label for imipramine states that CYP2D6 poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants when given usual doses. Their recommendations include monitoring tricyclic antidepressant plasma levels whenever a tricyclic antidepressant is going to be co-administered with another drug known to be an inhibitor of CYP2D6 (1).

In 2016, the Clinical Pharmacogenetics Implementation Consortium (CPIC) made dosing recommendations for tricyclic antidepressants based on *CYP2C19* and *CYP2D6* genotypes. Amitriptyline and nortriptyline were used as model drugs for this guideline because the majority of pharmacogenomic studies have focused on these two drugs. According to the CPIC guideline, because TCAs have comparable pharmacokinetic properties, it may be reasonable to apply the recommendations to other tricyclics, including imipramine (2).

For CYP2D6 ultrarapid metabolizers, CPIC recommends avoiding the use of a tricyclic due to the potential lack of efficacy, and to consider an alternative drug not metabolized by CYP2D6. If a TCA is still warranted, CPIC recommends considering titrating the TCA to a higher target dose (compared to normal metabolizers) and using therapeutic drug monitoring to guide dose adjustments. For CYP2D6 intermediate metabolizers, CPIC recommends considering a 25% reduction of the starting dose, and for CYP2D6 poor metabolizers, to avoid the use of tricyclics because of the potential for side effects. If a tricyclic is still warranted for CYP2D6 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects.

For CYP2C19 ultrarapid metabolizers, CPIC recommends avoiding the use of tertiary amines (e.g., imipramine) due to the potential for a sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19, such as the secondary amines nortriptyline or desipramine. For CYP2C19 poor metabolizers, CPIC

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recommends avoiding tertiary amine use due to the potential for sub-optimal response, and to consider an alternative drug not metabolized by CYP2C19. If a tertiary amine is still warranted for CYP2C19 poor metabolizers, CPIC recommends considering a 50% reduction of the starting dose while monitoring drug plasma concentrations to avoid side effects (2).

Drug Class: Tricyclic Antidepressants

Tricyclic antidepressants (TCAs) are mixed serotonin-norepinephrine reuptake inhibitors. They increase the amount of neurotransmitter in the synaptic cleft, thought to mediate their antidepressant effects.

From the 1960s to the 1980s, tricyclics were the first-line treatment for depression, until the introduction of SSRIs, which have fewer side effects and are safer. The common side effects of tricyclics include anticholinergic side effects (e.g., blurred vision, dry mouth, constipation, and sedation), cardiac effects, and orthostatic hypotension.

Today, the main therapeutic use of tricyclics is chronic pain management, such as neuropathic pain. However, tricyclics are still used in the treatment of depression as well as other psychiatric disorders including obsessive-compulsive disorder, panic attacks, generalized anxiety disorder, post-traumatic stress disorder, bulimia nervosa, smoking cessation, and enuresis (bedwetting).

Tricyclics are named after their chemical structure of three central rings and a side chain important for their function and activity. Its structure determines whether a drug is classified a tertiary amine (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine) or secondary amine (desipramine and nortriptyline).

Whereas tertiary amines are generally more potent in blocking reuptake of serotonin, the secondary amines are more potent in blocking the reuptake of norepinephrine. Secondary amines are better tolerated and are also associated with fewer anticholinergic side effects.

The CYP2C19 enzyme metabolizes tertiary amines to active metabolites, which include desipramine (the active metabolite of imipramine) and nortriptyline (the active metabolite of amitriptyline). Both the tertiary and secondary amines are metabolized by CYP2D6 to less active metabolites.

The effectiveness and tolerability of tricyclics are affected by CYP2D6 metabolism and partially by CYP2C19 metabolism. Individuals who carry *CYP2D6* or *CYP2C19* variants that influence enzyme activity may be at an increased risk of treatment failure (if plasma drug levels are decreased) or drug toxicity (if plasma drug levels are increased).

Drug: Imipramine

Imipramine was the first tricyclic used in the treatment of depression in the late 1950s. Imipramine is still used to relieve the symptoms of major depressive disorder, and it may be useful too as temporary adjunctive therapy in reducing enuresis (bedwetting) in children aged 6 years and older. Off-label uses of imipramine also include the treatment of neuropathic pain and attention deficit disorder.

Imipramine is a tertiary amine and is similar in structure to amitriptyline, another tertiary amine. Both drugs potently block the reuptake of serotonin and to a lesser degree norepinephrine. Imipramine has also strong affinities for alpha-1 adrenergic, histamine H1, and muscarinic M1 receptors, which account for its side effects of orthostatic hypotension, sedation, weight gain, and anticholinergic effects. However, the intensity of these side effects is generally less than it is for amitriptyline (3).

Imipramine is metabolized by CYP2C19 to desipramine, which is also a tricyclic antidepressant with distinct clinical features that differ from the imipramine. Desipramine is then metabolized by CYP2D6 to the less active

hydroxy-imipramine. For therapeutic drug monitoring, the levels of imipramine and hydroxy-imipramine should be monitored (4).

The optimal therapeutic range for imipramine is well-defined (5). Most individuals display an optimal response to imipramine when combined serum levels of imipramine and desipramine are between 175 and 300 ng/mL (6). However, individuals who are carriers of certain *CYP2D6* and/or *CYP2C19* variants may have drug levels that are outside this range even after being treated with standard doses of imipramine. As a result, they may have an increased risk of side effects (if the level of imipramine and its active metabolites are too high) or treatment failure (if drug levels are too low).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antipsychotics, analgesics, beta-blockers, and TCAs such as imipramine.

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described and currently catalogued at the Pharmacogene Variation Consortium database (7).

CYP2D6 is a particularly complex gene that is difficult to genotype, partly because of the large number of variants, but also because of the presence of gene deletions, duplications, and its neighboring pseudogenes. The complexity of genetic variation at this locus complicates the ability to interrogate *CYP2D6*.

There is substantial variation in *CYP2D6* allele frequencies among different populations (8). *CYP2D6*1* is the wild-type allele and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6* alleles **2*, **33*, and **35* are also considered to have normal activity.

Other alleles include no function variants that produce a non-functioning enzyme (e.g., **3*, **4*, **5*, **6*, **7*, **8*, and **12*) or an enzyme with decreased activity (e.g., **10*, **17*, *29, and **41*) (see Table 1) (9). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in the Caucasian population, **17* more common in Africans, and **10* more common in Asians (10).

Table 1: 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN* [b]
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 1 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

a For population-specific allele and phenotype frequencies, please see (2).
b Where xN represents the number of *CYP2D6* gene copies (N is 2 or more).
c Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Individuals who are intermediate or poor metabolizers carry copies of reduced-activity or no function *CYP2D6* alleles, respectively (Table 1). Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. In these populations, only half of *CYP2D6* alleles are fully functional, with the reduced function **10* variant being very common (~40%, compared to ~2% in Caucasians) (11). As a result, Asians are more likely to be intermediate metabolizers than Caucasians (12). Similarly, in Africans and African Americans, only half of *CYPD6* alleles are functional; however, a wider range of variants account for the remaining alleles (12-14).

Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to no function **4* and **5* alleles (12). Notably, less than 40% are homozygous normal metabolizers (carrying two copies of **1* allele) (15-17).

Individuals who are CYP2D6 poor metabolizers require a lower dose of imipramine to be in therapeutic range than CYP2D6 normal metabolizers (18). When treated with standard doses of imipramine, individuals who are CYP2D6 poor metabolizers will also have higher plasma concentrations of imipramine and desipramine compared to CYP2D6 normal metabolizers (19).

Because adverse effects are more likely due to elevated tricyclic plasma concentrations, CPIC recommends alternative agents for individuals who are CYP2D6 poor metabolizers. If a tricyclic is warranted, CPIC recommends considering a 50% reduction of the usual starting dose, and they strongly recommend therapeutic drug monitoring (4).

Individuals who have more than two copies of normal function *CYP2D6* alleles are *CYP2D6* ultrarapid metabolizers. These individuals require higher doses of imipramine to be within therapeutic range compared to normal metabolizers (18). However, increasing the dose of imipramine can lead to high plasma concentrations of desipramine, which may increase the risk for cardiotoxicity. Therefore, CPIC recommends that an alternative agent be used for CYP2D6 ultrarapid metabolizers. However, if a tricyclic is warranted, there is insufficient evidence to calculate a starting dose, and so therapeutic drug monitoring is strongly recommended (4) (Table 2).

Table 2. 2016 CPIC Dosing recommendations for tricyclic antidepressants based on *CYP2D6* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2D6 ultrarapid metabolizer	Increased metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6
	Lower plasma concentrations of active drugs will increase probability of pharmacotherapy failure	If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers)[a]. Utilize therapeutic drug monitoring to guide dose adjustments.
CYP2D6 normal metabolizer	Normal metabolism of TCAs	Initiate therapy with recommended starting dose[b].
CYP2D6 intermediate metabolizer	Reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Consider a 25% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Higher plasma concentrations of active drug will increase the probability of side effects	
CYP2D6 poor metabolizer	Greatly reduced metabolism of TCAs to less active compounds compared to normal metabolizers	Avoid tricyclic use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6
	Higher plasma concentrations will increase the probability of side effects	If a TCA is warranted, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].

TCAs: Tricyclic Antidepressants
Dosing recommendations only apply to higher initial doses of TCAs for treatment of conditions such as depression.
The therapeutic recommendations for amitriptyline and nortriptyline are classified as "moderate" for intermediate CYP2D6 metabolizers, and "strong" for ultrarapid, normal, and poor CYP2D6 metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2D6, including clomipramine, desipramine, doxepin, imipramine, and trimipramine.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects.
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Gene: CYP2C19

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as several proton pump inhibitors, clopidogrel, benzodiazepines, and several tricyclic antidepressants, including imipramine.

The *CYP2C19* gene is highly polymorphic as 35 variant star (*) alleles are currently catalogued at the Pharmacogene Variation Consortium database: (https://www.pharmvar.org/).

The *CYP2C19*1* wild-type allele is associated with normal enzyme activity and the "normal metabolizer" phenotype, whereas the *CYP2C19*17* allele is associated with increased enzyme activity and the "rapid" and "ultrarapid" metabolizer phenotypes (20).

The most common no function variant is *CYP2C19*2*, which is characterized by c.681G>A in exon 5 that results in an aberrant splice site and the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (20, 21).

Another commonly tested no function variant is *CYP2C19*3*, which is characterized by c.636G>A in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include *CYP2C19*4-*8* (20, 21).

"CYP2C19 intermediate metabolizers" carry one copy of a no function allele (e.g. **1/*2*), whereas "poor metabolizers" are homozygous or compound heterozygous for two no function alleles (e.g., **2/*2*, **2/*3*) (Table 3).

Table 3: 2016 Assignment of *CYP2C19* phenotypes by CPIC

Phenotype	Genotypes	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–35% of patients)[a]	An individual carrying two increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of patients)	An individual carrying one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of patients)	An individual carrying two normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of patients)	An individual carrying one normal function and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of patients)	An individual carrying two no function alleles	**2/*2* **2/*3* **3/*3*

[a] For population-specific allele and phenotype frequencies, please see (2).
[b] The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Studies have found that individuals who are CYP2C19 poor metabolizers have a lower plasma clearance of imipramine compared to normal metabolizers. When given standard doses of imipramine, CYP2C19 poor metabolizers have greater concentrations of imipramine and its active metabolite desipramine (22-24). Increased drug levels could potentially lead to an increased risk of adverse events. CPIC recommends considering a 50% reduction in the starting dose of tricyclics for CYP2C19 poor metabolizers (4)

Individuals who are CYP2C19 ultrarapid metabolizers may require an increased dose of tricyclics (25).

One study found that the imipramine plasma concentration was significantly lower in ultrarapid metabolizers (i.e., *CYP2C19*17/*17*) when compared to normal metabolizers (i.e., *CYP2C19*1/*1*) patients. However, the imipramine + desipramine plasma concentrations were not significantly different between *CYP2C19* genotypes (26). Because of the possibility of altered tricyclic plasma concentrations, CPIC recommends an alternative tricyclic or other drug for ultrarapid metabolizers (4) (Table 4).

Table 4. 2016 CPIC Dosing recommendations for tertiary amines based on *CYP2C19* phenotype

Phenotype	Implication	Therapeutic recommendation
CYP2C19 ultrarapid metabolizer and CYP2C19 rapid metabolizer	Increased metabolism of tertiary amines as compared to normal metabolizers Greater conversion of tertiary amines to secondary amines may affect response or side effects	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine.
		If a tertiary amine is warranted, utilize therapeutic drug monitoring to guide dose adjustments[a].
CYP2C19 normal metabolizer	Normal metabolism of tertiary amines	Initiate therapy with recommended starting dose[b].
CYP2C19 intermediate metabolizer	Reduced metabolism of tertiary amines compared to normal metabolizers	Initiate therapy with recommended starting dose[b].
CYP2C19 poor metabolizer	Greatly reduced metabolism of tertiary amines compared to normal metabolizers	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of recommended starting dose[b]. Utilize therapeutic drug monitoring to guide dose adjustments[a].
	Decreased conversion of tertiary amines to secondary amines may affect response or side effects	

Dosing recommendations apply only to higher initial doses of amitriptyline for treatment of conditions such as depression. The therapeutic recommendations for amitriptyline are classified as "strong" for normal and intermediate CYP2C19 metabolizers, "moderate" for poor metabolizers, and "optional" for ultrarapid metabolizers. CPIC state that it may be reasonable to apply these recommendations to other TCAs also metabolized by CYP2C19, including clomipramine, doxepin, imipramine, and trimipramine.
[a] Titrate dose to observed clinical response with symptom improvement and minimal (if any) side effects).
[b] Patients may receive an initial low dose of tricyclic, which is then increased over several days to the recommended steady-state dose. The starting dose in this guideline refers to the recommended steady-state dose.
Table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (2).

Genetic Testing

Clinical genotyping tests are available for many *CYP2D6* and *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides a list of test providers for "imipramine response," and the CYP2D6 and CYP2C19 genes.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (27). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (28).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for nonfunctional, 0.5 for reduced function, and 1 for each copy of a functional allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (2, 29)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): The biochemical activity of the drug metabolizing isozyme cytochrome P450 2D6 (debrisoquin hydroxylase) is reduced in a subset of the Caucasian population (about 7% to 10% of Caucasians are so-called "poor metabolizers"); reliable estimates of the prevalence of reduced P450 2D6 isozyme activity among Asian, African, and other populations are not yet available. Poor metabolizers have higher than expected plasma concentrations of tricyclic antidepressants (TCAs) when given usual doses. Depending on the fraction of drug metabolized by P450 2D6, the increase in plasma concentration may be small, or quite large (8-fold increase in plasma AUC of the TCA).

In addition, certain drugs inhibit the activity of this isozyme and make normal metabolizers resemble poor metabolizers. An individual who is stable on a given dose of TCA may become abruptly toxic when given one of these inhibiting drugs as concomitant therapy. The drugs that inhibit cytochrome P450 2D6 include some that are not metabolized by the enzyme (quinidine; cimetidine) and many that are substrates for P450 2D6 (many other antidepressants, phenothiazines, and the Type 1C antiarrhythmics propafenone and flecainide). While all the selective serotonin reuptake inhibitors (SSRIs), e.g., fluoxetine, sertraline, and paroxetine, inhibit P450 2D6, they may vary in the extent of inhibition. The extent to which SSRI-TCA interaction may pose clinical problems will depend on the degree of inhibition and the pharmacokinetics of the SSRI involved. Nevertheless, caution is indicated in the co-administration of TCAs with any of the SSRIs and also in switching from one class to the other. Of particular importance, sufficient time must elapse before initiating TCA treatment in a patient being withdrawn from fluoxetine, given the long half-life of the parent and active metabolite (at least 5 weeks may be necessary).

Concomitant use of tricyclic antidepressants with drugs that can inhibit cytochrome P450 2D6 may require lower doses than usually prescribed for either the tricyclic antidepressant or the other drug. Furthermore, whenever one of these other drugs is withdrawn from co-therapy, an increased dose of tricyclic antidepressant may be required. It is desirable to monitor TCA plasma levels whenever a TCA is going to be co-administered with another drug known to be an inhibitor of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

Because the TCAs have comparable pharmacokinetic properties, it may be reasonable to extrapolate this guideline to other TCAs including clomipramine, desipramine, doxepin, imipramine, and trimipramine, with the acknowledgement that there are fewer data supporting dose adjustments for these drugs than for amitriptyline or nortriptyline. […]

CYP2D6 dosing recommendations.

[…]. The recommended starting dose of amitriptyline or nortriptyline does not need adjustment for those with genotypes predictive of CYP2D6 normal metabolism. A 25% reduction of the recommended dose may be considered for CYP2D6 intermediate metabolizers. The strength of this recommendation is classified as "moderate" because patients with a CYP2D6 activity score of 1.0 are inconsistently categorized as intermediate or normal metabolizers in the literature, making these studies difficult to evaluate.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

CYP2D6 ultrarapid metabolizers have a higher probability of failing amitriptyline or nortriptyline pharmacotherapy due to subtherapeutic plasma concentrations, and alternate agents are preferred. There are documented cases of CYP2D6 ultrarapid metabolizers receiving large doses of nortriptyline in order to achieve therapeutic concentrations. However, very high plasma concentrations of the nortriptyline hydroxy-metabolite were present, which may increase the risk for cardiotoxicity. If a tricyclic is warranted, there are insufficient data in the literature to calculate a starting dose for a patient with CYP2D6 ultrarapid metabolizer status, and therapeutic drug monitoring is strongly recommended. Adverse effects are more likely in CYP2D6 poor metabolizers due to elevated tricyclic plasma concentrations; therefore, alternate agents are preferred. If a tricyclic is warranted, consider a 50% reduction of the usual dose, and therapeutic drug monitoring is strongly recommended.

CYP2C19 dosing recommendations.

[…]. The usual starting dose of amitriptyline may be used in CYP2C19 normal and intermediate metabolizers. Although CYP2C19 intermediate metabolizers would be expected to have a modest increase in the ratio of amitriptyline to nortriptyline plasma concentrations, the evidence does not indicate that CYP2C19 intermediate metabolizers should receive an alternate dose.

Patients taking amitriptyline who are CYP2C19 rapid or ultrarapid metabolizers may be at risk for having low plasma concentrations and an imbalance between parent drug and metabolites causing treatment failure and/or adverse events. Although the CYP2C19*17 allele did not alter the sum of amitriptyline plus nortriptyline plasma concentrations, it was associated with higher nortriptyline plasma concentrations, possibly increasing the risk of adverse events. For patients taking amitriptyline, extrapolated pharmacokinetic data suggest that CYP2C19 rapid or ultrarapid metabolizers may need a dose increase. Due to the need for further studies investigating the clinical importance of CYP2C19*17 regarding tricyclic metabolism and the possibility of altered concentrations, we recommend to consider an alternative tricyclic or other drug not affected by CYP2C19. This recommendation is classified as optional due to limited available data. If amitriptyline is administered to a CYP2C19 rapid or ultrarapid metabolizer, therapeutic drug monitoring is recommended.

CYP2C19 poor metabolizers are expected to have a greater ratio of amitriptyline to nortriptyline plasma concentrations. The elevated amitriptyline plasma concentrations may increase the chance of a patient experiencing side effects. Use an alternative agent not metabolized by CYP2C19 (e.g., nortriptyline and desipramine) or consider a 50% reduction of the usual amitriptyline starting dose along with therapeutic drug monitoring.

Please review the complete therapeutic recommendations that are located here: (2).

2011 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

For CYP2D6 poor metabolizers, defined as patients carrying two inactive alleles, reduce the dose of imipramine by 70% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 intermediate metabolizers, defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, reduce the dose of imipramine by 30% and monitor imipramine and desipramine plasma concentrations.

For CYP2D6 ultrarapid metabolizers, defined as patients carrying a gene duplication in the absence of inactive or decreased-activity alleles, select an alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration (Table 5).

For CYP2C19 poor metabolizers, reduce the dose of imipramine by 30% and monitor plasma concentration of imipramine and desipramine or select an alternative drug (e.g., fluvoxamine, mirtazapine).

For CYP2C19 intermediate metabolizers, there is insufficient data to allow calculation of dose adjustment for imipramine, select an alternative drug (e.g., fluvoxamine, mirtazapine)

There are no data for dose recommendations for CYP2C19 ultrarapid metabolizers (Table 6).

Table 5. *CYP2D6* phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

Phenotype	Recommendations for imipramine therapy
Ultrarapid metabolizer	Select alternative drug (e.g., citalopram, sertraline) or increase dose by 70% and monitor imipramine and desipramine plasma concentration
Intermediate metabolizer	Reduce dose by 30% and monitor imipramine and desipramine plasma concentrations
Poor metabolizer	Reduce dose by 70% and monitor imipramine and desipramine plasma concentrations

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. There are no data for ultrarapid metabolizers. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (30).

Table 6. *CYP2C19* phenotypes and the therapeutic recommendations for imipramine therapy, from The Dutch Pharmacogenetics Working Group (2011)

Phenotype	Recommendations for imipramine therapy
Ultrarapid metabolizer	No dose recommendations
Intermediate metabolizer	No dose recommendations
Poor metabolizer	Reduce dose by 70% and monitor plasma concentration of imipramine and desipramine or select alternative drug (e.g., fluvoxamine, mirtazapine)

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for all metabolizer types. The table is adapted from (31)

Please review the complete therapeutic recommendations that are located here: (30, 31).

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

* In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*17*	-806C>T	NM_000769.2:c.-806C>T	Not applicable—variant occurs in a non-coding region	rs12248560

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium database: https://www.pharmvar.org/

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Irinotecan Therapy and *UGT1A1* Genotype

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Introduction

Irinotecan (brand name Camptosar) is a topoisomerase I inhibitor widely used in the treatment of cancer. It is most frequently used in combination with other drugs to treat advanced or metastatic colorectal cancer. However, irinotecan therapy is associated with a high incidence of toxicity, including severe neutropenia and diarrhea (1).

Irinotecan is converted in the body to an active metabolite known as SN-38, which is then inactivated and detoxified by a UDP-glucuronosyltransferase (UGT) enzyme encoded by the *UGT1A1* gene. The UGT enzymes are responsible for glucuronidation, a process that transforms lipophilic metabolites into water-soluble metabolites that can be excreted from the body.

The risk of irinotecan toxicity increases with genetic variants associated with reduced UGT enzyme activity, such as *UGT1A1*28*. The presence of this variant results in reduced excretion of irinotecan metabolites, which leads to increased active irinotecan metabolites in the blood. Approximately 10% of North Americans carry 2 copies of the *UGT1A1*28* allele (homozygous, *UGT1A1 *28/*28*), and are more likely to develop neutropenia following irinotecan therapy (1, 2, 3).

The FDA-approved drug label for irinotecan states that "when administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment" (Table 1) (1).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) recommends starting with 70% of the standard dose for homozygous carriers of the *UGT1A1*28* allele. If the patient tolerates this initial dose, the dose can be increased guided by the neutrophil count. They state that no action is needed for heterozygous carriers of the *UGT1A1*28* allele (e.g., *UGT1A1 *1/*28*) (Table 2) (4). In addition, the French National Network of Pharmacogenetics (RNPGx) has proposed a decision tree for guiding irinotecan prescribing based on the *UGT1A1* genotype and irinotecan dose (Table 3) (5).

Table 1. FDA (2017) Drug Label for Irinotecan. Therapeutic Recommendations based on *UGT1A1* Genotype. Dosage and Administration.

Genotype	Recommendations
*UGT1A1 *28/*28*	When administered as a single-agent, a reduction in the starting dose by at least one level of irinotecan hydrochloride injection, USP[1] should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. Table adapted from (1).

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[1] USP stands for the United States Pharmacopeia. The USP establishes standards that promote safe medication use (e.g., procurement, prescribing, transcribing, order entry, preparation, dispensing, administration, and monitoring of medications).

Table 2. DPWG (2014) Recommendations for Irinotecan and *UGT1A1* Genotype.

Phenotype / genotype	Recommendations
UGT1A1 intermediate metabolizer (IM)	NO action is needed for this gene-drug interaction.
UGT1A1 poor metabolizer (PM)	Start with 70% of the standard dose If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.
*UGT1A1 *1/*28*	NO action is needed for this gene-drug interaction.
*UGT1A1 *28/*28*	Start with 70% of the standard dose If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Please see Therapeutic Recommendations based on Genotype for more information from the DPWG. Table is adapted from (4).

Table 3. RNPGx (2017) Recommendations for Irinotecan and *UGT1A1* Genotype.

Dose of irinotecan	Recommendation
low doses (<180 mg/m^2/week)	Presence of the *UGT1A1*28* allele is not a major risk factor (little difference in risk of hematological or digestive toxicity irrespective of the genotype)
180—230 mg/m^2 spaced by 2—3-week intervals	Patients who are homozygous for the *UGT1A1*28* allele have a higher risk of hematological and/or digestive toxicity than patients who are heterozygous or non-carriers. For these *28/*28* patients, a 25-30% dose reduction is recommended, especially if the patient presents other risk factors for toxicity. Dose can be adjusted for subsequent cycles depending on the tolerance.
240 mg/m^2 or higher spaced by 2—3-week intervals	Homozygous *UGT1A1*28* patients have a greatly increased risk of hematological toxicity (neutropenia) compared with other genotypes, contraindicating administration at this higher dose and leading to discussion of a standard dose depending on the associated risk factors. Administration of an intensive dose (240 mg/m^2) is recommended only for *1/*1* patients, or for *1/*28* patients who have no other risk factors and who benefit from intensive surveillance.

Please see Therapeutic Recommendations based on Genotype for more information from the RNPGx. Table is adapted from (5).

Drug: Irinotecan

Irinotecan is used to treat colorectal cancer, which is the third most common cancer worldwide (6). It is often used in combination with other drugs to treat patients with advanced or metastatic colorectal cancer, when the cancer has recurred, or has progressed following initial treatment. A common irinotecan-based combination therapy is referred to as FOLFIRI (FOLinic acid [also known as leucovorin], Fluorouracil, IRInotecan).

Irinotecan is a semisynthetic derivative of the antineoplastic agent camptothecin, which derives its name from the Camptotheca tree where it was first isolated. Like camptothecin, irinotecan is an inhibitor of the nuclear enzyme, topoisomerase I. This enzyme catalyzes a number of nuclear processes; regulation of DNA supercoiling, replication, recombination, and repair.

Topoisomerase I decreases the torsional strain in the helical strands of DNA by making single strand breaks in the DNA. Single strands of DNA pass through the breaks and bind to the topoisomerase to form a cleavable complex. Once the DNA is sufficiently relaxed and the passage of strands has been completed, topoisomerase re-ligates the broken DNA strands and allows for transcription to proceed (7, 8).

Irinotecan is a pro-drug, and is converted by carboxylesterase enzymes to the active metabolite SN-38, which is 100–1000 times more potent that its parent drug, after administration by intravenous injection (9). The SN-38 metabolite exerts its cytotoxic effects by binding to the cleavable complex to form a ternary drug-topoisomerase-DNA complex. This complex is thought to prevent the re-ligation of the single strand breaks, which interrupts the moving DNA replication fork. The arrest of replication and the interaction between replication enzymes and

the ternary complex introduces lethal double-stranded breaks in DNA causing irreparable DNA damage and subsequent cell apoptosis. (10, 11).

SN-38 is lipophilic, and it needs to be inactivated by undergoing phase II metabolism (glucuronidation). The resulting conjugated SN-38 glucuronide is water-soluble, and is mainly excreted through the bile, with about 30% excreted by the kidneys (12).

Irinotecan-based combination therapy has been found to be superior in overall response and survival when compared with the use of 5-fluorouracil/leucovorin therapy alone for patients with metastatic colorectal cancer (3). However, the use of irinotecan is limited by a high incidence of unpredictable and severe dose-limiting toxicity, including severe neutropenia, fever, and diarrhea (13). The rate of grade 3 or 4 adverse events is around 20-25% (14). Approximately 7% of patients who present with severe neutropenia and fever following treatment with irinotecan will die from these complications (3, 15, 16, 17, 18).

Gene: *UGT1A1*

The UGT enzymes (uridine diphosphate-glucuronosyltransferase, or UDP-glucuronosyltransferase) are a superfamily of enzymes that metabolize a wide range of lipophilic molecules such as bilirubin, steroids, toxins, and drugs—including irinotecan's active metabolite, SN-38. These enzymes mediate the process of glucuronidation, which is a phase II metabolic pathway during which glucuronic acid is conjugated to specific targets to convert them to water-soluble metabolites that can then be eliminated from the body.

The UGT genes are polymorphic, and genomic processes, such as copy-number variations, variant splicing, and epigenetic factors, likely contribute to their diversity. As a result, the substrates that the UGT enzymes catalyze are particularly variable (19).

The UGT superfamily contains at least 117 enzymes divided into 4 families, of which UGT1A is a member (20). The *UGT1A* gene locus, located on chromosome 2q37, is complex—it encodes multiple genes and pseudogenes, and alternatively spliced isoforms also exist (21).

The *UGT1A* locus contains multiple alternative first coding exons, each of which has its own promoter site, enabling the transcription of 9 unique UGT1A enzymes (22). One of these transcripts is *UGT1A1*, which encodes UGT1A1, the bilirubin-UGT enzyme. Whereas many UGT enzymes overlap in the substrates they glucuronidate, UGT1A1 is the only enzyme that glucuronidates bilirubin (23).

Bilirubin is a yellow waste product produced during the catabolism of heme, a constituent of hemoglobin. When old or damaged red blood cells are broken down in the spleen, their hemoglobin is broken down to heme, which is then converted into bilirubin. The UGT1A1 enzyme converts this toxic, insoluble form of bilirubin (unconjugated bilirubin) to its nontoxic form (conjugated bilirubin). Because conjugated bilirubin is water-soluble, it can be dissolved in bile and eliminated with solid waste. If bilirubin is not eliminated and instead accumulates to high levels (hyperbilirubinemia), it can cause a yellowish discoloration of the skin and eyes, a condition known as jaundice.

Variants of the *UGT1A1* gene that decrease UGT1A1 enzyme activity can lead to jaundice. The data suggests that one copy of *28 allele results in about a 35% decrease in transcriptional activity, and 2 copies (**28/*28*, homozygous) results in about a 70% decrease (24, 25).

The jaundice may be mild, as seen in Gilbert syndrome, or severe, as seen in Crigler-Najjar syndrome. Crigler-Najjar syndrome presents in 2 forms called type 1 and type 2. Type 1 is the extremely severe form where affected individuals can die in childhood due to kernicterus (bilirubin-induced brain injury), although they may survive for longer with treatment. Type 2 is less severe; the affected individuals are less likely to develop kernicterus and most survive into adulthood.

Currently, over 135 genetic variants of *UGT1A1* have been reported (23, 26). *UGT1A1*1* is the wild-type allele associated with normal enzyme activity. The most common variant allele is *UGT1A1*28*, which is commonly found in African-Americans (0.42–0.45 allele frequency) and Caucasians (0.26–0.31), and is less common in Asian populations (0.09–0.16) (27, 28). Within Caucasian and African American populations, the *UGT1A1*28* variant is a common cause of Gilbert syndrome and is also a cause of Crigler-Najjar syndrome types 1 and 2 (19, 27).

The *UGT1A1*28* [$(TA)_7TAA$] variant contains an extra thymine-adenine (TA) repeat within the TATA box promoter region (7 TA repeats compared with 6 in the wild-type allele) (29). This extra (TA) repeat decreases the rate of transcription initiation of the *UGT1A1* gene, leading to decreased enzyme activity and decreased glucuronidation of bilirubin to about 30% of wild-type levels (30).

Another variant allele, UGT1A1*37 [$(TA)_8TAA$], has 8 TA repeats at this site, and results in reduced promoter activity of the gene to levels lower than the UGT1A1*28 allele. In contrast, the UGT1A1*36 [$(TA)_5TAA$] allele only has 5 repeats and is associated with increased promoter activity and a reduced risk of neonatal hyperbilirubinemia (a common, and typically benign condition). Both UGT1A1*36 and UGT1A1*37 occur almost exclusively in populations of African origin, with estimated allele frequencies of 0.03–0.10 and 0.02–0.07, respectively.

The *UGT1A1*28* variant is also associated with drug toxicity. Approximately 10% of the North American population is homozygous for the **28* allele (**28/*28* genotype, also known as *UGT1A1 7/7* genotype) and are at an increased risk of neutropenia following intravenous irinotecan therapy (28). The rate of severe neutropenia in **28/*28* homozygous patients is as high as 36%, and is strongly associated with a higher hospitalization rate (7, 31, 32).

There is less evidence to support a link between *UGT1A1* genotype and irinotecan treatment-related diarrhea, and there is conflicting data on whether an individual's *UGT1A1* genotype influences their response to irinotecan therapy (8, 33).

Another variant allele, *UGT1A1*6*, is more prevalent in Asian populations, with an allele frequency of around 15–30% in Chinese, Korean, and Japanese populations (24, 34, 35). In this variant, there is a switch of amino acids, from a glycine to an arginine at position 71 within a coding region (p.Arg71Gly). Individuals who are homozygous for this allele have reduced UGT1A1 enzyme activity, which can cause Gilbert syndrome and prolonged neonatal jaundice (36, 37, 38, 39). This variant also appears to be an important predictor of severe toxicity to irinotecan therapy in Asian populations (35, 40, 41, 42, 43, 44, 45, 46, 47).

In addition to genetic variations in the *UGT1A1* gene, several other genetic markers may influence the risk of irinotecan toxicity. These include genetic variation in the adenosine triphosphate (ATP)-binding cassette (ABC) transporter genes, *ABCC1* and *ABCB2* (43, 48, 49), the solute carrier (*SLC*) transporter genes (48, 50, 51), the transforming growth factor (*TGFB*) gene (52), and the xenobiotic-sensing receptor, *NR1I2* (53).

The emerging data suggests that other variant alleles may have a protective effect. The newly discovered marker rs11563250 (NM_001287395.1:c.-1068A>G), located in the 3′-flanking region of *UGT1A1*, has a major *A* allele (rs11563250A) and a relatively common variant *G* allele (rs11563250G, found in 12% of the population). Carriers of the *G* allele have a lower risk of irinotecan-induced neutropenia. They also tend to have lower total plasma bilirubin levels, suggesting that this variant is associated with an enhanced capacity for glucuronidation. Evidence suggests that carriers of rs11563250G could tolerate a higher dose of irinotecan, especially if they also have the *UGT1A1*1/*1* genotype (54).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests currently available for irinotecan response and for the *UGT1A1* gene.

Genetic testing can be used to optimize irinotecan dosing. For example, the use of genotyping in selective cases may make the following patient choices possible:

• If the patient prefers aggressive treatment: genotyping might allow higher dosing for **1/*1* and **1/*28* genotypes (55, 56, 57, 58, 59).

- If the patient prefers maximizing quality of life: genotyping might allow lower dosing for **28/*28* genotype (7, 31, 32).

Genotyping may also enable irinotecan to be added to the treatment of other gastrointestinal tumors without the risk of hematologic toxicity (60). Genotyping may also be used as part of the management of Gilbert syndrome (15).

In the USA, the common **1* and **28 UGT1A1* alleles comprise 98–99% of genotypes (61). Routine genotyping typically tests for *UGT1A1 *1/*1, *1/*28*, and **28/*28 genotypes (*also known as *6/6*, *6/7*, and *7/7*, respectively).

Routine screening does not rule out other *UGT1A1* polymorphisms that are more common in specific populations (7). For example, the *UGT1A1*6* allele is common in Asian populations, and in Japan, a reduced dose of irinotecan is recommended for individuals with *UGT1A1 *6/*6*, **6/*28*, and **28/*28* genotypes (62). In addition, routine screening does not identify patients who are being under-dosed and could potentially tolerate a much higher dose of irinotecan.

The adoption of preemptive *UGT1A1*28* genotyping to increase irinotecan safety and efficacy in clinical practice is still limited and often not covered by health insurance, despite the significant costs of treating irinotecan-related toxicities (63, 64).

Part of the reason that healthcare providers forgo testing may be because the standard dose of irinotecan used in FOLFIRI is low (180 mg/m^2). A phase II trial is currently determining whether dosing irinotecan based on genotype, as part of a FOLFIRI treatment regime, is effective and safe. The standard irinotecan dose of 180 mg/m^2 is being used for patients with the *28/*28* genotype, a dose of 260 mg/m^2 is being used for patients with the **1/*28* genotype, and a dose of 310 mg/m^2 is being used for patients with the **1/*1* genotype (65).

Therapeutic Recommendations based on Genotype

This section contains excerpted[2] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Individuals who are homozygous for the *UGT1A1*28* allele (*UGT1A1 7/7* genotype) are at increased risk for neutropenia following initiation of Irinotecan Hydrochloride Injection, USP treatment.

In a study of 66 patients who received single-agent Irinotecan Hydrochloride Injection, USP (350 mg/m^2 once-every-3-weeks), the incidence of grade 4 neutropenia in patients homozygous for the *UGT1A1*28* allele was 50%, and in patients heterozygous for this allele (*UGT1A1 6/7* genotype) the incidence was 12.5%. No grade 4 neutropenia was observed in patients homozygous for the wild-type allele (*UGT1A1 6/6* genotype).

[2] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

When administered as a single-agent, a reduction in the starting dose by at least one level of Irinotecan Hydrochloride Injection, USP should be considered for patients known to be homozygous for the *UGT1A1*28* allele. However, the precise dose reduction in this patient population is not known and subsequent dose modifications should be considered based on individual patient tolerance to treatment.

UGT1A1 Testing

A laboratory test is available to determine the *UGT1A1* status of patients. Testing can detect the *UGT1A1 6/6*, *6/7* and *7/7* genotypes.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Recommendations from the French National Network of Pharmacogenetics (RNPGx)

Interpreting Results

The RNPGx has proposed a decision tree for guiding irinotecan prescription based on the *UGT1A1* genotype and the protocol's theoretical dose:

- for low doses (< 180 mg/m^2 /week), presence of the *UGT1A1*28* allele is not a major risk factor (little difference in risk of hematological or digestive toxicity irrespective of the genotype);
- for doses in the 180—230mg/m^2 spaced by 2—3-week intervals, patients who are homozygous for the *UGT1A1*28* allele have a higher risk of hematological and/or digestive toxicity than patients who are heterozygous or non-carriers. For these **28/*28* patients, a 25% to 30% dose reduction is recommended, especially if the patient presents other risk factors for toxicity. Dose can be adjusted for subsequent cycles depending on the tolerance;
- for doses of 240mg/m^2 or higher spaced by 2—3 weeks intervals, homozygous *UGT1A1*28* patients have a greatly increased risk of hematological toxicity (neutropenia) compared with other genotypes, contraindicating administration at this higher dose and leading to discussion of a standard dose depending on the associated risk factors. Administration of an intensive dose (240 mg/m^2) is recommended only for *1/*1 patients, or for *1/*28 patients who have no other risk factors and who benefit from intensive surveillance.

[...]

The first-intention of this strategy for analysis of *UGT1A1* status is to detect the *28 variant, the most common deficiency variant observed in the Caucasian population, to be performed before initiating treatment. Referring to the level of evidence classification for RNPGx recommendations detailed in the article by Picard et al. in this issue, *UGT1A1* genotyping is advisable for a standard dose (180—230mg/m^2) and essential for intensified dose (> 240 mg/m^2).

Thus, individualized treatment can be proposed based on the *UGT1A1* genotype, with either a dose reduction for **28/*28* homozygous patients, or possibly dose intensification for non-carriers of the *28 allele.

For the other *UGT1A1* alleles, genotyping is performed by a limited number of laboratories and is considered a second- intention test.

Moreover, the RNPGx suggests that this analysis could be performed concomitantly with other genetic explorations for colorectal cancer patients (search for *KRAS*, *BRAF* mutations. . .) and constitutional (search for *DYPD* variants) in order to guarantee optimal irinotecan therapy within adequate delay for optimal hospital practices.

Please review the complete therapeutic recommendations that are located here: (5).

2014 Recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

UGT1A1 Intermediate Metabolizers (IM)
NO action is needed for this gene-drug interaction.
This genetic variation (IM) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

UGT1A1 Poor Metabolizers (PM)
Genetic variation reduces conversion of irinotecan to inactive metabolites. This increases the risk of serious, life-threatening adverse events.
Recommendation:
1. Start with 70% of the standard dose
If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

UGT1A1 *1/*28
NO action is needed for this gene-drug interaction.
This genetic variation (*1/*28) is more common in Western populations than the wild-type (*1/*1). This means that treatment is largely geared to patients with this genetic variation. Adjustment of the treatment is therefore not useful.

UGT1A1 *28/*28
Genetic variation reduces conversion of irinotecan to inactive metabolites. This increases the risk of serious, life-threatening adverse events.
Recommendation:
1. Start with 70% of the standard dose
If the patient tolerates this initial dose, the dose can be increased, guided by the neutrophil count.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature of selected *UGT1A1* variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*1*	$(TA)_6TAA$	NM_000463.2:c.-53_-52TA[7]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347
*UGT1A1*6*	211G>A Gly71Arg	NM_000463.2:c.211G>A	NP_000454.1:p.Gly71Arg	rs4148323
*UGT1A1*36*	$(TA)_5TAA$	NM_000463.2:c.-53_-52TA[6]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347
*UGT1A1*28*	$(TA)_7TAA$	NM_000463.2:c.-53_-52[8]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*UGT1A1*37*	$(TA)_8TAA$	NM_000463.2:c.-53_-52TA[9]	Not applicable—variant occurs in a non-coding (TATA box promoter) region	rs8175347

*UGT1A1*1* is the wild-type allele and is associated with normal enzyme activity.
Note: The *UGT1A1*28* variant has 8 TA repeats, as shown by the "[8]" in the official HGVS term, "NM_000463.2:c.-53_-52[8]". In the medical literature, the term "$(TA)_7TAA$" is commonly used. Here, 7 of the TA repeats are shown in parentheses "$(TA)_7$", followed by the 8th repeat "(TAA)".
For an overview of the haplotypes for *UGT1A1*, please see the PharmGKB's Haplotype Translation Table.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Lacosamide Therapy and *CYP2C19* Genotype

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Introduction

Lacosamide (brand name Vimpat) is an antiseizure drug indicated for adjunctive therapy for partial-onset seizures in pediatric and adult patients with epilepsy. Lacosamide is thought to work by selectively enhancing slow inactivation of voltage-dependent sodium channels. This stabilizes the neuronal membrane and suppresses the repetitive neuronal firing associated with seizures.

Several cytochrome P450 (CYP) enzymes are involved in metabolizing active lacosamide to an inactive metabolite, including CYP2C19. Individuals who have no CYP2C19 enzyme activity are known as "CYP2C19 poor metabolizers".

The FDA-approved drug label for lacosamide cites a small study that found plasma levels of lacosamide were similar in CYP2C19 poor metabolizers (n=4) and normal (extensive) metabolizers (n=8) (Table 1). Therefore, the recommended standard doses of lacosamide may be used for CYP2C19 poor metabolizers (1).

Table 1. FDA (2016) Drug Label for Lacosamide. Recommendations for CYP2C19 Phenotype. Pharmacokinetics.

Phenotype	Recommendations
CYP2C19 Poor metabolizer	There are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor metabolizers and extensive metabolizers.

This table is adapted from (1).

Drug: Lacosamide

Lacosamide is an antiseizure drug that is used in the treatment of partial-onset (focal) seizures. It may be used as monotherapy, or as an adjunctive therapy. When lacosamide is taken orally, it can be used in pediatric patients (from age 4), or if given intravenously, it is indicated for adult patients (from age 17) (1).

Over 50 million people worldwide suffer from epilepsy, which is characterized by spontaneous recurrent epileptic seizures classified as generalized or focal. Generalized seizures appear to originate in all regions of the cortex simultaneously and include absence seizures (sudden impaired consciousness and staring) and general tonic-clonic seizures (loss of consciousness, stiffening of limbs in the tonic phase, and twitching or jerking muscles in the clonic phase). In contrast, symptoms of focal seizures depend upon where the focus of the seizure originates in the brain (e.g., jerking of a limb indicates a focus in the contralateral motor cortex).

Most currently available antiseizure medications target sodium channels (e.g., carbamazepine, phenytoin), calcium channels (e.g., ethosuximide), or the gamma-aminobutyric acid (GABA) system (e.g., clobazam). However, up to one-third of patients may not achieve seizure control, or they may not be able to tolerate the side effects. This has led to the development of newer antiseizure drugs with unconventional targets.

Lacosamide is a third-generation antiseizure drug that was designed to have a novel mechanism of action — it selectively enhances the slow inactivation of voltage-gated sodium channels. This leads to a stabilization of neuronal membranes and an inhibition of repetitive neuronal firing. This mode of action is fundamentally different to traditional sodium channel blocking drugs, which affect the fast inactivation of voltage-gated sodium channels (2, 3).

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In adult patients (aged 17 and older), the recommended initial dose for lacosamide monotherapy is 100 mg twice daily (50 mg twice daily for adjunctive therapy), which may be increased to a maximum dose of 200 mg twice daily, for both monotherapy and adjunctive therapy. At these doses, randomized controlled trials have reported that lacosamide reduces the frequency of focal seizures significantly more than placebo, while also being well tolerated (4-7). The most common adverse drug effects of lacosamide are diplopia (double vision), headache, dizziness, and nausea (2, 3, 8, 9).

Lacosamide is metabolized to a major inactive O-desmethyl metabolite by several cytochrome P (CYP) enzymes, including CYP3A4, CYP2C9, and CYP2C19. The role of CYP2C19 in lacosamide metabolism has been the most thoroughly studied. According to the FDA drug label for lacosamide, individuals who harbor 2 nonfunctional *CYP2C19* variant alleles ("CYP2C19 poor metabolizers") have lower concentrations of the inactive O-desmethyl metabolite in their plasma, compared to normal (extensive) metabolizers with 2 functional *CYP2C19* alleles.

However, the plasma concentration of active lacosamide was similar in both poor metabolizers and normal (extensive) metabolizers. Therefore, the label states that there are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor and normal metabolizers (1).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic and can result in no, decreased, normal, or increased enzyme activity.

Enzymes CYP2C19, CYP2C9, and CYP3A4 are involved in the metabolism of lacosamide to a major inactive O-desmethyl metabolite. The role of CYP2C19 in lacosamide metabolism has been the most thoroughly studied (10). According to the FDA drug label for lacosamide, individuals who harbor 2 nonfunctional *CYP2C19* variant alleles ("CYP2C19 poor metabolizers") have lower concentrations of the inactive O-desmethyl metabolite in their plasma and excreted in the urine compared with normal (extensive) metabolizers with 2 functional *CYP2C19* alleles.

Genetic Testing

The National Institutes of Health (NIH) Genetic Testing Registry (GTR) displays genetic tests that are currently available for the *CYP2C19* gene.

Given that currently there are no clinically significant differences in lacosamide pharmacokinetics between CYP2C19 poor and normal metabolizers, there is no evidence supporting clinical CYP2C19 pharmacogenetic testing prior to initiating lacosamide, and testing has not been addressed by currently available professional society practice guidelines.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

CYP2C19 Polymorphism

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

There are no clinically relevant differences in the pharmacokinetics of lacosamide between CYP2C19 poor metabolizers and extensive metabolizers. Results from a trial in poor metabolizers (PM) (N=4) and extensive metabolizers (EM) (N=8) of cytochrome P450 (CYP) 2C19 showed that lacosamide plasma concentrations were similar in PMs and EMs, but plasma concentrations and the amount excreted into urine of the O-desmethyl metabolite were about 70% reduced in PMs compared to EMs.

Please review the complete therapeutic recommendations that are located here: (1).

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Lecanemab Therapy and *APOE* Genotype

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Introduction

Lecanemab, brand name Leqembi, is a monoclonal antibody that targets amyloid beta (Aβ) aggregates for the treatment of Alzheimer disease (AD) (1). It is approved by the US Food and Drug Administration (FDA) for individuals with mild cognitive impairment (MCI) or mild dementia stage AD with confirmed amyloid pathology (1). Tests to confirm Aβ pathology in the clinical trials included positron emission tomography (PET) or cerebrospinal fluid (CSF) measurement of the Aβ42/Total Tau ratio (2). This disease-modifying medication is based on the amyloid cascade hypothesis, which suggests Aβ aggregates are a key driver in AD pathogenesis and that the removal of Aβ aggregates should slow cognitive decline.

Lecanemab is associated with amyloid-related imaging abnormalities (ARIA) due to edema (ARIA-E) or hemorrhage (ARIA-H) from blood vessels in the brain (3, 4). Individuals who have one or 2 copies of the AD risk-associated apolipoprotein E (*APOE)* ε4 (NM_000041.4:c.388T>C) allele have an increased risk of ARIA-E or -H (1) (Table 1). These individuals require additional monitoring during the first year of treatment (5). The FDA-approved label reports that concomitant antithrombotic medication (aspirin, antiplatelet, or anticoagulant) with lecanemab therapy resulted in intracerebral hemorrhage in 2.5% of individuals during clinical trials (1).

The appropriate use recommendations from the Alzheimer's Disease and Related Disorders Therapeutics Work Group state that individuals requiring anticoagulants should not be treated with lecanemab until additional data regarding this interaction are available (5). Both the FDA-approved label and Alzheimer's Disease and Related Disorders Therapeutics Work group encourage clinicians to consider participation in a registry for AD treatment to gather additional real-world data on lecanemab therapy (1, 5).

Table 1: Amyloid Related Imaging Abnormalities (ARIA) Risk Based on *APOE* Genotype

APOE ε4 status	Frequency of symptomatic ARIA-E[a]	Frequency of any ARIA[b]
Homozygous (ε4/ ε4)	9%	45%
Heterozygous (ε4/ εx)	2%	19%
No ε4 allele detected	1%	13%

ARIA-E Amyloid-related imaging abnormalities, edema; εx any other *APOE* ε allele other than 4.
APOE – Apolipoprotein E
[a] Percent of individuals within the CLARITY-AD (study 2) who experienced a symptomatic ARIA-E.
[b] Percent of individuals within the CLARITY-AD (study 2) who experienced any ARIA event.
Adapted from (1).

Drug: Lecanemab

Lecanemab is a humanized monoclonal antibody that targets Aβ protein in the soluble protofibril form and is indicated for the treatment of AD (1). Administered by infusion once every 2 weeks at a dose of 10 mg/kg body weight, it is approved by the FDA for individuals with MCI or mild dementia stage AD who have confirmed

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amyloid pathology (1). Tests to confirm Aβ pathology used in the clinical trials included PET or CSF measurement of the Aβ42/Total Tau ratio (2). Appropriate use criteria for amyloid PET imaging, CSF collection, and testing for biomarkers to diagnose AD have been issued by the Alzheimer's Association (6, 7, 8). Appropriate use recommendations for lecanemab have been provided by Alzheimer's Disease and Related Disorders Therapeutics Work Group (5).

The leading definition for AD diagnosis from a radiologic perspective relies on evidence of amyloid, tau, and neurodegeneration (ATN) biomarkers (9), though neurologic functional impairment is an included criterion in other definitions (10). Diagnosis and staging of AD have been covered in numerous guidelines, all include the utilization of both biomarkers and clinical assessment (11, 12, 13). Mild cognitive impairment clinical criteria include expressed concerns on changes in the individual's cognition, impairment in one or more cognitive domains (as measured by established cognitive assessments), and maintenance of independence in functional abilities (11). Determination of cognitive impairment in the lecanemab clinical trials was obtained via the Mini-Mental State Examination, and individuals qualified based on scores between 22 and 30 (5). Diagnosis based on a combination of cognitive assessment and fluid biomarkers or radiologic findings–or both–represents a significant advancement in the understanding of AD.

One of the leading hypotheses for AD pathology is that Aβ aggregates initiate and drive AD pathogenesis. The amyloid precursor protein (APP) normally undergoes proteolytic processing, but in AD, there is a cleavage into an abnormal length peptide (42 amino acid Aβ form). This protein assembles into toxic, soluble oligomers and protofibrils (14). The abnormal amyloid oligomers cause cell stress and damage, leading to the secretion of more abnormal Aβ proteins and aggregates, which in turn trigger other diseases processes; this model of AD pathology is named the amyloid cascade hypothesis (15). Protofibrils of Aβ can also be sequestered into insoluble amyloid fibrils and plaques. While plaque density has long been correlated with AD severity, some have proposed that the aggregates may protect neuronal cells (14). Notably, individuals homozygous for the "Osaka" variant (NM_000041.4: c.527G>C (p.R176P)) of *APP* present with typical Aβ and tau levels in CSF but no detectable plaques even after AD onset (16), supporting the role of soluble Aβ aggregates in disease pathogenesis.

Other pathological hallmarks of AD include higher levels of tau protein phosphorylation, the formation of insoluble tau aggregates and fibrils (neurofibrillary tangles), and neuroinflammation, which increase with disease severity (14, 17). Elevated levels of phosphorylated tau are associated with an increased risk of progression from MCI to AD dementia (18), and post-mortem studies have found a direct correlation between tau neurofibrillary tangles and the severity and progression of AD (17). Current neuropathological and PET findings also highlight that tau pathology is a strong correlate of neurodegeneration and symptoms (19, 20), and is an indicator of clinical prognosis in cognitively unimpaired individuals (21). Numerous additional hypotheses for AD pathology have also been proposed. One hypothesis includes cholesterol and lipid metabolic dysregulation as a contributing factor for AD pathogenesis, providing a potential mechanism to explain the role of variation in the *APOE* gene in AD risk (22). In this model, abnormal cholesterol levels and metabolism, as well as abnormal Aβ, contribute to other AD pathologies such as tau phosphorylation and loss of blood-brain-barrier integrity (22, 23). The *APOE4* variant is a risk-conferring allele for developing late-onset AD (22). Additional genetic risk factors include *SORL1*, *ABCA7*, and *TREM2* (24, 25, 26, 27). These genetic associations suggest that changes in immune response, endocytosis, and vascular factors may also contribute to AD pathogenesis (28). Dominantly inherited AD (also called early onset AD) has been associated with rare variants in *PSEN1*, *PSEN2*, and *APP*, all of which impact Aβ metabolism (29, 30).

Based on the predominant amyloid cascade hypothesis, anti-amyloid therapies target aggregated forms of Aβ with the goal to modify the course of AD (2, 4, 31, 32). After crossing the blood-brain barrier, lecanemab binds preferentially to Aβ soluble aggregates (oligomers and protofibrils) to facilitate immune clearance, leading to decreases in Aβ plaques (2, 3, 14, 33). The safe and effective dose for lecanemab was studied in a phase 2b trial,

BAN2401-G000-201 (Study 201), and found to be 10 mg/kg body weight every 2 weeks (33). The phase 3 CLARITY trial reported a smaller decrease in cognitive function in the study population taking lecanemab (1.21 point change from baseline on the Clinical Dementia Rating-Sum of Boxes [CDR-SB] score, maximum possible score of 18) than the placebo group (1.66 point change) (2). Additionally, the CLARITY study results indicated a significant reduction in brain amyloid levels over 18 months with lecanemab treatment, as well as a decrease in CSF levels of phosphorylated tau and neuroinflammatory markers (2). Despite these findings, there has been ongoing debate within the scientific community regarding the clinical significance of the cognitive outcomes from this trial (discussed below). However, these data supported the FDA decision to grant full approval of lecanemab in July 2023 (34) with a black box warning for ARIA (1).

Similar to other anti-amyloid therapies, lecanemab treatment can trigger ARIA-E, defined as "the extravasation of fluid resulting in interstitial vasogenic edema or sulcal effusion in the leptomeningeal/subpial space" (35), or hemosiderin deposits (ARIA-H), characterized as cerebral microhemorrhages and/or hemosiderosis (35), more frequently than placebo (2, 5, 31, 33). It is thought that Aβ plaques contribute to the loss of cerebral vascular integrity and are a prime target of anti-amyloid therapy, which may further disrupt vascular integrity in response to therapy (35). Clinically, ARIA can be asymptomatic but can sometimes lead to serious or fatal intracerebral hemorrhage (1, 36, 37, 38) or, in the case of ARIA-E, fatal inflammatory arteritis (39).

Sub-study analyses from the phase 2b clinical trial and post-hoc analysis of the phase 3 trial found that individuals with the *APOE* ε4 variant (*APOE4*) were significantly more likely to experience ARIA than individuals with other *APOE* alleles (2, 33, 40). Additional analysis in the phase 3 CLARITY trial found that the population with 2 *APOE4* alleles experienced ARIA more frequently than the study subpopulation with only one allele (2). The FDA-approved label for lecanemab advises testing for ApoE ε4 status "prior to initiation of treatment to inform the risk of developing ARIA" (41). Cummings and colleagues also recommend genotyping *APOE* before initiating lecanemab therapy (5).

Other factors contributing to the risk of ARIA include prior cerebral microhemorrhage, advanced age, antithrombotic medication use, and history of prior strokes (38, 42). Despite the exclusion of individuals with clinical findings suggesting cerebral amyloid angiopathy (CAA) (more than 4 microbleeds) from clinical trials with lecanemab (CLARITY, "study 2"), this is not an explicit contraindication in the FDA-approved drug label (1). However, the Appropriate Use Recommendations advise against using anticoagulants or acute thrombolytics while taking lecanemab due to the increased risk of hemorrhage (5). Preliminary results suggest that increasing the rate of antibody passage through the blood-brain barrier, thus bypassing vascular amyloid, may reduce ARIA incidence and increase the rate of amyloid clearance from the CSF (43).

It is recommended to perform a baseline MRI and then periodic monitoring for ARIA, particularly during the first 14 weeks of lecanemab therapy (1, 5). A baseline MRI is required to identify cerebral pathologies that indicate an increased risk of ARIA (5). It is recommended to obtain additional MRIs after the 5^{th}, $7^{th,}$ and 14^{th} infusions (1, 5). An additional scan at 52 weeks of therapy is also recommended, especially for individuals with an *APOE4*-positive genotype (5). If an ARIA occurs, the appropriate use recommendations are to suspend or discontinue dosing in nearly all cases, except for mild ARIA-E or -H with no symptoms (5).

Additional warnings for lecanemab include hypersensitivity reactions and infusion-related reactions. Hypersensitivity reactions can include angioedema, bronchospasm, and anaphylaxis and should result in prompt discontinuation of the infusion (1). Infusion reactions such as fever and flu-like symptoms, nausea, vomiting, and hyper- or hypotension led to medication discontinuation in 1% of participants in a clinical trial. Management to avoid discontinuation may include reducing the infusion rate or prophylactic treatment with antihistamines and other medications (see drug label for full details) (1).

Lecanemab has not been adequately evaluated in pregnant or nursing mothers to determine the risk of birth defects, miscarriage, or adverse effects on a breastfed infant. Additionally, the safety and efficacy of lecanemab in

a pediatric population have not been established. The age range of clinical study participants was between 50 and 90 years, and no overall differences in safety or efficacy were observed in individuals aged more than 65 years compared to younger individuals. (1) The clinical trials also did not include individuals with comedications or comorbidities that may increase bleeding risk or interfere with MRI (such as a pacemaker), which may be common in the Alzheimer population in real-world use (2). Conditions that require acute thrombolytics, such as ischemic stroke or myocardial infarction, present a unique challenge for clinicians and will require advance directives and planning (44).

The reported benefit of lecanemab therapy considering the risks of ARIA, intracranial hemorrhage, and other side effects, has led to significant debate in the medical community regarding the use of lecanemab. Many authors have been critical of the clinical significance reported by the CLARITY trial, including the small CDR-SB score differences in treatment versus placebo, calculation of the actual scores, or the limited benefit to subgroups (women or *APOE4* positive individuals) (45, 46, 47, 48, 49, 50, 51, 52, 53). Other concerns over the conclusions from CLARITY have been raised regarding the risk of bias due to functional unblinding of study participants based on ARIA occurrence (51, 54, 55, 56). Additionally, the increased risk of ARIA, intracranial hemorrhage, or vascular central nervous system (CNS) changes has been argued to be too great relative to the reported benefit of lecanemab, and some authors have suggested that real-world use could result in higher rates of these adverse events (47, 57). Planche and Villain have questioned the notion that these treatments, which were designed to modify the course of AD, have yet to produce convincing evidence of disease modification given the limited time on the medication relative to the 7–17 years observed for disease progression and lack of observed impact on all implicated biomarkers(58).

The authors of the CLARITY trial and others defend the conclusions and assessment of the trial results, citing differences in the definition of clinically meaningful CDR-SB values, the lack of power in the study to accurately perform subgroup analysis (such as those based on gender or *APOE* status), significant impact on amyloid pathology across multiple clinical trials (phases 2b and 3), and the predicted long-term benefit in slowing cognitive or functional decline on both health outcomes and quality of life (59, 60, 61). Other authors not affiliated with the study or sponsoring pharmaceutical company also expressed optimism for the benefits of anti-amyloid therapy, including lecanemab, while acknowledging the risk of ARIA (31, 62, 63, 64, 65, 66, 67). It is also noteworthy that most of the ARIA-E events (71%) were detected early in the course of therapy (5), and if appropriately managed, an individual may be able to resume therapy without experiencing future ARIA events (42). A correlation between amyloid clearance and clinical benefit has been made across the various anti-amyloid therapies trialed over the years, with more significant amyloid clearance associated with slowed cognitive decline (4). In line with the ATN definition of AD, CLARITY reported more reduction in biomarkers for all 3 categories with lecanemab therapy compared to placebo (2). Ongoing trials will assess the impact of lecanemab therapy before the onset of cognitive impairment in populations with intermediate (20–40 Centiloids) or elevated (>40 Centiloids) amyloid burden via PET (68). Additional questions remain regarding the appropriate duration of lecanemab therapy and whether there is a benefit to lecanemab co-medication with symptomatic therapies like cholinesterase inhibitor or memantine (69).

The appropriate use recommendations for lecanemab from the Alzheimer's Disease and Related Disorders Therapeutics Work Group state that before initiating lecanemab therapy, there must be established amyloid positivity, a baseline MRI, evaluation for vascular disease or other ARIA risk factors like anticoagulant therapy, as well as the recommended *APOE* testing and genetic counseling (5). Treatment itself is an hour-long infusion plus additional time for monitoring for infusion reactions, which must be performed in an appropriate clinical setting with the necessary training and equipment to manage infusion reactions. Monitoring MRIs must be performed 3 or 4 times during the first year of treatment, requiring clinical expertise in detecting ARIA (1, 5). Care teams are advised to develop a plan for responding to serious and severe ARIA and have sufficiently trained staff to manage seizures or other complications resulting from ARIA (5). There has been speculation that these aspects of clinical implementation may result in the use of lecanemab mostly in specialized care centers (69). All

these care components carry an expense, in addition to the estimated cost of the medication itself ($26,500 per individual annually) (70). The United States Veteran Health Authority announced in March 2023 that it would cover lecanemab costs when medically indicated for veterans (71). Medicare coverage was also extended beyond the scope of use in clinical trials when traditional FDA approval was granted (72). Lecanemab has been approved for use in Japan (73) and China (74). The Japanese Pharmaceutical and Medical Devices Agency review report states that "the safety data showed that the risk of lecanemab including ARIA can be managed irrespective of *APOE* genotype… and it is considered that lecanemab has a favorable risk-benefit balance" (75). Lecanemab review with the European Medicines Agency for marketing authorization was delayed in March 2024, and other country-specific applications are also pending at the time of writing (41).

Determination of real-world safety and benefits of lecanemab therapy will require additional time and research. Clinicians are encouraged to participate in a registry to collect information on AD treatment, including lecanemab, such as the Alzheimer's Network for Treatment and Diagnostics (ALZ-NET, www.alz-net.org). These data will, ideally, include a broader population than those eligible in the existing published studies with strict eligibility criteria. Based on the eligibility criteria from the lecanemab and aducanumab studies, researchers at the Mayo Clinic found that approximately 5–8% of the Mayo Clinic Study of Aging cohort with MCI or mild dementia and brain amyloid burden would have qualified for either medication (76). The specific amyloid burden requirement from anti-amyloid trials may also have resulted in the exclusion of some racial and ethnic groups, such as Hispanic and non-Hispanic Blacks, Hispanic Whites, and non-Hispanic Asians (terms utilized by the study authors) (77). Considering this, along with the limited data from individuals of Asian descent with AD (78), highlights the need for additional research in these underrepresented populations to understand disease mechanism, clinical presentation, and potential benefits and risks of anti-amyloid therapy.

Gene: *APOE*

The *APOE* gene encodes apolipoprotein E, which functions in lipid transport between cells and organs, binding to a variety of lipid-containing complexes including very low-density, intermediate-density, and a few subclasses of high-density lipoproteins, as well as chylomicron remnants (79, 80). It is expressed in various tissues, but it is one of the primary apolipoproteins in the CNS (79). The APOE protein facilitates the clearance of lipoproteins from the plasma or CSF by binding to both lipids and receptors in the LDL family or heparan sulfate proteoglycans (80). This is mediated by 2 functional domains in the APOE protein: the N-terminal portion of the protein interacts with receptor proteins, and the C-terminal portion interacts with lipids (80).

Variation in the *APOE* gene has been associated with AD, age-related macular degeneration, type III hyperlipoproteinemia, lipoprotein glomerulopathy, sea-blue histiocyte disease, and CAA (81, 82, 83, 84, 85, 86). Emerging evidence suggests that *APOE* variants or altered expression may also confer an increased risk for Parkinson disease dementia and dementia with Lewy bodies (23, 87). However, the specific association for an allele can vary among these diseases, with an allele conferring risk for one disorder but protecting against another. There are 3 common alleles of *APOE*: ε*2,* ε*3*, and ε*4*, with *APOE* ε*3* (also written as *APOE3*) representing the reference, normal function allele (78, 79, 80). The APOE4 protein (from *APOE* ε*4* or *APOE4* allele) is a well-established risk factor for AD. The *APOE2* allele appears to be a protective variant for AD development but is a causative allele for type III hyperlipoproteinemia (78, 80). Initially identified by differences in isoelectric focusing, subsequent studies have identified the underlying genomic changes. The *APOE2* allele has an additional cysteine residue (rs7412, NM_000041.4:c.526C>T, p.R176C) and *APOE4* has one less cysteine residue (rs429358, NM_000041.1:c.388T>C, p.C112R) (Table 2). These changes can impact dimerization, protein processing, and binding to other proteins (79).

Table 2: Common *APOE* Allele Minimum Variant Definitions and Associations

APOE allele	rs429358 genotype (cDNA and protein change)[a]	rs7412 genotype (cDNA and protein change)[a]	Protein function[b] and disease association
ε2	T (c.388T; p.Cys130=)	T (c.562C>T; p.Arg176Cys)	Decreased receptor binding, protective for AD; risk allele for ARMD and type III hyperlipoproteinemia
ε3	T (c.388T; p.Cys130=)	C (c.562C; p.Arg176=)	Normal receptor binding and reference disease risk
ε4	C (c.388T>C; p.Cys130Arg)	C (c.562C; p.Arg176=)	Normal receptor binding, likely toxic gain of function; risk for AD

AD - Alzheimer disease, ARMD - Age-related macular degeneration
[a] cDNA coordinates given for NM_000041.4; protein change in NP_000032.1
[b] Function relative to lipid receptor binding, adapted from (80).

The frequency of *APOE* alleles varies across populations; overall *APOE3* is the most common, followed by *APOE4* and then *APOE2* (88). The global minor allele frequency for the *APOE4* (rs429358) allele reported in the Allele Frequency Aggregation (ALFA) project is 0.074, though the 1000 Genomes data estimates it to be 0.15; in both data sets, the minor allele is more common in African ancestry than other groups (89). The global minor allele frequency reported in ALFA for *APOE2* (rs7412) allele is 0.083, though it is reported as high as 0.11 in some African descent populations and as low as 0.02 in South Asian populations (90). The frequency of the *APOE4*-defining allele (rs429358) has been reported to be 0.11 in a study population from India; a frequency that the authors note is lower than the European, African, and non-Finnish European population data reported in gnomAD (91). The frequency of *APOE4* in Hispanic ancestry is reported to be 0.12, though the frequency is 0.24 in Hispanic individuals with AD (92).

Despite the higher prevalence of the *APOE4* allele in populations of African descent, the surrounding genomic context may also have a significant impact on the expression of the allele. Studies suggest that the genomic context from a European ancestral background promotes higher expression of *APOE4* than an African ancestral background (78). This may be due to non-coding genetic variation at *APOE* enhancers, changes in chromatin accessibility, or both, resulting in a decreased risk of *APOE4*-assocated disorders for individuals with African ancestry (78). An *APOE4* genotype was associated with elevated Aβ levels in non-Hispanic White individuals, but not in individuals of African American descent (93). The association between *APOE4* and AD is also weaker in individuals of Hispanic ancestry compared to Caucasian ancestry (94, 95). Variation in the *APOE* region does seem to contribute to AD risk in Hispanic populations, but the *APOE4* allele alone showed a weaker association than polygenic risk scores that included other *APOE*-linked variants (96).

Whether *APOE4* is a toxic gain-of-function or loss-of-function allele has been studied using various approaches and could influence the utility of APOE4 as a disease target for future therapeutic efforts (78). Increased *APOE4* expression in African or European ancestry populations is associated with a higher frequency of AD or CAA and some studies have suggested that non-coding variants in a European ancestry haplotype may further contribute to increased *APOE4* expression and AD risk (78). Genetic analysis of the Alzheimer's Disease Sequencing Project identified putative loss-of-function variants as being associated with reduced risk of AD or later onset of AD (97). Mouse studies further support a model where increased *APOE* expression (either ε3 or ε4 variant forms) exacerbated Aβ accumulation and that downregulating *APOE4* expression could reduce amyloid deposits in the brain (98). The APOE4 variant protein is more susceptible to abnormal proteolytic cleavage, creating a C-terminal fragment that is neurotoxic in transgenic mouse models (80). Thus, the overall conclusion of the National Institute on Aging/Alzheimer's Disease Sequencing Project consortium is that reducing the levels of APOE4 in individuals of European or African ancestry would be an appropriate therapeutic target; additional studies are needed to confirm if this approach would also be beneficial to individuals of Asian ancestry (78).

Linking *APOE* Genetic Variation with Treatment Response

Clinical trial data from the lecanemab studies showed a clear, gene dose-dependent risk for ARIA in individuals with the *APOE* ε4 (*APOE4*) variant allele (2, 33). The frequency of symptomatic ARIA-E during the phase 3 CLARITY trial for individuals with 2 *APOE4* alleles was 9.2%, versus 1.7% in individuals with only one *APOE4* allele and 1.4% in individuals without an *APOE4* allele (2). The frequency of ARIA-H was 39% in *APOE4* homozygotes, 14% in heterozygotes and 11.9% in individuals without an *APOE4* allele (2). This increased frequency of ARIA in *APOE4* is the rationale behind Cummings et al's recommendation that *APOE4*-positive individuals receive an additional monitoring MRI scan during the first year of lecanemab therapy (5).

Given *APOE4*'s role as a genetic risk factor for sporadic AD, it is useful to test whether this variation may also impact the cognitive benefits from anti-amyloid therapies like lecanemab. A pooled analysis of published studies of the anti-amyloid medications lecanemab, aducanumab, solanezumab, and donanemab suggests that *APOE4*-positive individuals have the same or better response to amyloid-targeting therapies compared to individuals without an *APOE4* allele (99). However, *APOE4* is also associated with increased risk of ARIA for aducanumab and donanemab (37, 100). Specifically reviewing data from the lecanemab studies, some reports have suggested that *APOE4*-positive individuals experienced a reduced clinical benefit of lecanemab therapy (48, 54), though study authors state there is insufficient statistical power to perform this subgroup analysis from the CLARITY trial data (59). Additional data are needed to comprehensively assess the connection between *APOE4* status and anti-amyloid efficacy.

The proposed mechanisms underlying APOE4's contribution to AD and subsequent ARIA risk with anti-amyloid therapy are complex, with proposed Aβ-dependent and -independent mechanisms (78, 80, 101). Within the CNS, astrocytes are the primary producers of APOE protein and roughly 60% of the protein is secreted into the extracellular space (101). In a transgenic mouse model, abnormal APOE4 protein altered lipid metabolism in astrocytes and led to decreased astrocyte-blood vessel contacts, triggering increased leakiness in the blood-brain barrier (101). If this holds true in humans, it may explain, at least in part, the increased risk of ARIA observed in *APOE4*-positive individuals during from anti-amyloid trials. Other hypotheses for this observed ARIA association include the increased amyloid oligomer burden in *APOE4*-positive individuals, which leads to a higher level of disruption of blood vessel integrity during amyloid clearance (3). Finally, the potential link between ARIA and CAA-related inflammation suggests a shared pathophysiology between the 2 conditions (102). It known that *APOE4* increases the likelihood of CAA (49), suggesting that the APOE4 effect may be mediated by the increased presence of CAA among *APOE4*-positive individuals, even in the absence of radiological evidence.

Genetic Testing

The NIH Genetic Testing Registry (GTR) includes tests for *APOE* genotype. The appropriate use recommendations as well as the FDA-approved drug label recommend testing individuals for *APOE4* before initiating therapy (1, 5). While an *APOE4*-positive genetic result is not a contraindication for lecanemab therapy, it should prompt additional discussion regarding the risk of ARIA and the potential for related individuals to also have an *APOE4* allele (5). One suggested course of clinical care is to initiate *APOE* genetic testing only after AD biomarker (namely, amyloid levels) testing is complete and interest in anti-amyloid therapy is confirmed with the individual to be treated, since *APOE* genotype results are unlikely to impact clinical care with other therapeutic approaches (103).

Genetic testing for *APOE* comes with challenges. In the context of testing before initiating anti-amyloid therapy, both pretest and posttest counseling are recommended to ensure the individual with AD and their caregiver are informed of the risks and benefits of testing and treatment; this can be particularly challenging if the individual has MCI (104). In addition to the risks associated with *APOE4* genotype and ARIA, there are additional disease

risks for the individual, such as ischemic stroke, lobar intracerebral hemorrhage, and others, as well as the possibility of additional family members also having an *APOE4* allele (104). Diagnosis of an *APOE4* allele may also impact medical insurance decisions, potentially from the individual subscriber, the payer, or both, particularly with regard to life, disability, and long-term care insurance plans (104). Within the United States, federal laws are in place to protect from discriminatory practices based on genetic data (the Genetic Information and Nondiscrimination Act and Affordable Care Act), however, there are state-to-state differences in regulations for life, disability, or long-term care insurance (105). Legal or regulatory guidelines in other countries may warrant considerations as lecanemab or other anti-amyloid therapies with an *APOE*-associated risk genotype are introduced into clinical care elsewhere.

The *APOE* Gene Interactions with Medications Used for Additional Indications

Variation in *APOE* increases the risk of ARIA not only for lecanemab but for other anti-amyloid medications like aducanumab (3, 4, 100). Weak evidence links *APOE* and 3-Hydroxy-3-Methylglutaryl-CoA reductase variation to statin response, though it was insufficient to warrant actionable guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) (106).

Additional information on gene-drug interactions for *APOE* are available from PharmGKB, CPIC and the FDA (search for "APOE").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

Patients who are apolipoprotein E ε4 (ApoE ε4) homozygotes (approximately 15% of Alzheimer's disease patients) treated with this class of medications, including [lecanemab], have a higher incidence of ARIA, including symptomatic, serious, and severe radiographic ARIA, compared to heterozygotes and noncarriers. Testing for ApoE ε4 status should be performed prior to initiation of treatment to inform the risk of developing ARIA. Prior to testing, prescribers should discuss with patients the risk of ARIA across genotypes and the implications of genetic testing results. Prescribers should inform patients that if genotype testing is not performed they can still be treated with lecanemab; however, it cannot be determined if they are ApoE ε4 homozygotes and at higher risk for ARIA… Consider the benefit of lecanemab for the treatment of Alzheimer's disease and potential risk of serious adverse events associated with ARIA when decided to initiate treatment with lecanemab.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for Selected *APOE* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
APOE3	ε3	NM_000041.4: c.[388=;526=]	NP_000032.1	(none)

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes, and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
APOE2	ε2	NM_000041.4:c.562C>T	NP_000032.1:p.Arg176Cys	rs7412
APOE4	ε4	NM_000041.4:c.388T>C	NP_000032.1:p.Cys130Arg	rs429358

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Lesinurad Therapy and *CYP2C9* Genotype

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Introduction

Lesinurad (brand name Zurampic) is a urate transport inhibitor used in the treatment of gout. Gout is one of the most common types of inflammatory arthritis, affecting approximately 3% of adults worldwide. It is caused by the accumulation of urate crystals in joints. The long-term management of gout includes reducing risk factors (e.g., obesity, alcohol use, diuretic use, poor renal function), and medication to lower uric acid levels.

Lesinurad reduces the high level of uric acid (hyperuricemia) associated with gout. Lesinurad should only be used in combination with a xanthine oxidase inhibitor (e.g., allopurinol, febuxostat) –– the risk of acute renal failure is increased if lesinurad is used alone.

The addition of lesinurad to gout treatment is reserved for patients who have failed to achieve their target uric acid level despite being treated with a xanthine oxidase inhibitor. Xanthine oxidase inhibitors reduce uric acid by inhibiting its production, whereas lesinurad reduces uric acid by blocking its reabsorption in the kidney.

Lesinurad is primarily metabolized by CYP2C9 to several inactive metabolites. Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to lesinurad, and an increased risk of side effects. Adverse reactions of lesinurad therapy include kidney stones and other kidney problems. Lesinurad is also associated with an increased risk of cardiovascular events.

The FDA-approved drug label for lesinurad states that lesinurad should be used with caution in CYP2C9 poor metabolizers, but does not provide specific dose adjustments in this group (Table 1). The standard dose of lesinurad is 200 mg daily (1). Lesinurad is contraindicated in patients with severe impairment of kidney function (e.g., kidney transplant and hemodialysis patients) as well as individuals with tumor lysis syndrome or Lesch-Nyhan syndrome.

Table 1. The FDA (2018) Drug Label for Lesinurad. CYP2C9 Inhibitors, CYP2C9 Poor Metabolizers, and CYP2C9 Inducers.

Phenotype	Recommendations
CYP2C9 Poor metabolizer	Lesinurad exposure is increased when lesinurad is co-administered with inhibitors of CYP2C9 and in CYP2C9 poor metabolizers. Lesinurad should be used with caution in patients taking moderate inhibitors of CYP2C9 (e.g., fluconazole, amiodarone), and in CYP2C9 poor metabolizers.

This table is adapted from (1).

Drug: Lesinurad

Lesinurad is a urate transporter inhibitor used to treat the increased level of uric acid in patients with gout. Lesinurad should only be used for patients with gout who have high levels of uric acid despite being treated with a xanthine oxidase inhibitor, and lesinurad should only be used in combination with a xanthine oxidase inhibitor (1).

Gout is one of the most common types of inflammatory arthritis. It affects approximately 3% of adults worldwide, and the prevalence is increasing (2-5). Gout is caused by the body's inflammatory response to an accumulation of urate crystals. A high level of uric acid in the blood (above 6.8 mg/dl indicates hyperuricemia)

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always precedes gout. However, the majority of individuals with hyperuricemia do not develop urate crystal deposits and gout, and lesinurad should not be used to treat asymptomatic hyperuricemia.

Patients with gout usually have an extremely painful, swollen joint –– this is known as acute gouty arthritis. A single joint in the lower limb is most commonly affected (e.g., the base of the big toe, knee), and the joint will remain painful for at least several days. In a minority, persistent hyperuricemia leads to chronic gout, which is associated with deposits of urate crystals known as tophi.

Medications for gout focus on lowering uric acid levels. There are 3 main types of drugs:

- xanthine oxidase inhibitors that decrease the production of uric acid (e.g., allopurinol, febuxostat)
- uricosuric drugs that inhibit the reabsorption of uric acid in the kidneys (e.g., benzbromarone, probenecid, and lesinurad)
- uricase drugs that convert uric acid to a more soluble metabolite (e.g., pegloticase, rasburicase)

Lesinurad is the newest uricosuric drug to be approved for gout. However, since the introduction of allopurinol in the 1960s, uricosuric drugs have not been commonly used. This is because they are associated with numerous drug interactions and side effects (6-8).

Like other uricosuric drugs, lesinurad inhibits the urate transporter 1 (URAT1), which mediates reabsorption of uric acid in the kidney, and the organic anion transporter 4 (OAT4), which is implicated with hyperuricemia associated with diuretic use. But unlike probenecid, lesinurad does not appear to inhibit OAT1 or OAT3, and this may result in fewer drug interactions and adverse events (9). However, like all uricosuric agents, lesinurad is associated with the development of kidney stones (10-12).

There are many risk factors that may contribute to triggering a gout attack. These include dietary factors, dehydration, and alcohol use. In addition, medications that alter serum concentrations of uric acid, such as diuretics and gout medications, can trigger gout. Therefore, when starting medical therapy for gout, it is recommended that urate levels are reduced slowly (e.g., 1–2 mg/dl per month). To prevent flare-ups, an additional drug such as colchicine may be used to reduce swelling and pain until target serum levels have been achieved and maintained (13).

Allopurinol is the mainstay treatment for gout –– it is effective in lowering uric acid levels, reduces the frequency of gout attacks, and contributes to resolving tophi. However, in individuals who are carriers of the genetic variant *HLA-B*58:01*, allopurinol is associated with severe cutaneous adverse reactions (SCAR) (14, 15). For these individuals, febuxostat may be the safer choice –– it is a structurally different xanthine oxidase inhibitor that is not associated with SCAR (13).

In general, when allopurinol is used at an adequate dose, levels of uric acid can reach the target range of below 6 mg/dl (16, 17). If uric acid levels stay in this range, subsequent attacks of gout are unlikely. However, allopurinol therapy is needed long term; compliance is often poor; therefore, patient education is important (11, 18).

Several trials have shown that the addition of lesinurad to allopurinol therapy leads to a greater reduction in uric acid levels, and at the recommended dose of 200 mg daily, lesinurad is generally well tolerated (19-21).

Adverse effects associated with lesinurad therapy include rising creatinine levels, which are often reversible, nephrolithiasis (kidney stones), urolithiasis (stones in the bladder or urinary tract), and acute renal failure – which is associated more with lesinurad monotherapy (not recommended by the FDA) at the higher drug dose of 400 mg (twice the FDA-approved dose). Lesinurad is also associated with an increased risk of cardiovascular events (22).

Patients with moderate renal impairment experience a 150% increase in exposure to lesinurad, which should be used with caution in these patients (23). Lesinurad is contraindicated in individuals with severe renal

impairment, end stage renal disease, kidney transplant recipients, or patients on dialysis as well as individuals with tumor lysis syndrome or Lesch-Nyhan syndrome.

Lesinurad is primarily metabolized by CYP2C9 to several inactive metabolites. The co-administration of lesinurad with moderate inducers of CYP2C9 (e.g., rifampin, carbamazepine), may decrease the therapeutic effect of lesinurad by reducing its exposure.

In contrast, the co-administration of lesinurad with drugs that are CYP2C9 inhibitors (e.g., fluconazole, amiodarone), or the administration of lesinurad to patients who lack CYP2C9 activity ("CYP2C9 poor metabolizers"), will increase exposure to lesinurad. This may increase the risk of adverse reactions.

Therefore, lesinurad should be used with caution in patients with moderate kidney disease, patients taking CYP2C9 inhibitors, and patients who are CYP2C9 poor metabolizers (1, 24).

Gene: CYP2C9

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (25).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected and is categorized as normal enzyme activity (26). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual with one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual with 2 decreased-function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers.
a Global frequencies are approximate. Because haplotype frequencies vary considerably among popualations, please see (26) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (26) for activity ranges.
This table is adapted from (26). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (27).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10-20%) than Asian (1-3%) or African (0-6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (28-30).

Linking Gene Variation with Treatment Response

Currently, data are limited on the relationship between an individual's *CYP2C9* status and their response to lesinurad therapy.

The lesinurad drug label discusses an analysis of a small group of patients –– 2 patients were CYP2C9 poor metabolizers, and 41 were normal CYP2C9 metabolizers. At the 400 mg dose of lesinurad (which is twice the recommended dose of 200 mg daily), exposure to lesinurad was approximately 1.8 times higher in poor metabolizers compared to normal metabolizers. Therefore, the label states that lesinurad should be used with caution in CYP2C9 poor metabolizers.

The drug label also states that lesinurad should be used with caution in patients taking drugs that are CYP2C9 inhibitors (because of increased exposure and risk of side effects) and in patients taking drugs that are CYP2C9 inducers (because of decreased exposure and risk of reduced therapeutic effect). Drugs that inhibit CYP2C9 include fluconazole (antifungal agent) and amiodarone (antiarrhythmic); and drugs that induce CYP2C9 include rifampin (antibiotic) and carbamazepine (anti-seizure drug) (1).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH Genetic Testing Registry (GTR) displays genetic tests that are currently available for lesinurad response and for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Lesinurad exposure is increased when lesinurad is co-administered with inhibitors of CYP2C9, and in CYP2C9 poor metabolizers. Lesinurad should be used with caution in patients taking moderate inhibitors of CYP2C9 (e.g., fluconazole, amiodarone), and in CYP2C9 poor metabolizers.

Lesinurad exposure is decreased when lesinurad is co-administered with moderate inducers of CYP2C9 (e.g., rifampin, carbamazepine), which may decrease the therapeutic effect of lesinurad.

[…]

Patients who are CYP2C9 poor metabolizers are deficient in CYP2C9 enzyme activity. A cross-study pharmacogenomic analysis assessed the association between CYP2C9 polymorphism and lesinurad exposure in patients receiving single or multiple doses of lesinurad at 200 mg, 400 mg or 600 mg. At the 400 mg dose, lesinurad exposure was approximately 1.8-fold higher in CYP2C9 poor metabolizers (i.e., subjects with *CYP2C9 *2/*2* [N=1], and **3/*3* [N=1] genotype) compared to CYP2C9 extensive metabolizers (i.e., *CYP2C9 *1/*1* [N=41] genotype). Use with caution in CYP2C9 poor metabolizers, and in patients taking moderate inhibitors of CYP2C9.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (31).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Maraviroc Therapy and *CCR5* Genotype

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Introduction

Maraviroc is a chemokine receptor antagonist that is used in combination with other antiretroviral agents to treat human immunodeficiency virus type 1 (HIV-1) infection. Maraviroc exerts its therapeutic activity by blocking entry of the HIV-1 virus into immune cells—specifically the CD4-expressing T-helper cells, which play a major role in protecting the body from infection—precursor cells, and dendritic cells.

HIV-1 infection is classified in two major forms according to the co-receptor it employs to gain entry in to the cell, namely the chemokine receptor 5 (CCR5) or the CXC chemokine receptor 4 (CXCR4). These co-receptors are expressed on different types of cells, and HIV tropism refers to the types of cells and tissues in which the virus infects and replicates. A tropism assay is conducted to determine which co-receptor the HIV-1 virus uses, i.e., whether the virus is CCR5-tropic, CXCR4-tropic, dual tropic (i.e., HIV-1 virus that is able to use both receptors), or mixed tropic (i.e., a mixture of HIV-1 viruses, some of which use CCR5 and others that use CXCR4).

Maraviroc is only indicated for treatment of adults with CCR5 tropic HIV-1 and is not recommended when the CXCR4-tropic virus has been detected. The FDA-approved drug label for maraviroc states that "prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay" (1).

Drug: Maraviroc

Maraviroc is the first FDA-approved drug in a class of HIV drugs called entry and fusion inhibitors. Maraviroc blocks the interaction between HIV-1 and CCR5 in healthy immune cells, preventing certain strains (CCR5-tropic) of HIV from entering and infecting the cell. Maraviroc must be taken twice daily and must always be used with other HIV drugs. Taken in combination with these drugs, maraviroc may lower the HIV virus load in the blood.

Currently, maraviroc is the only CCR5 co-receptor inhibitor that has been approved for clinical use (2). It is used to treat HIV-1-infected patients who have a virus that uses CCR5 for entry, and either never received antiretroviral treatment before, or have experienced therapeutic failure following traditional antiretroviral therapies (3).

Maraviroc treatment regimens may be used less often than other regimens. Possible reasons include the requirement to test for tropism, which is time-consuming and expensive (see Genetic Testing). Furthermore, there is a large selection of potent and tolerable treatment regimens currently available that do not require genotyping prior to use. These treatment regimens may be based on nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI), boosted protease inhibitors (PI), and integrase inhibitors (2, 4).

The entry of HIV-1 into a host cell is a complex process, which begins when the viral envelope glycoprotein, gp120, binds to the cellular protein, CD4. Binding induces conformational changes in gp120 resulting in the exposure of gp4, another viral envelope protein that helps mediate the interaction between the virus and cellular co-receptors, and the fusion of viral and cellular membranes.

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The CD4 count is often used to determine the stages of HIV disease. CD4 is a glycoprotein found on the surface of T helper immune cells. HIV-1 infection leads to a progressive reduction in the number of T cells that express CD4, and a CD4 count of less than 200 cells/mm^3 is one of the qualifications for a diagnosis of AIDS (5, 6).

Measurement of the CD4 count is useful before HIV treatment is started because the CD4 count provides information on the overall immune function of the patient. In the United States, antiretroviral therapy (ART) is now recommended for all HIV-infected patients, regardless of their CD4 count or viral load (7), to keep viral loads at undetectable levels for as long as possible. In adults receiving optimized background treatment for infection with CCR5-tropic HIV-1, the addition of maraviroc leads to a greater increase in CD4 counts compared to the addition of placebo (1).

HIV-1 most commonly uses either the CCR5 or CXCR4 co-receptors to enter its target cells (8). Maraviroc is an effective antiretroviral agent in individuals who only harbor the CCR5-tropic HIV-1 virus. It is incapable of inhibiting infection against viruses that do not use CCR5 (i.e., CXCR-using virus or dual/mixed virus) (1).

Maraviroc is metabolized by the cytochrome P450 system, mainly CYP3A, in the liver to inactive metabolites (9, 10). As noted above, maraviroc must be used in combination with other antiretroviral medications; the recommended dosage of maraviroc depends on whether the co-medications are inhibitors or inducers of CYP3A (1).

Gene: *CCR5*

The chemokine (CC motif) receptor 5 (CCR5) is primarily expressed on the surface of white blood cells. Chemokines are a type of cytokine—they are small, secreted proteins that have a crucial role in the inflammatory response by helping immune cells migrate to areas of tissue damage. Other functions of chemokines include influencing the maturation of various immune cells and promoting the growth of new blood vessels.

Most chemokines have four characteristic cysteine residues in a conserved location, and they are classified into four families by the location of the first two cysteine residues: CXC, CC, C, and CX3C. For example, members of the "CC" cytokine family have two adjacent cysteine residues near their amino terminus.

The receptors for chemokines are G-protein coupled, seven-transmembrane domain receptors. Two of these receptors, CCR5 (binds CC chemokines) and CXCR4 (binds CXC chemokines), are also co-receptors used by HIV to enter human white blood cells. CCR5 is expressed on fewer cells (e.g., specific T cells, precursor cells (or macrophages) and dendritic cells) than CXCR4 (e.g., most immune cells, vascular endothelial cells, and neurons).

HIV-1 virus that uses the CCR5 co-receptor (CCR5-tropic) is more commonly found in the early stages of infection. It is also more common among individuals who have yet to receive treatment, and at least half of all infected individuals harbor only CCR5-tropic viruses throughout the course of infection. The CXCR4-tropic virus is more commonly found during later stages of disease and among individuals who have received HIV treatment. The presence of CXCR4-tropic virus is a predictor of lower CD4 count, a higher viral load, and a more rapid progression to AIDS (5).

A variant of *CCR5*, *CCR5-Δ32* (NM_000579.3:c.554_585del32), contains a 32 bp deletion and codes a nonfunctional receptor that hinders the entry of CCR5-tropic virus in to cells. Individuals who have two copies of this allele are highly resistant to HIV infection, and although individuals who have one copy of the allele remain susceptible to HIV infection, the progression of HIV infection to AIDS is delayed (11).

The *CCR5-Δ32* allele occurs at high frequency in European Caucasians (5%–14%) but is rare among African, Native American, and East Asian populations, suggesting that the allele may have conferred an evolutionary survival advantage (12). Possible causes of a positive selection pressure include protection against the bubonic

plague (*Yersinia pestis*) or smallpox (*Variola virus*) during the Middle Ages. However, other studies have found that the *CCR5-Δ32* allele arose long before this time and underwent neutral evolution (13).

Genetic Testing

Testing of the HIV-1 virus (i.e., the virus, not the patient) should be carried out prior to initiation of treatment with maraviroc. A tropism assay is needed to identify individuals with CCR5-tropic HIV-1. The assay must be highly sensitive to detect low levels of CXCR4-tropic viruses. Maraviroc should not be prescribed if non-CCR5 variants (CXCR4-tropic or dual/mixed-tropic) are detected (1, 9). HIV tropism can be determined by phenotype or genotype testing. Phenotypic assays can be performed using plasma RNA (if viral load is greater than 1000 copies/ml) or cell-associated DNA (if viral load is less than 1000 copies/ml). Phenotypic assays use replication-defective laboratory viruses that carry the complete cloned viral envelope proteins gp120 and gp41 derived from the patient. Phenotypic assays measure the ability of these pseudoviruses to infect CD4+ target cells that express either CCR5 or CXCR4 (7).

Genotyping methods are used to predict which co-receptors on the cell are used by the virus rather than directly assessing tropism. Genotyping methods involve sequencing the third variable region (V3) of gp120 and using algorithms to predict co-receptor usage.

While phenotypic assays are still considered to be the gold standard, the use of genotyping to determine patient eligibility for maraviroc is increasing due to low cost, greater accessibility, and faster turnaround time for the results as compared to the other methods (14, 15). Although there can be discrepancies between the results from phenotypic and genotypic assays, the correlation between genotypic assays and the clinical efficacy of maraviroc is improving (16).

The NIH's Genetic Testing Registry (GTR) displays genetic testing information for human genes and conditions, including tests for maraviroc response. These tests investigate the human genes that contribute to the pharmacokinetics of maraviroc, as opposed to the FDA-recommended genetic tests, which are tests for viral genes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Prior to initiation of maraviroc, test all patients for CCR5 tropism using a highly sensitive tropism assay. Maraviroc is recommended for patients with only CCR5-tropic HIV-1 infection. Outgrowth of pre-existing low-level CXCR4- or dual/mixed-tropic HIV-1 not detected by tropism testing at screening has been associated with virologic failure while on maraviroc.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Allele name	Other name(s)	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CCR5delta32		NM_000579.3:c.554_585del32 NM_001100168.1:c.554_585del32	NP_000570.1:p.Ser185Ilefs NP_001093638.1:p.Ser185Ilefs	rs333

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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To view an earlier version of this summary (18 March 2015), please click here.

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Mercaptopurine Therapy and *TPMT* and *NUDT15* Genotype

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Introduction

Mercaptopurine (brand names Purinethol, Purixan) is an immunosuppressant and anti-neoplastic agent that belongs to the drug class of thiopurines. It is used with other drugs to treat acute lymphoblastic leukemia, which is the most common form of cancer in children (1). Common off-label uses include the treatment of inflammatory bowel disease (IBD).

Mercaptopurine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), of which 6-thioguanine triphosphate (6-TGTP) is the major active metabolite. Two of the enzymes involved in the complex pathway of these metabolites are thiopurine S-methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15). Individuals with reduced activity of either enzyme will be exposed to higher levels of active metabolites, like 6-TGTP, and will be at a higher risk of side effects, such as severe bone marrow suppression (myelosuppression).

The FDA-approved drug label states that the initial dose of mercaptopurine should be reduced in individuals who are known to lack TPMT or NUDT15 activity ("homozygous deficiency") and that these individuals typically require 10% or less of the standard dose. In individuals who have reduced enzyme activity ("heterozygous deficiency"), the label states that the dose of mercaptopurine should be reduced based on tolerability. A more substantial dose reduction may be required in individuals who have reduced activity of both enzymes (Table 1) (1).

Dosing recommendations for mercaptopurine based on *TPMT* and *NUDT15* genotype have also been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 2, Table 3) and the Dutch Pharmacogenetics Working Group (DPWG). These recommendations include specific dose reductions for individuals who have low or deficient enzyme activity, including starting dose and more information on how and when to adjust the dose for example, the time allowed to reach steady-state after each dose adjustment (2, 3).

Table 1. FDA Drug Label Dosage and Administration of Mercaptopurine (2020)

Deficiency	Dosage and administration
Homozygous deficiency in either TPMT or NUDT15	Individuals with homozygous deficiency of either enzyme typically require 10% or less of the standard mercaptopurine oral suspension dosage. Reduce initial dosage in individuals who are known to have homozygous TPMT or NUDT15 deficiency.
Heterozygous deficiency in TPMT and/or NUDT15	Reduce the mercaptopurine oral suspension dosage based on tolerability. Most individuals with heterozygous TPMT or NUDT15 deficiency tolerate recommended mercaptopurine doses, but some require dose reduction based on toxicities. Individuals who are heterozygous for both TPMT and NUDT15 may require more substantial dosage reductions.

This FDA table is adapted from (1). TPMT, thiopurine S-methyltransferase; NUDT15, nudix hydrolase 15

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Table 2. CPIC Recommended Dosing of Mercaptopurine by *TPMT* Phenotype (2018 Update)

Phenotype	Implications for mercaptopurine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations[b]
TPMT normal metabolizer	Lower concentrations of TGN metabolites, higher MeTIMP, this is the "normal" pattern. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with normal starting dose[a] (for example, 75 mg/m^2/day or 1.5 mg/kg/day) and adjust doses of mercaptopurine (and of any other myelosuppressive therapy) without any special emphasis on mercaptopurine compared with other agents. Allow at least 2 weeks to reach steady-state after each dose adjustment.	Strong
TPMT intermediate metabolizer Or TPMT possible intermediate metabolizer	Moderate to high concentrations of TGN metabolites; low concentrations of MeTIMP. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] is ≥75 mg/m^2/day or ≥1.5 mg/kg/day (for example, start at 22.5–60 mg/m^2/day or 0.45–1.2 mg/kg/day) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing mercaptopurine over other agents. If normal starting dose is already <75 mg/m^2/day or <1.5 mg/kg/day, dose reduction may not be recommended.	Strong
TPMT poor metabolizer	Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease; no MeTIMP metabolites. Greatly increased risk of thiopurine- related leukopenia, neutropenia, myelosuppression.	For malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and reduce frequency to thrice weekly instead of daily (for example, 10 mg/m^2/day given just 3 days/week) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong

MeTIMP, metabolites of thiopurine methyltransferase; TGN, thioguanine nucleotides; TPMT, thiopurine methyltransferase.
[a]Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.
[b] Rating scheme described in Supplemental Material (2).
This CPIC table is adapted from (2).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both *TPMT* and *NUDT15* is known. Please see (2).

Table 3. CPIC Recommended Dosing of Mercaptopurine by *NUDT15* Phenotype (2018 Upate)

Phenotype	Implications for mercaptopurine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations[b]
NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with normal starting dose[a] (for example, 75 mg/m^2/day or 1.5 mg/kg/day) and adjust doses of mercaptopurine (and of any other myelosuppressive therapy) without any special emphasis on mercaptopurine compared with other agents. Allow at least 2 weeks to reach steady-state after each dose adjustment.	Strong
NUDT15 intermediate metabolizer OR NUDT15 possible intermediate metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] is ≥75 mg/m^2/day or ≥1.5 mg/kg/day (for example, start at 22.5–60 mg/m^2/day or 0.45–1.2 mg/kg/day) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing mercaptopurine over other agents. If the normal starting dose is already <75 mg/m^2/day or <1.5 mg/kg/day, dose reduction may not be recommended.	Strong
NUDT15 poor metabolizer	Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	For malignancy, initiate dose at 10 mg/m^2/day and adjust dose based on myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong

[a] Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.
[b] Rating scheme described in Supplemental Material.
This CPIC table is adapted from (3).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both *TPMT* and *NUDT15* is known. Please see (3). TPMT, thiopurine S-methyltransferase; NUDT15, nudix hydrolase 15

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in transplantation, inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurine derivatives are used in clinical practice: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine).

All 3 agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, whereas all 3 drugs are used for a variety of autoimmune conditions.

There is increasing evidence that DNA testing for *NUDT15* and *TPMT* before initiating thiopurine therapy is clinically useful. In Europeans and Africans, inherited TPMT deficiency is the primary genetic cause of

thiopurine intolerance, whereas for Asians, risk alleles in *NUDT15* explains most thiopurine-related myelosuppression (4, 5). Current clinical practice in some countries (in the absence of enzymatic testing) is to initiate therapy and monitor for changes in liver function and complete blood count parameters, which some studies suggest may be similarly beneficial to preemptive testing (6).

Drug: Mercaptopurine

Mercaptopurine is an anti-neoplastic agent and an immunosuppressive agent that is used in the treatment of acute lymphoblastic leukemia (ALL) as part of a combination regimen. Acute lymphoblastic leukemia is the most common form of cancer in children, accounting for approximately 30% of childhood malignancies with a peak incidence occurring at 3–5 years of age (1).

An off-label use of mercaptopurine is the treatment of IBD. Along with the closely related prodrug azathioprine (that is metabolized to mercaptopurine), mercaptopurine is used as an "immunomodulator" and as a "steroid-sparing agent" in the treatment of Crohn's disease and ulcerative colitis.

Mercaptopurine is a slow-acting drug used in IBD, which typically takes at least 3 months before a therapeutic effect is observed. Therefore, mercaptopurine is used as a maintenance therapy of IBD rather than as a monotherapy for induction of remission. Because the discontinuation of mercaptopurine is associated with a high rate of relapse of IBD, mercaptopurine is usually continued long term if there are no adverse effects (7-9).

The efficacy of mercaptopurine in individuals with IBD has been well established. However, there remain questions on the safety of long-term mercaptopurine treatment, as there have been reports of an increased risk of lymphoma in these individuals (10, 11).

Like all thiopurines, mercaptopurine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Activation of mercaptopurine occurs via hypoxanthine phosphoribosyltransferase (HPRT) followed by a series of reactions to form TGNs. The cytotoxicity of mercaptopurine is due, in part, to the incorporation of 6-thioguanosine triphosphate (6-TGTP) into DNA.

Inactivation of mercaptopurine occurs via 2 major pathways: via methylation, which is catalyzed by TPMT, and via oxidation, which is catalyzed by xanthine oxidase (XO). In individuals who take an XO inhibitor, such as allopurinol (used to manage gout), the dose of mercaptopurine must be reduced to one-third or one-quarter of the usual dose to avoid severe toxicity (1, 12, 13). In individuals with normal TPMT metabolization and myelo- or hepatotoxicity, allopurinol may be initiated to slow the breakdown of mercaptopurine, leading to higher concentrations of TGNs(14).

The NUDT15 enzyme has an impact on the incorporation of 6-TGTP into DNA –– this enzyme is involved in the breakdown of the deoxy-thioguanosine triphosphate metabolite 6-TGTP to the inactive monophosphate metabolite, 6-thioguanine monophosphate (6-TGMP) (1).

One of the most frequent adverse reactions to mercaptopurine is myelosuppression, which can occur in any individual and can usually be reversed by decreasing the dose of the drug. However, this risk is increased in individuals who have reduced or absent TPMT, NUDT15, or both, activity (1).

Determining genotype is helpful before initiating thiopurine therapy, but it does not replace the need for regular monitoring. One study reported that in individuals with IBD receiving thiopurine therapy, *TPMT* polymorphisms were associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions such as liver damage and pancreatitis. Therefore, regular blood tests to monitor for side effects are still needed during therapy (15).

Gene: *TPMT*

The *TPMT* gene encodes thiopurine S-methyltransferase, which is historically classified as a phase II metabolism enzyme. Importantly, TPMT is one of the main enzymes involved in the metabolism of thiopurines, including thioguanine.

The *TPMT* gene is highly polymorphic, with over 40 reported variant star (*) alleles (16-19). The *TPMT*1* allele is associated with normal enzyme activity (wild type).

The *TPMT*1* is considered the wild type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. Individuals who are normal metabolizers are more likely to have a typical response to thioguanine and a low risk of myelosuppression; however, all individuals receiving thioguanine require close monitoring (20-23).

Most individuals are TPMT normal metabolizers (~86–97%). A handful of variant *TPMT* alleles account for over 90% of the reduced or absent enzyme activity (2, 20, 21, 24):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (*TPMT*3B* c.460G>A and *TPMT*3C* c.719A>G in *cis*)
- *TPMT*3B* c.460G>A
- *TPMT*3C* (c.719A>G)
- *TPMT*4* (c. 626-1G>A)

Individuals who are TPMT poor metabolizers (~0.3% of individuals of European ancestry) have 2 non-functional *TPMT* alleles (Table 4). When treated with standard doses of azathioprine or mercaptopurine, these individuals will probably experience life-threatening bone marrow suppression because of high levels of TGNs (1).

Individuals who are TPMT intermediate metabolizers (approximately 3–14% of the general population) are heterozygous for one no function *TPMT* allele. These individuals may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs and are at an increased risk of moderate to severe bone marrow suppression. However, some of these individuals, approximately 40–70%, can tolerate the full dose of mercaptopurine or other thiopurines. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as methylmercaptopurine nucleotides (MeMPN), which is formed by TPMT, as compared with wild type individuals (20, 21). There are additional known *TPMT* alleles with uncertain function, including *TPMT*6, *7* and **8* (2). Individuals with these alleles in conjunction with an allele of known function are assigned to "possible intermediate metabolizer" or "indeterminate" categories as shown in Table 4. Additional details on these *TPMT* alleles is provided in the Nomenclature table below.

Table 4 Assignment of likely TPMT Phenotype based on Genotype (CPIC, 2018).

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*8, *3A/*7
Poor metabolizer	An individual with 2 no function alleles	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4, *2/*3C, *3A/*4

Table 4 continued from previous page.

Likely phenotype[a]	Genotype	Examples of diplotype
Indeterminate	An individual with 2 uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele	*6/*8 *1/*8

TPMT, thiopurine methyltransferase.
[a] See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (3). TPMT, thiopurine S-methyltransferase; NUDT15, nudix hydrolase 15

The frequency of *TPMT* variant alleles vary among different ethnic populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, though with a lower frequency (16).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs less commonly, and *TPMT*3B* is also rare (16, 25). The *TPMT*4* allele is seen in fewer than 0.01% of Europeans and not detected in other ethnic groups, as reported by CPIC (2).

Gene: *NUDT15*

The *NUDT15* gene encodes an enzyme that belongs to the nudix hydrolase superfamily. Members of this superfamily catalyze the hydrolysis of deoxynucleoside diphosphates and triphosphates, which are created as a result of oxidative damage.

The NUDT15 enzyme is directly involved in the metabolism of thiopurines, as it catalyzes the conversion of active metabolites 6-TGTP to the less toxic metabolites 6-TGMP and 6-thioguanine diphosphate (6-TGDP) and in doing so, prevents the incorporation of the toxic metabolites into DNA and RNA (26).

In individuals with reduced or absent NUDT15 activity (intermediate or poor metabolizers, Table 5), the reduction in NUDT15-mediated degradation of 6-TGTP results in more 6-TGTP available for incorporation into DNA, leading to increased DNA damage and cell death. These individuals subsequently have increased sensitivity to thiopurines at standard doses, including an increased risk of severe myelosuppression (27).

Similar to *TPMT*, *NUDT15* is polymorphic, as the PharmVar Consortium currently has catalogued 21 variant alleles. However, most variants are rare, and the clinical significance of many *NUDT15* star (*) alleles is currently unclear.

The first *NUDT15* variant associated with thiopurine toxicity is commonly known as p.R139C (rs116855232), which is present in both the *NUDT15**2 and *NUDT15**3 haplotypes. This amino acid change results in an unstable protein with almost no enzymatic activity. The *NUDT15*2* variant haplotype also includes an insertion (see Nomenclature table and (2)).

Deficiency of NUDT15 is rare among in individuals with European or African ancestry (found in less than 1%); however, NUDT15 deficiency is more common in individuals with East Asian ancestry (for example, Korea, China, Japan, Vietnam), with a complete deficiency found in as much as 2% of these populations (2).

Table 5 Assignment of likely *NUDT15* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1

Table 5 continued from previous page.

Likely phenotype[a]	Genotype	Examples of diplotype
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*5, *3/*6
Poor metabolizer	An individual with 2 no function alleles	*2/*2, *2/*3, *3/*3
Indeterminate	An individual with 2 uncertain function alleles OR one normal function allele plus one uncertain function allele	*1/*4, *1/*5 *4/*5, *5/*6

NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15
[a] See *TPMT* and *NUDT15* Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (3). TPMT, thiopurine S-methyltransferase; NUDT15, nudix hydrolase 15

Linking Gene Variation with Treatment Response

Genetic variation in the *TPMT* and *NUDT15* genes strongly influences the safety of thiopurine therapy, specifically, influencing the risk of treatment-related bone marrow suppression (28).

Thiopurine S-methyltransferase deficiency is the primary genetic cause of thiopurine intolerance in Europeans and Africans, and NUDT15 deficiency is a more common cause in Asians and Hispanics.

The clinical impact of variant *NUDT15* alleles was discovered more recently than for *TPMT*, and there is less evidence available to guide dose adjustments, but studies support genotyping *NUDT15* to improve the safety of thiopurine therapy. However, there is one clinical trial in progress that addresses azathioprine dosing guided by the status of both *TPMT* and *NUDT15* genotyping for the treatment of IBD (4, 5, 29-31).

Currently, *TPMT* and *NUDT15* testing is not required by the FDA before starting treatment with any thiopurine (azathioprine, mercaptopurine, or thioguanine); however, both genes were listed in the recently published FDA Association tables as pharmacogenetic associations with data supporting therapeutic management recommendations (32). Consequently, routine genotyping for *TPMT* and *NUDT15* variants has not been universally adopted (33). For homozygous or compound heterozygous deficiency of either *TPMT* or *NUDT15*, reconsider the use of thiopurines in non-neoplastic conditions, such as IBD, as potentially less toxic alternatives are available.(14)

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the azathioprine drug response, and the genes *TPMT* and *NUDT15*. The genes may be tested separately, or together, as part of test panel that evaluates the drug response to thiopurines.

As with many tests, only the most common variants are usually tested (for example, for *TPMT*, the *2, *3A*, *3B* and *3C* alleles, which account for more than 90% of known inactivating alleles). This means that rare or previously undiscovered variants will not be detected by variant-specific genotyping methods (20, 21, 34-37).

It is important to note that for *TPMT*3A*, 2 variants, c.460G>A and c.719A>G, are in *cis*. The variant, c.460G>A by itself is *TPMT*3B* and c.719A>G by itself is *TPMT*3C*. Most clinical laboratories are unable to phase the 2 variants. In most cases, especially if the individual is of European ancestry, the laboratory will assume the 2 variants are in *cis*, though the possibility of the variants being in *trans* cannot be ruled out.

For TPMT, phenotype testing is also available. Phenotype tests directly measure TPMT enzyme activity in red blood cells but accurate phenotyping is not possible in individuals who have recently received blood transfusions (22). However, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying individuals at risk of adverse reactions from thiopurine treatment, and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (23, 38-40).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA):

Dosage Modifications in Patients with TPMT and NUDT15 Deficiency

Consider testing for TPMT and NUDT15 deficiency in patients who experience severe myelosuppression or repeated episodes of myelosuppression.

Homozygous Deficiency in either TPMT or NUDT15

Patients with homozygous deficiency of either enzyme typically require 10% or less of the recommended dosage. Reduce the recommended starting dosage of mercaptopurine tablets in patients who are known to have homozygous TPMT or NUDT15 deficiency.

Heterozygous Deficiency in TPMT and/or NUDT15

Reduce the mercaptopurine tablets dose based on tolerability. Most patients with heterozygous TPMT or NUDT15 deficiency tolerate the recommended dosage, but some require a dose reduction based on adverse reactions. Patients who are heterozygous for both TPMT and NUDT15 may require more substantial dose reductions.

[…]

Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of mercaptopurine, accumulate excessive cellular concentrations of active 6-TGNs, and are at higher risk for severe myelosuppression. In a study of 1028 children with ALL, the approximate tolerated mercaptopurine dosage range for patients with TPMT and/or NUDT15 deficiency on mercaptopurine maintenance therapy (as a percentage of the planned dosage) was as follows: heterozygous for either TPMT or NUDT15, 50-90%; heterozygous for both TPMT and NUDT15, 30- 50%; homozygous for either TPMT or NUDT15, 5-10%.

Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the *TPMT* gene and have little or no TPMT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The *TPMT*2*, *TPMT*3A*, and *TPMT*3C* alleles account for about 95% of individuals with reduced levels of TPMT activity. NUDT15 deficiency is detected in < 1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the *NUDT15* gene, and approximately 21% have one loss-

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

of-function allele. The p.R139C variant of *NUDT15* (present on the *2 and *3 alleles) is the most commonly observed, but other less common loss-of-function *NUDT15* alleles have been observed.

Consider all clinical information when interpreting results from phenotypic testing used to determine the level of thiopurine nucleotides or TPMT activity in erythrocytes, since some coadministered drugs can influence measurement of TPMT activity in blood, and blood from recent transfusions will misrepresent a patient's actual TPMT activity.

[…]

Consider testing for TPMT or NUDT15 deficiency in patients with severe myelosuppression or repeated episodes of myelosuppression. *TPMT* genotyping or phenotyping (red blood cell TPMT activity) and *NUDT15* genotyping can identify patients who have reduced activity of these enzymes. Patients with heterozygous or homozygous TPMT or NUDT15 deficiency may require a dose reduction.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

***TPMT* recommendation**

If starting doses are already high (e.g., 75 mg/m^2 of mercaptopurine), as is true in some ALL treatment regimens, lower than normal starting doses should be considered in TPMT intermediate metabolizers and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers. This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL. Even at these markedly reduced dosages, erythrocyte TGN concentrations in TPMT poor metabolizers remain well above those tolerated and achieved by the majority of patients (who are TPMT normal metabolizers).

In some nonmalignant conditions, alternative agents may be chosen for TPMT intermediate or poor metabolizers rather than reduced doses of thiopurines; if thiopurines are used, full starting doses are recommended for TPMT normal metabolizers, reduced doses (30–80% of target dose) in TPMT intermediate metabolizers, and substantially reduced doses (or use of an alternative agent) in TPMT poor metabolizers.

Some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype, these recommendations apply regardless of the method used to assess TPMT status.

***NUDT15* recommendation**

Similar to *TPMT*, tolerated mercaptopurine dosage is also correlated with the number of nonfunctional alleles of the *NUDT15* gene. In fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of TPMT vs. NUDT15 decreased function alleles, there remains a paucity of multi-ethnic studies examining both TPMT and NUDT15 variants.

Therefore, our *NUDT15* recommendations parallel those for *TPMT*. For NUDT15 normal metabolizers (NUDT15*1/*1), starting doses do not need to be altered. For NUDT15 intermediate metabolizers (e.g., NUDT15*1/*3), reduced starting doses should be considered to minimize toxicity, particularly if the starting doses are high (e.g., 75 mg/m^2/ day for mercaptopurine). For NUDT15 poor metabolizers (e.g., NUDT15*3/*3), substantially reduced doses (e.g., 10 mg/m^2/ day of mercaptopurine) or the use of an alternative agent should be considered.

As for TPMT, there is substantial variability in the tolerated thiopurine dosages within NUDT15 intermediate metabolizers, with a minority of individuals who do not seem to require significant dose reduction. Therefore,

genotype-guided prescribing recommendations apply primarily to starting doses; subsequent dosing adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines). In contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in NUDT15 poor metabolizers and pre-emptive dose reductions are strongly recommended.

The NUDT15 poor metabolizer phenotype is observed at a frequency of about 1 in every 50 patients of East Asian descent, which is more common than the TPMT poor metabolizer phenotype in Europeans, and, thus, genotyping NUDT15 in the Asian populations may be of particular clinical importance. NUDT15 deficiency is also more prevalent in individuals of Hispanic ethnicity, particularly those with high levels of Native American genetic ancestry.

Please review the complete therapeutic recommendations, which include CPIC's recommended course of action if both TPMT and NUDT15 genotypes are known, located here: (2).

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

The Dutch Pharmacogenetics Working Group considers genotyping before starting azathioprine or 6-mercaptopurine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

TPMT Intermediate Metabolizer

Grade 2 leukopenia occurs in 23% of these patients with normal therapy for immunosuppression. The genetic variation increases the quantity of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

IMMUNOSUPPRESSION

- Start with 50% of the standard dose

Adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

Dose adjustment is not required for doses lower than 1.5 mg/kg per day for azathioprine or 0.75 mg/kg per day for mercaptopurine.

LEUKEMIA

- Start with 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

The initial dose should be adjusted based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also NUDT15 IM or NUDT15 PM.

TPMT Poor Metabolizer

Grade 2 leukopenia and intolerance occurred in 98% of these patients with standard therapy. The gene variation increases the quantities of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

- Choose an alternative or use 10% of the standard dose.

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

If the dose is decreased: advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

Background information:

Azathioprine is converted in the body to mercaptopurine. Mercaptopurine is an inactive pro-drug, which is converted to the active metabolites - thioguanine nucleotides - in the body.

Two catabolic routes reduce mercaptopurine bio-availability for thioguanine nucleotide formation. Thiopurine methyltransferase (TPMT) catalyses S-methylation of both mercaptopurine and the 6- mercaptopurine ribonucleotides formed in the metabolic pathway. In addition to this, mercaptopurine is oxidised to the inactive 6-thiouric acid by the enzyme xanthine oxidase (XO), which occurs primarily in the liver and intestines.

For more information about the TPMT phenotypes: see the general background information about TPMT on the KNMP Knowledge Bank or on www.knmp.nl (search for TPMT).

NUDT15 Intermediate Metabolizer

Grade ≥ 2 leukopenia occurs in 42% of these patients with standard immunosuppression therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

IMMUNOSUPPRESSION

- start with 50% of the standard dose

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

LEUKAEMIA

- start at 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also TPMT IM or TPMT PM.

NUDT15 Poor Metabolizer

Grade ≥ 2 leukopenia occurs in 96% of these patients with standard therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

- avoid azathioprine and mercaptopurine
- if it is not possible to avoid azathioprine and mercaptopurine: use 10% of the standard dose and advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and efficacy.

Background information:

NUDT15 reverses the last step in the formation of the active metabolite of mercaptopurine and its precursor azathioprine. It converts 6-thiodeoxyguanosine triphosphate (6-thio-dGTP), which is incorporated in DNA, to 6-thiodeoxyguanosine monophosphate (6-thio-dGMP). Lower metabolic activity of NUDT15 therefore leads to increased intracellular concentrations of the active metabolite 6- thio-dGTP. This increases the risk of side effects, such as myelosuppression.

For more information about TPMT and NUDT15 phenotypes: see the general background information in the KNMP Knowledge Bank or on www.knmp.nl (search for TPMT or NUDT15).

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature for Selected *TPMT* and *NUDT15* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.4:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains 2 variants in *cis*: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.4:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.4:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345
*TPMT*4*	626-1G>A	NM_000367.4:c.626-1G>A	(Splice acceptor variant)	rs1800584
*TPMT*6*	539A>T	NM_000367.4:c.539A>T	NP_000358.1:p.Tyr180Cys	rs75543815
*TPMT*7*	681T>G	NM_000367.4:c.681T>G	NP_000358.1:p.His227Gln	rs72552736
*TPMT*8*	644G>A	NM_000367.4:c.644G>A	NP_000358.1:p.Arg215His	rs56161402
*NUDT15*2*	p.R139C p.13_14GV[5]	NM_018283.4:c.415C>T NM_018283.4:c.38_43GAGTCG[4]	NP_060753.1:p.Arg139Cys NP_060753.1:p.13_14GV[5]	rs116855232 rs746071566
*NUDT15*3*	p.R139C	NM_018283.4:c.415C>T	NP_060753.1:p.Arg139Cys	rs116855232
*NUDT15*4*	p.R139H c.416G>A	NM_018283.4:c.416G>A	NP_060753.1:p.Arg139His	rs147390019
*NUDT15*5*	Val18Ile	NM_018283.4:c.52G>A	NP_060753.1:p.Val18Ile	rs186364861
*NUDT15*6*	p.13_14GV[4]	NM_018283.4:c.38_43GAGTCG[2]	NP_060753.1:p.13_14GV[4]	rs746071566

Note: the p.R139C variant of *NUDT15* is present on the *NUDT15*2* and *NUDT*3* alleles.
The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants.
Nomenclature for *NUDT15* is available from the Pharmacogene Variation (PharmVar) Consortium.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS)

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To view the second edition of this summary (Update: May 3, 2016), please click here.
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Metoprolol Therapy and *CYP2D6* Genotype

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Introduction

Metoprolol is a beta-blocker indicated for the treatment of various cardiovascular diseases, including hypertension, arrhythmias, angina, myocardial infarction, and heart failure (HF). Metoprolol selectively blocks $beta_1$-adrenoreceptors, which are expressed predominantly in cardiac tissue. The primary therapeutic effect resulting from the blockade of these receptors is a reduction in heart rate and a decrease in the force of heart contractions.

Metoprolol is metabolized extensively by the hepatic CYP2D6 enzyme. Approximately 8% of Caucasians and 2% of most other populations have absent CYP2D6 activity and are known as "CYP2D6 poor metabolizers (PM)." In addition, several drugs inhibit CYP2D6 activity, such as bupropion, quinidine, fluoxetine, paroxetine, and propafenone.

The FDA-approved drug label for metoprolol states that CYP2D6 PM and normal metabolizers (NM) who concomitantly take drugs that inhibit CYP2D6 will have increased metoprolol blood levels, decreasing metoprolol's cardioselectivity; co-medication with CYP2D6 inhibitors warrants close monitoring (1). (Table 1) Beta-blockers, such as metoprolol, have been demonstrated in several large clinical trials to be safe and effective for the treatment of individuals with cardiovascular disease. As a mainstay of therapy associated with improvements in quality of life, hospitalization rates, and survival (2, 3), clinical care pathways that might lead to the underutilization of beta-blockers require scrutiny. It is common clinical practice to adjust the dose of metoprolol according to individual heart rate until either the target or maximum tolerated dose is reached. The FDA does not specifically comment on the role of genetic testing for initiating therapy.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that CYP2D6 PM should initiate metoprolol therapy at the lowest recommended starting dose, and titration should be performed with care and close monitoring for bradycardia. (Table 2) Standard dosing and care are recommended for intermediate metabolizers (IM) and NM of CYP2D6, but no recommendation is made for ultrarapid metabolizers (UM) given the limited data on this phenotype and beta-blocker response. (4)

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP) has also published metoprolol dosing recommendations based on *CYP2D6* genotype. For individuals who have a *CYP2D6* gene variation that reduces the conversion of metoprolol to inactive metabolites (namely, the IM and PM phenotype), DPWG states that the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia. For CYP2D6 PM or IM, if a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia, DPWG recommends increasing the dose of metoprolol in smaller steps, prescribing no more than 25% (PM) or 50% (IM) of the standard dose, or both. For CYP2D6 UM, DPWG indicates that clinical response is hardly decreased at a dose of 200 mg/day. However, if efficacy is insufficient at this maximum dose, the DPWG recommends increasing the dose based on effectiveness and side effects up to a maximum of 2.5 times the normal dose, or selecting an alternative. (Table 3) (5).

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Table 1: Statement on CYP2D6-based Interactions with Metoprolol from the Food and Drug Administration (FDA)

Interaction	Effect	Recommendation
CYP2D6 inhibition by other medication(s)	Strong inhibitors of CYP2D6 were shown to double metoprolol concentrations; increases in plasma concentration decrease the cardioselectivity of metoprolol	Monitor individuals closely when the combination cannot be avoided.
CYP2D6 poor metabolizers	Will have increased (several-fold) metoprolol blood levels, decreasing metoprolol's cardioselectivity.	(None)

Table adapted from (1)

Table 2: Recommendations from the Clinical Pharmacogenetic Implementation Consortium (CPIC) for Beta-Blocker Therapy by Genotype

CYP2D6 phenotype	Implications[a]	Recommendation	Classification of recommendation[b]
Ultrarapid metabolizer	Increased metabolism of metoprolol leading to decreased drug concentrations; however, it is unclear whether this results in clinically significant changes in heart rate, blood pressure, or clinical outcomes.	No recommendation for metoprolol therapy due to insufficient evidence regarding diminished metoprolol effectiveness clinically.	No recommendation
Normal metabolizer	Normal metabolism of metoprolol	Initiate standard dosing	Strong
Intermediate metabolizer	Decreased metabolism of metoprolol leading to increased drug concentrations; however, this does not appear to translate into clinically significant changes in heart rate, blood pressure, or clinical outcomes.	Initiate standard dosing	Moderate
Poor metabolizer	Decreased metabolism of metoprolol leading to markedly increased drug concentrations; this leads to greater heart rate and blood pressure reductions. The effect on clinical outcomes is unclear.	Initiate therapy with lowest recommended starting dose. Carefully titrate dose upward to clinical effect or guideline-recommended dose; monitor more closely for bradycardia. Alternatively, consider selecting another beta-blocker.	Moderate
Indeterminate	n/a	No recommendation	No recommendation

n/a, not applicable. Indeterminate: Genotype data for *CYP2D6* is either unavailable or cannot be converted to a metabolizer phenotype due to unknown or uncertain allele function.
[a] Metoprolol has no known active metabolites formed by CYP2D6.
[b] No recommendation: There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice. Moderate: There is a close or uncertain balance as to whether the evidence is high quality, and the desirable effects clearly outweigh the undesirable effects. Strong: The evidence is high quality, and the desirable effects clearly outweigh the undesirable effects.
Table adapted from (4).

Table 3: Recommendations for Metoprolol and CYP2D6 Phenotype from the Dutch Pharmacogenetics Working Group (DPWG)

CYP2D6 phenotype	Effect	Recommendation
UM	Increases the conversion of metoprolol to inactive metabolites. This can increase the dose requirement. However, with a target dose of 200 mg/day, there was no effect on the blood pressure and hardly any effect on the reduction of the heart rate.	Use the maximum dose for the relevant indication as a target dose. If the effectiveness is still insufficient: increase the dose based on effectiveness and side effects to 2.5 times the standard dose or select an alternative. Possible alternatives include: -Heart failure: bisoprolol or carvedilol. -Other indications: atenolol or bisoprolol.
IM	Reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia	If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia: use smaller steps in dose titration, prescribe no more than 50% of the standard dose or both. Other cases: no action required.
PM	Reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia	If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia: use smaller steps in dose titration, prescribe no more than 25% of the standard dose or both. Other cases: no action required.

UM: Ultrarapid metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer
Table adapted from (5).

Drug: Metoprolol

Metoprolol is a commonly prescribed drug that belongs to the drug class of beta-adrenoreceptor antagonists, also known as "beta-blockers". Metoprolol is indicated to treat hypertension, angina, myocardial infarction, and HF (stable, symptomatic [New York Heart Association Class II or III] HF). Metoprolol selectively blocks the $beta_1$-adrenoreceptor (1). Beta-blockers are a recommended first-line therapy for cardiac conditions, including various arrhythmias, and as second-line therapy for hypertension by professional medical societies in the US and Europe (6, 7, 8).

There are 2 main types of adrenoreceptors, alpha and beta, each of which has numbered subtypes. The beta adrenoreceptors have 3 subtypes: $beta_1$, $beta_2$, and $beta_3$. All 3 subtypes are coupled to the G_s protein, which in turn activates adenylate cyclase, an enzyme that catalyzes the production of cyclic adenosine monophosphate (cAMP) from ATP. The binding of an agonist, such as the catecholamines adrenaline and noradrenaline, to beta receptors leads to a rise in the intracellular concentration of cAMP, which triggers signaling pathways via protein kinase A (9). Stimulation of the $beta_1$ receptor, which is predominantly expressed in cardiac tissue, leads to an increase in heart rate and the contractility of the atria and ventricles. It also leads to the increased secretion of hormones from other tissues, including renin (from the kidneys), ghrelin (from the stomach), and amylase (from the salivary glands).

Metoprolol exerts its therapeutic effects by reducing the impact of catecholamine stimulation. Metoprolol reduces heart rate, improves contractile function by stimulating the upregulation of $beta_1$ receptors, reduces vasoconstriction, and possibly also reduces the risk of arrhythmias (2, 10, 11, 12). In the treatment of HF, certain beta-blockers, such as extended-release metoprolol succinate, are thought to protect the heart from increased catecholamine stimulation. In the short term, adrenergic activation can help the heart maintain cardiac performance, but over time, continued activation can be detrimental. Harmful effects include a persistently increased heart rate, down-regulation and impaired functioning of the beta receptors, and myocyte hypertrophy and death, which lead to adverse remodeling of heart tissue (10, 13).

Metoprolol is a racemic mixture of R- and S-enantiomers (in equal amounts). S-metoprolol has a higher affinity for the $beta_2$ receptors than the R-enantiomer (14). R-metoprolol is predominantly metabolized by O-

demethylation, whereas S-metoprolol primarily undergoes alpha-hydroxylation (15, 16, 17). Both pathways are metabolized extensively by CYP2D6, though CYP2D6 seems to metabolize R-metoprolol more efficiently (18). The CYP2D6 enzyme is absent in approximately 8% of Caucasians (PM) and approximately 2% of most other populations. Individuals who lack CYP2D6 activity will have plasma concentrations of metoprolol roughly 5 times higher and may be at an increased risk of side effects (18, 19, 20, 21). Individuals with hepatic or renal failure have also been reported to experience elevated plasma levels of metoprolol and increased exposure (22). Metoprolol has a relatively low (approximately 12%) albumin-bound fraction in plasma, can cross the blood-brain barrier, and shows a dose-related increase in bioavailability, though this increase is not directly proportional (1). At higher plasma concentrations, metoprolol is less cardioselective. Metoprolol can inhibit $beta_2$ receptors, which are mainly located in the bronchial and vascular musculature. The FDA-approved drug label advises that individuals with bronchospastic disease should not take beta-blockers, except for individuals who cannot tolerate another antihypertensive treatment; in such cases, the lowest possible dose should be used (1).

In a geriatric population, plasma levels of metoprolol above the median were associated with an increased risk of falls; however, this association was not seen for non-selective beta-blockers (23). The drug label in Canada and the FDA label for metoprolol tartrate recommend lower starting and maintenance doses, as well as safety monitoring in geriatric individuals on metoprolol therapy (24, 25).

The safety and effectiveness of metoprolol have not been established in individuals under 6 years of age, according to the FDA-approved label (1). Metoprolol has not been authorized for pediatric use in Canada (24). Even though beta-blockers are typically not recommended for use in pediatric individuals, off-label use in pediatric heart failure does occur (26). The few studies available provide conflicting outcomes regarding the benefit of beta-blockers in a pediatric HF population. Although this pharmacologic management approach did not appear to cause a significant rate of adverse events, more studies are needed to determine the efficacy of beta-blockers for pediatric HF (26). Much of the use of beta-blockers in a pediatric population is extrapolated from experiences in adults, and CPIC states that their recommendations can, with caution, be extrapolated from adult to pediatric use (4, 27). Changes in gene expression over the lifespan of an individual can impact drug responses; the primary metabolic enzyme for metoprolol, CYP2D6, is absent in fetal development but reaches mature expression within the first several post-natal weeks of life. Less is known regarding the beta-adrenergic receptors (27).

Metoprolol can cross the placenta and is secreted in breast milk (28). There are notable risks to a pregnant mother with untreated hypertension during pregnancy, including pre-eclampsia, gestational diabetes, premature delivery and delivery complications. Likewise, hypertension also increases the risk of adverse fetal outcomes (1). Observational studies have not definitively established nor excluded any drug-associated risk of major congenital malformations during pregnancy for metoprolol tartrate or succinate (1, 25). The combined levels of metoprolol and the alpha-OH-metoprolol metabolite secreted into breast milk are reported to be low (less than 2% of the mother's weight-adjusted dose), and the amounts ingested by the infant are not expected to cause adverse effects, with studies to date finding no adverse reactions in breastfed infants (29). Changes in gene expression or metabolism can occur during pregnancy; an increase in the clearance rate of metoprolol has been reported during mid and late pregnancy, which may require changes in medication selection or dosing (30). However, the use of metoprolol during pregnancy or nursing is not recommended by Health Canada (24), and the US FDA advises monitoring of a breastfed infant for bradycardia or listlessness (signs of beta-blockade) (1).

Gene: *CYP2D6*

The CYP450s are a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are polymorphic and can result in decreased, absent,

or increased enzyme activity. One prominent CYP450 member, CYP2D6, is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

The *CYP2D6* Alleles

The *CYP2D6* gene is highly polymorphic, as over 170 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 4) (31, 32). Star alleles are defined by the variants detected on one chromosome (haplotype).

The combination of *CYP2D6* haplotypes that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on their impact on enzyme function, each allele can be assigned an activity score from 0 to 1, which is then used to assign a phenotype (for example, CYP2D6 PM). To promote harmonization, the CPIC and DPWG standardized their *CYP2D6* genotype-to-phenotype methods in October 2019, creating a consensus activity scoring guideline. The CYP2D6 phenotype is predicted from the diplotype activity score, defined by the sum of the allele score values, which usually ranges from 0 to 3.0. (33)

- An UM has an activity score greater than 2.25
- A NM phenotype has an activity score of 1.25–2.25
- An IM has an activity score of >0–<1.25
- A PM has an activity score of 0

Table 4. Activity Status of Selected CYP2D6 Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See the Pharmacogene Variation Consortium . Activity scores from (34).

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the NM phenotype. The *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, and **6*) (35, 36, 37) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (32, 38) (see Table 4). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **6*, and **41* being more common in individuals with European ancestry, **17* more common in those with African ancestry, and **10* more common in individuals with Asian ancestry. (39)

Larger structural variants at the *CYP2D6* locus have also been described, including gene duplications, deletions, tandem alleles, gene hybrids (namely, *CYP2D6-CYP2D7*), and gene conversions. The *CYP2D6* gene deletions result in a no-function allele (for example, the **5* allele is a deletion). Duplications have been reported for alleles with both normal and decreased function. For allele duplications, the activity scores for the full complement of *CYP2D6* alleles are summed to determine the predicted metabolizer phenotype. Additional details on structural variants are available from PharmVar (see the document Structural Variation for CYP2D6) (40).

The frequency of *CYP2D6* star alleles with altered function varies across global populations, resulting in different frequencies of the resulting metabolizer phenotypes. Given CYP2D6's role in the metabolism of many drugs, the

literature on allele and phenotype frequency is expansive. Most populations have a high frequency of normal-function star alleles, and thus a high proportion of the population are NMs. However, reduced-function alleles like *CYP2D6*10* are highly prevalent in East Asian populations, leading to a higher proportion of IM phenotype individuals in this ancestral group. Many groups in sub-Saharan Africa have higher frequencies of decreased-function alleles like *CYP2D6*17* and **29*, which can correlate with lower metabolizer scores in these individuals. More details regarding published allele and phenotype frequencies are available in the CYP2D6 supplemental chapter.

Pharmacologic Conversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and, thus, the metabolizer phenotype of any individual. Administration of an interacting medication can lead to phenoconversion, whereby an individual with one metabolizer genotype can exhibit the enzymatic activity of a different metabolizer group (higher or lower, depending on the medications). The enzymatic activity of CYP2D6 can be inhibited or reduced by medications, including but not limited to strong inhibitors such as paroxetine, fluoxetine, bupropion, and quinidine, and moderate inhibitors such as duloxetine (24, 41, 42, 43). This can potentially result in NMs or IMs responding to medications as if they were PMs, depending on the strength of the enzyme inhibition. Strong inhibitors can completely inhibit CYP2D6, while moderate inhibitors can reduce activity by 50%. Thus, co-medication with multiple CYP2D6 strong or moderate inhibitors may result in reduced metabolism of drug substrates, as has been observed in psychiatric pharmacotherapy (44, 45 2023). In contrast, discontinuing a concomitant CYP2D6 inhibitor can then revert the individual's CYP2D6 activity back to the genetically predicted baseline phenotype. Coadministration of metoprolol and amiodarone has shown a significant increase in metoprolol concentrations and lower heart rate, despite no significant changes in metoprolol doses (46). The product monograph reviewed by Health Canada for metoprolol states that "strong inhibition of CYP2D6 would result in the change of phenotype into poor metabolizer… caution should therefore be exercised when co-administering potent CYP2D6 inhibitors with metoprolol" (24). The metoprolol tartrate drug labeling approved by the FDA states that strong CYP2D6 inhibitors (such as quinidine, fluoxetine, paroxetine, and propafenone) have been shown to double metoprolol concentrations (25). Integration of CYP2D6 phenoconversion into clinical practice requires knowledge of multiple clinical factors, and tools have been developed to support clinicians (47).

Other Genes of Interest: *ADRB1, ADRB2, OR10P1, SNX9, GRK5*

The beta-adrenergic receptors mediate signaling via coupled G-protein signaling. In the heart, beta$_1$-adrenergic receptors are the primary target for beta-blockers and represent the major adrenergic receptor (27). Encoded by *ADRB1* on chromosome 10q25, the beta$_1$-adrenergic receptor is a 7-transmembrane domain protein with intracellular and extracellular portions that facilitate ligand and G-protein binding (48, 49). Binding of a ligand to the receptor induces a conformational change that allows the G-alpha subunit to activate adenylyl cyclase, creating cAMP signaling molecules, which in turn leads to increased intracellular calcium ion levels. Calcium influx drives increased contractility and heart rate, as well as increased electrical automaticity (27).

There are known polymorphisms in *ADRB1* that have been associated with disease and altered drug response in some studies. The 2 commonly studied variants are ADRB1 p.Ser49Gly (rs1801252, c.145A>G) and p.Arg389Gly (rs1801253, c.1165G>C). The p.389 residue falls within the G-protein binding intracellular loop, and the p.49 residue occurs in an extracellular loop (27). The C>G single nucleotide polymorphism (SNP) at rs1801253 (p.Arg389Gly) has been reported to occur at a frequency of 27% in individuals of European descent, 40–42% in those of African descent, 32–41% in individuals of Asian descent, and 19–32% in individuals of Latin American descent (50, 51). The variation at p.49 of ADRB1 (c.145A>G, rs1801252) occurs less frequently: 10–12.5% in Asian populations, 13% in European populations, 20–25% in African and African descent populations, and 17–24% in Latin American populations. These 2 variants are in negative linkage disequilibrium, such that it is

unlikely for an individual to have a Gly49-Gly389 allele (27). Individuals homozygous for arginine at p.389 have been reported to have an increased risk of hypertension (50), and this variation may interact with other variants in the G-protein signaling pathway to negatively impact survival, particularly in individuals of African ancestry (52).

The beta$_2$-adrenergic receptor, encoded by *ADRB2* and located on chromosome 5q32, has been associated with nocturnal asthma, obesity, type 2 diabetes, cardiovascular disease, and Parkinson's disease risk (53). The ADRB2 protein is abundantly expressed in bronchial smooth muscle cells, where activation of the receptor leads to bronchodilation (54). It is also expressed, to a lesser extent, in the cardiac myocytes and vascular smooth muscle cells, contributing to increased heart rate and contractility following adrenergic stimulation (54). There are 2 common variants in *ADRB2* that encode amino acid changes: rs1042713 (p.Gly16Arg) and rs1042714 (p.Glu27Gln, c.79G>C), with the minor allele frequencies ranging between 40–50% (54). The Gly16 protein isoform is more susceptible to agonist-stimulated downregulation in vitro (54). The Gln27 variant has been associated with increases in systolic blood pressure and reduced response to isoproterenol (54). A third variant, rs1800888 (p.Thr164Ile), is relatively rare, with a minor allele frequency range of ~1–2% in individuals of European or African ancestry, and it is not detected in Chinese populations (54). This rare allele is associated with reduced response to beta$_2$-agonists such as salmeterol or albuterol (54, 55).

A genome-wide association study found that *OR10P1* and *SNX9*-linked variants were associated with changes in heart rate in response to beta-blocker therapy for individuals classified as 'Black' in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) and PEAR-2 studies (56). The variants g.56022983T>G (rs17117817, near the olfactory receptor family 10 subfamily-p-member 1 gene *OR10P1*) or rs2364349 c.1648+912G>A (rs2364349) (linked to the sorting nexin-9 gene *SNX9*) were associated with a decreased heart rate-lowering response in this population. These loci were found to yield significant association signals in 'White' study participants as well (56). There is evidence that OR10P1 is expressed in cardiac tissue and may indirectly interact with ADRB2, though the specific mechanism by which olfactory receptors impact heart rate is unknown. Sorting nexin-9 is associated with endocytosis of transmembrane signaling proteins and may be involved in regulating the endocytosis of ADRB2.

One study also found a connection between *GRK5* variation and improved survival in HF. p.Gln41Leu (c.122A>T) in the G-protein-coupled receptor kinase 5 protein (rs17098707, now rs2230345) was reported to be more common in individuals of African ancestry than in Caucasians and may attenuate beta-adrenergic signaling, similar to beta-blocker medication (57). This same allele was examined in a Chinese population with an allele frequency of 0.008, but it was not associated with differences in systolic HF mortality compared with the reference allele (58).

Linking *CYP2D6* and *ADRB1/2* Genetic Variation with Treatment Response

Genetic variants of the *CYP2D6* gene have been found to influence the ratio of enantiomers, the dose and dose titration of metoprolol, and heart rate—CYP2D6 PM have an increased risk of bradycardia (59, 60, 61, 62, 63, 64). However, in one study, *CYP2D6* did not appear to influence the efficacy of metoprolol when used to treat hypertension (65). Reduced function of CYP2D6 enzyme alters the pharmacokinetics of metoprolol, resulting in increased exposure (measured by plasma area under the concentration-time curve levels) in PMs and IMs (*10/*10) and this is correlated with a higher risk of bradycardia in PMs (66). In a genome-wide association biobank study using 'white' (self-reported) study participants, metoprolol and alpha-OH-metoprolol concentrations were significantly associated with genetic variation located only in the *CYP2D6* locus (67).

A meta-analysis of 21 studies reported a significant difference in the BP-lowering effects of metoprolol between CYP2D6 PMs and all other phenotypes (68). At similar doses, individuals with a PM CYP2D6 phenotype

experienced a larger decrease in heart rate (measured in beats per minute, BPM), blood pressure (both systolic and diastolic), and more cases of bradycardia (heart rate of less than 60 BPM) across the relevant studies (68).

Variants within the $beta_1$ receptor have also been found to influence the treatment response to specific beta-blockers. The most studied is a reduced-function variant, p.Gly389Arg (rs1801253), which leads to reduced levels of cAMP and diminished $beta_1$ receptor signaling cascades (69). Individuals who are homozygous p.Arg389 may have a more favorable response to metoprolol treatment than individuals who are homozygous for the reference sequence p.Gly389 (69, 70, 71, 72, 73). The p.Ser49Gly (rs1801252) variant has been reported to be associated with increased ventricular ectopic beats and favorable beta-blocker response in a cohort study of hypertrophic cardiomyopathy (HCM), though the p.Gly389Arg polymorphism did not show significant impacts on beta-blocker response in this HCM cohort (74). The *ADRB1* p.Gly389Arg (rs1801253) variant, which was associated with decreased response to beta-blockers, was detected in roughly half the cohort from the Alabama Genomic Health Initiative that had a prescription for one of the affected medicines, including metoprolol (75). One study found that individuals with HF and a variant allele (p.389Arg) at rs1801253 benefited from higher doses of beta-blockers (76). In a separate study, p.389Arg homozygous individuals had greater improvement in left ventricular ejection fraction compared with individuals with the homozygous reference allele (77). However, the CPIC guideline writing committee found the data insufficient to issue recommendations regarding genetic variations in *ADRB1* and beta-blocker therapy, citing a need for additional research to refute or confirm the reported findings to date (4).

Additional studies have examined the role of $beta_2$ receptor variants and heart rate responses to beta-blockers (78). The PEAR-2 trial examined variants in metoprolol response for individuals with hypertension, finding a significant association of a more pronounced heart rate-lowering response with *ADRB2* rs1042714 C/C genotype (encoding Gln at p.27) and a "trending toward significant" association with rs1042713 A-allele in *ADRB2* (78). In contrast, the p.27Glu genotype was associated with longer survival for individuals with HF who were taking beta-blockers, and no association was observed with the p.Arg16Gly variants and clinical outcomes (76). This literature was also insufficient to justify clinical recommendations from the CPIC writing committee for beta-blockers and *ADRB2* variation (4).

Other studies have failed to find associations between *ADRB1* or *ADRB2* common variants and blood pressure responses to beta-blockers (79), though it is unclear if the underlying cardiac disorder may impact the specific drug response (or lack of response) associated with genetic variants.

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests available for metoprolol response and the CYP2D6 gene.

The available *CYP2D6* tests include targeted single-gene tests as well as multi-gene panels. In addition, variant *CYP2D6* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP) (80). For *CYP2D6*, the AMP recommends that the minimum panel of variant alleles should include *2, *3, *4, *5, *6, *9, *10, *17, *29, *41, and a copy number interrogation. Results are reported as a diplotype, such as *CYP2D6 *1/*1*; however, copy number testing is crucial when interpreting *CYP2D6* results (81). When individuals have more than 2 copies of *CYP2D6*, the copies of the allele are denoted by an "xN", where the "N" can either be quantified or unquantified (for example, *CYP2D6 *1/*2x2* or *CYP2D6 *1/*2xN*). Some laboratories also use the notation of duplication ("DUP") to indicate an increase in copy number, but the report does not always specify the number of duplications or the allele that has been duplicated due to technical limitations. The test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be confirmed by checking the diplotype and calculating the *CYP2D6* activity score, as described in the "*CYP2D6* Alleles" section above.

Multiple studies have reported successful implementation of pharmacogenetic testing to guide medication selection or dosing in real-world clinical settings (82, 83, 84). Of particular concern are cases where individuals are prescribed multiple medications for chronic health conditions, where gene-drug or gene-drug-drug interactions may negatively impact the individual's response to medications (85).

The *CYP2D6* Gene Interactions with Medications Used for Additional Indications

The CYP family of enzymes is involved in the metabolism of many substances, and CYP2D6 has been implicated in altered pharmacologic responses for many compounds. The drugs can be categorized into many different classes:

- Antipsychotics—for example, aripiprazole, risperidone, and thioridazine, and to a lesser extent, clozapine, are metabolized by CYP2D6. According to the FDA, aripiprazole dosage should be reduced for PMs, and thioridazine is contraindicated for individuals known to have reduced CYP2D6 activity due to an increased risk of potentially fatal side effects. The UMs may have a decreased plasma concentration of risperidone.

- Tricyclic antidepressants—for example, amitriptyline and imipramine may require dosage adjustments, potentially guided by therapeutic drug monitoring, to achieve the desired therapeutic range in UMs or PMs. Ultimately, tricyclic antidepressants may be ineffective in CYP2D6 UMs.

- Serotonin and norepinephrine reuptake inhibitors, for example atomoxetine and venlafaxine may have reduced efficacy in UMs at standard doses, while PMs are at risk of elevated plasma concentrations for both medications. The DPWG advises against the use of venlafaxine in CYP2D6 PMs and IMs.

- Antimalarial medications—for example, primaquine is activated by CYP2D6 and CYP450 Nicotinamide Adenine Dinucleotide Phosphate oxidoreductase.

- Anticancer medications—for example, tamoxifen is activated by CYP2D6, and IMs or PMs may have reduced benefit from tamoxifen therapy.

- Pain management—for example, codeine and tramadol are pro-drugs that require activation by CYP2D6 to achieve the desired analgesic effect.

- Various therapies for genetic disorders, for example, eliglustat used in the treatment of Gaucher disease, and deutetrabenazine, used in the treatment of Huntington disease, have reduced dose recommendations for CYP2D6 PMs. The CYP2D6 UMs may not achieve adequate concentrations of eliglustat, and therefore *CYP2D6* genotyping is required before initiating eliglustat therapy.

It is important to note that *CYP2D6* is the most common biomarker in drug responses for FDA drug labels. The lists provided here is by no means exhaustive. Additional information on gene-drug interactions for *CYP2D6* is available from PharmGKB, CPIC, and the FDA (search for "CYP2D6").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2024 Statement from the US Food and Drug Administration (FDA):

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

CYP2D6 Inhibitors are likely to increase metoprolol concentration. […] Drugs that are strong inhibitors of CYP2D6 such as quinidine, fluoxetine, paroxetine, and propafenone were shown to double metoprolol concentrations. While there is no information about moderate or weak inhibitors, these too are likely to increase metoprolol concentration. Increases in plasma concentration decrease the cardioselectivity of metoprolol … Monitor patients closely when the combination cannot be avoided.

[…]

Drug Interactions

CYP2D6

Metoprolol is metabolized predominantly by CYP2D6. In healthy subjects with CYP2D6 extensive metabolizer phenotype, coadministration of quinidine 100 mg, a potent CYP2D6 inhibitor, and immediate-release metoprolol 200 mg tripled the concentration of S-metoprolol and doubled the metoprolol elimination half-life.

[…]

CYP2D6 is absent in about 8% of Caucasians (poor metabolizers) and about 2% of most other populations. CYP2D6 can be inhibited by several drugs. Poor metabolizers of CYP2D6 will have increased (several-fold) metoprolol blood levels, decreasing metoprolol's cardioselectivity.

Please review the complete therapeutic recommendations that are located here: (1)

2024 Summary of recommendations from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

The evidence supporting the association of CYP2D6 genotype with metoprolol exposure and response included participants with a variety of health statuses (e.g., healthy, hypertension, heart failure, etc.). Therefore, it may be reasonable to assume that the pharmacokinetic effects of CYP2D6 variation would affect clinical metoprolol response similarly across a variety of indications, and the dosing recommendations provided could be utilized for most cardiovascular indications …

Recommendations primarily focus on minimizing the risk of adverse effects in CYP2D6 poor metabolizers related to the greater observed reductions in heart rate and blood pressure stemming from increased metoprolol systemic exposure. In addition, the maximally tolerated metoprolol dose may be lower in poor metabolizers compared with normal metabolizers due to these pharmacokinetic differences… We found insufficient evidence to support recommendations related to CYP2D6 genotype and other clinical outcomes.

While the evidence suggests metoprolol plasma concentrations are also increased in CYP2D6 intermediate metabolizers compared with normal metabolizers, these effects appear smaller in magnitude than those observed with poor metabolizers, and there was insufficient evidence to clarify whether these smaller pharmacokinetic differences significantly affect clinical response.

… Most of the data available regarding associations between CYP2D6 genotype and metoprolol response are related to oral formulations; limited evidence exists regarding pharmacogenetic effects with intravenous formulations.

….

CYP2D6 normal and intermediate metabolizers: Recommendations: Initiate standard dosing.

CYP2D6 poor metabolizers: Recommendations: Initiate therapy with lowest recommended starting dose. Carefully titrate dose upward to clinical effect or guideline-recommended dose; monitor more closely for bradycardia. Alternatively, consider selecting another beta-blocker.

CYP2D6 ultrarapid metabolizers: No recommendation for metoprolol therapy due to insufficient evidence regarding diminished metoprolol effectiveness clinically.

Please review the complete therapeutic recommendations that are located here: (4)

2022 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

CYP2D6 Poor Metabolizers:

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1. Use smaller steps in dose titration and/or prescribe no more than 25% of the standard dose

Other cases:

1 No action required

CYP2D6 Intermediate Metabolizers:

The gene variation reduces the conversion of metoprolol to inactive metabolites. However, the clinical consequences are limited mainly to the occurrence of asymptomatic bradycardia.

Recommendation:

If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia:

1. Use smaller steps in dose titration and/or prescribe no more than 50% of the standard dose.

Other cases:

1. No action required

CYP2D6 Ultrarapid Metabolizers:

The gene variation increases the conversion of metoprolol to inactive metabolites. This can increase the dose requirement. However, with a target dose of 200 mg/day, there was no effect on the blood pressure and hardly any effect on the reduction of the heart rate.

Recommendation:

1. Use the maximum dose for the relevant indication as a target dose

2. If the effectiveness is still insufficient: increase the dose based on effectiveness and side effects to 2.5 times the standard dose or select an alternative

Possible alternatives include:

- Heart failure: bisoprolol or carvedilol. Bisoprolol: advantage: not metabolised by CYP2D6; disadvantage: elimination depends on the kidney function. Carvedilol: advantage: elimination does not depend on the kidney function; disadvantage: is metabolised (to a lesser extent than metoprolol) by CYP2D6.
- Other indications: atenolol or bisoprolol. Neither is metabolised by CYP2D6.

Please review the complete therapeutic recommendations that are located here: (5)

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*2	2851C>T	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*3	2550delA	NM_000106.6:c.775del	NP_000097.3:p.Arg259fs	rs35742686
CYP2D6*4	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
CYP2D6*5	Gene deletion			
CYP2D6*6	1707 del T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
CYP2D6*10	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*17	1022C>T	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*27	3854G>A	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
CYP2D6*31	2851C>T	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*36[1]	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C	NM_000106.6:c.1432C>T+ NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735+ rs766507177
	4159G>C	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G	NM_000106.6:c.1444G>A+ NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221+ rs75467367
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*41	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2989G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*49	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] CYP2D6*36 is a gene conversion with CYP2D7; variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Nomenclature for Cytochrome P450 enzymes is available from PharmVar (28).

Nomenclature of Selected *ADRB1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
Ser49Gly	ADRB1 Ser49Gly	NM_000684.3:c.145A>G	NP_000675.1:p.Ser49Gly	rs1801252
Arg389Gly	ADRB1 Arg389Gly	NM_000684.3:c.1165G>C	NP_000675.1:p.Gly389Arg	rs1801253

Nomenclature of Selected *ADRB2* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
Gly16Arg	ADRB2 Gly16Arg	NM_000024.6:c.46G>A	NP_000015.2:p.Gly16Arg	rs1042713
Glu27Gln	ADRB2 Glu27Gln	NM_000024.6:c.79G>C	NP_000015.2:p.Glu27Gln	rs1042714

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Version History

The April 4, 2017 version (1.0) of this chapter is available as a PDF here.

Version 2.0 published on September 19, 2024 encompasses new recommendations from CPIC that were not previously available as well as expanded recommendations from DPWG that include dosing adjustment guidelines for CYP2D6 IM and UM individuals. Language from the FDA-approved drug label discussing drug-drug interactions via CYP metabolism is also included in version 2.0. Updated *CYP2D6* allele information, activity scores and translation from genotype-based activity scores to predicted metabolizer phenotype are also provided in this version based on harmonized international standards. The Genetic Testing section also includes the recommendations from AMP regarding Tier 1 and Tier 2 alleles for *CYP2D6* pharmacogenetic testing.

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Omeprazole Therapy and *CYP2C19* Genotype

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Introduction

Omeprazole (brand name Prilosec) is a first-generation proton pump inhibitor (PPI) used to treat gastroesophageal reflux disease (GERD), gastric ulcers, duodenal ulcers, upper gastrointestinal (GI) tract inflammatory conditions, eosinophilic esophagitis, and erosive esophagitis. Omeprazole is also used in the treatment of hypersecretory conditions, such as Zollinger-Ellison syndrome, and is used with antibiotics to eradicate *Helicobacter pylori (H. pylori)*.

Omeprazole reduces the acidity (raises the pH) in the stomach by inhibiting the secretion of gastric acid. The level of individual omeprazole exposure is influenced by several factors, such as the dose administered, amount of drug absorbed, as well as the kinetics of drug metabolism and drug inactivation.

Omeprazole is primarily metabolized by the CYP2C19 enzyme. Individuals with increased CYP2C19 enzyme activity ("CYP2C19 rapid and ultrarapid metabolizers") may have an insufficient response to standard doses of omeprazole because the drug is inactivated at a faster rate. In contrast, individuals who have reduced or absent CYP2C19 enzyme activity (namely, CYP2C19 intermediate and poor metabolizers) have greater plasma concentrations of omeprazole, which is associated with more potent acid suppression. The frequencies of CYP2C19 metabolizer phenotypes vary among global populations.

The FDA-approved drug label does not give dosing guidance for CYP2C19 intermediate or ultrarapid metabolizers (1) (Table 1); however, it does recommend a reduced dosage for individuals of Asian descent without regard for CYP2C19 metabolizer status. It is important to note that early PPI research studies investigating the *CYP2C19* gene were conducted in Asian populations with ultrarapid and rapid metabolizer phenotypes making up only 1.3–4% of Asian populations compared with approximately 20% of non-Asian populations.

In 2018, PPI dosing recommendations for all *CYP2C19* metabolizer phenotypes were published by the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) (Table 2) (2, 3). For poor and intermediate metabolizers, DPWG recommends no alteration in dosing based on their consensus that higher plasma concentration of omeprazole results in an increase in the therapeutic effectiveness without an increase in side effects. For CYP2C19 ultrarapid metabolizers with *H. pylori* infection, DPWG states that the dose of omeprazole should be increased 3-fold for the eradication of infection. For other indications (for example, GERD), the physician should be aware of possible reduced effectiveness if individuals are rapid or ultrarapid metabolizers and consider increasing the dose 3-fold. The CYP2C19 ultrarapid metabolizers should also be advised to contact their doctor if symptoms of dyspepsia persist.

In 2020, the Clinical Pharmacogenetics Implementation Consortium (CPIC) evaluated additional data and now recommends that CYP2C19 ultrarapid and rapid metabolizers may require increased doses of omeprazole to achieve desired therapeutic outcomes, whereas CYP2C19 intermediate and poor metabolizers may require reduced dosage for chronic therapy once efficacy has been established (Table 3) (4).

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Table 1. The FDA Drug Label for Omeprazole: *CYP2C19* Pharmacogenomics (2020)

Phenotype	Omeprazole exposure
CYP2C19 metabolism	The systemic exposure to omeprazole varies with an individual's metabolism status: poor metabolizers > intermediate metabolizers > normal metabolizers. Approximately 3% of Caucasians and 15–20% of Asians are CYP2C19 poor metabolizers. In studies of healthy subjects, Asians had approximately a four-fold higher exposure than Caucasians. Dosage reduction of Omeprazole delayed-release capsules to 10 mg once daily is recommended for Asian individuals for maintenance of healing of erosive esophagitis.

Please see Therapeutic Recommendations based on Genotype for more information from FDA. This FDA table is adapted from (1).

Table 2. The DPWG Recommendations for Omeprazole and *CYP2C19* Genotype (2018)

Phenotype	Action	Pharmacist text
CYP2C19 poor metabolizer	No action is required for this gene-drug interaction.	The higher plasma concentration of omeprazole results in an increase in the therapeutic effectiveness, without an increase in the side effects.
CYP2C19 intermediate metabolizer	No action is required for this gene-drug interaction.	The higher plasma concentration of omeprazole results in an increase in the therapeutic effectiveness, without an increase in the side effects.
CYP2C19 ultrarapid metabolizer	For *Helicobacter pylori* eradication therapy: • use a 3-fold higher dose • advise the individual to report persisting symptoms of dyspepsia	The genetic variation may lead to a reduced omeprazole plasma concentration and therefore reduced effectiveness.
	Other indications • be alerted to reduced effectiveness • if necessary, use a 3-fold higher dose • advise the individual to contact their doctor if symptoms of dyspepsia persist	

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This Dutch Pharmacogenetics Working Group (DPWG) table is adapted from (2).

Table 3. The CPIC Dosing Recommendations for Omeprazole based on CYP2C19 Phenotype (2020)

CYP2C19 phenotype[a]	Implications for phenotypic measures	Therapeutic recommendation	Classification of recommendation[b]
CYP2C19 ultrarapid metabolizer	Decreased plasma concentrations of PPIs compared with CYP2C19 NMs; increased risk of therapeutic failure	Increase starting daily dose by 100%. Daily dose may be given in divided doses. Monitor for efficacy.	Optional
CYP2C19 rapid metabolizer	Decreased plasma concentrations of PPIs compared with CYP2C19 NMs; increased risk of therapeutic failure	Initiate standard starting daily dose. Consider increasing dose by 50–100% for the treatment of *H. pylori* infection and erosive esophagitis. Daily dose may be given in divided doses. Monitor for efficacy.	Moderate

Table 3. continued from previous page.

CYP2C19 phenotype[a]	Implications for phenotypic measures	Therapeutic recommendation	Classification of recommendation[b]
CYP2C19 normal metabolizer	Normal PPI metabolism: may be at increased risk of therapeutic failure compared with CYP2C19 IMs and PMs	Initiate standard starting daily dose. Consider increasing dose by 50–100% for the treatment of *H. pylori* infection and erosive esophagitis. Daily dose may be given in divided doses. Monitor for efficacy.	Moderate
CYP2C19 likely intermediate metabolizer	Likely increased plasma concentration of PPI compared with CYP2C19 NMs; likely increased chance of efficacy and potential toxicity	Initiate standard starting daily dose. For chronic therapy (>12 weeks) and efficacy achieved, consider 50% reduction in daily dose, and monitor for continued efficacy.	Optional[c]
CYP2C19 intermediate metabolizer	Increased plasma concentration of PPI compared with CYP2C19 NMs; increased chance of efficacy and potential toxicity	Initiate standard starting daily dose. For chronic therapy (>12 weeks) and efficacy achieved, consider 50% reduction in daily dose, and monitor for continued efficacy.	Optional
CYP2C19 likely poor metabolizer	Likely increased plasma concentration of PPI compared with CYP2C19 NMs; likely increased chance of efficacy and potential toxicity	Initiate standard starting daily dose. For chronic therapy (>12 weeks) and efficacy achieved, consider 50% reduction in daily dose, and monitor for continued efficacy.	Moderate[c]
CYP2C19 poor metabolizer	Increased plasma concentration of PPI compared with CYP2C19 NMs; increased chance of efficacy and potential toxicity	Initiate standard starting daily dose. For chronic therapy (>12 weeks) and efficacy achieved, consider 50% reduction in daily dose, and monitor for continued efficacy.	Moderate

Table adapted from (4).
IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; PPI, proton pump inhibitor *H. pylori*, *Helicobacter pylori*.
[a] The online *CYP2C19* Frequency Table provides phenotype frequencies for major race/ethnic groups, and the online *CYP2C19* Diplotype-Phenotype Table provides a complete list of possible diplotype and phenotype assignments (3, 4).
[b] Rating scheme described in the Supplemental Material online.
[c] The strength of recommendation for "likely" phenotypes are the same as their respective confirmed phenotypes. "Likely" indicates the uncertainty in the phenotype assignment, but it is reasonable to apply the recommendation for the confirmed phenotype to the corresponding "likely" phenotype.

Drug class: Proton Pump Inhibitors

Proton pump inhibitors block the secretion of gastric acid through irreversible inhibition of the H+-K+ ATPase on gastric parietal cells. They are among the most prescribed drugs in the US and globally, and some PPI formulations are available without a prescription.

According to FDA labeling, PPIs can be used to treat several conditions in adults:

- Peptic ulcer disease, including gastric and duodenal ulcers
- Eradication of *H. pylori* infection (in combination with antibiotics)
- Hypersecretory conditions (for example, Zollinger-Ellison syndrome)

According to FDA labeling, PPIs are also used in children and adults to treat:

- Symptomatic GERD
- Complications of GERD, including erosive esophagitis, peptic stricture, Barrett's esophagus
- Eosinophilic esophagitis (along with other therapeutic interventions)

Additional off-label indications for PPIs in infants, children and adults to treat:

- Acute and chronic GERD
- Acute and chronic eosinophilic esophagitis
- Acute and chronic upper GI tract inflammatory conditions

The human stomach contains approximately one billion parietal cells that secrete hydrochloric acid into the stomach (gastric lumen). Gastric acid aids digestion by hydrolyzing dietary protein and facilitating the absorption of calcium, iron, and vitamin B12. Gastric acid is also relevant to maintaining normal gastric microbiota diversity (5).

Hydrogen ions (H+) are actively secreted into the gastric lumen in exchange for potassium ions (K+) via an H^+/K^+-ATPase, which is also known as a "proton pump". Located on the luminal surface of gastric parietal cells, the proton pump controls the last step in acid secretion. The PPIs potently suppress gastric acid secretion by covalently binding to and irreversibly inactivating this proton pump.

Six PPIs are FDA-approved for clinical use in the US: esomeprazole (brand name Nexium), dexlansoprazole (Dexilant, Kapidex), lansoprazole (Prevacid), omeprazole (Prilosec), pantoprazole (Protonix), and rabeprazole (Aciphex). All PPIs are similarly potent at inhibiting gastric acid secretion and are thought to be similarly efficacious (6, 7). The available PPIs are generally grouped by first-generation (omeprazole, pantoprazole, lansoprazole) and second-generation designations (esomeprazole, dexlansoprazole, rabeprazole).

In adults, PPIs are used in the treatment of ulcers (gastric and duodenal), GERD, and to maintain healing of erosive esophagitis. Omeprazole is also used in the long-term treatment of hypersecretory conditions such as Zollinger-Ellison syndrome, multiple endocrine adenomas, and systemic mastocytosis. In children age 2 and over, omeprazole is used in the treatment of GERD and erosive esophagitis. Importantly, although FDA labeling for PPIs may have some variation, in clinical practice, PPIs are often used interchangeably and commonly for non-FDA labeled conditions such as eosinophilic esophagitis.

There are a few differences between the FDA-approved indications of different PPIs. For example, for the treatment of GERD in young children, only esomeprazole is indicated for infants from one month old (lansoprazole is licensed from one year of age, omeprazole and dexlansoprazole from 2 years of age, and rabeprazole from age 12) (8).

Nearly all PPIs, to varying degrees, are metabolized and inactivated by CYP2C19 (and to a lesser extent by CYP3A4). Additionally, given that PPIs are also inhibitors of CYP2C19 and that CYP2C19 is involved in the metabolism of many drugs, PPI administration can lead to clinically significant drug interactions. For example, the concomitant use of a PPI and clopidogrel, which requires CYP2C19 for bioactivation, has been associated with reduced antiplatelet activity, indicating that the concurrent administration of omeprazole with clopidogrel must balance overall risks and benefits, considering both cardiovascular and GI complications (9, 10, 11, 12, 13). The FDA specifically addresses interactions between omeprazole and multiple drugs that interact with CYP2C19 and CYP3A4 (including St. John's Wort or rifampin). These drug-drug interactions may result in altered drug exposure for all CYP2C19 substrate medications (1).

Genetic variation in the *CYP2C19* gene influences the clearance of PPIs that may in turn, influence treatment outcomes. First-generation PPIs (omeprazole, lansoprazole, and pantoprazole) and second-generation dexlansopraozle are dependent on CYP2C19 metabolism. In contrast, second-generation PPIs of esomeprazole and rabeprazole are less likely to be influenced by *CYP2C19* genotype (14, 15, 16, 17).

Drug: Omeprazole

Omeprazole was the first PPI to be introduced to the US market in 1989. Today, omeprazole is one of the PPIs that are available both as prescription and over-the-counter medications.

Omeprazole is metabolized and inactivated in the liver by the cytochrome P450 system. The CYP2C19 enzyme is the principal enzyme involved, although other enzymes such as CYP3A4 also contribute to a lesser degree. Omeprazole is metabolized to hydroxy and desmethyl metabolites, which is thought to have no effect on gastric acid secretion.

The long-term use of PPIs has been associated with some adverse effects, which may include but are not limited to infections, kidney disease, bone fractures and electrolyte disturbances. Many of these may stem from longstanding hypochlorhydria/achlorhydria, including B12 deficiency or iron deficiency. There can also be an increased risk of enteric infections, including *Salmonella*, *Campylobacter*, and the vegetative form of *Clostridium difficile* (17). There are mixed data from large epidemiological studies of the association with PPIs and other adverse outcomes, but these have largely not been substantiated and instead may represent residual confounding. Nevertheless, as with most drugs, the lowest effective dose for the shortest duration appropriate to the condition is applicable to PPIs. (1) Notably, there are only a few clinical diseases, such as Barrett's esophagus with dysplasia, that necessitate chronic PPI use.

Studies have not adequately assessed the safety of omeprazole therapy during pregnancy. Epidemiology studies failed to find an increased risk of major congenital malformations or other adverse pregnancy outcomes when omeprazole was used during pregnancy.

Gene: *CYP2C19*

The CYP superfamily is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, including antidepressants, antiplatelet agents, anti-fungal agents, some proton pump inhibitors, and benzodiazepines such as diazepam.

The *CYP2C19* gene is highly polymorphic, as there are over 35 variant star (*) alleles cataloged by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with 'rapid' (**1/*17*) and 'ultrarapid' (**17/*17*) metabolizer phenotypes. Individuals who have one copy of non-functional alleles (for example, **2* and **3*) are classified as 'intermediate metabolizers' (for example, **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (for example, **2/*2*, **2/*3*) (Table 4).

Table 4. The CPIC Assignment of *CYP2C19* Phenotype based on Genotype (2017)

Phenotype	Genotype	Examples of diplotype
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual with 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual with one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual with 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual with one normal function allele and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]

Table 4. continued from previous page.

Phenotype	Genotype	Examples of diplotype
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual with 2 no function alleles	**2/*2* **2/*3* **3/*3*

CPIC: Clinical Pharmacogenetics Implementation Consortium
[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (18).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (18).

It has been reported that approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are CYP2C19 poor metabolizers; and up to 45% of individuals are CYP2C19 intermediate metabolizers (19). Other studies have found poor metabolizer phenotypes to range between 10.8–16.4% in Asian populations, 3% in African descendants, and 1.6% in Middle-Eastern populations (20, 21). Pacific Islanders have been reported to have higher frequencies of poor metabolizers—11.8% (21). The frequency of intermediate metabolizers is similarly distributed, higher in East and South Asian and Pacific Islander, lower in African or Middle-Eastern populations (20).

The 2018 FDA-approved drug label for omeprazole states that approximately 15–20% of Asians are CYP2C19 poor metabolizers, compared with 3% of Caucasians. And the label states that studies have shown that Asians have an approximately four-fold higher exposure to omeprazole than Caucasians.

The most common no function allele is *CYP2C19*2*, which contains a c.681G>A variant in exon 5 that results in an aberrant splice site. This leads to the production of a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians of European descent and Africans, and ~27–36% in Asians (20, 22).

*CYP2C19*3* is another commonly identified no function variant, which contains a c.636G>A variant in exon 4 that causes a premature stop codon. The *CYP2C19*3* allele frequencies are ~2–7% in Asian populations (22), but rare in other racial groups. Other no function variants occur in less than 1% of the general population and include *CYP2C19*4-*8* (23, 24).

The *CYP2C19*17* allele, which results in rapid and ultrarapid metabolizers, has frequencies of only 1.3–4% among Asian populations compared with approximately 20–33.7% of African,-European and Near-Eastern populations (20, 21).

Linking Gene Variation with Treatment Response

In adults, it has been established that genetic variation in *CYP2C19* influences PPI clearance and exposure, which are heavily metabolized by CYP2C19. Recent CPIC guidelines support increasing treatment failures in rapid and ultrarapid metabolizers and emerging adverse events in intermediate and poor metabolizers, particularly for chronic use (4). However, DPWG recommendations based on an independent review of the literature does not find compelling evidence for an increased risk to intermediate or poor metabolizers (2). Multiple reviews have recently been published describing the risks and potential adverse effects for long-term PPI use, supporting genotype-guided dosage and limited duration of PPI administration (25, 26, 27).

In pediatric studies, there are less data, yet some trends are emerging with decreased omeprazole efficacy (more acidic gastric pH) in CYP2C19 ultrarapid metabolizers (8, 28, 29, 30) and less adverse events with genotype-guided dosing. Pediatric individuals with increased function **17* alleles were more likely to experience GERD treatment failure with PPIs, requiring surgical intervention (31). One study observed a lower frequency of PPI-

associated infection in pediatric rapid and ultrarapid metabolizers compared with normal metabolizers (32). Additional pediatric studies are needed.

Response of CYP2C19 Poor Metabolizers

Individuals with reduced CYP2C19 enzyme activity may have up to 2-fold higher plasma concentration of omeprazole with standard doses compared with individuals with normal enzyme function. Studies report that poor metabolizers have less acidic gastric pH than normal metabolizers, indicating higher activity (33, 34).

Some studies support a model whereby reduced or absent CYP2C19 enzyme activity has a positive effect on clinical outcomes and because PPIs are generally regarded as safe drugs, especially in the short-term (less than 6 months), this can have a beneficial effect without an increased risk of omeprazole toxicity (35, 36, 37, 38). However, emerging evidence links long-term PPI use with a higher risk of adverse events including bone fracture, GI infections—such as *Clostridium difficile*—hypomagnesemia, fundic gland polyps, interstitial nephritis, and vitamin B-12 deficiency (1, 26, 27).

One study reported that when using omeprazole as part of the treatment to eradicate *H. pylori*, success was achieved in all individuals who had little or no CYP2C19 activity, but in only 29% of individuals who had "normal" CYP2C19 activity. Similar results were found in another study that evaluated lansoprazole in the treatment of GERD: the response rate was 85% for individuals with little or no CYP2C19 activity, compared with 16% for individuals with normal CYP2C19 activity (39, 40, 41).

The emerging risk profile for PPI medications is of particular concern for CYP2C19 poor and intermediate metabolizers. Therefore, recent CPIC guidelines recommend a 50% reduction in daily dose of omeprazole for chronic therapy among poor and intermediate metabolizers (4). The DPWG guidelines do not recommend the reduced dose for these individuals (2). This discordance between CPIC and DPWG may stem from the recent emergence of data showing increased adverse effects with long-term PPI use.

Response of CYP2C19 Rapid and Ultrarapid Metabolizers

Individuals with increased CYP2C19 enzyme activity may experience subtherapeutic exposure to standard doses of omeprazole, compared with individuals with normal enzyme function (42). Several studies have reported an association between CYP2C19 ultrarapid metabolizers and incomplete acid suppression ("PPI resistance") and a decreased rate of eradication of *H. pylori* (37, 43, 44, 45). Based on the concerns of *CYP2C19* influenced treatment failure, recent CPIC guidelines recommend an increase in daily PPI dose by 100% in ultrarapid metabolizers and 50–100% in rapid metabolizers (4). The DPWG guidelines recommend that up to a 3-fold increase in dosage can be utilized for ultrarapid metabolizers for *H. pylori* eradication (2), this recommendation is based on review of pharmacokinetics reports for rapid and ultrarapid metabolizers. For more information on DPWG recommendations and justifications, please see (3).

Genotype-guided therapy for PPI administration has been shown to have higher success rates than empirical or standard dosing. Rapid and ultrarapid metabolizers with genotype-guided dosing showed a higher rate of efficacy in *H. pylori* eradication following triple therapy than standard dosing (46). Combined testing for bacterial drug-resistance and *CYP2C19* genotype also shows promise for improving therapeutic efficacy in the pediatric population (47).

The *CYP2C19* Gene Interactions with Medications Used for Additional Indications

Genetic variation in the *CYP2C19* gene influences the metabolism of other medications used for the treatment of several conditions such as:

- Acute coronary syndrome –– individuals who are CYP2C19 poor metabolizers and undergoing percutaneous coronary intervention have an increased risk of cardiovascular events if they are treated with the antiplatelet drug clopidogrel (a pro-drug that is activated by CYP2C19-mediated metabolism).
- Depression –– CYP2C19 influences the metabolism of tricyclic antidepressants (amitriptyline, imipramine); SSRIs (citalopram) and other serotonin receptor agonists (flibanserin). Individuals who are CYP2C19 poor metabolizers may have an increased risk of side effects, whereas CYP2C19 ultrarapid metabolizers may have an increased risk of treatment failure.
- Epilepsy — Brivaracetam, lacosamide, and clobazam are antiseizure drugs that are metabolized by CYP2C19, and poor metabolizers are at an increased risk of adverse events due to higher drug plasma concentrations. Diazepam is another drug with indications for seizure management that is metabolized by CYP2C19.
- Muscle pain—Carisoprodol is a muscle relaxant that is metabolized by CYP2C19, and poor metabolizers are at an increased risk of adverse events due to higher plasma concentration of carisoprodol, accompanied by lower active metabolite concentrations.
- Anti-fungal treatment—Voriconazole is a broad spectrum anti-fungal agent that is metabolized by CYP2C19 and both ultrarapid and poor metabolizers may require alternative medications.

Additional information on gene-drug interactions for *CYP2C19* are available from PharmGKB, CPIC and the FDA (search for "CYP2C19").

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are available for the omeprazole response and the *CYP2C19* gene.

Individual results are typically reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation with the predicted metabolizer phenotype (ultrarapid, rapid, normal, intermediate, or poor). Table 3 summarizes common CYP2C19 phenotypes.

In 2018, the Association for Molecular Pathology published recommendations for *CYP2C19* genotyping allele selection. The recommendations determined varying tiers of alleles, based on the strength of evidence supporting drug-response, minor allele frequencies and availability of reference materials. The Association's tier 1 group represent the core alleles recommended for genotyping panels: **2, *3,* and **17* (48). These guidelines provide information for laboratories performing *CYP2C19* genotype testing and are a useful complement to CPIC prescribing recommendations.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Asian Population

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

In studies of healthy subjects, Asians had approximately a four-fold higher exposure than Caucasians. Dosage reduction of Omeprazole delayed-release capsules to 10 mg once daily is recommended for Asian patients for maintenance of healing of erosive esophagitis.

[…]

Pharmacogenomics

CYP2C19, a polymorphic enzyme, is involved in the metabolism of omeprazole. The *CYP2C19*1* allele is fully functional while the *CYP2C19*2* and **3* alleles are nonfunctional. There are other alleles associated with no or reduced enzymatic function. Patients carrying two fully functional alleles are normal metabolizers and those carrying two loss-of-function alleles are poor metabolizers. In normal metabolizers, omeprazole is primarily metabolized by CYP2C19. The systemic exposure to omeprazole varies with a patient's metabolism status: poor metabolizers > intermediate metabolizers > normal metabolizers. Approximately 3% of Caucasians and 15 to 20% of Asians are CYP2C19 poor metabolizers.

In a pharmacokinetic study of single 20 mg omeprazole dose, the AUC of omeprazole in Asian subjects was approximately four-fold of that in Caucasians.

[…]

Interaction with Clopidogrel

Avoid concomitant use of Omeprazole delayed-release capsules with clopidogrel. Clopidogrel is a prodrug. Inhibition of platelet aggregation by clopidogrel is entirely due to an active metabolite. The metabolism of clopidogrel to its active metabolite can be impaired by use with concomitant medications, such as omeprazole, that inhibit CYP2C19 activity. Concomitant use of clopidogrel with 80 mg omeprazole reduces the pharmacological activity of clopidogrel, even when administered 12 hours apart. When using Omeprazole delayed-release capsules, consider alternative anti-platelet therapy.

[…]

Clinically relevant interactions affecting Omeprazole delayed-release capsules when co-administered with other drugs

CYP2C19 or CYP3A4 Inducers	
Clinical Impact:	Decreased exposure of omeprazole when used concomitantly with strong inducers
Intervention:	St. John's Wort, rifampin: Avoid concomitant use with Omeprazole delayed-release capsules Ritonavir-containing products: see prescribing information for specific drugs.
CYP2C19 or CYP3A4 Inhibitors	
Clinical Impact:	Increased exposure of omeprazole
Intervention:	Voriconazole: Dose adjustment of Omeprazole delayed-release capsules are not normally required. However, in patients with Zollinger-Ellison syndrome, who may require higher doses, dose adjustment may be considered. See prescribing information for voriconazole.

Please review the complete therapeutic recommendations located here: (1).

2020 Summary of recommendations from the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for *CYP2C19* and Proton Pump Inhibitor Dosing

Therapeutic Recommendations

Table 2 summarizes therapeutic recommendations for PPI prescribing in adults and pediatric patients based on CYP2C19 phenotype, specifically for the first-generation PPIs (omeprazole, lansoprazole, pantoprazole) and dexlansoprazole. These recommendations apply to both oral and intravenous PPI use. While CYP2C19 [normal metabolizers] NMs are expected to have normal PPI metabolism and clearance, a large body of literature from studies in Asian populations reported an association between CYP2C19 NMs and decreased therapeutic effectiveness with these PPIs (e.g., failure to eradicate *H. pylori* infection and lower healing rates of erosive esophagitis) compared to CYP2C19 [intermediate metabolizers] IMs and [poor metabolizers] PMs (Tables S1-S4). Therefore, for CYP2C19 NMs, initiating these PPIs at standard daily doses (e.g., label recommended doses) is generally recommended; however, for *H. pylori* infection or erosive esophagitis, clinicians may consider increasing the recommended dose for these indications by 50- 100% to optimize therapeutic efficacy.

[…]

It has been suggested that continued inhibition of acid secretion in individuals taking PPIs chronically who are genotyped as CYP2C19 IMs or PMs may have a higher risk of PPI-related adverse events compared to NM, [rapid metabolizer] RM, or [ultrarapid metabolizer] UM phenotypes (1). While the current data are insufficient to make strong dosing recommendations, potential associations of CYP2C19 phenotype and incidence of adverse events (e.g., infections) are emerging (24). Therefore, for CYP2C19 IMs and PMs, it is recommended to initiate standard daily dosing to maximize the likelihood of efficacy and, once efficacy is achieved, consider a 50% reduction in the daily dose in the setting of chronic PPI therapy (beyond 12 weeks) to minimize the risk of adverse events from prolonged acid suppression. If a dose reduction is made, monitoring for continued efficacy is recommended. Additional studies that investigate the relationship between *CYP2C19* genotype and incidence of PPI-related adverse events are needed.

The RM and UM phenotypes are driven by the presence of the increased function *CYP2C19*17* allele. Due to the relatively recent discovery of this variant (11) and because the majority of studies describing associations between *CYP2C19* genotype, pharmacokinetics, and pharmacodynamics of PPIs were conducted in Asian populations in whom the *CYP2C19*17* allele occurs less frequently, there are limited data on the relationship between *CYP2C19*17*, pharmacokinetic parameters, acid secretion indices and therapeutic outcomes in CYP2C19 RMs and UMs. … Therefore, it is recommended to increase the starting daily dose by 100% in CYP2C19 UMs. For RMs, standard dosing should be initiated, but a 50-100% dose increase could be considered for the treatment of H. pylori infection and erosive esophagitis to maximize the likelihood of therapeutic plasma concentrations and therapeutic effect. These patients should be monitored for efficacy.

[…]

Pediatrics

The *CYP2C19*-guided PPI recommendations presented in Table 2 also apply to pediatric patients.

Please review the complete therapeutic recommendations located here: (4).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 Poor Metabolizer

NO action is required for this gene-drug interaction.

The higher plasma concentration of omeprazole results in an increase in the therapeutic effectiveness, without an increase in the side effects.

CYP2C19 Intermediate Metabolizer

NO action is required for this gene-drug interaction.

The higher plasma concentration of omeprazole results in an increase in the therapeutic effectiveness, without an increase in the side effects.

CYP2C19 Ultrarapid Metabolizer

The genetic variation may lead to a reduced omeprazole plasma concentration and therefore reduced effectiveness.

Recommendation:

For *Helicobacter pylori* ERADICATION THERAPY:

- use a 3-fold higher dose
- advise the patient to report persisting symptoms of dyspepsia

OTHER INDICATIONS:

- be alert to reduced effectiveness
- if necessary, use a 3-fold higher dose
- advise the patient to contact their doctor if symptoms of dyspepsia persist

Background information

Mechanism:

Omeprazole is primarily converted by CYP2C19 to inactive metabolites.

Omeprazole inhibits CYP2C19 and therefore its own metabolism. This leads to non-linear pharmacokinetics. The AUC response to dose increases is greater than linear in normal metabolizer patients at doses exceeding 40 mg.

For more information about the UM phenotype: see the general background information about CYP2C19 on the KNMP Knowledge Bank or on www.knmp.nl (search for CYP2C19).

Please review the complete therapeutic recommendations that are located here: (2, 3).

Nomenclature for Selected *CYP2C19* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.4:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.4:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893
*CYP2C19*4*	1A>G Met1Val	NM_000769.4:c.1A>G	NP_000760.1:p.Met1Val	rs28399504
*CYP2C19*5*	90033C>T Arg433Trp	NM_000769.4:c.1297C>T	NP_000760.1:p.Arg433Trp	rs56337013
*CYP2C19*6*	12748G>A Arg132Gln	NM_000769.4:c.395G>A	NP_000760.1:p.Arg132Gln	rs72552267

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*7*	19294T>A	NM_000769.4:c.819+2T>A	(Splice donor variant)	rs72558186
*CYP2C19*8*	12711T>C Trp120Arg	NM_000769.4:c.358T>C	NP_000760.1:p.Trp120Arg	rs41291556
*CYP2C19*9*	12784G>A Arg144His	NM_000769.4:c.431G>A	NP_000760.1:p.Arg144His	rs17884712
*CYP2C19*17*	-806C>T	NM_000769.4:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

Note: when no variants are detected the genotype is designated as *CYP2C19*1* and is considered the normal "wild-type" allele.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (49).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Previous Versions

To view the version of this Summary from 8 March 2016, please click here.

To view the version of this Summary from 18 March 2013, please click here.

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Oxycodone Therapy and *CYP2D6* Genotype

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Introduction

Oxycodone (brand names OxyContin, Roxicodone, Xtampza ER, and Oxaydo), is an opioid analgesic used for moderate to severe pain caused by various conditions for which alternative analgesic treatments are inadequate. (1) Oxycodone exerts its analgesic affects by binding to the mu-opioid receptors (MOR) in the central and peripheral nervous system. While it is an effective pain reliever, this agent also has a high potential for addiction, abuse, and misuse.

Oxycodone is metabolized by members of the cytochrome P450 (CYP) enzyme superfamily. The CYP3A4, CYP3A5, and CYP2D6 enzymes convert oxycodone to either less-active (CYP3A4 and CYP3A5) or more-active (CYP2D6) metabolites. Most of the analgesic effect is mediated by oxycodone itself, rather than its metabolites. Variation at the *CYP3A4* and *CYP3A5* loci leading to altered enzyme activity is rare. A handful of altered-function alleles are known, but there is no documented evidence to support altered oxycodone response in the presence of these variant alleles. The FDA approved drug label for oxycodone cautions that co-medication with CYP3A inhibitors or inducers may lead to altered pharmacokinetics and analgesia, but does not discuss genotype-based recommendations for prescribing (1).

Genetic variation at the *CYP2D6* locus has conflicting evidence regarding altered response of individuals to oxycodone therapy. Thus, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has determined that there is insufficient evidence to recommend alterations to standard clinical use based on *CYP2D6* genotype (2). Similarly, the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) recognizes the drug-gene interaction between *CYP2D6* and oxycodone but states that the interaction does not affect analgesia achieved by the medication (3, 4). The PharmGKB online resource reports that drug labels in Switzerland (regulated by Swissmedic) state that *CYP2D6* variation can alter oxycodone response (5, 6).

Interactions among drugs from polypharmacy may be further enhanced by genetic variation, but there are no professional recommendations to alter prescribing based on drug-drug-gene interactions. Regardless of genotype, oxycodone is contraindicated in individuals with significant respiratory depression, acute or severe bronchial asthma, known or suspect gastrointestinal obstruction, or known hypersensitivity to the medication (1).

Drug: Oxycodone

Oxycodone is a semi-synthetic derivative of thebaine, belonging to the drug class of opioid agonists. It is used to treat both chronic and acute pain of moderate to severe intensity when alternative treatments are inadequate. (1) Oxycodone has a similar half-life (2–4 hours) as morphine and approximately twice the bioavailability (7, 8). However, oxycodone is 4-fold less potent than morphine as a MOR agonist, with similar receptor activation efficiency (9). Oxycodone is approximately twice as analgesic as morphine, perhaps reflecting the increased bioavailability, but may be less effective for some pain conditions, such as diabetic neuropathy (10, 11).

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However, oxycodone has a high abuse potential (11, 12). It is one of the most widely abused opioid analgesics, with increased abuse reported among all ethnic and economic groups since the 1960s (12, 13). Oxycodone is classified as a Schedule II substance by the US Drug Enforcement Agency (DEA) due the high potential for abuse leading to psychological or physical dependance (1, 13). The factors predisposing any individual to addiction are complex and as such the risk of opioid addiction should be assessed on a case-by-case basis. Clinicians are advised to ensure that the analgesic benefits outweigh the addiction, abuse, or misuse risks for each individual. A risk evaluation and mitigation strategy educational program may be offered as a part of continuing education for prescribing clinicians. (1) Clinicians should be advised that naloxone, an opioid antagonist medication is available to counter opioid overdose and individuals taking oxycodone should be at least aware of this medication. Current guidelines from the Substance Abuse and mental Health Services Administration (SAMHSA) recommend naloxone prescription to anyone on high doses of opioids or using long acting/extended release opioids.(14)

Oxycodone has multiple administration modalities, including intravenous, epidural, rectal, or oral. Oral formulations come in the form of liquid medications or tablets for immediate or extended release. Regardless of administration route, the pharmacokinetics are dose dependent, and most of the oxycodone metabolism occurs in the liver.

Oxycodone is metabolized by members of the CYP enzyme superfamily, with a small amount of oxycodone being excreted without undergoing metabolic processing. Most of the hepatic oxycodone metabolism (roughly 45–50% of the total dose) is performed by the CYP3A enzymes (CYP3A4 and CYP3A5) to form noroxycodone, a largely inactive metabolite. (11, 12, 15) Approximately 10–19% of an oxycodone dose is also metabolized by the CYP2D6 enzyme to form a potent opioid oxymorphone. Oxycodone and its metabolites can be further reduced or can undergo glucuronidation by UDP-glucuronosyltransferase (UGT) enzymes. (11)

Inhibition of the CYP2D6 enzyme by concomitant medications (such as paroxetine) reduces oxycodone analgesia (16) due to reduced oxymorphone formation. Similarly, CYP3A inhibition results in increased oxycodone and oxymorphone exposure and analgesia (17); (16). Where CYP3A4 or CYP3A5 inhibition or induction is a concern due to multiple co-medications, oxymorphone can be substituted.

Many opioids undergo CYP2D6 metabolism to varying degrees. Oxycodone is an active analgesic with minimal metabolism by CYP2D6, whereas codeine and tramadol are pro-drugs that require activation by CYP2D6 and thus are more directly affected by *CYP2D6* enzyme activity. (18, 19) Another opioid, hydrocodone, is also metabolized by CYP2D6 into an active analgesic—hydromorphone—but like oxycodone, the parent and metabolite compounds can both provide analgesic effect, though with differing potency (2, 20, 21, 22).

Oxycodone and oxymorphone both activate MOR in the central nervous system and in peripheral tissues. At pharmacologically relevant concentrations oxycodone and oxymorphone act selectively through MOR, with oxymorphone being 8-fold more potent as a MOR activator than oxycodone. Unlike oxycodone, oxymorphone has also been shown to bind to delta and kappa opioid receptors, but the demonstrated affinities greatly exceed therapeutic plasma concentrations. Following oxycodone administration, oxymorphone has been reported to only account for a small portion of total opioid exposure, while the parent, oxycodone, accounts for roughly 90% of the total analgesic effect (23). Conversely, some experts have concluded that the small amount of oxymorphone produced following oxycodone administration may account for most of the analgesic effect (15). The relative role of the parent and oxymorphone metabolite may depend on the route of drug administration with more oxymorphone being produced following oral dosing than seen with parenteral dosing (8). In addition, oxymorphone is a higher potency MOR agonist and exhibits a longer half-life than oxycodone—it is the predominate MOR activator 6 hours after oxycodone dosing. Given the conflicting views regarding the contribution of oxymorphone to analgesia following oxycodone administration, the role of CYP2D6 activity in an individual's response to oxycodone is also debated and is discussed further below. In contrast, the CYP3A

metabolite noroxycodone, exhibits a 3-fold lower reduced binding affinity than the parent and appears to be an antagonist/very weak partial agonist. (11)

Clearance of oxycodone, either unmodified or following its metabolism by cytochrome P450 enzymes and UGT enzymes, is partially dependent upon renal function, hepatic metabolism, and seems to vary with age and gender. The plasma half-life for oxycodone is 3–5 hours in healthy adults(12), it decreases by 25% in geriatric individuals (24). Plasma protein binding of oxycodone is approximately 45% and so unlikely to be a significant variable affecting free oxycodone exposure in the elderly (25). Repetitive bolus simulations suggest that geriatric individuals have a 20% higher exposure and thus some increased risk of adverse effects from oxycodone at standard dosage (1). In neonates the clearance rate increases from birth to 6 months (11) .

Oxycodone use can cause life-threatening or fatal respiratory depression. Risk of this adverse reaction is greatest at initiation of therapy, following a dose increase, or due to initiation or cessation of co-medications that alter CYP2D6 or CYP3A enzyme activities. Accidental ingestion by children can result in respiratory depression and death following a single dose of oxycodone. Respiratory depression risk is also elevated for individuals who are elderly, cachectic or debilitated due to altered pharmacokinetics and drug clearance (1).

Medications that inhibit CYP3A or CYP2D6 enzyme activities will result in an increased exposure to oxycodone. Notably, CYP3A4 inhibitors such as macrolide antibiotics, azole-antifungals and protease inhibitors may prolong opioid adverse reactions. Furthermore, discontinuing a CYP3A inducer—rifampin, carbamazepine, or phenytoin—can also increase oxycodone exposure. The FDA approved drug label specifically notes the importance of CYP3A4- associated drug interactions in the black box warning on the drug label (1).

The FDA recommends monitoring for serotonin syndrome symptoms if concomitant use is warranted with oxycodone and selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), or other drugs that affect the serotonin neurotransmitter system (1, 26). Furthermore, the FDA approved label says to discontinue oxycodone if serotonin syndrome is suspected and advises against the use of oxycodone with monoamine oxidase inhibitors (MAOIs) or for 14 days following the completion of MAOI therapy (1).

The use of oxycodone during pregnancy can result in opioid withdrawal symptoms in the neonate, which can be life threatening. There are insufficient data to determine if oxycodone use during pregnancy leads to increased rates of birth defects or miscarriages. However, animal studies suggest that the neonate may experience adverse neurobehavioral effects following in utero exposure. Additionally, data suggests that oxycodone crosses the placenta and maternal plasma levels correlate with neonate exposure (1, 11). Chronic opioid use may cause reduced fertility in both males and females that is potentially irreversible (1).

Opioids are present in breastmilk, though one study estimated that an exclusively breast-fed infant would receive, at most, 8% of the maternal weight-adjusted dose (1, 26). However, infants are particularly sensitive to opioids and are at a significant risk for respiratory depression. As such, other analgesics are preferred over oxycodone in breastfeeding mothers (26).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity. CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers (27).

The CYP2D6 Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either increased, normal, decreased, or absent enzyme function (Table 1). (28)

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on their impact on enzyme function, each allele can be assigned an activity score from 0 to 1, which in turn is then used to assign a phenotype (for example, CYP2D6 PM). However, the activity score system is not standardized across all clinical laboratories or *CYP2D6* genotyping platforms. The CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is predicted from the diplotype activity score defined by the sum of the allele score values, which usually ranges from 0 to 3.0: (29)

- An ultrarapid metabolizer (UM) has an activity score greater than 2.25
- A normal metabolizer phenotype (NM) has an activity score of 1.25–2.25
- An intermediate metabolizer (IM) has an activity score of >0–<1.25
- A poor metabolizer (PM) has an activity score of 0

Table 1. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See PharmVar. Activity scores from (29).

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the NM phenotype. The *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (30, 31, 32, 33) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (34, 35, 36) (see Table 1). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in individuals with European ancestry, **17* more common in Africans, and **10* more common in Asians. (37)

Larger structural variants at the *CYP2D6* locus have also been described, including gene duplications, deletions, tandem alleles, and gene conversions. As one might expect, deletions result in a no-function allele (for example, the **5* allele is a deletion). Duplications have been reported for alleles with normal function and decreased function, as well. In the case of allele duplications, the activity scores for the full complement of *CYP2D6* alleles are summed to determine the predicted metabolizer phenotype. Additional details on structural variants are available from PharmVar (38).

The frequency of the *CYP2D6* star alleles with altered function varies across global populations, resulting in different frequencies of the resulting metabolizer phenotype(s). Given CYP2D6's role in metabolism of many drugs, the literature on allele and phenotype frequency is expansive. Most populations have a high frequency for normal-function star alleles and thus a high proportion of the population are NMs. However, reduced-function alleles like *CYP2D6*10* are highly prevalent in east and southeast Asian populations, leading to a higher proportion of IM phenotype individuals in this ancestral group. Many nations in sub-Saharan Africa have higher

frequencies of decreased-function alleles like *CYP2D6*17* and **29*, which can correlate with lower metabolizer scores in these individuals. More details regarding published allele and phenotype frequencies are available in the CYP2D6 supplemental chapter.

Pharmacologic Conversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and thus the metabolizer phenotype of any individual. Administration of multiple drugs, sometimes called polypharmacy or co-medications, can lead to a phenomenon called phenoconversion whereby an individual with one metabolizer genotype can have the enzymatic activity of a different metabolizer group (higher or lower, depending on the medications). The enzymatic activity of CYP2D6 can be inhibited or reduced by medications including duloxetine, paroxetine, fluoxetine, bupropion, and quinidine (21, 39, 40, 41). This can result in NMs or IMs responding to medications as if they were PMs. Thus, co-medication with multiple CYP2D6 strong or moderate inhibitors may result in reduced metabolism of drug substrates. In contrast, discontinuing a co-medication can increase the rate of CYP2D6 metabolism.

Other Genes of Note

The *CYP3A4* and *CYP3A5* Genes

Other cytochrome P450 enzymes are involved in the metabolism of oxycodone. The CYP3A enzymes, encoded by *CYP3A4* and *CYP3A5*, perform most of the oxycodone metabolism. Similar to CYP26, the CYP3A enzymes are also susceptible to phenoconversion due to medications that inhibit or activate these enzymes, as described above. (1)

Variation at the *CYP3A4* locus is relatively uncommon and CPIC has not assigned a functional status to most variants (28). Although around 40 variant *CYP3A4* alleles have been reported, most have not been shown to alter CYP3A4 activity (42, 43). To date, only 3 loss-of-function *CYP3A4* alleles have been identified (*CYP3A4*6, CYP3A4*20* and *CYP3A4*26*) (Table 2) (44, 45).

The *CYP3A4*22* allele has decreased function and explains 12% of the variation in CYP3A4 activity (46). This variant is present in 3.2–10.6% of the Dutch population and 5.2–8.3% of the population in America (47). The Allele Frequency Aggregator project reports this reduced-function allele to be present in approximately 5% of the global population, with the lowest prevalence in Asian and African populations (48). The 1000 Genomes Project phase 3 data release estimates global prevalence to be slightly lower (~1%); a minor allele frequency of 5% is reported for the European average (49).

The *CYP3A4*20* allele has a premature stop codon that results in a loss-of-function of *CYP3A4*. It appears to be the most common *CYP3A4*-defective allele but is still relatively rare, with approximately 0.2% of European Americans and 0.05% African Americans who are heterozygous. However, in Spain, the *CYP3A4*20* allele is present in 1.2% of the population, and up to 3.8% in specific Spanish regions (44).

Table 2. Activity Status of Selected *CYP3A4* Alleles

Allele type[#]	*CYP3A4* alleles
Normal function	**1*
Decreased function	**22*
No function	**6*, **20*, **26*

For a comprehensive list of *CYP3A4* alleles, please see PharmVar.
[#]As of the date of publication, there is no "CPIC Clinical Function" assessment provided for the *CYP3A4* alleles within PharmVar. The activity status provided here is based on the literature and historic assessment. In the event of a discrepancy between the functional classifications provided herein and PharmVar's data, the authors defer to PharmVar and CPIC.

The *CYP3A5* locus has less than 10 known genetic variants. The *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7* alleles are important no-function alleles and the **1* allele is the normal-function allele (Table 3) (28). The combination of alleles present predicts either normal (homozygous **1*), intermediate (at least one copy of **1* or compound heterozygous for no-function alleles), or PM phenotypes (homozygous for a single no-function allele) (50, 51). The PM phenotypes have been seen more commonly in individuals identified as "White" than African American or Black (52, 53). The *CYP3A5*3* allele has been observed at a high frequency in Egyptian and Italian populations (54, 55).

Table 3. Activity Status of Selected *CYP3A5* Alleles

Allele type	*CYP3A5* alleles
Normal function	**1*
No function	**3, *6, *7*

For a comprehensive list of *CYP3A5* alleles, please see PharmVar.

The *OPRM1* Gene

The MOR is encoded by the *OPRM1* gene. The MOR is a G-coupled protein receptor and is a key signal transducer for the desired analgesic effect of opioids such as tramadol and codeine. There are more than 200 known variant alleles of *OPRM1*, and some variants have been suggested to have a role in opioid response or predisposition to opioid use disorders (56, 57). However, CPIC's expert review found inconsistent evidence linking any of these alleles to post-operative dose requirements for some opioids and the effect on morphine dose adjustment was deemed not to be clinically actionable (2).

The *COMT* Gene

The catechol-o-methyltransferase (COMT) enzyme is involved in the methylation and degradation of adrenaline, noradrenaline, and dopamine. This enzyme regulates the concentration of catecholamines and thus is a key regulator of the pain perception pathways (58). The variant rs4680 (p.Val158Met) in *COMT* has been suggested to result in decreased levels of methylation activity (2, 58). However, CPIC's review found variable evidence associating this variant with analgesia response or opioid dose requirements and thus makes no recommendations based on *COMT* genotype (2).

Linking Gene Variation with Treatment Response

Altered CYP2D6 enzyme activity has been associated with altered levels and ratios of oxycodone, noroxycodone, and oxymorphone levels in the blood; lower CYP2D6 activity correlated with a decrease in this ratio in plasma and urine (11, 59, 60, 61, 62). However, there are conflicting reports regarding the associated impact on analgesia or adverse outcomes.

Several studies report improved analgesia, or higher rates of adverse reactions, or both in individuals with higher levels of CYP2D6 activity. One study in 33 healthy volunteers reported a modest but significant decrease in analgesic effect of oxycodone in CYP2D6 PM genotyped individuals in 3 out of 5 tests. Genotyping in this study was limited to analysis of variants for *CYP2D6*3, *4, *6* and **9* with no detection of duplication nor deletion; individuals were classified as CYP2D6 PMs if they had 2 no-function alleles based on this limited genotyping. (63) A small study of 10 healthy volunteers reported a correlation between CYP2D6 activity and oxycodone analgesia, with CYP2D6 UM participants also reporting an increased incidence of negative side effects and more intense adverse reactions to oxycodone compared with other metabolizer phenotypes. This group also found inhibition of CYP2D6 by co-medication with quinidine reduced the peak analgesic effect along with the oxymorphone exposure. This study genotyped *CYP2D6* variation by microarray and thus reported testing for a total of 32 alleles, including the *CYP2D6*5* deletion and duplication of a subset of alleles (64, 65). A study of 121

post-operative individuals found a direct correlation between CYP2D6 predicted enzyme activity and oxymorphone/oxycodone ratio as well as higher oxycodone consumption in CYP2D6 PMs for 48 hours post-operative self-controlled analgesia, though the pain scores were similar across metabolizer groups. This study interrogated 8 defined *CYP2D6* alleles, including the *5 deletion and gene duplication (66). Similarly, Deodhar and colleagues support the phenotypic assessment of CYP2D6 activity when oxycodone is prescribed for pain management, which could include pharmacogenomics testing to enable identification of CYP2D6 PMs or evaluation of polypharmacy leading to phenoconversion or both. (15) Another recent review suggests that within European populations, individuals who are CYP2D6 UMs have an increased risk of adverse events and additionally noted the potential impact of phenoconversion due to CYP2D6 inhibitors, which may reduce analgesic effect (67).

In contrast, multiple studies suggest the differences in oxymorphone/oxycodone ratios due to CYP2D6 activity do not impact pain management or other symptoms. A larger study of 270 individuals who had recently undergone surgical procedures were genotyped for CYP2D6 and intravenous oxycodone use for 24 hours post-surgery was monitored. This study found no significant differences in the frequency of oxycodone non-responders between CYP2D6 PM and other metabolizer phenotypes, nor differences in average oxycodone consumption between the groups, indicating that CYP2D6 metabolism did not affect oxycodone analgesia even though oxymorphone/oxycodone ratios were lower in the CYP2D6 PMs group. It should be noted that genotyping in this study was limited to detection of the **3, *4, *6* and **9* alleles, **5* was specifically excluded in the analysis and the **1* allele was assigned in the absence of any detected variants (68). A similar study in 450 individuals who were being treated for cancer pain observed changes in oxymorphone/oxycodone ratios but no difference in pain intensity, nausea, tiredness nor cognitive function, however the scope of CYP2D6 genotyping in this study was limited and the **2* duplication, **3, *4, *5, *6, *7* and **8* alleles were the only alleles specifically examined (59).

Because of the conflicting and limited evidence for either CYP2D6 metabolizer phenotypes, COMT function, or OPRM1 function being involved with altered oxycodone response, both CPIC and DPWG have no recommendations regarding dosing or selection when oxycodone is considered (2, 3).

As reported by PharmGKB, the Swiss drug labels for oxycodone state that *CYP2D6* polymorphism can alter the efficacy of the medication or lead to undesired effects; "slow" metabolizers (PMs) may experience weaker analgesia and "ultra-fast" metabolizers (UMs) may have higher analgesia and increased risk of adverse effects (69).

A large study of urine drug test samples found an association between another member of the CYP450 family: CYP2C19. The CYP2C19 UMs had a higher oxymorphone/oxycodone ratio than PMs, like CYP2D6, suggesting that CYP2C19 may play a minor role in oxycodone metabolism. However, these observations warrant further research to determine if CYP2C19 genetic variations are associated with oxycodone response (60).

Genetic variation in the *CYP3A4* locus is exceedingly rare and has not been associated with altered oxycodone analgesia. However, many reports have stated that induction of CYP3A4 and CYP3A5 by co-medications such as rifampin and carbamazepine are associated with decreased analgesia, though at least one report found no effect by co-medication (11, 70).

Drug-drug interactions have been reported to influence treatment responses, most likely due to enzyme inhibition or induction. Adverse reactions were more common in elderly individuals taking oxycodone concomitantly with CYP2D6 or CYP3A4 inhibitor medications (71). Rifampin has been reported to reduce analgesia from multiple opioid medications, including oxycodone, though the data is limited (72).

Genetic Testing

Genetic testing is available for many (~30) of the variant *CYP2D6* alleles. Usually, an individual's result is reported as a diplotype, which includes one maternal and one paternal allele, for example, *CYP2D6 *1/*2*. When individuals have more than 2 copies of the *CYP2D6* allele, the copies are denoted by an "xN", for example, *CYP2D6*1/*2x2*. Some laboratories also use the notation of DUP to indicate an increase in copy number. Depending on the testing methodology and platform used, a laboratory may or may not be able to specify the number of duplicated CYP2D6 alleles nor the allele that has been duplicated.

Studies in oncology and cardiovascular surgical intervention have estimated that 25–56% of these populations may be prescribed oxycodone or other opioids to manage pain during their treatment; multiple authors recommend pharmacogenomic testing in these individuals to optimize management of pain or other symptoms (20, 73, 74).

Genetic tests for oxycodone response, the *CYP2D6* gene, the *CYP3A4* gene, and the *CYP3A5* gene can be found on the NIH Genetic Testing Registry (GTR). The available tests include targeted single-gene tests as well as multi-gene panels or genome-wide sequencing tests.

The test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be confirmed by checking the diplotype and calculating the CYP2D6 activity score, as described in the "*CYP2D6* Alleles" section above. Variant *CYP2D6* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (75).

Variants in other genes, such as *COMT* and *OPRM1*, may also influence an individual's response to oxycodone, though there are no established guidelines for dose alterations or drug selection based on genetic variation at any of the loci described in this summary.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2021 Statement from the US Food and Drug Administration (FDA):

Cytochrome P450 3A4 Interaction [drug-drug interactions]

The concomitant use of oxycodone hydrochloride tablets with all cytochrome P450 3A4 inhibitors may result in an increase in oxycodone plasma concentrations, which could increase or prolong adverse reactions and may cause potentially fatal respiratory depression. In addition, discontinuation of a concomitantly used cytochrome P450 3A4 inducer may result in an increase in oxycodone plasma concentration. Monitor patients receiving oxycodone hydrochloride tablets and any CYP3A4 inhibitor or inducer.

[…]

Drug interactions: Inhibitors of CYP3A4 and CYP2D6, Clinical Impact [drug-drug interactions]

The concomitant use of oxycodone hydrochloride and CYP3A4 inhibitors can increase the plasma concentration of oxycodone, resulting in increased or prolonged opioid effects. These effects could be more pronounced with

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

concomitant use of oxycodone hydrochloride and CYP2D6 and CYP3A4 inhibitors, particularly when an inhibitor is added after a stable dose of oxycodone hydrochloride is achieved… After stopping a CYP3A4 inhibitor, as the effects of the inhibitor decline, the oxycodone plasma concentration will decrease, resulting in decreased opioid efficacy or a withdrawal syndrome in patients who had developed physical dependence to oxycodone.

[…]

Drug Interactions: CYP3A Inducers, Clinical Impact [drug-drug interactions]

The concomitant use of oxycodone hydrochloride and CYP3A4 inducers can decrease the plasma concentration of oxycodone, resulting in decreased efficacy or onset of a withdrawal syndrome in patients who have developed physical dependence to oxycodone. After stopping a CYP3A4 inducer, as the effects of the inducer decline, the oxycodone plasma concentration will increase, which could increase or prolong both the therapeutic effects and adverse reactions, and may cause serious respiratory depression.

[…]

Pharmacokinetics: Metabolism

A high portion of oxycodone is N-dealkylated to noroxycodone during first-pass metabolism, and is catalyzed by CYP3A4. Oxymorphone is formed by the O-demethylation of oxycodone. The metabolism of oxycodone to oxymorphone is catalyzed by CYP2D6.

Please review the complete therapeutic recommendations that are located here: (1).

2021 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

There is insufficient evidence and confidence to provide a recommendation to guide clinical practice at this time for oxycodone or methadone based on *CYP2D6* genotype or *COMT* genotype or *OPRM1* genotype (Tables S5 and S6, no recommendation, CPIC level C).

Please review the complete therapeutic recommendations that are located here: (2).

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 IM: oxycodone[e]

[and] CYP2D6 PM: oxycodone[e]

NO action is required for this gene-drug interaction.

The reduced conversion of oxycodone to the more active metabolite oxymorphone does not result in reduced analgesia for patients.

CYP2D6 UM: oxycodone[e]

NO action is required for this gene-drug interaction.

The increased conversion of oxycodone to the more active metabolite oxymorphone does not result in an increase in side effects in patients.

Please review the complete therapeutic recommendations that are located here: (3, 4)

Nomenclature for Selected Alleles

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*3*	2550delA	NM_000106.6:c.775del	NP_000097.3:p.Arg259fs	rs35742686
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Gene deletion			
*CYP2D6*6*	1707 del T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*17*	1022C>T	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*36*[1]	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2989G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

Nomenclature of Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*49*	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] *CYP2D6*36* is a gene conversion with *CYP2D7*; variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.

Nomenclature of Selected *CYP3A4* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A4*6*	17661_17662insA 277Frameshift	NM_017460.5:c.830_831insA	NP_059488.2:p.Asp277Glufs	rs4646438
*CYP3A4*20*	1461_1462insA 488Frameshift	NM_017460.5:c.1461dup	NP_059488.2:p.Pro488Thrfs	rs67666821
*CYP3A4*22*	15389C>T	NM_017460.6:c.522-191C>T	Not applicable—variant occurs in a non-coding region	rs35599367
*CYP3A4*26*	17642C>T R268Stop	NM_017460.6:c.802C>T	NP_059488.2:p.Arg268Ter	rs138105638

*CYP3A4*1.001* is the wild-type allele and is determined to be present with no variants are detected.

Nomenclature of Selected *CYP3A5* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP3A5*3*	6981A>G	NM_000777.5:c.219-237A>G	Not applicable—variant occurs in a non-coding region	rs776746
*CYP3A5*6*	14685G>A	NM_000777.5:c.624G>A	NP_000768.1:p.Lys208= (Alters mRNA splicing)	rs10264272
*CYP3A5*7*	27126_27127insT	NM_000777.5:c.1035dup	NP_000768.1:p.Thr346fs	rs41303343

*CYP3A5*1* is the wild-type allele and is determined to be present with no variants are detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (76).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium; the authors defer to that authority with regards to any discrepancies in allele definitions.

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Panitumumab Therapy and *RAS* and *BRAF* Genotype

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Introduction

Panitumumab (brand name Vectibix) is a monoclonal antibody used for the treatment of metastatic colorectal cancer (mCRC). Panitumumab is an epidermal growth factor receptor (EGFR) antagonist, which works by blocking the growth of cancer cells. It is administered every 14 days as an intravenous (IV) infusion, often with chemotherapy. Panitumumab is approved for first-line therapy with folinic acid, fluorouracil, and oxaliplatin (FOLFOX) and as monotherapy following disease progression after prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy (1).

The location of the primary tumor correlates whether an individual with mCRC is likely respond to anti-EGFR therapy. Individuals with left-sided tumors are more likely to respond well to anti-EGFR therapy and have a better prognosis. Individuals with right-sided tumors have a worse prognosis and respond poorly to anti-EGFR therapy. However, only the genetic variation status of the tumor, and not the location of the tumor, is discussed in the FDA drug label's dosing recommendations.

Resistance to panitumumab is associated with specific RAS mutations. The RAS is a family of oncogenes that includes the *KRAS* and *NRAS* genes. When mutated, these genes have the ability to transform normal cells into cancerous cells by providing a continual growth stimulus to cells. The *KRAS* mutations are particularly common, being detectable in 40% of metastatic colorectal tumors.

The *KRAS* mutations often lead to constitutive activation of the EGFR and are associated with resistance to anti-EGFR drugs such as panitumumab. Mutations in *NRAS* and another gene, *BRAF*, have also been associated with poor response to anti-EGFR therapy.

The 2017 FDA-approved label states that panitumumab is indicated for wild-type RAS (no mutations in either *KRAS* or *NRAS*) mCRC (Table 1). The label states that an FDA-approved test must be used to confirm the absence of RAS mutations before starting panitumumab, and that panitumumab is not indicated for the treatment of individuals with colorectal cancer with RAS mutations (in either *NRAS* or *KRAS*), or when the RAS genetic variation status is unknown (1).

Similarly, the 2015 Update from the American Society of Clinical Oncology (ASCO) states that anti-EGFR therapy should only be considered for the treatment of individuals whose tumor is determined to not have variations detected after extended RAS testing (Table 2) (2).

The 2020 National Comprehensive Cancer Network (NCCN) guideline also strongly recommends *KRAS/NRAS* genotyping of tumor tissue in all individuals with mCRC. In addition, the guideline states the V600E mutation in the *BRAF* gene makes a response to panitumumab highly unlikely, unless given with a BRAF inhibitor (Table 3) (3).

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Table 1. The FDA Drug Label for Panitumumab: Dosage and Administration (2017)

Genes to be tested	Recommendations for metastatic colorectal cancer
KRAS *NRAS*	Panitumumab is not indicated for the treatment of individuals with colorectal cancer that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either *KRAS* or *NRAS* and hereafter is referred to as "RAS". Prior to initiation of treatment with panitumumab, assess RAS mutational status in colorectal tumors and confirm the absence of a RAS mutation in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of both *KRAS* and *NRAS*. Information on FDA-approved tests for the detection of *KRAS* mutations in individuals with metastatic colorectal cancer is available at: http://www.fda.gov/CompanionDiagnostics.

This FDA table is adapted from (1).

Table 2. The ASCO RAS Mutational Testing of Colorectal Carcinoma Tissue (2015)

Genes to be tested	Recommendation
KRAS *NRAS*	RAS mutational testing of colorectal carcinoma tissue should be performed for all individuals who are being considered for anti-EGFR monoclonal antibody therapy (cetuximab and panitumumab). Before treatment with anti-EGFR antibody therapy, individuals with mCRC should have their tumor tested for mutations in: • *KRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) • *NRAS* exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146) Anti-EGFR antibody therapy should only be considered for treatment of individuals with metastatic colorectal carcinoma who are identified as having tumors with no mutations detected after such extended RAS mutation analysis.

This ASCO table is adapted from (2). EGFR, epidermal growth factor receptor; mCRC, metastatic colorectal cancer; ASCO, American Society of Clinical Oncology

Table 3. The NCCN *KRAS*, *NRAS*, and *BRAF* Mutation Testing (2020)

Genes to be tested	Recommendations for colorectal cancer
KRAS *NRAS*	All individuals with metastatic colorectal cancer should have tumor tissue genotyped for RAS (KRAS and NRAS) and BRAF mutations. Individuals with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab.
BRAF	BRAF V600E mutation makes response to cetuximab or panitumumab highly unlikely unless given with a BRAF inhibitor.

This NCCN table is adapted from (3). NCCN, National Comprehensive Cancer Network

Drug: Panitumumab

Panitumumab is an EGFR antagonist and is used for the treatment of mCRC. Panitumumab, and the related drug cetuximab (brand name Erbitux), are monoclonal antibodies that specifically target the extracellular domain of EGFR. They act by blocking endogenous ligand binding to the extracellular domain of EGFR, and by enhancing receptor internalization and degradation (4).

Panitumumab is a fully human monoclonal antibody, whereas cetuximab a chimeric monoclonal antibody, being composed of regions of both murine and human antibody. Both drugs have been shown to provide a clear clinical benefit in the treatment of RAS wild-type mCRC (5, 6).

Colorectal cancer is the second leading cause of cancer death for men and women in the US, and the second in Europe (7, 8). Surgery is the most common treatment for localized colorectal cancer that has not spread.

Chemotherapy alone, or with radiation, is given before (neoadjuvant) or after (adjuvant) surgery to most individuals with cancer that has penetrated the bowel wall deeply or spread to the lymph nodes (9). In the context of localized colon cancer, surgery first with adjuvant chemotherapy is standard. For localized rectal cancer, chemotherapy and chemoradiation can be used before or after surgery; standard therapy is either chemoradiation followed by surgery and adjuvant chemotherapy or chemoradiation and chemotherapy followed by surgery (10). National Comprehensive Cancer Network (NCCN) guidelines state there is a lack of definitive data or clear benefit from multiple types of adjuvant therapy for stage II or III colon cancer (3).

Treatment regimens for advanced or metastatic colorectal carcinoma include drugs such as folinic acid, fluorouracil, irinotecan, capecitabine, and oxaliplatin. Targeted biological agents may be added to such regimens, such as panitumumab, cetuximab, bevacizumab, ziv-aflibercept and ramucirumab. Bevacizumab (brand name Avastin) is a monoclonal antibody that targets vascular endothelial growth factor, VEGF. The NCCN guidelines provide further detail regarding which combinations are recommended based on tumor pathology (3).

Panitumumab is used with FOLFOX (FOLinic acid, Fluorouracil, and OXaliplatin) as a first-line treatment. It can also be combined with second-line chemotherapy (11). Additionally, it is used a single agent (monotherapy) following disease progression after chemotherapy including fluoropyrimidine, oxaliplatin, and irinotecan (with or without anti-VEGF therapy) (12, 13, 14, 15, 16). Although panitumumab is not indicated as first-line therapy in combination with irinotecan-based regimes, this may be an appropriate therapy for specific individuals (17, 18, 19). However, the NCCN advises against combining panitumumab with a bevacizumab-containing regimen due to inferior outcomes and toxicity (3, 20, 21).

Of note, the location of the primary colorectal tumor is a predictor of the prognosis for metastatic disease. Left-sided tumors derive from the embryonic hindgut (which gives rise to the splenic flexure, descending colon, sigmoid colon, rectum, and one-third of the transverse colon). Whereas right-sided tumors derive from the embryonic midgut (which gives rise to the appendix, cecum, ascending colon, hepatic flexure, and two-thirds of the transverse colon) (22). The ASCO recommends that individuals with left-sided tumors *versus* right-sided tumors are more likely to receive benefit of anti-EGFR therapy in a first-line, maximal-resourced setting (23).

Individuals with left-sided tumors benefit more from EGFR therapy than individuals with right-sided tumors. Panitumumab appears to have no meaningful activity for right-sided tumors, except perhaps where early response and tumor shrinkage is an important indicator (24). Right-sided tumors may respond to bevacizumab (25, 26, 27, 28, 29).

Administration of intravenous anti-EGFR therapy is associated with severe infusion reactions, including anaphylaxis (1% for panitumumab and 3% for cetuximab). Others include cardiopulmonary arrest, severe skin rashes (the severity of which may predict an increased response and survival (30, 31)), and an increased risk of venous thrombosis and embolism (2, 9)

Pregnant women and females of childbearing age should be advised that panitumumab can cause fetal harm. Based on data from animal studies (cynomolgus monkeys), panitumumab can be lethal to embryos. Therefore, females of reproductive potential should use effective contraception during panitumumab therapy, and for at least 2 months after the last dose of panitumumab. Individuals who know or suspect they are pregnant should inform their healthcare provider.

An important role in the progression of mCRC is thought to involve the impaired regulation of EGFR function, resulting in activation of the associated mitogen-activated protein kinase (MAPK) pathway. Panitumumab and cetuximab are important drugs in metastatic disease because they can block the activation of the MAPK pathway. However, drug resistance can arise through constitutive activation of the MAPK pathway, caused by variation in downstream signaling proteins, such as KRAS, NRAS and BRAF. Approximately 40% of cases of mCRC are found to have activating mutations in *KRAS* (32, 33).

The efficacy of panitumumab in treating mCRC is confined to individuals with wild-type *KRAS* tumors. Specifically, tumors that do not harbor specific mutations in exons 2, 3, and 4 of the *KRAS* gene. The *NRAS* gene is highly similar (homologous) to *KRAS*, and has mutations in the same exons—2, 3, and 4—which are also associated with a lack of response to panitumumab (33, 34, 35).

Therefore, expanded RAS testing (of KRAS and NRAS) is the standard of care to determine which individuals with mCRC will benefit from anti-EGFR therapy (36, 37).

Proto-oncogenes

Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene.

Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (9). Members of the RAS family and the *EGFR* gene are all proto-oncogenes.

The RAS family contains 3 genes, *HRAS*, *NRAS*, and *KRAS*, and they are essential components of signaling pathways. They act as signal transducers –– coupling cell surface receptors to intracellular signaling pathways.

The RAS proteins regulate cell signal transduction by acting as a switch –– they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily by the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Variation in *RAS* genes leads to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" –– providing a continual growth stimulus to cells. Such activating RAS mutations are common in colorectal cancers.

Gene: *KRAS*

The *KRAS* gene is the most frequently mutated RAS gene found in metastatic colorectal carcinoma. The most frequent individual mutations occur in *KRAS* exon 2, in codons 12 (G12D, G12V) and 13 (G13D). Collectively, these mutations account for more than 60% of all RAS mutations in mCRC (38). Individuals with mCRC that harbor *KRAS* mutations do not benefit from anti–EGFR therapy (either panitumumab or cetuximab therapy) (3, 5, 33, 34, 39).

Gene: *NRAS*

The *NRAS* gene is highly homologous to *KRAS*, and mutations have been reported in exons 2, 3, and 4. Although NRAS mutations are not as frequent as *KRAS* in mCRC, occurring in approximately 2% of tumors, *NRAS* influences the response to treatment with anti-EGFR drugs (2, 40, 41, 42).

Individuals with *NRAS*-mutated tumors are less likely to respond to panitumumab or cetuximab (34, 39, 43). Furthermore, panitumumab may even have a detrimental effect in individuals with *NRAS* or *KRAS* mutations (2, 39) .

Gene: *BRAF*

The RAF proteins are a family of serine/threonine kinases that are downstream effectors of KRAS, within the MAPK signaling pathway. The RAF family has 3 members, *ARAF*, *BRAF* and *CRAF* (44).

The *BRAF* mutations are detectable in approximately 5–15% of mCRC individuals. They tend to only occur in tumors that do not have *KRAS* exon 2 mutations (45). It is therefore unlikely that tumors with *KRAS* mutations will respond to either anti-BRAF treatment (which targets mutant BRAF) or anti-EGFR treatment (because of the presence of *KRAS* mutations) (46).

By far the most common *BRAF* mutation is known as V600E, which accounts for approximately 90% of *BRAF* mutations. The resulting BRAF V600E protein is constitutively active and is a highly potent oncogene, acting downstream in the EGFR pathway, thus bypassing inhibition of EGFR by panitumumab or cetuximab (7). Constitutively active BRAF can then activate the downstream kinases MEK1 and MEK2, which ultimately activate ERK kinases at the terminus of the MAP kinase signaling pathway (47).

The *BRAF* V600E mutation is associated with a poorer diagnosis for individuals with mCRC, as well as with resistance to anti-EGFR treatment. It is also possible that other *BRAF* mutations contribute to anti-EGFR resistance. In *BRAF* V600E-mutant mCRC, BRAF inhibition results in rapid feedback activation of EGFR, a likely mechanistic explanation for limited clinical utility of this monotherapy (48). Alternative treatments may include the use of drug combinations, such as the addition of a BRAF inhibitor to anti-EGFR, to overcome resistance (36, 49). Indeed, utilization of BRAF inhibitor therapy with anti-EGFR (with or without additional targeting of MEK kinases) showed improved survival in the BEACON trial, with the greatest overall survival in the group targeting BRAF, EGFR and MEK simultaneously (48, 50). Guidelines from the NCCN recommend this triple therapy as one approach for *BRAF* V600E mutation-positive disease (3). The NCCN guidelines recommend additional combination therapies for *BRAF* V600E positive CRC of either vemurafenib, irinotecan and anti-EGFR monoclonal antibodies (cetuximab or panitumumab) or dabrafenib, trametinib and anti-EGFR monoclonal antibodies (3).

The NCCN Colon/Rectal Cancer Panel states that evidence increasingly suggests that the *BRAF* V600E variant makes response to panitumumab or cetuximab, as single agents or with cytotoxic chemotherapy, highly unlikely unless it is also given with a BRAF inhibitor. Therefore, the panel recommends *BRAF* genotyping of tumor tissue (either primary tumor or metastasis) at diagnosis of stage IV disease (3).

Gene: *EGFR*

The human epidermal growth factor receptor (HER) family consists of 4 members: the *EGFR*, *ERBB2 (HER2)*, *ERBB3 (HER3)*, and *ERBB4 (HER4)*. All 4 members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation.

The EGFR is expressed in many different tissues, and is activated by the binding of a ligand, such as epithelial growth factor or transforming growth factor α. Binding induces receptor dimerization, either homodimers or heterodimers with other HER family members, and triggers autophosphorylation of the intracellular tyrosine kinase domain.

By activating downstream signaling pathways, EGFR has many different biological roles, including stimulating the cell cycle, cell growth, division, differentiation, as well as increased cell invasiveness, apoptosis, and angiogenesis. Therefore, overexpression of EGFR is thought to be an important step in tumor progression, making the EGFR a target for anticancer drugs (51, 52, 53).

There are 2 classes of drug that target EGFR: tyrosine kinase inhibitors (for example, gefitinib and erlotinib) and anti-EGFR monoclonal antibodies (for example, cetuximab and panitumumab) (4).

The EGFR is overexpressed in several cancers, including squamous cell carcinoma of the head and neck, squamous cell lung cancer, and colorectal cancer. The EGFR is overexpressed in approximately 50–80% of colorectal tumors (2, 51). However, for colorectal cancer, EGFR expression has not been associated with efficacy of anti-EGFR therapy (54).

The NCCN Colon/Rectal Cancer Panel states that EGFR testing of colorectal tumor cells has no proven predictive value in determining likelihood of response to either panitumumab or cetuximab. Therefore, the panel does not recommend routine EGFR testing, and states that no individual should be considered for or excluded from cetuximab or panitumumab therapy based on EGFR test results (3).

Gene: *HER2/ERBB2*

The HER2 receptor belongs to the same family of signaling kinase receptors as EGFR and is encoded by the gene *ERBB2*, also called *HER2*. Monoclonal antibodies that target HER2, such as pertuzumab and trastuzumab, are used in the treatment of breast cancer. However, HER2 is rarely expressed in colorectal tumors (approximately 3% overall), though the prevalence is higher in *RAS/BRAF* wild-type tumors (5–14%) (3). Initial evidence suggested that HER2 overexpression may be predictive of resistance to anti-EGFR therapy, yet some evidence suggested that HER2 status is not a biomarker for anti-EGFR response (36, 55) A recent review of HER2 retrospective studies found a consistent correlation between HER2 amplification and resistance to anti-EGFR treatment (56).

The NCCN Colon/Rectal Cancer Panel recommends *HER2* amplification/overexpression testing for individuals with mCRC. However, if the tumor is known to have a *RAS* or *BRAF* mutation, *HER2* testing is not required. Based on the outcome of *HER2* testing, the individual may be eligible for enrollment in one of the on-going clinical trials investigating targeted HER2 therapy in mCRC (3). The NCCN guidelines emphasize that *HER2* overexpression is not prognostic, but can be used to predict success of HER2-targeted therapy and resistance to anti-EGFR antibodies, including panitumumab (3).

Linking gene variation with treatment response

Specific mutations in the genes *KRAS* and *NRAS* result in resistance to panitumumab therapy. In addition, the presence of the *BRAF* V600E mutation makes a beneficial response to monotherapy treatment unlikely, combination therapy targeting BRAF and EGFR is recommended in these circumstances. Mutations in *EGFR* do not appear to be associated with panitumumab resistance. Initial results suggest *HER2* amplification may predict resistance to panitumumab (reviewed in (3)).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the panitumumab drug response, and the genes *KRAS*, *NRAS*, *BRAF*, *HER2*, and *EGFR*

The 2020 NCCN Guideline for Colon Cancer (Version 4.2020) provides the following recommendations for genetic testing:

KRAS, NRAS, and BRAF Mutation Testing

- All [individuals] with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* [variants] individually or as part of an NGS panel. [Individuals] with any known *KRAS* mutation (exon 2, 3, 4) or *NRAS* mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab. *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor.
- No specific methodology is recommended (e.g., sequencing, hybridization) for testing *KRAS*, *NRAS*, and *BRAF* mutations.

- The testing can be performed on formalin-fixed paraffin-embedded tissue. The testing can be performed on the primary colorectal cancers and/or the metastasis, as literature has shown that the *KRAS*, *NRAS*, and *BRAF* mutations are similar in both specimen types.

Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing

- Universal MMR* or MSI* testing is recommended in all newly diagnosed [individuals] with colon cancer. See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal (*IHC for MMR and DNA analysis for MSI are different assays and measure different biological effects caused by deficient MMR function)
- The presence of a *BRAF* V600E mutation in the setting of *MLH1* absence would preclude the diagnosis of Lynch syndrome (LS) in the vast majority of cases. However, approximately 1% of cancers with *BRAF* V600E mutation (and loss of MLH-1) are LS. Caution should be exercised in excluding cases with a strong family history from germline screening in the case of *BRAF* V600E mutations.
- Stage II MSI-H [individuals] may have a good prognosis […]
- Testing for MSI may be accomplished by polymerase chain reaction or a validated NGS panel, especially in [individuals] with metastatic disease who require genotyping of *RAS* and *BRAF* (3).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA):

Prior to initiation of treatment with panitumumab, assess RAS mutational status in colorectal tumors and confirm the absence of a RAS mutation. Information on FDA-approved tests for the detection of KRAS mutations in individuals with metastatic colorectal cancer is available at: http://www.fda.gov/CompanionDiagnostics.

[…]

Panitumumab is not indicated for the treatment of individuals with colorectal cancer that harbor somatic mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) of either KRAS or NRAS and hereafter is referred to as "RAS".

Please review the complete therapeutic recommendations that are located here: (1)

2015 Provisional Clinical Opinion from the American Society of Clinical Oncology (ASCO) and 2020 Late-Stage Colorectal Cancer ASCO Resource-Stratified Guidelines
All individuals with metastatic colorectal cancer who are candidates for anti-EGFR antibody therapy should have their tumor tested in a Clinical Laboratory Improvement Amendments–certified laboratory for mutations in both KRAS and NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146). The weight of current evidence indicates that anti-EGFR monoclonal antibody therapy should only be considered for treatment of individuals whose tumor is determined to not have mutations detected after such extended RAS testing.

What's New and Different?

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

In addition to testing for mutations in KRAS exon 2 (codons 12 and 13) as recommended previously, before treatment with anti-EGFR antibody therapy, individuals with mCRC should have their tumor tested for mutations in:

- KRAS exons 3 (codons 59 and 61) and 4 (codons 117 and 146)
- NRAS exons 2 (codons 12 and 13), 3 (codons 59 and 61), and 4 (codons 117 and 146)

Targeted therapies such as anti-VEGF and anti-EGFR agents may be added to doublet chemotherapies in maximal settings. […] If molecular testing results for *RAS* (*KRAS/NRAS*) are available, this guideline provides recommendations according to the status of these markers. In maximal (-resource) settings, for individuals with left-sided colon cancer and known *KRAS/NRAS* wild type (WT) molecular status, anti-EGFR antibodies such as cetuximab or panitumumab may be added to chemotherapy doublet, with a moderate-strength recommendation. However, individuals with right-sided colon cancer and *RAS* WT status should not be offered treatment with anti-EGFR antibodies in the first-line setting. Anti-EGFR therapies have increased response rates and conversion from unresectable to resectable metastatic disease when added to chemotherapy with FOLFOC or FOLFIRI for individuals with *RAS* wildtype, but more recent data suggest that debenit with anti-EGFR therapies seems to be limited to individuals whose primary tumors are left-sided.

Please review the complete therapeutic recommendations that are located here: (2 , 23)

2020 Clinical Practice Guidelines in Oncology: Colon Cancer, from the National Comprehensive Cancer Network (NCCN)

Version 4.2020 – Discussion update in progress.

A sizable body of literature has shown that tumors with a mutation in codon 12 or 13 of exon 2 of the *KRAS* gene are essentially insensitive to cetuximab or panitumumab therapy. More recent evidence shows mutations in *KRAS* outside of exon 2 and mutations in *NRAS* are also predictive for a lack of benefit to cetuximab and panitumumab.

The panel therefore strongly recommends *RAS* (*KRAS/NRAS)* genotyping of tumor tissue (either primary tumor or metastasis) in all individuals with metastatic colorectal cancer. Individuals with known *KRAS* or *NRAS* mutations should not be treated with either cetuximab or panitumumab, either alone or in combination with other anticancer agents, because they have virtually no chance of benefit and the exposure to toxicity and expense cannot be justified. It is implied throughout the guidelines that NCCN recommendations involving cetuximab or panitumumab relate only to individuals with disease characterized by *KRAS/NRAS* wild-type genes. ASCO released a Provisional Clinical Opinion Update on extended *RAS* testing in individuals with metastatic colorectal cancer that is consistent with the NCCN panel's recommendations. A guideline on molecular biomarkers for colorectal cancer developed by the ASCP, CAP, AMP and ASCO also recommends *RAS* testing consistent with the NCCN recommendations.

The recommendation for KRAS/NRAS testing, at this point, is not meant to indicate a preference regarding regimen selection in the first-line setting. Rather, this early establishment of KRAS/NRAS status is appropriate to plan for the treatment continuum, so that the information may be obtained in a non- time–sensitive manner and the individual and provider can discuss the implications of a KRAS/NRAS mutation, if present, while other treatment options still exist. Note that because anti-EGFR agents have no role in the management of stage I, II, or III disease, KRAS/NRAS genotyping of colorectal cancers at these earlier stages is not recommended. […] The NCCN Colon/Rectal Cancer Panel believes that *RAS* mutation status should be determined at diagnosis of stage IV disease. Individuals with any known *RAS* mutation should not be treated with either cetuximab or panitumumab.

KRAS mutations are early events in colorectal cancer formation, and therefore a very tight correlation exists between mutation status in the primary tumor and the metastases. For this reason, *KRAS/NRAS* genotyping can be performed on archived specimens of either the primary tumor or a metastasis. Fresh biopsies should not be obtained solely for the purpose of *KRAS/NRAS* genotyping unless an archived specimen from either the primary tumor or a metastasis is unavailable.

Approximately 5% to 9% of colorectal cancers are characterized by a specific mutation in the *BRAF* gene (V600E). *BRAF* mutations are, for all practical purposes, limited to tumors that do not have *KRAS* exon 2 mutations. Activation of the protein product of the non-mutated *BRAF* gene occurs downstream of the activated KRAS protein in the EGFR pathway. The mutated BRAF protein product is believed to be constitutively active, thereby putatively bypassing inhibition of EGFR by cetuximab or panitumumab.

The panel recommends that *KRAS*, *NRAS*, and *BRAF* gene testing be performed only in laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform highly complex molecular pathology testing. No specific testing methodology is recommended.

Please review the complete therapeutic recommendations that are located here:(3)

Allele Nomenclature

Selected *KRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
G12D	p.Gly12Asp	NM_004985.5:c.35G>A	NP_004976.2:p.Gly12Asp	rs121913529
G12V	p.Gly12Val	NM_004985.5:c.35G>T	NP_004976.2:p.Gly12Val	rs121913529
G13D	p.Gly13Asp	NM_033360.4:c.38G>A	NP_004976.2:p.Gly13Asp	rs112445441

Selected *NRAS* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
NRAS G12V	p.Gly12Val	NM_002524.5:c.35G>T	NP_002515.1:p.Gly12Val	rs121913237
NRAS G13R	p.Gly13Arg	NM_002524.5:c.37G>C	NP_002515.1:p.Gly13Arg	rs121434595
NRAS Q61R	p.Gln61Arg	NM_002524.5:c.182A>G	NP_002515.1:p.Gln61Arg	rs11554290
NRAS Q61K	p.Gln61Lys	NM_002524.5:c.181C>A	NP_002515.1:p.Gln61Lys	rs121913254

Selected *BRAF* Somatic Variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.6:c.1799T>C	NP_004324.2:p.Val600Glu	rs113488022

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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Pegloticase Therapy and *G6PD* Genotype

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Introduction

Pegloticase (brand name Krystexxa) is used to treat the high levels of uric acid associated with refractory gout. The use of pegloticase is reserved for individuals with symptomatic, chronic gout who have not responded to, or are unable to take, conventional gout treatments. Pegloticase is given once every 2 weeks as an intravenous infusion, and is given in a healthcare setting that is prepared to manage infusion reactions and anaphylaxis (1).

Pegloticase is a pegylated urate oxidase – a modified version of the enzyme that catalyzes the oxidation of uric acid to 5-hydroxyisourate and hydrogen peroxide in most mammalian species. However, urate oxidase is not active in humans due to an inactivating mutation in the gene (2). Subsequent hydrolysis and decarboxylation of 5-hydroxyisourate leads to the formation of a more soluble metabolite (allantoin), which is then excreted by the kidneys.

Red blood cells that lack the glucose-6-phosphate dehydrogenase (G6PD) enzyme are sensitive to oxidative damage caused by agents like hydrogen peroxide. Once exposed, the red blood cells become rigid, trapped, and are rapidly broken down (hemolysis). This can lead to a deficiency of mature red blood cells (hemolytic anemia) and the production of red blood cells with abnormally high levels of methemoglobin (methemoglobinemia).

Approximately 400 million people worldwide have G6PD deficiency. Most of these individuals are asymptomatic. However, they are at risk of life-threating hemolytic reactions and methemoglobinemia if given oxidizing drugs such as pegloticase.

Pegloticase is contraindicated in individuals with G6PD deficiency. The FDA-approved label states that individuals at higher risk for G6PD deficiency should be screened before starting pegloticase therapy, with specific examples including individuals of African, Mediterranean (including Southern European and Middle Eastern), and South Asian ancestry (Table 1) (1). Importantly, approximately 12% of African-Americans have G6PD deficiency.

Table 1. The FDA Drug Label for Pegloticase. Glucose-6-phosphate dehydrogenase Deficiency Associated Hemolysis and Methemoglobinemia (2020)

Phenotype	Recommendations
G6PD deficiency	Screen individuals at risk for G6PD deficiency before starting pegloticase. For example, individuals of African, Mediterranean (including Southern European and Middle Eastern), and Southern Asian ancestry are at increased risk for G6PD deficiency. Life threatening hemolytic reactions and methemoglobinemia have been reported with pegloticase in individuals with G6PD deficiency. Because of the risk of hemolysis and methemoglobinemia, do not administer pegloticase to individuals with G6PD deficiency.

This table is adapted from (1).

Drug: Pegloticase

Pegloticase is a urate-lowering drug for adults with severe, chronic refractory gout, which is administered intravenously every 2 weeks. Pegloticase is a member of the drug class of uricases (urate oxidases), which also includes rasburicase. While rasburicase (brand name Elitrek) is licensed for use in managing tumor lysis

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syndrome, pegloticase is used to treat adults with severe, refractory chronic gout who cannot tolerate or have failed to respond to adequate doses and combinations of available uricostatic or uricosuric urate-lowering drugs.

Gout is one of the most common types of inflammatory arthritis. It affects approximately 4% of adults in the USA and though its global incidence and prevalence are increasing, they have now stabilized in high income Western countries (3-6). Gout is caused by an inflammatory response to urate crystals. Prolonged asymptomatic elevation of serum urate levels (hyperuricemia) above a solubility saturation threshold of approximately 6.8 mg/dL always precedes the development of gout. However, most individuals with hyperuricemia do not develop clinical gouty arthritis, and urate-lowering drugs including pegloticase are not used to treat asymptomatic hyperuricemia.

Patients with gouty arthritis usually present for the first time with an extremely painful acute inflammatory monoarthritis (gout flare) in a lower limb joint such as the first metatarsophalangeal joint in the great toe (podagra), the ankle, or knee. Acute gout flares can occur in the elbows, wrists or small joints of the hands but flares in joints of the upper limbs and acute flares in multiple joints (polyarticular gout) are usually restricted to individuals with longstanding poorly controlled disease. Without treatment, acute gout attacks are self-limiting and will settle in 7–14 days. Following resolution, individuals have a pain-free period of variable length (intercritical gout) before experiencing a further flare. In a few individuals, persistent hyperuricemia is associated with palpable or visible, or both, sub-cutaneous granulomata containing masses of urate crystals known as tophi.

While acute gout flares are treated with anti-inflammatory medications, definitive treatment of gout requires continuous medication with urate-lowering drugs at doses that maintain the serum urate level below that required to prevent urate crystal formation and dissolve existing urate deposits.

There are 3 main types of urate-lowering drugs:

- Uricostatic xanthine oxidase inhibitors that decrease the production of uric acid (for example, allopurinol, febuxostat)
- Uricosuric drugs that inhibit the reabsorption of uric acid in the kidneys (for example, benzbromarone, probenecid, and lesinurad)
- Uricase drugs that convert uric acid to a more soluble metabolite (for example, pegloticase, rasburicase)

Xanthine oxidase inhibitors are the mainstay treatment for gout, with the addition of uricosuric drugs being reserved for individuals who do not have an adequate response.

Despite conventional urate-lowering therapy for gout with uricostatic or uricosuric, or both drugs, a few individuals may continue to have symptomatic, chronic gouty arthritis, with tophi, and high serum uric acid levels. Intravenous infusions of pegloticase can lead to rapid and persistent lowering of urate levels and significant clinical improvement of symptoms in approximately 45% of these individuals (7-9).

Pegloticase is a pegylated urate oxidase –– a modified version of the enzyme that catalyzes the oxidation of uric acid to 5-hydroxyisourate and hydrogen peroxide in most mammalian species, but which is not active in humans. Subsequent hydrolysis and decarboxylation lead to the formation of a more soluble metabolite (allantoin), which is then excreted by the kidneys. Pegloticase is a predominantly porcine-like recombinant uricase, which is modified ("pegylated") to increase its half-life and to reduce the development of anti-drug protein antibodies. However, clinically significant anti-pegloticase antibodies are detected in approximately 40% of individuals treated with pegloticase, and a high level of antibodies is associated with a higher incidence of infusion reactions (10). However, most of the anti-pegloticase antibodies are directed at the polyethylene glycol component of the drug, rather than the uricase, leading to reduced efficacy as a result of increased drug clearance, and reduction in drug concentration associated with a secondary rise in serum urate levels.

The safety of pegloticase in pregnant women has not been studied, but animal studies showed no evidence of fetal harm. In pregnant rats and rabbits, no fetal structural abnormalities were seen after sub-cutaneous injections of pegloticase during the organogenesis stages of pregnancy.

Gout flares are the most common adverse reactions associated with pegloticase therapy despite flare prophylaxis with colchicine, nonsteroidal anti-inflammatory drugs, or corticosteroids. Infusion reactions and—very rarely—reactions fulfilling criteria for anaphylaxis can occur despite pre-treatment with antihistamines and corticosteroids. Approximately a quarter of individuals experience a mild to moderate infusion reaction with a pruritic urticarial rash, dyspnea, or chest discomfort. More severe anaphylactoid reactions with wheezing, swelling around the mouth and lips, and reduced blood pressure occur in less than 1% of individuals, usually within 2 hours of the infusion. Because of the risk of infusion reactions and anaphylaxis, pegloticase should only be administered in a healthcare setting with monitoring of the individual during the infusion and reducing or stopping treatment if necessary, and subsequently monitoring the individual after the infusion for any signs of anaphylaxis (7, 8, 11). The severity and frequency of these infusion reactions remains an area of debate within the field. The current FDA drug label includes a boxed warning regarding the possibility of anaphylaxis and infusion reactions that could occur during or after pegloticase administration, including the first infusion; thus pegloticase is recommended only for administration in a healthcare setting by providers prepared to manage anaphylaxis and infusion reactions (1, 12).

The use of pegloticase is contraindicated in individuals with G6PD deficiency because of the risk of acute hemolytic anemia and methemoglobinemia (1, 13, 14). This is because a byproduct of the conversion of uric acid to 5-hydroxyisourate is hydrogen peroxide, an oxidizing agent.

Individuals who have G6PD deficiency have red blood cells that are susceptible to oxidative damage. If exposed to agents such as hydrogen peroxide, the red blood cells become rigid, get trapped, and are subsequently destroyed by macrophages in the spleen, bone marrow, and liver. The rapid destruction of red blood cells is called hemolysis, and it may result in hemolytic anemia (a deficiency of red blood cells or hemoglobin, caused by hemolysis).

In addition, hemoglobin may be oxidized to methemoglobin. Hemoglobin binds oxygen and delivers oxygen to the body's tissues, while methemoglobin does not. Normally, approximately 1% of red blood cells contain methemoglobin. When the levels of methemoglobin increase, red blood cells are less able to delivery oxygen to tissues, resulting in cyanosis (bluish skin color), and potentially life-threatening arrhythmias and seizures (15).

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on chromosome Xq28. As such, males are hemizygous for one *G6PD* allele, making them more susceptible to this X-linked disorder. Females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene.

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme deficit in humans, affecting 400 million people worldwide (16), with a worldwide prevalence of approximately 5%. Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once was, endemic (for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean) (17-19). In the US, G6PD deficiency is more common among African-Americans, affecting approximately 12% (20).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate

(NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. Red blood cells that lack G6PD also have a deficiency of NADPH.(21)

Red blood cells that are G6PD and NADPH deficient are more susceptible to oxidative stress by oxygen free radicals and hydrogen peroxide. Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism) and is an adverse effect of several drugs (for example, the uric acid lowering drugs pegloticase and rasburicase, the antimalarial drugs primaquine and tafenoquine, the skin cancer drug dabrafenib, and the antibacterials, dapsone and sulfamethoxazole).

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. However, at birth, they are predisposed to neonatal jaundice, and throughout life, they are sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid oxidizing agents when possible, including drugs such as pegloticase.

Symptomatic individuals with G6PD deficiency may suffer from episodes of acute hemolytic anemia, jaundice, and hemoglobinuria or chronic, non-spherocytic, hemolytic anemia. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency and to induce formation of new red blood cells.

More than 180 genetic variants of the *G6PD* gene have been identified, with approximately 400 biochemical and enzyme variants (22). Most known *G6PD* variants are missense, which can also be inherited as haplotypes that are comprised of more than one variant allele (23). Large deletions are rare, and a complete lack of G6PD activity is thought to be fatal in utero.

The normal (wild-type) copy of the *G6PD* gene is known as *G6PD B*, and is found in most individuals with European, Asian, or African ancestry. Common *G6PD* variants include:

- *G6PD* A+ (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of blacks from Africa (24).
- *G6PD* A- (p.Asn126Asp and p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (25). Additional *A*-haplotypes have also been identified, both with the A+ variant with a second single nucleotide polymorphisms (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (26).
- *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is the most common abnormal variant in Caucasians (27).
- *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in Asians (28).

The World Health Organization categorized *G6PD* variants into 5 classes according to the level of enzyme activity and severity of hemolysis. Class 1 variants are the most severe, but rare in the general population. These variants have less than 10% of normal GP6D enzyme activity and are associated with chronic hemolytic anemia.

Most individuals with G6PD deficiency have Class II (enzyme activity less than 10% but no chronic hemolytic anemia) or Class III (enzyme activity between 10–60%) variants. Class II and III variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but for most of the time, affected individuals have no symptoms. Class IV and V variants are not considered to be clinically significant, as they are associated with normal (class IV) or increased (class V) enzyme activity (29).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has assigned *G6PD* phenotypes based on *G6PD* genotypes (Table 2) (29).

Table 2. Assignment of likely *G6PD* Phenotype based on Genotype/Diplotype (CPIC 2014)

Likely phenotype	Definition	Genotype	Who class for *G6PD* variants[a]	Example of diplotype[b]
Normal	Very mild or no enzyme deficiency (less than 60% of normal enzyme levels)	A male who has a non-deficient (class IV) allele	IV	B, Sao Boria
		A female who has 2 non-deficient (class IV) alleles	IV/IV	B/B, B/Sao Boria
Deficient	Less than 10–60% of normal enzyme activity	A male who has a deficient (class II–III) allele	II, III	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		A female who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA	A male who has a class I allele	I	Bangkok, Villeurbanne
		A female who has 2 deficient (class I variants) alleles	I/I	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[c]	Normal or deficient enzyme activity[c]	A female who has one non-deficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III	B/A–, B/Mediterranean, B/Bangkok

CNSHA, chronic nonspherocytic hemolytic anemia
WHO, World Health Organization
[a] WHO classifications (Ref. 14 and Ref. 17, from (29)). Class I variants are extremely rare; the distinction between class II and III variants is not clear, and the "class V" very high activity variant has been reported in only a single case. Therefore, almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.
[b] Due to the large number of *G6PD* variants, many other diplotypes may be possible besides those given as examples here; see Supplementary Table S1 online for a more comprehensive list of variant alleles with their assigned WHO class (29). For HGVS terms, please see the Nomenclature table below.
[c] Due to X-linked mosaicism, females heterozygous for one non-deficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online (*G6PD* heterozygotes)) (29).
This table is adapted from (29).

Linking Gene Variation with Treatment Response

Although evidence that directly links G6PD status with an increased risk of hemolytic anemia is limited to case reports of individuals taking pegloticase, it is well established that hydrogen peroxide, which is produced during pegloticase therapy, can cause acute hemolysis in individuals with G6PD deficiency.

The first case of severe hemolysis and methemoglobinemia associated pegloticase therapy was reported in 2014, and a later case of hemolytic anemia was reported in 2016. In both cases, the individuals were later diagnosed to have G6PD deficiency (13-15).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for pegloticase response and the *G6PD* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD and may also be used to screen females with a family history of G6PD to see if they are carriers.

Glucose-6-phosphate dehydrogenase deficiency is an X-linked recessive trait and most individuals are asymptomatic throughout life.

X-linked disorders affect males at a much higher rate than females because males only have one copy of the X chromosome (hemizygous, XY). Since females have 2 copies of the X chromosome (XX) they tend to be less affected. However, female carriers can present with a range of phenotypes from no symptoms through a severe deficiency due to the high frequency of *G6PD* variants. Females randomly inactivate one X chromosome in somatic cells during development, resulting in a mixed population of somatic cells expressing one *G6PD* allele or the other.

Glucose-6-phosphate dehydrogenase deficiency occurs in homozygous and compound heterozygous females (who have inherited 2 copies of *G6PD* deficiency alleles) and in heterozygous females (one normal *G6PD* allele and one deficiency *G6PD* allele) with skewed X-chromosome inactivation of the functional allele (18). Genetic testing alone is insufficient for heterozygous females with one normal function *G6PD* allele, as the expression of the 2 alleles will vary between blood cells and over time (29).

A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons (21, 30).

The FDA recommends that individuals at risk of G6PD deficiency be screened for G6PD deficiency before starting pegloticase therapy. However, individuals of all ancestries may be G6PD deficient (worldwide prevalence of 5%). Therefore, caution must be taken in all individuals when initiating pegloticase therapy.

In routine clinical practice, G6PD deficiency is diagnosed by measuring the level of G6PD activity in red blood cells. Two different types of enzyme activity tests are used: qualitative and quantitative. Often, qualitative tests do not accurately detect individuals with intermediate G6PD activity. False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, screening for G6PD should be performed 2–3 months after a blood transfusion or hemolytic episode. Of note, G6PD activity false negatives have been reported (14, 21, 29, 31).

In men, if genetic testing determined that an individual was positive for G6PD deficiency, the use of pegloticase would be contraindicated. However, a negative result cannot be entirely relied upon because only a small subset of *G6PD* variants are routinely tested (21, 29, 30). In addition, *G6PD* phenotypes may be unpredictable in heterozygous females due to random X-chromosome inactivation.

Universal neonatal screening programs for G6PD deficiency are employed in some countries with a high incidence of G6PD deficiency (more than 3–5% of males) (32). These populations are primarily in Asia, Africa, along the Mediterranean and in the Middle East. Screening either uses quantitative enzyme activity assays, or the fluorescent spot test that visually identifies NADPH, which is produced by G6PD (if the blood spot does not fluoresce, the test is positive for G6PD deficiency) (29).

Relatively inexpensive rapid point of care qualitative tests are used in countries where G6PD deficiency is frequent and can identify individuals with severe G6PD deficiency who would be at risk of severe hemolysis with commencement of antimalarial drug therapy. Unfortunately, however, they are insufficiently sensitive for the detection of individuals with less severe G6PD deficiency in whom pegloticase is also contraindicated. (31)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA):

Contraindications: Glucose-6-phosphate dehydrogenase (G6PD) deficiency

[...]

Life threatening hemolytic reactions and methemoglobinemia have been reported with pegloticase in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Because of the risk of hemolysis and methemoglobinemia, do not administer pegloticase to patients with G6PD deficiency. Screen patients at risk for G6PD deficiency prior to starting pegloticase. For example, patients of African, Mediterranean (including Southern European and Middle Eastern), and Southern Asian ancestry are at increased risk for G6PD deficiency.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for Selected *G6PD* Variants

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Boria	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3: c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3: c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient	rs5030868

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Table continued from previous page.

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
* WHO classifications based on (33)

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Pertuzumab Therapy and *ERBB2* Genotype

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Introduction

Pertuzumab (brand name, Perjeta) is a monoclonal antibody used in the treatment of breast cancer. Pertuzumab was designed to target an epidermal growth factor receptor encoded by the *ERBB2* gene, commonly referred to as the *HER2* gene.

The *ERBB2* gene is overexpressed in 15–20% of breast cancers and is also overexpressed in some cases of other cancer types (gastric, colon, head, and neck). Historically, "HER2-positive" tumors are associated with a faster rate of growth and a poorer prognosis than other breast cancer subtypes. The use of pertuzumab in treatment regimens improves outcomes, with limited adverse effects that include cardiac toxicity.

Pertuzumab is used with other drugs as an advanced breast cancer treatment, a neoadjuvant treatment, and an adjuvant treatment for HER2-positive breast cancer. In the advanced/metastatic setting, pertuzumab added to trastuzumab and a taxane is used to increase long-term progression-free and overall survival when administered in the first line setting. As neoadjuvant treatment, pertuzumab is given with trastuzumab and chemotherapy before surgery in individuals with early breast cancer to increase pathologic complete response rates. And as an adjuvant treatment, pertuzumab is given with trastuzumab and chemotherapy to reduce the risk of cancer reoccurrence in individuals with early breast cancer (Table 1).

The 2020 FDA-approved drug label states that pertuzumab should only be used to treat individuals with tumors that have either HER2 protein overexpression or *ERBB2* gene amplification, as determined by an accurate and validated FDA-approved assay. This is because these are the only individuals studied for whom benefit has been shown (1).

The most recent update (2018) American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) guidelines continue to state that all newly diagnosed individuals with breast cancer must have an HER2 test performed. Individuals who then develop metastatic disease must have an HER2 test performed in a metastatic site, if a tissue sample is available (2).

Table 1. The FDA Indications and Usage of Pertuzumab in HER2-positive Breast Cancer (2020)

Individual selection*	Metastatic breast cancer (treatment)	Breast cancer (neoadjuvant treatment)	Early breast cancer (adjuvant treatment)
Tumor HER2 status	HER2-positive	HER2-positive	HER2-positive
Usage	Use pertuzumab with trastuzumab and docetaxel	Use pertuzumab with trastuzumab and chemotherapy	

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Table 1. continued from previous page.

Individual selection*	Metastatic breast cancer (treatment)	Breast cancer (neoadjuvant treatment)	Early breast cancer (adjuvant treatment)
Indications	Indicated for individuals with metastatic breast cancer who have not received before anti-HER2 therapy or chemotherapy for metastatic disease	Indicated for individuals with locally advanced, inflammatory, or early-stage breast cancer (either greater than 2 cm in diameter or node positive) as part of a treatment regime for early breast cancer	Indicated for individuals with early breast cancer at high risk of recurrence

* Select individuals based on HER2 protein overexpression or *HER2* gene amplification in tumor specimens. Assessment of HER2 protein overexpression and *ERBB2* gene amplification should be performed using FDA-approved tests specific for breast cancer by laboratories with demonstrated proficiency. Information on the FDA-approved tests for the detection of HER2 protein overexpression and *ERBB2* gene amplification is available at: http://www.fda.gov/CompanionDiagnostics.
Improper assay performance, including use of sub optimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.
This FDA table was created from (1).

Drug Class: HER2 Inhibitors

Human epidermal growth factor receptor 2 (commonly referred to as HER2 or HER-2/neu) is encoded by the gene *ERBB2,* which is a transmembrane receptor tyrosine kinase. Overexpression of *ERBB2* leads to rapid cell growth in multiple types of solid tumors. The HER2 can be inactivated by a class of chemicals known as tyrosine kinase inhibitors or via targeted monoclonal antibodies. An increasing number of HER2-targeted therapies have been approved to treat HER2-positive breast cancer, including:

- Pertuzumab –– monoclonal antibody (brand name Perjeta)
- Trastuzumab –– monoclonal antibody (brand name Herceptin)
- Ado-trastuzumab emtansine –– antibody-drug-conjugate (monoclonal antibody attached to a chemotherapy drug (brand name Kadcyla, also called TDM-1)
- Fam-trastuzumab deruxtecan –– antibody-drug-conjugate (brand name Enhertu)
- Neratinib –– a kinase inhibitor (brand name Nerlynx)
- Lapatinib –– a kinase inhibitor (brand name Tykerb)
- Tucatinib –– a kinase inhibitor (brand name Tukysa)
- Dacomitinib –– a kinase inhibitor (brand name Vizimpro)

There are more anti-HER2 drugs progressing through clinical trials, and some trials are looking at whether HER2-targeted therapies could be used to treat other tumors that overexpress HER2, such as colorectal and non-small-cell lung cancer. However, early results are not replicating the success of HER2-targeted therapies in breast and gastric cancer (3, 4, 5). One exception is the use of trastuzumab, which has been approved for use in HER2-positive metastatic gastric cancer (6).

Drug: Pertuzumab

Pertuzumab is a monoclonal antibody that targets HER2 (a tyrosine kinase receptor, encoded by the *ERBB2* gene). Pertuzumab is only used to treat specific tumors that overexpress or are amplified for *ERBB2*, which are known as “HER2-positive” tumors.

In 2012, the FDA approved the use of pertuzumab in the treatment of HER2-positive metastatic breast cancer to increase the chance of long-term disease-free survival. Pertuzumab is used with trastuzumab (another monoclonal antibody that targets *ERBB2*) and docetaxel (a chemotherapy drug) to treat individuals who have not previously had anti-HER2 therapy or chemotherapy for HER2-positive metastatic breast cancer.

In 2013, the FDA granted an accelerated approval to pertuzumab, again with trastuzumab and docetaxel, as a neoadjuvant treatment of HER2-positive breast cancer. In the neoadjuvant setting, pertuzumab is given before surgical therapy in women with HER2-positive breast cancer that is locally advanced, inflammatory, or at an early stage (either greater than 2 cm in diameter or lymph node positive) (1, 7, 8, 9). In 2017, the FDA approved pertuzumab for use with trastuzumab and chemotherapy as an adjuvant treatment for individuals with HER2-positive early breast cancer with a high risk of recurrence, to reduce the risk of recurrence (10, 11).

Before treatment with pertuzumab begins, overexpression of the HER-2 protein or amplification of the *ERBB2* gene must first be determined. In clinical studies of pertuzumab, individuals with breast cancer were required to have evidence of HER2 overexpression defined as 3+ immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH) amplification ratio of 2 or greater (see Tumor Testing for *ERBB2* (HER2)).

The FDA recommends that testing be performed using an FDA-approved test, in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of pertuzumab have only been proven in individuals with HER2 positive tumors (12).

In addition, although pertuzumab is well tolerated, the risks of treatment include infusion reactions, diarrhea, and cardiomyopathy that can result in cardiac failure. The mechanism of how anti-HER2 drugs such as pertuzumab can cause cardiotoxicity is not known, but evidence from mouse models suggests HER2 signaling plays a key role in heart development and cardiomyocyte survival during stress (reviewed by (13). Additional evidence suggests that using cardiac drugs, such as beta blockers and ace inhibitors, may help prevent subsequent cardiomyopathy. Data on the safety of anti-HER2 drugs in older individuals, who are most likely to take these drugs, are lacking, having been mostly derived from subgroup analysis of larger trials (14, 15, 16).

The pregnancy status of women of child-bearing age should be checked, and women should be warned that exposure to pertuzumab during pregnancy, or within 7 months before conception, can result in fetal harm and potentially, fetal death. Therefore, women should use effective contraception for 7 months following their last dose of pertuzumab (1).

Pertuzumab targets the HER2 receptor by binding to a specific region in its extracellular domain. The HER2 receptor is an epidermal growth factor receptor, consisting of an intracellular tyrosine kinase domain, a single transmembrane spanning region, and an extracellular domain, made up of 4 subdomains (I–IV). Pertuzumab binds to subdomain II and trastuzumab binds to subdomain IV. This binding limits the receptor's ability to activate its intrinsic kinase, which in turn, limits the activation of numerous signaling pathways that can promote cell growth.

A number of proposed mechanisms may underlie the anti-tumor effects of pertuzumab and trastuzumab. One such mechanism is that these drugs block the HER3 receptor from binding to HER2. The HER2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a "true" ligand (17, 18, 19, 20).

Another proposed mechanism is antibody-dependent cellular cytotoxicity. Once pertuzumab or trastuzumab have bound to a cancer cell, immune cells (typically activated natural killer cells) bind to the drug and initiate lysis of the cancer cell (21).

Unfortunately, breast cancer may start to progress again during HER2 targeted therapy. Mechanisms that may facilitate drug resistance and disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor-1 receptor (22, 23). In the instance of metastatic disease, ASCO practice guidelines suggest obtaining a tissue sample from the metastatic site to confirm HER2-status in case of relapse after curative treatment (24).

As a new drug, there is not enough data on the risk of developing resistance to pertuzumab therapy. However, there is a lower response rate to pertuzumab among individuals previously treated with trastuzumab. Resistance is particularly problematic because pertuzumab is now being used earlier, to treat of early-stage disease (25, 26).

Gene: *ERBB2* (*HER2*)

The human epidermal growth factor receptor (HER) protein family consists of 4 members: the epidermal growth factor receptor (EGFR), *ERBB2* (HER2), *ERBB3* (HER3), and *ERBB4* (HER4) (see Nomenclature for Selected Genes). All 4 members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (27).

The *ERBB2* gene, along with *EGFR*, are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to abnormal cell growth. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (28).

The official gene symbol for HER2 is *ERBB2*, which is derived from a viral oncogene with which the receptor shares homology; "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2." However, clinicians commonly refer to the *ERBB2* gene as "*HER2*" (Human Epidermal growth factor Receptor 2) or "*HER2/neu*" (neu was the name given to the gene that caused cancer derived from a rodent neuro/glioblastoma). It is the legacy gene symbol for *ERBB2* and may be more commonly used by the community in clinical care, it is also commonly used to describe the protein encoded by the *ERBB2* gene.

One unique feature of HER2 compared with the other receptors in the HER family is the absence of a known ligand. This receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (29). Additionally, one unique feature of HER3 is that it has little enzymatic activity compared with the other tyrosine kinase receptors in the HER family. An important role of HER3 is to act as a heterodimerization partner for HER2 (30, 31).

When a partner such as HER3 binds to HER2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (17, 32). Impaired HER2 signaling is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer disease, while excessive HER2 signaling is associated with the development of cancers.

The *ERBB2* gene is overexpressed in approximately 15–20% of breast tumors, as a result of amplification of the *ERBB2* gene, and tumors with increased HER2 typically have a higher growth rate and more aggressive clinical behavior (33, 34, 35, 36). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remains largely unknown (37). In the case of *ERBB2*, although sequence variants have been identified, it is most commonly a wildtype *ERBB2* gene copy that is overexpressed in tumors (23). In approximately 1% of breast cancers, activating variants in *ERBB2* can be identified that are likely to drive tumorigenesis, without *ERBB2* amplification (38).

Genes: *ESR1* and *PR*

The *ESR1* gene encodes the estrogen receptor, and the *PR* gene encodes the progesterone receptor. Breast cancer cells may have:

- a receptor for estrogen (ER-positive)

- a receptor for progesterone (PR-positive)
- receptors for both estrogen and progesterone (hormone receptor positive)
- nether receptor (hormone receptor negative)
- or receptors for both hormones and HER2 overexpression (triple positive).

Endocrine (hormone) therapy is typically indicated for hormone receptor positive tumors. Some evidence suggests that HER2 status influences the response of hormone receptor positive tumors to endocrine therapy. In one study, individuals with ER-positive tumors and low-level *ERBB2* amplification (as defined by quantitative FISH) had significantly less benefit from adjuvant anti-HER2 therapy after chemotherapy (39). However, ASCO guidelines clearly state that HER2 status should not be used to withhold endocrine therapy for an individual with a hormone receptor-positive breast cancer, nor to select one specific type of endocrine therapy over another (40).

The drug label discuses 2 trials that included analyses of how hormone receptor status influenced the response of HER2-positive tumors to pertuzumab. The NeoSphere clinical trial (NCT00545688) reported that individuals with hormone receptor-positive tumors responded less well to pertuzumab therapy compared with individuals with hormone receptor-negative tumors. The CLEOPATRA clinical trial (NCT00567190) reported that individuals with hormone receptor–positive disease had a higher hazard ratio than individuals with hormone receptor-negative disease. (1, 41, 42) Furthermore, in the interim overall survival analysis of the APHINITY clinical trial (NCT01358877), a treatment benefit of pertuzumab was seen in the node positive, hormone receptor positive cohort (invasive disease-free survival hazard ratio for HR positive is 0.73 [95% confidence interval 0.59–0.92]). Subgroup analysis of the APHINITY trial further suggests that ER-negative individuals benefit more from dual blockade with trastuzumab and pertuzumab. (43) Early results from another study (NCT02564900) suggest that trastuzumab deruxtecan may be effective as an anti-HER2 therapy in HER2-low expressing (HER2 "negative" scores of 1+ or 2+ by standard IHC testing) tumors (44).

Linking Gene Overexpression with Treatment Response

The HER2 overexpression or amplification is strongly linked to a beneficial treatment response to pertuzumab. This is to be expected, given that pertuzumab was developed to target HER2. Consequently, current guidelines for breast cancer treatment limit the use of HER2-blocking agents to tumors with HER2 gene amplification (2, 35, 36).

Genetic Testing

The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for the *ERBB2* gene and the pertuzumab drug response.

Tumor Testing for *ERBB2* (HER2) Gene and Protein

There are 2 main methods used for *ERBB2*/HER2 testing: testing for overexpression of the HER2 protein using IHC or testing for gene amplification using *in situ* hybridization (ISH). Each assay type has diagnostic pitfalls that must be avoided, and so the pathologist who reviews the histologic findings should decide the optimal assay (IHC or ISH) for the determination of HER2 status (35, 36).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that contains high levels of HER2. The tumor sample is then examined by light microscopy to assess the intensity of membrane staining—the amount of staining correlates with the quantity of HER2 protein and is typically graded from 0 to 3+:

- IHC 0 means no visible staining or membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells

- IHC 1+ is also an "HER2 negative" result—there is a staining pattern of incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells
- IHC 2+ is an "HER2 equivocal result"— invasive breast cancer with "weak to moderate complete membrane staining observed in >10% of tumor cells."
- IHC 3+ is an "HER2 positive result"—there is a staining pattern with circumferential membrane staining that is complete, intense and in >10% of tumor cells. This should be readily appreciated using a low-power objective and observed within a homogenous and continuous invasive cell population.

For an equivocal (IHC 2+) result, either a reflex test must be ordered (same specimen using ISH), or a new test must be ordered (using a new specimen, if available, using ISH or FISH) to confirm the results.

The ISH assay, or FISH/CISH assay (fluorescence or chromogenic *in situ* hybridization), measures *ERBB2* gene amplification by measuring *ERBB2* DNA—the actual number of copies of the *ERBB2* genes are counted. Using the FISH assay, under the microscope, the genes appear as red signals or dots, in a blue-stained cancer cell nucleus. The result is usually either FISH negative (normal level of *ERBB2* gene) or FISH positive (at least twice as much as the normal level of *ERBB2* gene), but in a small number of cases the FISH result will be equivocal due to a low level of *ERBB2* amplification. The use of a control helps distinguish between a negative result and a non-informative result caused by an error. Approximately 25% of individuals who have an IHC 2+ result will have a FISH positive result (45).

For the complete algorithms for evaluation of HER2 protein expression using IHC or ISH, please see the ASCO guidelines, located here: (2).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Pertuzumab is a HER2/neu receptor antagonist indicated for:

- Use in combination with trastuzumab and docetaxel for treatment of patients with HER2-positive metastatic breast cancer (MBC) who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease.
- Use in combination with trastuzumab and chemotherapy as
  - neoadjuvant treatment of patients with HER2-positive, locally advanced, inflammatory, or early stage breast cancer (either greater than 2 cm in diameter or node positive) as part of a complete treatment regimen for early breast cancer.
  - adjuvant treatment of patients with HER2-positive early breast cancer at high risk of recurrence.

[…]

Select patients based on HER2 protein overexpression or *HER2* gene amplification in tumor specimens. Assessment of HER2 protein overexpression and *HER2* gene amplification should be performed using FDA-approved tests specific for breast cancer by laboratories with demonstrated proficiency. Information on the FDA-approved tests for the detection of HER2 protein overexpression and *HER2* gene amplification is available at: http://www.fda.gov/CompanionDiagnostics.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Update: American Society of Clinical Oncology (ASCO) /College of American Pathologists (CAP) Recommendations

First released in 2007 and updated in 2013 and 2018, the recommendations by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) human epidermal growth factor receptor 2 (HER2) testing Expert Panel are aimed at improving the analytic validity of HER2 testing and the clinical utility of HER2 as a predictive biomarker for potential responsiveness to therapies targeting the HER2 protein.

2013: ASCO/CAP Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for a patient who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
- Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
- Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen.
- Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.
- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of patient status (comorbidities, prognosis, and so on) and patient preferences after discussing available clinical evidence.

2018: ASCO/CAP Updated Key Recommendations for HER2 testing

[…]

Two recommendations addressed via correspondence in 2015 are included. First, immunohistochemistry (IHC) 2+ is defined as invasive breast cancer with weak to moderate complete membrane staining observed in >10% of tumor cells. Second, if the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may (not "must") be ordered on the excision specimen based on specific clinical criteria.

Please review the complete ASCO/CAP recommendations in the 2013 update (20) and 2018 update (2).

Nomenclature for Selected Genes Associated with Pertuzumab Response

Common gene symbols	Alternative gene symbols
EGFR	*ERBB1* *ERBB* *HER1*
ERBB2	*HER2* *HER-2* *HER-2/neu* *NEU*
ERBB3	*HER3*
ERBB4	*HER4*

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Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotype

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Introduction

Phenytoin (brand name Dilantin) is an anticonvulsant medication used for the treatment of seizures (1).

Phenytoin has a narrow therapeutic index—individuals that have supratherapeutic blood concentrations of phenytoin have increased risks of acute side effects. Dosing can be complex due to pharmacokinetic factors, including individual weight, age, gender, concomitant medications, plasma binding protein status, the presence of uremia or hyperbilirubinemia, and specific pharmacogenetic variants. As such, therapeutic drug monitoring is often used to adjust dose and maintain serum concentrations within the therapeutic range (10–20 µg/mL).

The CYP2C9 enzyme is one of the main enzymes involved in the metabolism of phenytoin, and variant *CYP2C9* alleles are known to influence phenytoin drug levels. Individuals who have decreased activity *CYP2C9* variants may have reduced clearance rates of phenytoin and be at greater risk for dose-related side effects (2).

An individual's human leukocyte antigen B (*HLA-B*) genotype is a known risk factor for drug-induced hypersensitivity reactions. The HLA-B protein has an important immunological role in pathogen recognition and response, as well as to non-pathogens such as drugs. Individuals who have the *HLA-B*15:02* allele are at high risk of developing potentially life-threatening phenytoin-induced Stevens-Johnson syndrome (SJS) and the related toxic epidermal necrolysis (TEN).

The *HLA-B*15:02* allele is most often found among individuals of Southeast Asian descent, where there is a strong association between SJS/TEN and exposure to carbamazepine. Carbamazepine is an antiseizure medication used to treat the same types of seizures as phenytoin, as well as trigeminal neuralgia and bipolar disorder.

The FDA-approved drug label for phenytoin states that consideration should be given to avoiding phenytoin as an alternative for carbamazepine in individuals positive for *HLA-B*15:02* (Table 1). The label also mentions that variant *CYP2C9* alleles may contribute to unusually high levels of phenytoin (1).

Dosing recommendations for phenytoin based on *HLA-B* and *CYP2C9* genotype have also been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 2, Figure 1) and the Dutch Pharmacogenetics Working Group (DPWG, Table 3, Table 4). These recommendations include the use of an antiseizure medication other than carbamazepine, phenytoin (or its prodrug fosphenytoin) for any *HLA-B*15:02* positive individual regardless of *CYP2C9* genotype, individual ancestry, or age. These recommendations also include specific dose reductions of phenytoin for individuals who have low or deficient enzyme activity (2, 3).

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Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C9 and HLA-B Genotypes and Phenytoin Dosing: 2020 Update

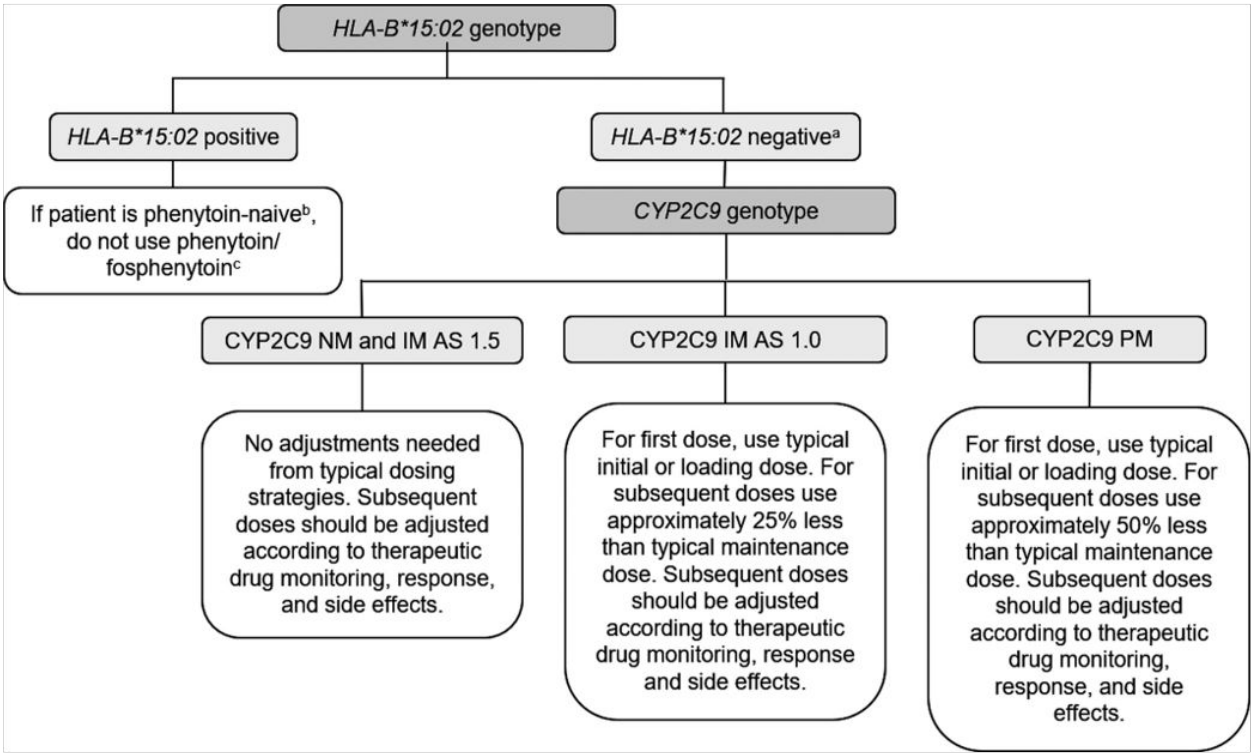

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Figure 1: Dosage Guidelines from the CPIC for Phenytoin based on *HLA-B* and *CYP2C9* Genotype. Figure reproduced with permission from the authors.

Table 1. FDA Phenytoin Dosage and Administration (2019)

Gene or gene variant	Dosing considerations
*HLA-B*15:02*	Consideration should be given to avoiding phenytoin as an alternative for carbamazepine in individuals positive for *HLA-B*15:02*. The use of *HLA-B*15:02* genotyping has important limitations and must never substitute for appropriate clinical vigilance and individual management.
CYP2C9 and *CYP2C19*	If individual is phenytoin naïve, do not use phenytoin/fosphenytoin. Avoid carbamazepine and oxcarbazepine.[a] If the individual has previously used phenytoin continuously for longer than 3 months without incidence of cutaneous adverse reactions, cautiously consider use of phenytoin in the future. The latency period for drug-induced SJS/TEN is short with continuous dosing and adherence to therapy (4–28 days), and cases usually occur within 3 months of dosing.[b]

This FDA table is adapted from (1).

Table 2. The CPIC Recommended Dosing of Phenytoin Based on *HLA-B*15:02* and *CYP2C9* Phenotype/Genotype (2020)

CYP2C9 phenotype and *HLA-B* genotype	Implication	Therapeutic recommendation
Any *CYP2C9* phenotype and *HLA-B*15:02* positive[#]	Increased risk of phenytoin-induced SJS/ TEN	If the individual is phenytoin-naïve, do not use phenytoin/ fosphenytoin. Avoid carbamazepine and oxcarbazepine.[a] If the individual has previously used phenytoin continuously for longer than 3 months without incidence of cutaneous adverse reactions, cautiously consider use of phenytoin in the future. The latency period for drug-induced SJS/TEN is short with continuous dosing and adherence to therapy (428 days), and cases usually occur within 3 months of dosing.[b]
CYP2C9 normal metabolizer and *HLA-B*15:02* negative	Normal phenytoin metabolism	No adjustments needed from typical dosing strategies. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An *HLA-B*15:02* negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and individuals should be carefully monitored according to standard practice.[**a**]
CYP2C9 intermediate metabolizer (activity score 1.5) and *HLA-B*15:02* negative	Slightly reduced phenytoin metabolism: however, this does not appear to translate into increased side effects.	No adjustments needed from typical dosing strategies. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An *HLA-B*15:02* negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and individuals should be carefully monitored according to standard practice.[c]
CYP2C9 intermediate metabolizer (Activity score 1.0) and *HLA-B*15:02* negative	Reduced phenytoin metabolism: Higher plasma concentrations will increase probability of toxicities.	For first dose, use typical initial or loading dose. For subsequent doses, use approximately 25% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An *HLA-B*15:02* negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and individuals should be carefully monitored according to standard practice.[c]
CYP2C9 poor metabolizer and *HLA-B*15:02* negative	Reduced phenytoin metabolism: Higher plasma concentrations will increase probability of toxicities.	For first dose, use typical initial or loading dose. For subsequent doses use approximately 50% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An *HLA-B*15:02* negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and individuals should be carefully monitored according to standard practice.[a]

SJS/TEN: Stevens-Johnson syndrome/toxic epidermal necrolysis.

[#] Considerations: Other aromatic anticonvulsants, including eslicarbazepine, lamotrigine, and phenobarbital, have weaker evidence linking SJS/TEN with the *HLA-B*15:02* allele; however, caution should still be used in choosing an alternative agent. Previous tolerance of phenytoin is not indicative of tolerance to other aromatic anticonvulsants.

a The strength of the therapeutic recommendation is classified as "strong".

b The strength of the therapeutic recommendation is classified as "optional".

c The strength of the therapeutic recommendation is classified as "moderate".

This CPIC table is adapted from (2).

Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (4).

Table 3. The DPWG Phenytoin Dosing based on *HLA-B*15:02* Genotype (2017)

Genotype	Implication	Dosing recommendations
Positive for *HLA-B*15:02*	Increased risk of the life-threatening cutaneous side effect Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). The calculated risk of phenytoin-induced SJS/TEN in individuals with *HLA-B*15:02* is 0.65%.	Carefully weigh the risk of SJS/TEN against the benefits Avoid phenytoin if an alternative is possible • Carbamazepine has a 10-fold higher risk of SJS/TEN for these individuals and is therefore not an alternative. • A comparable risk has been reported for lamotrigine as for phenytoin. The same applies for oxcarbazepine, but the most severe forms (SJS/TEN overlap and TEN) are not observed with oxcarbazepine. If it is not possible to avoid this medication, then advise the individual to report any skin rash immediately

This DPWG table is adapted from (3).

Table 4. The DPWG Phenytoin Dosing based on *CYP2C9* Genotype (2018)

Metabolizer type	Genotype	Side effects	Recommendations		
			Loading dose	Other doses	Advise the individual
CYP2C9 IM	*1/*2	Genetic variation reduces conversion of phenytoin to inactive metabolites. This increases the risk of side effects. For the genotype group *1/*3+*3/*3, an increased risk of the life-threatening cutaneous side effects Stevens-Johnson Syndrome and toxic epidermal necrolysis has been observed, especially in Asians.	The loading dose does not need to be adjusted.	Use 75% of the standard dose*	Advise the individual to report side effects (such as ataxia, nystagmus, slurred speech, sedation or, especially in Asian individuals, rash) occur.
	*1/*3				
	other				
CYP2C9 PM	*2/*2			Use 50% of the standard dose*	
	*2/*3			Use 50% of the standard dose*	
	*3/*3			Use 40% of the standard dose*	
	other			Use 40–50% of the standard dose*	

* Assess the dose based on effect and serum concentration after 7–10 days.
This DPWG table is adapted from (5).
IM – intermediate metabolizer
PM – poor metabolizer

Drug: Phenytoin

Phenytoin is a generic antiseizure drug that is rarely prescribed to newly diagnosed individuals due to its propensity for long-term side effects. Nevertheless, it continues to be used by many individuals who initiated treatment before the availability of newer medications that have fewer side effects and drug-drug interactions. Phenytoin is used for the control of partial seizures and generalized tonic-clonic convulsions. It is also used in the treatment of status epilepticus and may be used to prevent or treat seizures that occur during and following neurosurgery (1).

Phenytoin belongs to the sodium channel blocker class of antiseizure drugs, which are thought to suppress seizure activity by blocking voltage-gated sodium channels that are responsible for the upstroke of action potentials (6, 7). The block by phenytoin and other members of this class of antiseizure drugs occurs in a state-dependent fashion, with preferential binding and block of the inactivated state of the channel. This results in

voltage- and frequency-dependent block in which high frequency action potential firing, which occurs during epileptic activity, is preferentially inhibited. (1, 8)

The dosing of phenytoin can be complex, as treatment is typically initiated at a low starting dose, which considers individual age, weight, and the presence of concomitant medications that may influence phenytoin metabolism or protein binding. The dose is then carefully escalated to obtain the desired therapeutic effect. There is a wide variation in how individuals respond to phenytoin (2). Therapeutic drug monitoring is often used to adjust the dose to ensure that plasma levels are within therapeutic range (10–20 μg/ml in adults). Measurement of plasma levels is useful when adding or discontinuing concomitant medications that effect phenytoin levels. Periodic measurement of plasma phenytoin concentrations may also be valuable in pregnancy because altered phenytoin pharmacokinetics increases the risk of seizures.

Phenytoin use during pregnancy has been associated with an 11% risk of fetal hydantoin syndrome in the offspring, which is characterized by dysmorphism, hypoplasia, and irregular ossification of the distal phalanges. Facial dysmorphism includes epicanthal folds, hypertelorism, broad flat nasal bridges, an upturned nasal tip, wide prominent lips, and, in addition, distal digital hypoplasia, intrauterine growth retardation, and mental retardation. An additional 30% of the in utero-exposed children express fetal hydantoin effects, in which there is a more limited pattern of dysmorphic characteristics. Some studies have found significant associations between in utero exposure to phenytoin and major congenital abnormalities (mainly, cardiac malformations and cleft palate) whereas others have failed to find such associations (9, 10).

The adverse effects of phenytoin fall into 2 categories, types A and B. (11)

Type A adverse drug reactions account for up to 90% of reactions. They are predictable and can occur in any individual if their drug exposure is high enough. Some of these reactions occur rapidly and are reversible when the dose is reduced. These include acute central nervous system adverse effects such as sedation, nystagmus, and ataxia. Other common side effects occur with long-term exposure and include changes to the physical appearance, such as gingival hyperplasia, coarsening of the facial features, hirsutism, and acne.

Type B adverse drug reactions include idiosyncratic hypersensitivity reactions, such as severe cutaneous adverse reaction (SCAR). Such reactions can occur at any dose and develop through a mechanism that is unrelated to the mechanism of action of the drug. A rare but life-threatening hypersensitivity reaction associated with phenytoin treatment is SJS and the related TEN. Both are severe cutaneous reactions to specific drugs, and are characterized by fever and lesions of the skin and mucous membranes, with a mortality rate of up to 30% (12).

It is difficult to predict in whom a drug-induced hypersensitivity reaction is likely to occur. However, for phenytoin individuals who are positive for a specific *HLA* allele are known to be susceptible to phenytoin-induced SJS/TEN. Human leukocyte antigen testing of individuals can identify at-risk individuals so that an alternative drug can be used.

The *HLA* Gene Family

The human leukocyte antigen (*HLA*) genes are members of the major histocompatibility complex (*MHC)* gene family, which includes more than 200 genes. The *MHC* family has been subdivided into 3 subgroups based on the structure and function of the encoded proteins: Class I, Class II, and Class III. The class I region contains the genes encoding the HLA molecules HLA-A, HLA-B, and HLA-C. These molecules are expressed on the surfaces of almost all cells and play an important role in processing and presenting antigens. The class I gene region also contains a variety of other genes, many of which are not known to be involved in immune function.

An important role of HLA class I molecules is to present peptide fragments to immune cells (CD8+ T cells). Most of these peptides originate from the breakdown of normal cellular proteins (“self”). However, if foreign peptide fragments are presented, for example, from a pathogen, CD8+T cells will recognize the peptides as “non-

self" and will be activated to release inflammatory cytokines and launch an immune response to dispose of the pathogen (or foreign body).

Because HLA molecules need to present such a wide variety of "self" and "non-self" peptides, the HLA genes are both numerous and highly polymorphic. More than 1,500 *HLA-B* alleles have been identified (13). The *HLA* allele nomenclature includes the HLA prefix, followed by the gene, an asterisk and a 2 digit number that corresponds to antigen specificity, and the assigned allele number (14). For example, the *HLA-B*15:02* allele is composed of:

- HLA: the HLA prefix (the HLA region on chromosome 6)
- B: the B gene (a particular HLA gene in this region)
- 15: the allele group (historically determined by serotyping, namely, a group of alleles that share the same serotype)
- 02: the specific HLA allele (a specific protein sequence; determined by genetic analysis).

Additional digits have recently been added to the nomenclature to discriminate alleles that do not differ in the protein amino acid sequence, but differ in their genetic sequence (namely, due to synonymous and noncoding genetic variants).

Variation in *HLA* genes plays an important role in the susceptibility to autoimmune disease and infections, and they are also critical in the context of transplant surgery where better outcomes are observed if the donor and recipient are HLA-compatible.

More recently, *HLA* alleles have been associated with susceptibility to Type B adverse drug reactions. For example, *HLA-B* alleles have been associated with severe hypersensitivity reactions to abacavir (used to treat HIV), allopurinol (used to treat gout), and the antiepileptic drugs carbamazepine and phenytoin.

Gene: *HLA-B*15:02*

Individuals who have one or 2 copies of the high-risk *HLA-B*15:02* allele are known as *HLA-B*15:02* positive (Table 5).

Table 5. The CPIC Assignment of Likely *HLA-B* Phenotype Based on Genotype (2020)

HLA phenotype	Genotype	Examples of diplotypes
*HLA-B*15:02* negative	Homozygous for any allele(s) other than *HLA-B*15:02*	**X/*X* [a]
*HLA-B*15:02* positive	Homozygous or heterozygous variant	**15:02/*X* [a] , **15:02/*15:02*

[a] Where **X* = any *HLA-B* allele other than **15:02*.
Table is adapted from (2).
Note: The nomenclature used in this table reflect the standardized pharmacogenetic terms proposed by CPIC (4).

The association between the *HLA-B*15:02* allele and SJS/TEN was first reported with the use of carbamazepine in the Han Chinese population. In the initial study, all individuals who had carbamazepine-induced SJS/TEN were found to have *HLA-B*15:02* (44/44, 100%), whereas the allele was much less common among carbamazepine-tolerant individuals (3/101, 3%)(15). In subsequent studies, this association was replicated, with an *HLA-B*15:02* positivity frequency of 70–100% among cases of carbamazepine-induced SJS/TEN (16).

The *HLA-B*15:02* allele was later associated with phenytoin-induced hypersensitivity reactions, including phenytoin-induced SJS in a Thai population and phenytoin-induced SJS/TEN in Chinese Asians (17, 18).

There are fewer studies on phenytoin-induced hypersensitivity than carbamazepine, and the strength of association between phenytoin and SJS/TEN is weaker than that of carbamazepine and SJS/TEN. However, from

the evidence available, the FDA recommends consideration of avoiding phenytoin as an alternative treatment to carbamazepine in individuals who have *HLA-B*15:02* (2).

The prevalence of carbamazepine-induced SJS/TEN is higher in populations where *HLA-B*15:02* is more common. Of note, the *HLA-B*15:02* allele frequency is highest in Southeast Asia, as populations from Hong Kong, Thailand, Malaysia, Vietnam, and parts of the Philippines have an allele frequency >15%. It is slightly lower (~10–13%) in Taiwan and Singapore, and around 4% in North China. South Asians, including Indians, appear to have an *HLA-B*15:02* allele frequency of ~2–4%, with higher frequencies in some subpopulations. (15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30)

The *HLA-B*15:02* allele is rare (<1%) in East Asia (Japan and Korea) and among individuals who are not of Asian descent. For example, the allele is rare in Europeans, Hispanics, Africans, African Americans, and Native Americans. (16, 21)

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2C9 enzyme metabolizes approximately 15% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the *CYP2C9* gene can play a major role in adverse drug reactions (31, 32).

The *CYP2C9* gene is polymorphic, with more than 50 known alleles. Variation in *CYP2C9* is thought to contribute to the pharmacogenetic variability in phenytoin metabolism. *CYP2C9*1* is considered the wild-type allele when no variants are detected and is categorized as normal enzyme activity (2). Individuals who have 2 normal-function alleles (for example, *CYP2C9* *1/*1) are classified as "normal metabolizers" (Table 6).

Table 6. The CPIC Assignment of Likely *CYP2C9* Phenotype Based on Genotype (2020)

CYP2C9 phenotype[a,b]	Activity score	Genotype	Examples of diplotypes
Normal metabolizer	2	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer	1.5 1	An individual with one normal-function allele plus one decreased-function allele OR one normal-function allele plus one no-function allele OR 2 decreased-function alleles	*1/*2 *1/*3 *2/*2
Poor metabolizer	0.5 0	An individual with one no-function allele plus one decreased-function allele; OR 2 no-function alleles	*2/*3 *3/*3

[a] Assignment of allele function and associated citations can be found at the CPIC website, also see (2).
[b] See the *CYP2C9* Frequency Table in refs. 3 and 4 from (2) for population-specific allele and phenotype frequencies.
Note: There are no known cases of CYP2C9 ultrarapid metabolizers
This CPIC table has been adapted from (2).

For individuals who are CYP2C9 normal metabolizers, the recommended starting maintenance dose of phenytoin does not need to be adjusted based on genotype (2).

Two common allelic variants associated with reduced enzyme activity are *CYP2C9*2* (p.Arg144Cys) and *CYP2C9*3* (p.Ile359Leu). The *2* allele is more common in Caucasian (10–20%), than Asian (1–3%) or African (0–6%) populations. The *3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African Americans, the *CYP2C9*5*, *6*, *8* and *11* alleles are more common (33, 34, 35).

Linking *HLA-B and CYP2C9* Genetic Variation with the Risk of Side Effects and Treatment Response

Reduced activity *CYP2C9* alleles, in particular *CYP2C9*3*, influence phenytoin dosage, individual response, and predict adverse drug reactions (36, 37, 38, 39, 40). Individuals with reduced-function *CYP2C9* alleles have reduced clearance of phenytoin and have an increased risk of side effects.

Specific HLA alleles, namely, *HLA-B*15:02,* are also strongly associated with SCAR (41).

To guide the optimal dose of phenytoin and reduce the risk, both genetic factors (*CYP2C9* and *HLA* alleles) and non-genetic factors (for example, omeprazole co-medication) need to be considered (41, 42, 43, 44, 45).

The *HLA-B* and *CYP2C9* Gene Interactions with Medications Used for Additional Indications

Other medications with multiple indications are known to interact with the *HLA-B* alleles or to be metabolized by CYP2C9.

- Other seizure medications—Carbamazepine has similar interactions with HLA variation and hypersensitivity reactions: individuals with one or more copies of *HLA-B*15:02* are at risk of SJS/TEN and the *HLA-A*31:01* is strongly associated with a potentially life-threatening condition known as drug reaction with eosinophilia and systemic symptoms (DRESS) and a milder reaction maculopapular exanthema (MPE).
- Uric acid reduction medications—Allopurinol is a xanthine oxidase inhibitor to treat high uric acid levels seen in gout, tumor lysis syndrome, and cases of symptomatic hyperuricemia. The *HLA-B*58:01* allele is associated with SCAR during allopurinol treatment. Lesinurad is a urate transport inhibitor, also used in the treatment of gout and it is metabolized by CYP2C9 to inactive metabolites. Individuals who are CYP2C9 poor metabolizers will have an increased exposure to the active drug and thus have an increased risk of side effects such as kidney stones or cardiovascular events.
- Anti-retroviral medication—Abacavir, a nucleoside/nucleotide reverse transcriptase inhibitor used in the treatment of HIV, is also associated with hypersensitivity reactions, the risk of which increases when an individual has the *HLA-B*57:01* allele.
- Non-steroidal anti-inflammatory drugs (NSAIDs)—Celecoxib, Flurbiprofen, and Piroxicam are used for pain management in osteoarthritis, rheumatoid arthritis, and other conditions; they are all metabolized by CYP2C9 and poor metabolizers will experience higher levels of exposure to these NSAIDs and have higher risk of side effects.
- Anti-emetics—Dronabinol, a synthetic cannabinoid, is used in the treatment of chemotherapy-induced nausea and vomiting for individuals who had poor responses to traditional anti-emetics. It also is used to treat anorexia-associated weight loss in individuals with acquired immunodeficiency syndrome. Dronabinol is activated by CYP2C9 metabolism and individuals with poor metabolizer phenotypes will have an increased exposure to dronabinol and increased risk of side effects.

Additional information on gene-drug interactions for HLA-B and CYP2C9 are available from PharmGKB, CPIC, and the FDA (search for "HLA-B" or "CYP2C9").

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are available for the phenytoin drug response, the *HLA-B* gene, and the *CYP2C9* gene.

The genotype results for an *HLA* allele such as *HLA-B*15:02* can either be "positive" or "negative". There are no intermediate phenotypes because the *HLA* genes are expressed in a codominant manner.

A positive result indicates the individual is either "heterozygous" or "homozygous" for the variant, depending upon whether they have one or 2 copies of the **15:02* allele.

A negative result indicates that the individual does not have the *HLA-B*15:02* allele. However, a negative result does not rule out the possibility of an individual developing phenytoin-induced SJS/TEN. Therefore, clinicians should carefully monitor all individuals according to standard practices.

For *CYP2C9*, alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology. (46) Results are typically reported as a diplotype, such as *CYP2C9 *1/*2,* and may include an interpretation of the individual's predicted metabolizer phenotype (normal, intermediate, or poor) and an activity score (Table 6).

Therapeutic Recommendations based on Genotype

This section contains excerpted [1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

Regarding HLA-B:

Studies in individuals of Chinese ancestry have found a strong association between the risk of developing SJS/TEN and the presence of *HLA-B*15:02*, an inherited allelic variant of the *HLA-B* gene, in individuals using carbamazepine. Limited evidence suggests that *HLA-B*15:02* may be a risk factor for the development of SJS/TEN in individuals of Asian ancestry taking other antiepileptic drugs associated with SJS/TEN, including phenytoin. Consideration should be given to avoiding phenytoin as an alternative for carbamazepine in individuals positive for *HLA-B*15:02*.

The use of *HLA-B*15:02* genotyping has important limitations and must never substitute for appropriate clinical vigilance and individual management. The role of other possible factors in the development of, and morbidity from, SJS/TEN, such as antiepileptic drug (AED) dose, compliance, concomitant medications, comorbidities, and the level of dermatologic monitoring have not been studied.

Regarding CYP2C9 and CYP2C19:

In most individuals maintained at a steady dosage, stable phenytoin serum levels are achieved. There may be wide interindividual variability in phenytoin serum levels with equivalent dosages. Individuals with unusually low levels may be noncompliant or hypermetabolizers of phenytoin.

Unusually high levels result from liver disease, variant *CYP2C9* and *CYP2C19* alleles, or drug interactions which result in metabolic interference. The individual with large variations in phenytoin serum levels, despite standard doses, presents a difficult clinical problem. Serum level determinations in such individuals may be particularly helpful. As phenytoin is highly protein bound, free phenytoin levels may be altered in individuals whose protein binding characteristics differ from normal.

Please review the complete therapeutic recommendations that are located here: (1).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

2018 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

HLA-B*1502

The life-threatening cutaneous side effect Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) occurs more frequently in patients with this genetic variation. The calculated risk of phenytoin-induced SJS/TEN in patients with *HLA-B*15:02* is 0.65%.

- Carefully weigh the risk of SJS/TEN against the benefits
- Avoid phenytoin if an alternative is possible
  - Carbamazepine carries a 10-fold higher risk of SJS/TEN for these individuals and is therefore not an alternative.
  - A comparable risk has been reported for lamotrigine as for phenytoin. The same applies for oxcarbazepine, but the most severe forms (SJS/TEN overlap and TEN) are not observed with oxcarbazepine.
- If it is not possible to avoid this medication, then advise the individual to report any skin rash immediately (Table 2)

CYP2C9 genotypes *1/*2, *1/*3 and other IMs

Genetic variation reduces conversion of phenytoin to inactive metabolites. This increases the risk of side effects.

Recommendation:

1. The loading dose does not need to be adjusted.
2. For the other doses, use 75% of the standard dose and assess the dose based on effect and serum concentration after 7-10 days.
3. Advise the patient to report if side effects (such as ataxia, nystagmus, slurred speech, sedation or rash) occur.

CYP2C9 genotypes *2/*2 and *2/*3 and other PMs

Genetic variation reduces conversion of phenytoin to inactive metabolites. This increases the risk of side effects.

Recommendation:

1. The loading dose does not need to be adjusted.
2. For the other doses, use 50% of the standard dose and assess the dose based on effect and serum concentration after 7-10 days.
3. Advise the patient to report if side effects (such as ataxia, nystagmus, slurred speech, sedation or rash) occur.

CYP2C9 genotype *3/*3 (PM)

Genetic variation reduces conversion of phenytoin to inactive metabolites. This increases the risk of side effects, including SJS/TEN in Asian patients.

Recommendation:

1. The loading dose does not need to be adjusted.
2. For the other doses, use 40% of the standard dose and assess the dose based on effect and serum concentration after 7-10 days.

3. Advise the patient to report if side effects (such as ataxia, nystagmus, slurred speech,sedation or, especially in Asian individuals, rash) occur.

Please review the complete therapeutic recommendations that are located here (3).

2020 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

***HLA-B*15:02* recommendations**

[…] If a individual is phenytoin-naïve and *HLA-B*15:02* positive, the individual has an increased risk of SJS/TEN and the recommendation is to consider using an anticonvulsant other than phenytoin unless the benefits of treating the underlying disease clearly outweigh the risks (see Table 3). Carbamazepine and oxcarbazepine should also be avoided if a individual is *HLA-B*15:02* positive. Alternative medications such as eslicarbazepine acetate and lamotrigine have limited evidence linking SJS/TEN with the *HLA-B*15:02* allele.

[…]

If a individual is phenytoin-naïve and *HLA-B*15:02* negative, the individual has a normal risk of phenytoin-induced SJS/TEN and the recommendation is to use phenytoin with dosage adjustments based on *CYP2C9* genotype (if known) or standard dosing guidelines (if *CYP2C9* genotype is unknown). However, an *HLA-B*15:02* negative test does not eliminate the risk of phenytoin-induced SJS/TEN.

***CYP2C9* recommendations**.

The recommended phenytoin initial or loading and maintenance doses do not need adjustments based on genotype for CYP2C9 NMs and IMs with an AS of ≥1.5. Available evidence does not clearly indicate the extent of dose reduction needed to prevent phenytoin-related toxicities in CYP2C9 IMs with an AS of 1.0 and PMs with an AS of 0 or 0.5. Furthermore, multiple case studies have observed that CYP2C9 PMs are at increased risk for exposure-related phenytoin toxicities, and multiple studies have observed an association between the *CYP2C9**3 allele and SJS/TEN. Although presence of the *CYP2C9**3 allele is insufficient to predict phenytoin-induced SJS/TEN, these and other data suggest that the risk of SJS/TEN is dose-related and provide an additional rationale for reducing phenytoin dose in CYP2C9 PMs . Thus, our recommendations are conservative given the variability surrounding phenytoin dosing. Based on the doses reported in the pharmacokinetic and pharmacogenetic studies mentioned above and in Table S2, a typical initial or loading dose followed by at least a 25% reduction in the recommended starting maintenance dose may be considered for CYP2C9 IMs with AS of 1.0. Subsequent maintenance doses should be adjusted based on therapeutic drug monitoring and response. For CYP2C9 PMs, use a typical initial or loading dose then consider at least a 50% reduction of starting maintenance dose with subsequent maintenance doses adjusted based on therapeutic drug monitoring and response.

Pediatrics

Much of the evidence (summarized in Table S1) linking *HLA-B*15:02* to phenytoin induced SJS/TEN was generated in both children and adults. Therefore, the above recommendation is made regardless of *CYP2C9* genotype, individual age, race or ancestry.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature of Selected *HLA-B* Alleles

Allele name	dbSNP reference identifier for allele location
*HLA-B*15:02*	Tagged variants cannot be reliably used to detect this allele. Sequencing is the most accurate technique for allele detection.

For the *major histocompatibility complex* region, variations in genes such as *HLA-B* occur across the whole sequence of the gene, not a single locus. Therefore, the *HLA-B*15:02* allele is defined by its sequence rather than single coding or protein variations. If there is strong linkage disequilibrium between one or more SNPs and a specific *HLA* allele, the presence of these SNPs (tag SNPs) may be used for *HLA* typing in some populations; however, genotyping tagged variants should not be considered diagnostic or equivalent to actual HLA testing. For *HLA-B*15:02*, rs2844682 and rs3909184 were previously considered the tagged variants (47), however, these variants have shown to be less accurate in other studies (48). Other tagged variants have been suggested, however, the sensitivity and accuracy of these variants to detect the *HLA-B*15:02* allele is limited (49, 50). Sequence for the full *HLA-B*15:02* allele (and subtypes) can be accessed here.
Guidelines on nomenclature of the HLA system are available from HLA Nomenclature: http://hla.alleles.org/

Nomenclature of Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.3:c.818del	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

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Piroxicam Therapy and *CYP2C9* Genotype

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Introduction

Piroxicam (brand name Feldene) is a nonsteroidal anti-inflammatory drug (NSAID) used to treat osteoarthritis and rheumatoid arthritis. Piroxicam provides pain relief and reduces inflammation.

Piroxicam is primarily metabolized by CYP2C9. Individuals who lack CYP2C9 activity ("CYP2C9 poor metabolizers") have an increased exposure to piroxicam, and an increased risk of side effects.

Like all NSAIDs, piroxicam increases the risk of serious cardiovascular events, including myocardial infarction and stroke, and serious gastrointestinal (GI) adverse events such as bleeding, ulceration, and perforation.

The standard dose of piroxicam for osteoarthritis and rheumatoid arthritis in adults is 20 mg once daily. But for all patients, the lowest effective dose of piroxicam should be used for the shortest length of time, consistent with the treatment goals of each individual (1).

The FDA-approved drug label for piroxicam states that a dose reduction should be considered in "patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin)". Dose reductions should be considered because these patients may have abnormally high plasma levels of piroxicam caused by reduced metabolic clearance. However, specific dose reductions based on CYP2C9 phenotype are not provided (Table 1) (1).

As for all NSAIDs, piroxicam is contraindicated in patients with a known hypersensitivity, a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID, and following coronary artery bypass graft (CABG) surgery. Piroxicam should also be avoided by pregnant women starting at 30 weeks gestation.

Table 1. The FDA (2018) Drug Label for Piroxicam. Recommendations for CYP2C9 Phenotype. Pharmacogenomics.

Phenotype	Recommendations
CYP2C19 poor metabolizers	In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin) consider dose reduction as they may have abnormally high plasma levels due to reduced metabolic clearance.

This table is adapted from (1).

Drug Class: NSAIDs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation, fever, and pain. They are one of the most commonly used classes of medicine. Worldwide, it is estimated that more than 30 million people receive NSAIDs daily (2).

Currently, more than 20 NSAIDs are licensed for use. Several NSAIDs (e.g., aspirin, ibuprofen, and naproxen) are available over-the-counter, but stronger doses and other types of NSAIDs, such as celecoxib and piroxicam, are only available via prescription.

The main action of NSAIDs is to inhibit cyclooxygenase (COX). Cyclooxygenase is the central enzyme in the synthesis of prostaglandins, prostacyclin, and thromboxanes from arachidonic acid. Prostaglandins can be

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protective (e.g., protect the gastric mucosal lining and aid platelet aggregation) or inflammatory (e.g., recruiting inflammatory white blood cells).

There are 2 main isoforms of COX, and the safety, and effectiveness of NSAIDs may be influenced by the degree they inhibit the 2 different forms. Cyclooxygenase-1 (COX-1) is a "housekeeping enzyme" which is expressed in most tissues. It protects the GI tract and induces platelet aggregation in response to injury. In contrast, COX-2 is often undetectable in tissues. However, the expression of COX-2 is increased during inflammation.

Most NSAIDs are non-selective COX inhibitors that inhibit both COX-1 and COX-2. There are exceptions, such as celecoxib, which is a selective COX-2 inhibitor that appears to be associated with less adverse GI events. However, GI adverse events still occur.

Approximately 25% of the exposed US population has experienced NSAID-related side effects that required medical care (3). All NSAIDs carry a boxed warning regarding the risk of serious GI and cardiovascular adverse events; e.g.,

"NSAIDs cause an increased risk of serious cardiovascular thrombotic events, including myocardial infarction and stroke, which can be fatal. This risk may occur early in treatment and may increase with duration of use.

NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients and patients with a prior history of peptic ulcer disease and/or GI bleeding are at greater risk for serious GI events" (1).

Drug: Piroxicam

Piroxicam is an NSAID used for the relief of osteoarthritis and rheumatoid arthritis. The recommended dose in adults is 20 mg daily, and although therapeutic effects are seen early, it takes up to 12 days for steady-state levels to be reached. Therefore, the effect of therapy should not be assessed for the first 2 weeks.

Because of the adverse events associated with any type of NSAID, the lowest effective dose of piroxicam should be used for the shortest duration. And, as for all NSAIDs, piroxicam is contraindicated in patients with a known hypersensitivity, or a history of asthma, urticaria, or other allergic-type reactions after taking aspirin or another NSAID. Piroxicam is also contraindicated to treat pain in the days following CABG surgery (NSAIDs cause an increased risk of myocardial infarction and stroke post-operatively), and piroxicam should be avoided by pregnant women starting at 30 weeks gestation (NSAID use in the third trimester causes an increased risk of premature closure of the fetal ductus arteriosus).

A subset of NSAIDs, known as oxicams, are highly potent and share a similar structure with a new binding fold that is different to typical NSAIDs. Piroxicam was the first oxicam to be licensed, other oxicams include isoxicam, meloxicam, tenoxicam, and lornoxicam (4, 5).

One study found that oxicams (piroxicam and tenoxicam) had a higher risk of Stevens -Johnson Syndrome (SJS), and toxic epidermal necrolysis (TEN) (relative risk [RR] of 34) than diclofenac (RR 4.1) and ibuprofen (RR 5.3). However, the absolute risk of SJS or TEN during piroxicam is still thought to be low –– the incidence of SJS or TEN during the first 8 weeks of piroxicam or tenoxicam therapy is one per 100,000 patients (6).

CYP2C9 is the main enzyme involved in the metabolism of piroxicam to its major inactive metabolite: 5′-hydroxy-piroxicam. Individuals with low CYP2C9 activity ("CYP2C9 poor metabolizers") have a higher exposure to piroxicam (1).

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The CYP450 genes are very polymorphic and can result in reduced, absent, or increased enzyme activity (7).

The *CYP2C9* gene is highly polymorphic, with approximately 60 known alleles. *CYP2C9*1* is considered the wild-type allele when no variants are detected, and is categorized by normal enzyme activity (8). Individuals who have 2 normal-function alleles (e.g., *CYP2C9 *1/*1*) are classified as "normal metabolizers" (Table 2).

Table 2. Assignment of likely *CYP2C9* Phenotype based on Genotype (CPIC, 2014)

Likely phenotype[a]	Genotype	Examples of diplotypes
Ultrarapid metabolizer (increased activity) (frequency unknown)	Unknown – currently there are no known increased activity alleles	Unknown
Normal metabolizer (normal activity) (approximately 91% of individuals)	An individual with 2 normal-function alleles	*1/*1
Intermediate metabolizer (heterozygote or intermediate activity) (approximately 8% of individuals)[b]	An individual with one normal-function allele plus one decreased-function allele	*1/*3, *1/*2
Poor metabolizer (homozygous variant, low or deficient activity) (approximately 1% of individuals)	An individual with 2 decreased function alleles	*2/*2, *3/*3, *2/*3

Note: There are no known cases of CYP2C9 ultrarapid metabolizers
[a] Global frequencies are approximate. Because haplotype frequencies vary considerably among popualations, please see (8) for individual population frequencies.
[b] The enzyme activity in this grouping varies widely. Please see (8) for activity ranges.
This table is adapted from (8). Note: The nomenclature used in this table reflects the standardized pharmacogenetic terms proposed by CPIC (9).

Two allelic variants associated with reduced enzyme activity are *CYP2C9*2* and **3*. The **2* allele is more common in Caucasian (10-20%), than Asian (1-3%) or African (0-6%) populations. The **3* allele is less common (<10% in most populations) and is extremely rare in African populations. In African-Americans, the *CYP2C9*5*, **6*, **8* and **11* alleles are more common (10-12).

Linking Gene Variation with Treatment Response

Studies have shown that CYP2C9 poor metabolizers have increased exposure and reduced clearance when taking standard doses of piroxicam (1, 13). A gene-dose effect was proposed recently to explain the gradual increase in piroxicam exposure in an individual with a *CYP2C9* *3/*3 genotype compared with those with the *1/*1 and *1/*3 genotypes. And although data are lacking, overall it appears that the decreased function alleles *CYP2C9*2*, *CYP2C9*3* are associated with an increased risk of acute GI bleeding in patients receiving NSAID therapy. The *CYP2C8* variant, *CYP2C8*3*, may also contribute to this increased risk (3, 14, 15).

A recent small study (n=102 volunteers heterozygous for *CYP2C8*3* and *CYP2C9*3*) reported that the administration of 20 mg oral piroxicam for 4 days was effective in the control of pain following molar surgery regardless of the CYP haplotype (16). However, this study was not specifically designed to address the risk of adverse events across genotype groups, and the study used a low dose for a short duration (20 mg once daily for 4 days).

In addition to increased exposure of piroxicam by decreased CYP2C9 activity in poor metabolizers, CYP2C9 may also impact cardiovascular morbidity by altering the metabolism of fatty acids, prostanoids, and steroid hormones, especially in poor metabolizers of CYP2C9 (7).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C9* alleles. The NIH Genetic Testing Registry (GTR) displays genetic tests that are currently available for the *CYP2C9* gene.

The *CYP2C9* variants that are routinely tested for include *CYP2C9*2* and **3*. Usually, the results are reported as a diplotype, such as *CYP2C9 *1/*1*, and may also include an interpretation of the patient's predicted metabolizer phenotype (normal, intermediate, or poor). Table 2 summarizes common CYP2C9 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Higher systemic exposure of piroxicam has been noted in subjects with *CYP2C9* polymorphisms compared to normal metabolizer type subjects.

[...]

CYP2C9 activity is reduced in individuals with genetic polymorphisms, such as the *CYP2C9*2* and *CYP2C9*3* polymorphisms. Limited data from two published reports showed that subjects with heterozygous *CYP2C9*1/*2* (n=9), heterozygous *CYP2C9*1/*3* (n=9), and homozygous *CYP2C9*3/*3* (n=1) genotypes showed 1.7-, 1.7-, and 5.3-fold higher piroxicam systemic levels, respectively, than the subjects with *CYP2C9*1/*1* (n=17, normal metabolizer genotype) following administration of a single oral dose. The mean elimination half-life values of piroxicam for subjects with *CYP2C9*1/*3* (n=9) and *CYP2C9*3/*3* (n=1) genotypes were 1.7- and 8.8-fold higher than subjects with *CYP2C9*1/*1* (n=17). It is estimated that the frequency of the homozygous **3/*3* genotype is 0% to 1% in the population at large; however, frequencies as high as 5.7% have been reported in certain ethnic groups.

Poor Metabolizers of CYP2C9 Substrates: In patients who are known or suspected to be poor CYP2C9 metabolizers based on genotype or previous history/experience with other CYP2C9 substrates (such as warfarin and phenytoin) consider dose reduction as they may have abnormally high plasma levels due to reduced metabolic clearance.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature for selected *CYP2C9* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	818delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Note: the normal "wild-type" allele is *CYP2C9*1* and is reported when no variant is detected.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (17).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Prasugrel Therapy and *CYP* Genotype

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Introduction

Prasugrel (also known as Efient) is a third-generation thienopyridine platelet inhibitor used in individuals with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI). Prasugrel is prescribed to reduce thrombotic cardiovascular events, such as stent thrombosis, myocardial infarction, and stroke in these individuals. Along with other antiplatelet agents such as clopidogrel and ticagrelor, prasugrel inhibits platelet activation by irreversibly binding to the platelet receptor, P2RY12. (1)

Prasugrel is metabolized into its active metabolite primarily by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19. The FDA-approved label for prasugrel states that genetic variations in *CYP2B6*, *CYP2C9*, *CYP2C19*, or *CYP3A5* genes do not significantly affect prasugrel's pharmacokinetics, the generation of its active metabolite, or its inhibition of platelet aggregation in healthy subjects, individuals with stable atherosclerosis, or those with ACS (1).

Another commonly prescribed antiplatelet agent is the second-generation thienopyridine clopidogrel, which is bioactivated primarily by CYP2C19. As a result,, clopidogrel is less effective in individuals with decreased or non-function variant alleles of the *CYP2C19* gene. In contrast, *CYP2C19* variants do not decrease the effectiveness of prasugrel, which is a more potent antiplatelet agent compared to clopidogrel, though it carries a higher risk of bleeding (2, 3, 4, 5).

Drug: Prasugrel

Prasugrel is a third-generation thienopyridine antiplatelet agent that binds irreversibly to the P2RY12 receptor and inhibits adenosine diphosphate (ADP)-mediated platelet activation and aggregation. Other P2RY12 receptor blockers include clopidogrel and ticagrelor.

As an antiplatelet agent, prasugrel inhibits the formation of blood clots in the coronary, peripheral, and cerebrovascular arteries among individuals with ACS.

A decrease in blood flow in the coronary arteries, known as ACS, includes unstable angina, which occurs suddenly, often at rest or with minimal exertion. Unstable angina may be new in onset or may occur with less exertion than previously. Another form of ACS is a myocardial infarction (MI), which may be classified as ST-segment elevation myocardial infarction (STEMI), or non-ST-segment elevation myocardial infarction (NSTEMI) based on electrocardiogram (EKG) findings. A STEMI is identified when EKG findings show ST-segment elevation. If no ST-segment elevation is present but myocardial biomarkers such as troponin I or T are increased, the term NSTEMI is applied.

Individuals with ACS are usually treated with a P2Y12 receptor blocker and aspirin (dual antiplatelet therapy, DAPT) to reduce the risk of developing a coronary artery thrombus. Platelet adhesion and aggregation are early stages in thrombus formation, which may occlude the coronary artery. Individuals who undergo PCI are at risk of stent occlusion via this mechanism.

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The TRITON-TMI 38 trial compared prasugrel with clopidogrel in 13,608 individuals with ACS undergoing PCI. Prasugrel provided more potent platelet inhibition than clopidogrel: after 15 months, individuals treated with prasugrel had a lower incidence of cardiovascular death, nonfatal MI, or nonfatal stroke as compared with those treated with clopidogrel (9.9% versus 12.1%) (2, 3). However, prasugrel was associated with a higher risk of bleeding, leading to the FDA warning that its use is contraindicated in individuals with active pathological bleeding or a history of stroke or transient ischemic attack (TIA) (4, 5).

Prasugrel inhibits ADP-induced platelet aggregation by selectively binding to P2RY12. As a pro-drug, prasugrel requires conversion into its active metabolite to function as an antiplatelet agent. It is rapidly metabolized to thioacetone, which is further converted to an active metabolite by CYP3A5 and CYP2B6, and to a lesser extent by CYP2C9 and CYP2C19.

The active prasugrel metabolite (R-138727) contains a reactive thiol group that forms a disulfide bridge with a free cysteine residue on the P2RY12 receptor. Once irreversibly bound to prasugrel, the receptor is unable to bind ADP, and platelet activation via this pathway is prevented for the platelet's lifespan, approximately 10 days (6).

Despite the general efficacy of clopidogrel, interindividual variability in metabolite levels, platelet inhibition, and clinical response has been observed. It has been estimated that between 16–50% of individuals treated with clopidogrel exhibit high on-treatment platelet reactivity (HTPR), meaning that some P2RY12 receptors remain unblocked (7). This is partially due to genetic variants in the *CYP2C19* gene, which encodes the enzyme responsible for converting clopidogrel to its active metabolite. Individuals with no-function *CYP2C19* alleles (for example, *CYP2C19*2*) have reduced plasma levels of the active clopidogrel metabolites and an increased risk for HTPR.

In contrast, genetic variation in *CYP3A5*, *CYP2B6*, *CYP2C9*, or *CYP2C19* does not have a relevant effect on the prasugrel pharmacokinetics, active metabolite formation, or platelet aggregation inhibition(8, 9, 10, 11, 12). Therefore, although both clopidogrel and prasugrel form active metabolites with similar potency, prasugrel is a more potent antiplatelet agent due to its more efficient active metabolite formation (13).

While prasugrel is more effective than standard-dose clopidogrel, DAPT with clopidogrel and aspirin remains the standard of care at some institutions for certain ACS individuals undergoing PCI (14). This preference is mainly because clopidogrel has a lower bleeding risk and is less expensive (15). However, the availability of *CYP2C19* genetic testing allows for personalized antiplatelet therapy, where individuals with impaired CYP2C19 activity can be identified and offered an alternative antiplatelet agent, such as prasugrel (16, 17, 18, 19). Recent studies have shown that *CYP2C19*-genotype guided antiplatelet therapy results in the therapeutic goal of reduced on-treatment platelet reactivity more frequently than standard therapy (20, 21, 22), which may also be cost-effective in ACS individuals undergoing PCI (23).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic, which can result in no, decreased, normal, or increased enzyme activity.

The CYP2C19, CYP2C9, CYP3A5, and CYP2B6 enzymes are involved in the metabolism of prasugrel, but genetic variations in these genes do not appear to influence the pharmacokinetics of prasugrel. In contrast, genetic variation in the *CYP2C19* gene may lead to decreased effectiveness of the related drug clopidogrel. For more information on CYP variants and the clopidogrel drug response, see "Clopidogrel Therapy and *CYP2C19* Genotype".

Conversely, administration of prasugrel is not expected to impact the efficacy of other medications that depend on CYP2C19, CYP2C9, CYP3A5, or CYP2B6 metabolism, despite in vitro assays showing an induction of CYP3A enzymes (1, 24, 25).

Genetic Testing

The NIH Genetic Testing Registry (GTR) lists genetic tests available for *CYP2C19*, *CYP2C9*, *CYP3A5*, and *CYP2B6* genes. Since the formation of the active metabolite of prasugrel is not known to be affected by CYP variants, genetic testing before prasugrel use is not recommended.

For clopidogrel, its effectiveness depends on its activation to an active metabolite, primarily by CYP2C19. Therefore, the FDA states that tests identifying an individual's CYP2C19 genotype can be used as an aid in determining therapeutic strategy.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2024 Statement from the US Food and Drug Administration (FDA): There is no relevant effect of genetic variation in CYP2B6, CYP2C9, CYP2C19, or CYP3A5 on the pharmacokinetics of prasugrel's active metabolite or its inhibition of platelet aggregation.

[...]

Prasugrel can be administered with drugs that are inducers or inhibitors of cytochrome P450 enzymes.

Please review the complete therapeutic recommendations that are located here: (1)

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Version 1.1 of this chapter was published on October 15, 2024. This minor revision features an update to a more recent FDA-approved drug label reference, inclusion of references for international drug package labelling (Canada, Japan). There are no changes to the recommendations or guidelines for genotype-guided dosing of this medication relative to the prior version.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Primaquine Therapy and *G6PD* and *CYP2D6* Genotype

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Introduction

Primaquine is a potent antimalarial medication indicated for the radical cure of malaria caused by *Plasmodium vivax (P. vivax)* and *Plasmodium ovale (P. ovale)* species (1, 2). Malaria is a blood borne infection caused by infection of *Plasmodium* parasites that is spread by mosquitos. The *P. vivax* and *P. ovale* species present a particular challenge to treat because the parasitic life cycle includes a dormant, liver-specific stage that is not susceptible to other antimalarial medications. Thus, primaquine is often used with other therapies such as chloroquine or artemisinin-based medicines that target the reproductive, active forms of the parasite. Primaquine is also used to prevent transmission of malaria caused by *Plasmodium falciparum* (*P. falciparum)* species. A single, low dose (SLD) of primaquine has gametocidal activity, which does not cure the individual but does provide malaria transmission control.

Primaquine is a pro-drug that must be activated by the cytochrome P450 (CYP) enzyme system. Metabolism by the cytochrome P450 member 2D6 (CYP2D6) and cytochrome P450 nicotinamide adenine dinucleotide phosphate (NADPH):oxidoreductase (CPR) generates 2 hydroxylated active metabolites that generate hydrogen peroxide (H_2O_2). This causes significant oxidative stress to the malarial parasite and the host human cells. Individuals who are glucose-6-phosphate dehydrogenase (G6PD) deficient are particularly susceptible to oxidative stress and may experience acute hemolytic anemia (AHA). Before starting a course of primaquine, individuals should be tested for G6PD deficiency to ensure safe administration (1, 2). According to the FDA-approved drug label, individuals with severe G6PD deficiency should not take primaquine (Table 1) (1).

The World Health Organization (WHO) recommends that individuals with G6PD deficiency should be treated with a modified course of primaquine therapy. The recommended course for individuals with G6PD deficiency is a single dose once per week for 8 weeks, while the standard course is daily administration for 14 days (Table 2) (2). The Clinical Pharmacogenetics Implementation Consortium (CPIC) reports that the risk of adverse effects of primaquine therapy for G6PD-deficient individuals is dose-dependent, with the SLD regimen presenting the least risk (Table 3) (3).

Primaquine is contraindicated during pregnancy and is not recommended for breastfeeding individuals when the G6PD status of the baby is unknown (1, 2). Primaquine is not approved for individuals under 6 months of age. Individuals with acute illness that are prone to granulocytopenia or individuals taking another hemolytic medication are also contraindicated from taking primaquine. (1)

Table 1. The FDA Drug Label for Primaquine Phosphate (2021)

G6PD status	Risk	Recommendation
Deficient	Hemolytic anemia	G6PD testing has to be performed before using primaquine. Due to the limitations of G6PD tests, physicians need to be aware of residual risk of hemolysis
Severe deficiency	Hemolytic anemia	Primaquine should not be prescribed

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Table 1. continued from previous page.

G6PD status	Risk	Recommendation
Mild to moderate deficiency	Hemolytic anemia	A decision to prescribe primaquine must be based on an assessment of the risks and benefits of using primaquine. If primaquine administration is considered, baseline hematocrit and hemoglobin must be checked before treatment and close hematological monitoring (for example, at day 3 and 8) is required
Unknown, testing unavailable	Hemolytic anemia	A decision to prescribe primaquine must be based on an assessment of the risks and benefits of using primaquine. Risk factors for G6PD deficiency or favism must be assessed. Baseline hematocrit and hemoglobin must be checked before treatment and close hematological monitoring (for example, at day 3 and 8) is required

This table is adapted from (1). G6PD - glucose-6-phosphate dehydrogenase

Table 2. The WHO Recommended Dosing Regimen for Primaquine Phosphate and G6PD Deficiency

Dosing regimen	G6PD testing	Recommendation strength, evidence certainty	G6PD status	Therapeutic goal and recommendations
Single low dose (0.25 mg/kg bw)	Not required	Strong, low	All	Reducing the transmissibility of treated *P. falciparum* infections in low-transmission areas. Recommended course does not apply to pregnant women, infants aged <6 months, women breastfeeding infants aged <6 months
14-day course (0.25–0.5[a] mg/kg bw per day)	Recommended to guide administration	Strong, high	Known G6PD normal	Treating uncomplicated malaria caused by *P. vivax, P. ovale, P. malariae* or *P. knowlesi*, preventing relapse. Excludes pregnant women, infants aged <6 months, women breastfeeding infants aged <6 months, women breastfeeding older infants unless they are known not to be G6PD deficient, and people with G6PD deficiency
0.75 mg/kg bw once weekly for 8 weeks	Recommended to guide administration	Conditional, very low	G6PD deficient	Treating uncomplicated malaria caused by *P. vivax, P. ovale, P. malariae* or *P. knowlesi*, preventing relapse. When G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine

This table adapted from (2). Mg/kg bw - milligrams per kilogram of body weight. G6PD - glucose-6-phosphate dehydrogenase
[a] The WHO advises that "temperate" strains be treated with 0.25 mg/kg bw dose, while tropical, frequent-relapsing *P. vivax* prevalent in East Asia and Oceania may require the higher 0.5 mg/kg bw daily dose. *P. falciparum - Plasmodium falciparum, P. vivax – Plasmodium vivax, P. ovale – Plasmodium ovale, P. malariae - Plasmodium malariae, P. knowlesi – Plasmodium knowlesi*

Table 3. The CPIC Guidelines for Primaquine based on G6PD Phenotype

G6PD status (predicted from genotype)[a]	Dosing recommendation	Risk	Classification of recommendation
Normal	No reason to avoid primaquine based on G6PD status	Low	Strong
Deficient	Avoid primaquine at ≥ standard dose (0.25–0.5 mg/kg daily for 14 days)	High	Strong

Table 3. continued from previous page.

G6PD status (predicted from genotype)[a]	Dosing recommendation	Risk	Classification of recommendation
Deficient	Medium dose (0.75 mg/kg or 45 mg, once weekly for 8 weeks) for *P. vivax* malaria; monitor individuals closely for hemolysis	Medium	Strong
Deficient	Single low dose (0.25 mg/kg) for *P. falciparum* malaria	Low to no	Strong
Deficient with CNSHA	Avoid primaquine	High	Strong
Variable or indeterminant	Ascertain G6PD status by enzyme activity; drug use should be guided by activity-based phenotype.[b]	Variable or unknown	Moderate

This table is adapted from (3). Mg/kg - milligram per kilogram of the individual's body weight. CNSHA - chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phosephate dehydrogenase, *P. vivax - Plasmodium vivax, P. falciparum - Plasmodium falciparum*
[a] Definition of G6PD status based on data from the World Health Organization and US Centers for Disease Control.
[b] X-linked mosaicism in individuals with more than one X chromosome (who are heterozygous for *G6PD* alleles of different functional status) can lead to variable G6PD function, enzyme-based assays should be used to determine G6PD activity and guide dosing. An enzyme activity-based test is also recommended for individuals with any allele of unknown function.

Drug: Primaquine

Primaquine is an 8-aminoquinoline antimalarial medication, indicated for the radical cure of *P. vivax* and *P. ovale* caused malaria (1, 4). Primaquine is approved by the WHO to prevent relapse of *P. vivax* and *P. ovale* malaria, often with chloroquine (in areas with chloroquine-sensitive *P. vivax*) (2). Though once not recommended in high-transmission settings, the WHO 2022 Malaria Guidelines state that "given the benefits of preventing relapse and in the light of changing epidemiology worldwide and more aggressive targets for malaria control and elimination, the [WHO Global Malaria Programme] group now recommends that primaquine be used in all settings" (2). In severe *P. vivax* malaria, primaquine therapy should be administered after the completion of other antimalarial therapies that target the active parasites, such as artesunate or chloroquine (2).

The standard administration of primaquine is a daily dose of 0.25 mg base per kg of body weight (mg/kg bw) for 14 days (1, 2). However, some sources recommend a standard adult dose of 15 mg base daily for 14 days, with increased dosing based on either weight over 70 kg or known infection with a frequently relapsing strain of *Plasmodium* (5, 6). In individuals with G6PD deficiency, either standard dosage presents a significant risk of life-threatening hemolysis, so an adjusted regimen of 0.75 mg/kg bw once a week for 8 weeks with close medical supervision is conditionally recommended by the WHO (2). Primaquine may also be administered as a SLD (0.25 mg/kg bw) in addition to artemisinin combination therapy (ACT) to eliminate malaria caused by *P. falciparum* in low-transmission areas (2). The benefit of SLD primaquine is primarily targeted to the community level as a means to reduce transmission, as this dose causes sterilization of the mature *P. falciparum* parasite, rather than curing the infected individual (2, 7, 8).

Primaquine is a pro-drug that needs to be metabolized to exert the desired antimalarial effect. Primaquine is metabolized by 2 different pathways: monoamine oxidase-A (MAO-A) generates the inactive metabolite carboxyprimaquine, while CYP2D6 and CPR generates the 2 active metabolites 5-hydroxyprimaquine and 5-hydroxy-6-desmethylprimaquine (9, 10). Spontaneous oxidation of the active metabolites generates quinoneimine and H_2O_2 that contribute to the antiparasitic activity of primaquine. The enzyme CPR then mediates redox cycling of quinoneimine to the primaquine active metabolites (9, 10). Primaquine has an estimated systemic half-life of 6 hours, necessitating multiple doses for effective radical cure of *P. vivax* malaria (11).

Primaquine can cause significant oxidative stress due to the accumulation of H_2O_2 in red blood cells, leading to AHA. Under normal homeostatic conditions, NADPH protects cells from oxidative stress. The enzyme G6PD generates NADPH and is particularly critical in red blood cells where it is the only source of NADPH. Individuals who G6PD deficient are especially sensitive to oxidative stress, whether due to endogenous or exogenous sources. As a result, primaquine is contraindicated at standard doses in individuals with G6PD deficiency (1). As discussed below, the residual amount of G6PD activity can vary based on the specific underlying genotype of an individual, so the risk of AHA varies based on the degree of deficiency. The FDA-approved drug label for primaquine advises monitoring blood cell counts and hemoglobin routinely during therapy even in individuals with normal levels of G6PD activity (1).

Contraindications for primaquine treatment include pregnancy, acute illness with a predisposition to granulocytopenia (often seen in rheumatoid arthritis or systemic lupus erythematosus), and medication with other potentially hemolytic drugs (1). The WHO further advise to avoid primaquine therapy in breastfeeding women unless the G6PD status of the breastfed infant is known to be within the normal range (2). One small study found that the estimated primaquine dose that a nursing infant receives following maternal dosing with 0.5 mg/kg/day primaquine was estimated to be 0.6% of the infant daily dose (0.5 mg/day) (12). The amount of primaquine excreted into breastmilk is low, and some sources suggest that G6PD-deficient infants over 28 days of age have a low risk of hemolysis due to primaquine exposure in breastmilk (13); however avoidance of primaquine by nursing mothers when the infants G6PD status is unknown is recommended by WHO, FDA, the US Centers for Disease Control and Prevention, as well as the United Kingdom malaria treatment guidelines (1, 2, 6, 14).

Primaquine is not approved for use in children younger than 6 months of age (2), though one study of infants in Indonesia found that severe clinical outcomes following primaquine treatment in infants under 12 months of age were rare (15). On-going studies suggest that younger children (14 years of age and younger) may require a higher weight-adjusted dose due to lower exposure primaquine and its metabolites (16, 17, 18). The FDA-approved label recommends caution with dose selection for geriatric individuals, as this population has a higher frequency of decreased hepatic, renal, and cardiac function. Initiating therapy at the low end of recommended dosing range is recommended (1). However, both the FDA and the Health Canada approved drug labels clearly state that efficacy and safety of primaquine has not been assessed in individuals over age 65 (1, 5).

In addition to the hemolysis risks, primaquine therapy may also trigger QT prolongation. As such, it should be avoided in conjunction with other medications that prolong the QT interval, in individuals with cardiac conditions such as long QT syndrome, ventricular arrhythmias or bradycardia (1). Other adverse reactions to primaquine can include nausea, vomiting, epigastric distress, abdominal cramps, dizziness, rash, and pruritus. Overdosage of primaquine phosphate can cause these adverse reactions as well as central nervous system and cardiovascular disturbances, cyanosis, granulocytopenia and AHA, among others (1).

Disease: Malaria

Malaria is a serious tropical disease caused by a parasite (*Plasmodium*) that spreads to humans by infected mosquitos. The only available vaccine is moderately effective and acts only against *P. falciparum* species (19). Widely recommended antimalarial drugs such as mefloquine or atovaquone-proguanil can be used for prevention –– this is known as chemoprophylaxis. The type of chemoprophylaxis recommended depends upon the individual taking the prophylaxis (namely, age, pregnancy status, and medical comorbidities) and the nature of their exposure –– specifically, the country of residence or traveled to, the length of stay, the species of *Plasmodium* that are most prevalent, and the level of drug resistance. For individuals residing in malaria-endemic regions, the WHO recommends a variety of preventative chemotherapies that can be used in infants, children, during pregnancy or collectively for the population of endemic areas (2).

Despite chemoprophylaxis, travel to malaria-endemic areas is not without risk. Individuals at elevated risk for malaria complications include pregnant women (20) and adults who have had their spleen removed (21). If travel cannot be avoided, chemoprophylaxis should be combined with additional precautions to avoid mosquito bites, such as bed nets and repellents. In 2021, the WHO estimated 247 million cases of malaria occurred worldwide, and malaria was responsible for 619,000 deaths. (22)

Malaria is found in over 100 countries and occurs throughout most tropical regions in the world. These regions include large parts of Africa, Asia, Central and South America, and parts of the Middle East and Pacific islands (22, 23). Individuals who are heterozygous carriers for sickle cell disease and G6PD deficiency have a protective advantage against malaria, and as a result, the frequency of such genetic conditions is higher in countries where malaria is endemic (24).

Malaria is transmitted to humans by the bite of an infected *Anopheles* mosquito. Only female mosquitos spread the infection (females feed on human blood, males feed on nectar). Although malaria can also be spread by sharing contaminated needles or via a contaminated blood transfusion, these are rare means of transmission.

There are several different *Plasmodium* species, but only a few species cause the most malaria cases:

● *P. falciparum*

o The most common cause of malaria, and death from malaria

o Predominates in sub-Saharan Africa

o Also found in regions of Australasia (Papua New Guinea, Southeast Asia), and the Caribbean (Haiti and the Dominican Republic)

● *P. vivax*

o A common cause of malaria outside of Africa

o Most frequent species found in Central and South America

o Parasite has a dormant, hypnozoite stage

o Early gametocytes that infect mosquitos

● *Plasmodium malariae*

o Less common

o Found in most areas where malaria is endemic

● *P. ovale*

o Less common

o Parasite has a dormant, hypnozoite stage

● *Plasmodium knowlesi*

o Less common

o Found in some Southeast Asia areas

The first stage of malaria infection begins when an infected mosquito bites the human host. Typically, mosquitos bite at dusk, or during the night. As the mosquito feeds, infective parasite sporozoites (the motile spore-like stage in the life cycle of this parasitic sporozoan, which is the infective agent) are inoculated into humans. The

sporozoites travel to the liver, where they invade liver cells and asexually reproduce to form schizonts. The liver schizonts contain daughter merozoites. This process is asymptomatic, and because it occurs outside of the red blood cell (erythrocyte), it is known as the exoerythrocytic stage.

Some species of the parasite (*P. vivax* and *P. ovale*) have an additional dormant stage in the liver. The parasite exists as hypnozoites, which can stay in the liver for weeks or months without causing any clinical symptoms.

The second stage of malaria infection is the erythrocytic stage. It begins when the liver schizonts rupture and release the daughter merozoites into the bloodstream. The merozoites invade red blood cells, digest hemoglobin, produce a toxic metabolite (hemozoin), and damage red blood cell membranes. Infected, brittle red blood cells are rapidly broken down (hemolysis) and if too many damaged red blood cells get trapped in the spleen, the spleen can rapidly enlarge (splenic sequestration).

Some of the daughter merozoites differentiate into male or female gametocytes (sexual forms). When they are ingested by a mosquito, they mature, fertilize, reproduce, and develop into sporozoites. When the mosquito feeds again, the sporozoites are inoculated into another human host and the cycle of malaria transmission is complete.

The erythrocytic stage of malaria is usually associated with fever, and malaria should always be suspected in anyone with a fever who has recently returned from a malaria-endemic region, even if antimalarial chemoprophylaxis was correctly followed. Other symptoms and signs include nausea, vomiting, abdominal pain, tachycardia (fast heart rate), diaphoresis (sweating), chills, and myalgia (muscle pain). The complications of malaria infection include severe anemia, cerebral malaria, and multi-organ failure. Without correct diagnosis and prompt treatment, malaria can be fatal.

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on the long arm of X chromosome (Xq28). Variants in the *G6PD* gene that result in a complete loss of enzymatic activity are not viable; variants observed in living humans generally impact the stability of the enzyme. As such, males can only be hemizygous (have one *G6PD* allele) while females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene. Males with Klinefelter syndrome have an additional X chromosome (47, XXY) and thus 2 *G6PD* alleles. Thus, it is important to consider the number of X chromosomes for an individual when determining *G6PD* genotype or phenotype.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide, with a worldwide prevalence of approximately 5% (25). Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once was, endemic; for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean (26, 27, 28). In the US, G6PD deficiency is more common among African Americans, affecting approximately 12% (29).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step NADP+ is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by H_2O_2 and oxygen radicals. Red blood cells that lack G6PD also have a deficiency of NADPH. (30)

Red blood cells that are G6PD deficient have a normal function but are more susceptible to increased oxidative stress (for example, by reactive oxygen species and H_2O_2). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the antimalarial drugs primaquine and tafenoquine, the antibacterials dapsone and sulfamethoxazole, the skin cancer drug dabrafenib, and the uric acid lowering drugs pegloticase and rasburicase.

While some reports estimate the frequency of G6PD deficiency to be <0.3% in individuals of European, Finish, or Amish descent, other more targeted population analyses have estimated the frequency of G6PD deficiency to be 0.5% in Portuguese males, 6.4% in males from Cyprus, and 8.3% in newborn males in Greece (31, 32, 33, 34). Among Asian populations, estimates broadly range from 2.7–3.5% of individuals will be G6PD deficient (31). However, a study in Cambodia observed 16% of their male study participants were G6PD deficient (<30% activity) while 32% of their female study participants demonstrated an intermediate level of G6PD activity (30–80%) and 4% were deficient (35). Other studies in Asia report the frequency of G6PD deficiency to be approximately 9–31% in Thailand, almost 30% among the Kachin ethnic group from Myanmar and China, 8% in Lao PDR, 9% in Vietnam, and 15.8% in Myanmar (36, 37, 38, 39, 40). Thus, it is difficult to predict the likelihood of an individual being G6PD deficient based solely on their geographic ancestry, as it can vary significantly within commonly used ancestral designations.

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. However, at birth, they may be predisposed to neonatal jaundice, and throughout life, they will be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid exposure to oxidizing agents when possible, including drugs such as primaquine.

Symptomatic individuals with G6PD deficiency may suffer from episodes of AHA or, the more severe condition, chronic non-spherocytic hemolytic anemia (CNSHA). The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency and to induce formation of new red blood cells.

More than 200 genetic variants of the *G6PD* gene have been identified so far (41), with approximately 400 biochemical and enzyme variants (42). Most known *G6PD* variants are missense, which can also be inherited as haplotypes that are comprised of more than one variant allele (43). Large deletions are rare, and a complete lack of G6PD activity is thought to be fatal in utero.

The normal (wild-type) copy of the *G6PD* gene is known as *G6PD* B, and is found in most Caucasians, Asians, and Africans. Common *G6PD* variants include:

● *G6PD* A+ (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of individuals of African descent and approximately 1.5% of Latinos (44, 45)

● *G6PD* A- (p.Asn126Asp with p.Val68Met) is associated with mild to moderate hemolysis and is found in up to 15% of African Americans (46). Additional *A-* haplotypes have also been identified, both with the A+ variant with a second variant (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (47)

● *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is a common pathogenic variant in Caucasians (48)

● *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in Asians (49)

● *G6PD* Viangchan (p.Val291Met) is the most common *G6PD* variant among Thais, Laotians, Cambodians, and Malaysians (50, 51)

The WHO recently updated its categorization of *G6PD* variants into 4 classes based on the median residual enzyme activity in males (expressed as a percentage of normal activity) (52). Class A variants have <20% activity and are associated with CNSHA.

Most individuals with G6PD deficiency have variants that belong to class B (enzyme activity less than 45%). Class B variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but most of the time, affected individuals are asymptomatic. Class C variants show median G6PD activity from 60–150% and are not associated with hemolysis. In class U are all the variants with unknown clinical significance, regardless of activity level. The CPIC has assigned G6PD phenotypes based on *G6PD* genotypes; the updated WHO categories are provided in Table 4 for completeness (3).

Table 4. Assignment of likely G6PD Phenotype based on Genotype/Diplotype (CPIC, 2022)

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Normal	Very mild or no enzyme deficiency (no less than 60% of normal enzyme levels) (60–150% of normal activity)	An X chromosome hemizygote who has a nondeficient (class IV) allele	IV (C)	B, Sao Borja
		An individual who has 2 nondeficient (class IV) alleles	IV/IV (C)	B/B, B/Sao Borja
Deficient	Less than 10–60% of normal enzyme activity (20–45% of normal activity)	An X chromosome hemizygote who has a deficient (class II–III) allele	II, III (B)	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		An individual who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III (B)	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNSHA (<20% of normal activity)	An X chromosome hemizygote who has a class I allele	I (A)	Bangkok, Villeurbanne
		An individual who has 2 deficient (class I variants) alleles	I/I (A)	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[d]	Normal or deficient enzyme activity[c]	An individual who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III (U)	B/A–, B/Mediterranean, B/Bangkok

Table 4. continued from previous page.

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Indeterminant	Uncertain		(U)	

CNSHA - chronic non-spherocytic hemolytic anemia, WHO - World Health Organization, G6PD - glucose-6-phosphate dehydrogenase

[a] The traditional (Class I–IV) and updated (A, B, C, and U) activity levels are both provided, with the updated activity ranges provided in parentheses where relevant.

[b] WHO classifications were under revision at the time of Clinical Pharmacogenetics Implementation Consortium publication, updated classifications (using A, B, C, and U designations) have been proposed based on enzyme activity levels and are provided in parenthesis here (52).

Class I alleles are extremely rare; the distinction between class II and III alleles is not clear. Almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.

[c] Due to the large number of *G6PD* variants, many other diplotypes may be possible besides those given as examples here; see Supplementary data from(3) for a more comprehensive list of alleles with their assigned WHO class. For Human Genome Variation Society terms, please see the Nomenclature table below. The alleles and diplotypes provided here are based upon the historic class I-IV definitions and may not fit the updated WHO classification.

[d] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is, therefore, difficult to predict the phenotype of these individuals (Supplementary Material online (*G6PD* heterozygotes)) (3).

This table is adapted from (3).

Phenoconversion of G6PD Phenotype

Increased turnover of red blood cells may lead to a temporary increase in G6PD activity as measured by enzyme activity assays. One study of 335 individuals with acute malaria infection (either *P. vivax* or *P. falciparum*) found that, on average, G6PD enzyme activity was 10.4% lower in the convalescent, post-infection state than during acute malarial infection. Furthermore, 66–87% of individuals who, following resolution of their malarial infection, had intermediate to severe G6PD deficiency yet they had presented with normal levels of G6PD activity during the acute infection stage (53).

Gene: *CYP2D6*

The CYP450 superfamily is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity. One prominent member, CYP2D6, is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers (54).

The *CYP2D6* Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 5). (55) Star alleles are defined by the variants detected on one chromosome (haplotype).

The combination of *CYP2D6* haplotypes that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on their impact on enzyme function, each allele can be assigned an activity score from 0 to 1, which in turn is then used to assign a phenotype (for example, CYP2D6 PM). However, the activity score system is not standardized across all clinical laboratories or *CYP2D6* genotyping platforms. The CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is

predicted from the diplotype activity score defined by the sum of the allele score values, which usually ranges from 0 to 3.0: (56)

- An ultrarapid metabolizer (UM) has an activity score greater than 2.25
- A normal metabolizer phenotype (NM) has an activity score of 1.25–2.25
- An intermediate metabolizer (IM) has an activity score of >0–<1.25
- A poor metabolizer (PM) has an activity score of 0

Table 5. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See the Pharmacogene Variation Consortium . Activity scores from (56).

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the NM phenotype. The *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (57, 58, 59, 60) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) (61, 62, 63) (see Table 5). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in individuals with European ancestry, **17* more common in Africans, and **10* more common in Asians. (64)

Larger structural variants at the *CYP2D6* locus have also been described, including gene duplications, deletions, tandem alleles, and gene conversions. As one might expect, deletions result in a no-function allele (for example, the **5* allele is a deletion). Duplications have been reported for alleles with normal function and decreased function, as well. In the case of allele duplications, the activity scores for the full complement of *CYP2D6* alleles are summed to determine the predicted metabolizer phenotype. Additional details on structural variants are available from PharmVar (65).

The frequency of the *CYP2D6* star alleles with altered function varies across global populations, resulting in different frequencies of the resulting metabolizer phenotype(s). Given CYP2D6's role in the metabolism of many drugs, the literature on allele and phenotype frequency is expansive. Most populations have a high frequency for normal-function star alleles, and thus a high proportion of the population are NMs. However, reduced-function alleles like *CYP2D6*10* are highly prevalent in East Asian populations, leading to a higher proportion of IM phenotype individuals in this ancestral group. Many groups in sub-Saharan Africa have higher frequencies of decreased-function alleles like *CYP2D6*17* and **29*, which can correlate with lower metabolizer scores in these individuals. More details regarding published allele and phenotype frequencies are available in the CYP2D6 supplemental chapter.

Phenoconversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and, thus the metabolizer phenotype of any individual. Administration of multiple drugs, sometimes called polypharmacy or co-medications, can lead to a phenomenon called phenoconversion, whereby an individual with one metabolizer genotype can have the enzymatic activity of a different metabolizer group (higher or lower, depending on the medications). The

enzymatic activity of CYP2D6 can be inhibited or reduced by medications including duloxetine, paroxetine, fluoxetine, bupropion, and quinidine (66, 67, 68, 69). This can result in NMs or IMs responding to medications as if they were PMs. Thus, co-medication with multiple CYP2D6 strong or moderate inhibitors may result in reduced metabolism of drug substrates. In contrast, discontinuing a concomitant CYP2D6 inhibitor can then revert the individual's CYP2D6 activity back to genetically predicted phenotype baseline. Both chloroquine and primaquine are used to treat malaria in regions with chloroquine-sensitive *Plasmodium* species, however both inhibit CYP2D6 enzyme activity and co-administration was found to inhibit CYP2D6-mediated hydroxylation (70).

Other Genes of Interest

The *P450 oxidoreductase* (*POR)* gene encodes the CPR enzyme that is involved in primaquine metabolism. Deficiency of *POR* presents with a variety of phenotypes; potential clinical presentations include 21-hydroxylase deficiency, polycystic ovary syndrome, and Antley-Bixler syndrome, as well as a distinct disorder of sexual development (71, 72). Genetic variants in *POR* have been shown to affect the enzymatic activity of many members of the CYP450 family, including 3A4 and 2D6 (73).

Genetic variation in *MAO-A* (rs6323, NM_000240.4:c.891G>T) has been found to be associated with reduced metabolism of primaquine to carboxyprimaquine in healthy volunteers (74). Variation in *CYP2C19* was also shown to influence primaquine metabolism in healthy volunteers, though the clinical impact of the variation was unclear (74).

Drug transport proteins can also impact the efficacy of various medications. Notably, variations in transport proteins encoded by solute carrier organic anion transporter (*SLCO)1A2* and *SLCO1B1* (75), as well as *CYP2C8* (76) variants associated with decreased enzymatic activity, have been associated with altered clearance of *P. vivax* parasites and a higher frequency of relapse after primaquine and chloroquine therapy. It is possible that the apparent decrease in therapeutic effect associated with these genetic variations is due to altered chloroquine transport or metabolism, particularly as chloroquine is known to be transported by SLCOs and metabolized by CYP2C8.

Linking *G6PD* and *CYP2D6* Genetic Variation with Treatment Response

Individuals with G6PD deficiency (<20% activity) or intermediate deficiency (20–45% activity) (2) are at a significant risk of hemolysis when treated with the standard primaquine course (daily for 14 days) but have shown tolerance for an extended course with a single dose of primaquine per week for 8 weeks (2). One study in Cambodia found that 95% of the individuals with reduced G6PD activity were able to complete the 8-week course of primaquine and no severe adverse events were recorded (35). A meta-analysis of 20 different trials, based in Africa or Asia, of SLD primaquine found that the proposed WHO regimen (0.25 mg/kg) was, indeed, safe, even in the context of G6PD deficiency (7). While individuals with G6PD deficiency demonstrated a more significant drop in hemoglobin concentration in the 2–3 days immediately following treatment and were more likely to experience hemoglobinuria within 72 hours of primaquine treatment, these effects were transitory and only 2 (out of 194) individuals required further intervention (7). Other risk factors for anemia following SLD primaquine, aside from G6PD deficiency, were high parasite density, young age, and the primary anemia risk factor: low baseline hemoglobin levels (7).

Based on non-clinical metabolism data and limited clinical data, the Health Canada approved drug label for primaquine advises that *CYP2D6* polymorphism may be associated with variable clinical response and suggests it may be useful to consider drug-drug interaction or CYP2D6 metabolizer status; it further states that for CYP2D6 PMs, alternative treatment should be considered (5). Several studies in the literature suggest that individuals who are CYP2D6 IM or PMs may not respond well to standard primaquine therapy for the radical

cure of *P. vivax* malaria, which may result in a relapse of malaria symptoms and positive malaria tests weeks to months later (77). There are several case reports that link CYP2D6 reduced enzymatic activity or IM/PM genotypes with malaria relapse after primaquine therapy (78, 79, 80, 81, 82, 83). A study of 25 individuals found that IM or PM phenotypes were associated with malaria relapse, while individuals with CYP2D6 NM phenotype did not experience relapse following treatment with chloroquine and primaquine (84). Additionally, a case-control study of 57 individuals found that CYP2D6 IM or PM phenotype (determined either by genotype or inferred based on reduced dextromethorphan metabolism) strongly correlated with increased frequency of malaria relapse (85). A prospective cohort study with 190 individuals found *P. vivax* malaria relapse was more common among individuals with reduced *CYP2D6* activity alleles (86). A study of 260 individuals living in a *P. vivax* endemic region of the Amazon found a significant correlation between *CYP2D6* reduced-function genotype (AS<1) and risk of malaria recurrence (87). In Korea, individuals with CYP2D6 IM phenotype were more likely (an odds ratio of 2.33) to have *P. vivax* malaria relapse even after treatment with primaquine (82). Similar results were observed in a study with 120 individuals in the Yunnan Province of China, where the c.886C>T and *CYP2D6*2* variants were associated with relapse of *P. vivax* malaria after treatment with chloroquine and an 8-day course of primaquine (88). A meta-analysis of 9 studies that included a total of 970 individuals from Asia, Brazil, and Oceania found that CYP2D6 IM and PMs were nearly twice as likely to experience malaria relapse following primaquine therapy as compared to NM or UM individuals (82). In contrast, a small study of 51 individuals treated with chloroquine and primaquine combination therapy did not observe any significant enrichment for CYP2D6 IM or PMs among the relapse group, though the limited number of relapses and sample size may have left this study underpowered (89). A larger study with 157 subjects from Australia also found no association between CYP2D6 activity and malaria relapse (90).

Reduced CYP2D6 activity was found to be associated with reduced clearance of *P. falciparum* gametocytes in SLD primaquine therapy in a study of 774 individuals from Africa; however, even with reduced CYP2D6 activity, the addition of SLD primaquine was more effective to clear gametocytes than ACT alone (91). This same study found no significant impact of CYP2D6 activity on the frequency or degree of anemia following SLD primaquine in G6PD deficient individuals (91). Similarly, a study of 157 children, aged 1–10 years, found no difference in the incidence or severity of acute hemolysis nor in efficacy against the *Plasmodium* parasites among individuals with reduced CYP2D6 or G6PD activity treated with ACT with SLD primaquine, leading the authors to conclude that this treatment regimen is both safe and sufficient to reduce *P. falciparum* transmission (7). The WHO guidelines do not require G6PD testing when administering SLD primaquine (2).

The *G6PD* and *CYP2D6* Gene Interactions with Medications Used for Additional Indications

Medications that can induce oxidative stress in red blood cells can trigger hemolysis readily in individuals with G6PD enzyme deficiency. Many of these medications are antimalarials (tafenoquine, for example) but many more medications pose a hazard for G6PD deficient individuals.

- Urate-lowering medications: both refractory gout and tumor lysis syndrome can cause systemic elevation of urate levels, medications such as rasburicase and pegloticase are uricase enzymes that aid in the breakdown of uric acid into more soluble metabolites. These reactions produce H_2O_2 as a byproduct, thus increasing oxidative stress in the body.

- Kinase inhibitors: anticancer medications such as dabrafenib may also increase oxidative stress.

- Antimicrobial medications: nitrofurantoin, often used for urinary tract infections, was determined to be a medication of moderate risk for AHA in G6PD-deficient individuals by CPIC and may call for additional monitoring. In contrast, CPIC found sulfamethoxazole to be a medication with low-to-no risk in G6PD deficient individuals. (3)

Additional information on gene-drug interactions for *G6PD* are available from PharmGKB, CPIC and the FDA (search for "G6PD").

The CYP family of enzymes is involved in the metabolism of many substances and CYP2D6 especially has been implicated in altered pharmacologic responses for many compounds. The drugs can be categorized into many different classes:

● Antipsychotics—for example, aripiprazole, risperidone, thioridazine and—to a lesser extent—clozapine is metabolized by CYP2D6. According to the FDA, aripiprazole dosage should be reduced for PMs and thioridazine is contraindicated for individuals who are known to have reduced CYP2D6 activity due to increased risk of potentially fatal side effects. The UMs may have a decreased plasma concentration of risperidone.

● Tricyclic antidepressants—for example, amitriptyline, and imipramine may require dosage adjustments, potentially guided by therapeutic drug monitoring, to achieve the desired therapeutic range in UMs or PMs. Ultimately, tricyclic antidepressants may be ineffective in CYP2D6 UMs.

● Serotonin and norepinephrine reuptake inhibitors—for example atomoxetine and venlafaxine may have reduced efficacy in UMs at standard doses while PMs are at risk of elevated plasma concentrations for both medications. The Dutch Pharmacogenetics Working Group advises against the use of venlafaxine in CYP2D6 PMs and IMs.

● Cardiovascular dysfunction—for example, carvedilol, metoprolol, and propafenone are all metabolized by CYP2D6, and PMs will have higher plasma concentrations of these medications compared with NMs resulting in potentially undesired side effects or (in the case of metoprolol) extensive slowing of the heart rate.

● Anticancer medications—for example, tamoxifen is activated by CYP2D6, and IMs or PMs may have reduced benefit from tamoxifen therapy.

● Pain management—for example, codeine and tramadol are pro-drugs that require activation by CYP2D6 to achieve the desired analgesic effect.

● Various therapies for genetic disorders—for example eliglustat used in the treatment of Gaucher disease, and deutetrabenazine used in the treatment of Huntington disease—have reduced dose recommendations for CYP2D6 PMs. The CYP2D6 UMs may not achieve adequate concentrations of eliglustat and therefore *CYP2D6* genotyping is required before initiation of eliglustat therapy.

It is important to note that *CYP2D6* is the most common biomarker in drug responses for FDA drug labels, the list provided here is by no means exhaustive. Additional information on gene-drug interactions for *CYP2D6* are available from PharmGKB, CPIC and the FDA (search for "CYP2D6").

Genetic Testing

The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for primaquine response, the *G6PD* gene, and the *CYP2D6* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD deficiency, and testing may also be used to screen females with a family history of G6PD deficiency to see if they are carriers.

While many biochemical *G6PD* variants are known, the genetic underpinnings of some of these variants may still be unknown. Additionally, quantitative, or semi-quantitative tests for G6PD enzyme activity may be more readily available in some settings. Whether the clinical test is biochemical or molecular, assessment of G6PD enzyme activity is required before administering primaquine for the radical cure of *P. vivax* or *P. ovale* malaria, per the FDA (1). A number of point-of-care tests have been developed and tested (92, 93, 94) to improve the

accessibility of *G6PD* genetic testing before administration of medications like primaquine, though the availability of such testing in areas with the highest malarial burden is still lacking (95).

The available *CYP2D6* tests include targeted single-gene tests as well as multi-gene panels or genome-wide sequencing tests. In addition, variant *CYP2D6* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (96). The test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be confirmed by checking the diplotype and calculating the *CYP2D6* activity score, as described in the "*CYP2D6* Alleles" section above. When individuals have more than 2 copies of the *CYP2D6*, the copies of the allele are denoted by an "xN", for example, *CYP2D6*1/*2x2*. Some laboratories also use the notation of DUP to indicate an increase in copy number, but the report does not always specify the number of duplications nor the allele that has been duplicated due to technical limitations.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2021 Statement from the US Food and Drug Administration (FDA):

Due to the risk of hemolytic anemia in patients with G6PD deficiency, G6PD testing has to be performed before using primaquine. Due to the limitations of G6PD tests, physicians need to be aware of residual risk of hemolysis and adequate medical support and follow-up to manage hemolytic risk should be available.

Primaquine should not be prescribed for patients with severe G6PD deficiency…

In case of mild to moderate G6PD deficiency, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of using primaquine. If primaquine administration is considered, baseline hematocrit and hemoglobin must be checked before treatment and close hematological monitoring (e.g. at day 3 and 8) is required. Adequate medical support to manage hemolytic risk should be available.

When the G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of using primaquine. Risk factors for G6PD deficiency or favism must be assessed. Baseline hematocrit and hemoglobin must be checked before treatment and close hematological monitoring (e.g. at day 3 and 8) is required. Adequate medical support to manage hemolytic risk should be available.

Please review the complete therapeutic recommendations that are located here: (1).

2022 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

It is recommended to avoid primaquine at standard (or higher) anti-relapse dosages of 0.25–0.5 mg/kg daily for 14 days for the treatment of P. vivax or *P. ovale* in G6PD deficiency. For the anti-gametocyte treatment of *Plasmodium falciparum* malaria, the single-dose regimen of 0.25 mg/kg is considered safe and effective (low to no risk in G6PD deficiency). For the treatment of *P. vivax* or *P. ovale* malaria for radical cure of liver-stage infections, 0.75 mg/kg once weekly for 8 weeks (WHO) or 45 mg for adults once weekly for 8 weeks (CDC) is

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

considered in the medium risk category, and patients should be monitored closely for hemolysis… No dose of primaquine is considered safe in patients who are G6PD deficient with CNSHA and thus should be avoided.

Please review the complete therapeutic recommendations that are located here: (3).

2020 Statement from Health Canada:

Hemolytic anemia and G6PD deficiency

Due to the risk of hemolytic anemia in G6PD deficient patients, G6PD testing has to be performed before using primaquine. […] Due to the limitations of G6PD tests, physicians need to be aware of residual risk of hemolysis and adequate medical support and follow-up to manage hemolytic risk should be available. Observe particular caution in individuals with a personal or family history of hemolytic anemia.

In case of mild to moderate G6PD deficiency, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of using primaquine; if primaquine administration is considered, the dosage regimen should be adapted accordingly (see DOSAGE AND ADMINISTRATION) and close hematological monitoring is required.

[….]

CYP2D6 genotype:

Based on non-clinical data, primaquine activity probably depends on the formation CYP2D6 metabolite(s). Therefore, CYP2D6 polymorphism may be associated with variability in clinical response to primaquine.

Limited clinical data reported more elevated treatment failure rates in patients with CYP2D6 poor or intermediate metabolizer status than in patients with normal/extensive metabolizer status (see ACTION AND CLINICAL PHARMACOLOGY).

In case of treatment failure, after checking patient's compliance to treatment, it may be useful to reconsider potential concomitant use of CYP2D6 inhibitors (see DRUG INTERACTIONS) and to assess the patient's CYP2D6 status if feasible. For poor CYP2D6 metabolizers, alternative treatment should be considered.

Please review the complete therapeutic recommendations that are located here: (5).

Nomenclature for Selected *G6PD* and *CYP2D6* Alleles

Nomenclature of Selected *G6PD* Alleles

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Borja	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3:c.680G>T	NP_001035810.1:p.Arg227Leu		

Nomenclature of Selected continued from previous page.

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3:c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	n/a

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
* WHO classifications based on (97) WHO - World Health Organization; PharmGKB - Pharmacogenomics Knowledgebase; CPIC - Clinical Pharmacogenetics Implementation Consortium; CNSHA - chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phosphate dehydrogenase

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*2	2851C>T	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*3	2550delA	NM_000106.6:c.775del	NP_000097.3:p.Arg259fs	rs35742686
CYP2D6*4	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
CYP2D6*5	Gene deletion			
CYP2D6*6	1707 del T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
CYP2D6*10	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*17	1022C>T	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*27	3854G>A	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652

Nomenclature of Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*31	2851C>T	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*36[1]	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C	NM_000106.6:c.1432C>T+ NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735+ rs766507177
	4159G>C	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G	NM_000106.6:c.1444G>A+ NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221+ rs75467367
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*41	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2989G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*49	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] *CYP2D6*36* is a gene conversion with *CYP2D7;* variants provided here are from the Pharmacogene Variation Consortium.
CYP2D6 - cytochrome P450 member 2D6, dbSNP - database of single nucleotide polymorphisms
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (98).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Propafenone Therapy and *CYP2D6* Genotype

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Introduction

Propafenone is an antiarrhythmic medication. It is used to prevent the reoccurrence of atrial fibrillation in patients with episodic atrial fibrillation who do not have underlying structural heart disease (propafenone may provoke proarrhythmic events in patients with structural heart disease).

Propafenone belongs to class IC of antiarrhythmic agents and acts on cardiac sodium channels to inhibit action potentials. In general, because of the lack of evidence that antiarrhythmic agents improve survival, they should only be used to treat arrhythmias that are thought to be life-threatening.

Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 enzymes. Approximately 6% of Caucasians in the US lack CYP2D6 activity, and are known as "CYP2D6 poor metabolizers" (Table 1) (1). Standard doses of propafenone will lead to higher plasma drug concentrations in poor metabolizers, compared to normal metabolizers. In addition, drugs that inhibit CYP2D6, CYP3A4, and CYP1A2 may also increase propafenone levels, which may lead to cardiac arrhythmia episodes.

The FDA-approved drug label for propafenone states that the recommended dosing regimen of propafenone is the same for all patients (CYP2D6 poor metabolizers and normal metabolizers). However, the label also cautions that the simultaneous use of propafenone with both a CYP2D6 inhibitor (or in patients with CYP2D6 deficiency) and a CYP3A4 inhibitor should be avoided, because of the increased risk of causing arrhythmias and other adverse events (1).

A guideline from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP) provides dosing recommendations for propafenone, based on *CYP2D6* genotype. For CYP2D6 poor metabolizers, the guideline recommends reducing the initial dose of propafenone by 70%, ECG monitoring, and monitoring plasma concentrations. For intermediate and ultrarapid metabolizers, the guideline states there is insufficient data to allow for a calculation of dose adjustment. Therefore, it is recommended to adjust the dose in response to plasma concentration and to monitor with ECG, or select an alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone) (2, 3) (Table 2).

Drug class: Antiarrhythmics

Antiarrhythmic agents suppress abnormal heart rhythms (cardiac arrhythmias), which can originate from the atria (e.g., atrial fibrillation, atrial flutter) or the ventricles (e.g., ventricular tachycardia, ventricular fibrillation).

There are five main classes of antiarrhythmic agents, based on their primary site of action:

- Class I: block sodium (Na+) channels e.g., quinidine (class IA), lidocaine (class IB), propafenone (class IC)
- Class II: block beta adrenoreceptors e.g., carvedilol, metoprolol, propranolol
- Class III: block potassium (K+) channels e.g., amiodarone, sotalol
- Class IV: block calcium (Ca2+) channels e.g., verapamil, diltiazem
- Class V: work by other or unknown mechanisms e.g., adenosine, digoxin

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Drug: Propafenone

Propafenone is an antiarrhythmic used to prevent the recurrence of atrial fibrillation in patients who have episodic atrial fibrillation and no underlying structural heart disease. Propafenone is also used in the management of paroxysmal supraventricular tachycardia and atrial flutter (1).

Because there are no well-controlled studies in pregnant women, the FDA-approved drug label states that propafenone should only be used during pregnancy if the benefit justifies the potential risk to the fetus. The label also states that the safety and effectiveness of propafenone in pediatric patients have not been established.

Atrial fibrillation is the most common type of harmful cardiac arrhythmias. It is more common in men than women, and the risk of developing atrial fibrillation increases with age. Atrial fibrillation may be paroxysmal (intermittent), persistent (persists for at least 7 days), long-standing (more than 12 months), or permanent.

The symptoms of atrial fibrillation range from no symptoms, to feeling dizzy, short of breath, and experiencing palpitations. The pulse feels irregular, and an ECG will show an absence of P waves and an irregular QRS complex. Atrial fibrillation can lead to reduced cardiac output, increase the risk of thrombosis and stroke, and affected patients may be at an increased risk for mortality (4). Management typically includes antithrombotic therapy and rhythm control.

Propafenone is a class IC *antiarrhythmic* agent. All class I agents have a "membrane stabilizing effect"—by reducing the fast influx of sodium ions into the cardiac muscle cells, they inhibit the propagation of action potentials. Propafenone also has some Class II activity—it can act as a beta blocker. Side effects of this action include bradycardia and bronchospasm (5, 6).

The class IC agents encainide and flecainide have been associated with increasing the risk of cardiac arrest or death, compared to placebo. Consequently all class IC agents, including propafenone, are considered to have a significant risk of provoking proarrhythmic events in patients with structural heart disease. Therefore, propafenone should not be used in patients with underlying structural heart disease. Its use is contraindicated in a number of conditions, including heart failure, conduction disorders, bradycardia, and recent myocardial infarction (within the last 3 months) (1, 7, 8, 9).

Propafenone is metabolized into two active metabolites: 5-hydroxypropafenone, which is formed by CYP2D6, and norpropafenone, which is formed by both CYP3A4 and CYP1A2. Multiple studies have found that genetic variants in the *CYP2D6* gene influence the plasma drug levels of propafenone (10, 11, 12, 13).

In patients who lack CYP2D6 activity, metabolism of propafenone is slower, so the 5-hydroxy metabolite is not formed or is formed at very slow rates. In these patients, high doses of propafenone (850mg daily) lead to plasma concentrations of propafenone that are about twice those of patients who have normal CYP2D6 activity. At lower initial doses, the difference between propafenone and 5-hydroxy metabolite concentrations is even greater (1, 14).

However, the FDA recommends that the dosing regimen of propafenone should be the same for all patients, regardless of their CYP2D6 activity levels. This is because even at high doses, the effects of high propafenone levels are mitigated by the lack of the active 5-hydroxy metabolite in the slow metabolizers, and also because steady-state conditions are achieved after 4 to 5 days of titrating the dose in all patients. But the FDA also recommends that because of the large variation in plasma drug levels between individuals, the dose of propafenone should be individually titrated on the basis of response and tolerance, with close attention paid to clinical and ECG evidence of toxicity (1).

The FDA-approved drug label for propafenone cautions against the simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor. This is because the combination of CYP3A4 inhibition and either

CYP2D6 inhibition or deficiency may increase propafenone exposure, which may trigger new cardiac arrhythmias and exaggerate beta adrenoreceptor blockage (1).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (15).

Individuals who have more than two normal function copies of the *CYP2D6* (*CYP2D6*xN*) gene are "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers."

Individuals with one normal and one no function allele or two decreased function alleles are categorized as "normal metabolizers" by recent nomenclature guidelines (16), but have also been categorized as "intermediate metabolizers" in the literature. Subjects with one decreased and one no function allele are predicted to be intermediate metabolizers and those with two no function alleles are classified as poor metabolizers.

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (17, 18, 19, 20), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (5, 6, 18, 20, 21) (Table 1).

There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (22).

Consequently, the phenotype frequencies vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (17, 23).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) lists genetic tests currently available for propafenone response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1* (wild type). A result for copy number, if available, is also important when interpreting *CYP2D6* results (19).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Propafenone is metabolized by CYP2D6, CYP3A4, and CYP1A2 isoenzymes. Approximately 6% of Caucasians in the US population are naturally deficient in CYP2D6 activity and other demographic groups are deficient to a somewhat lesser extent. Drugs that inhibit these CYP pathways (such as desipramine, paroxetine, ritonavir, sertraline for CYP2D6; ketoconazole, erythromycin, saquinavir, and grapefruit juice for CYP3A4; and amiodarone and tobacco smoke for CYP1A2) can be expected to cause increased plasma levels of propafenone.

Increased exposure to propafenone may lead to cardiac arrhythmias and exaggerated beta-adrenergic blocking activity. Because of its metabolism, the combination of CYP3A4 inhibition and either CYP2D6 deficiency or CYP2D6 inhibition in users of propafenone is potentially hazardous. Therefore, avoid simultaneous use of propafenone with both a CYP2D6 inhibitor and a CYP3A4 inhibitor.

Please review the complete therapeutic recommendations that are located here: (1).

2016 Summary of recommendations from The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP): For CYP2D6 poor metabolizers (PMs), defined as patients carrying two defective alleles, dose reductions are recommended for clomipramine, flecainide, haloperidol, zuclopenthixol (all 50%); doxepin, nortriptyline (both 60%); imipramine, propafenone (both 70%); and metoprolol (75%).

[…].

For CYP2D6 intermediate metabolizers (IMs), defined as patients carrying two decreased-activity alleles or one active/decreased-activity allele and one inactive allele, dose reductions ranging from 20 to 50% are advised for doxepin, amitriptyline, zuclopenthixol, imipramine, nortriptyline, and metoprolol. There were insufficient data to calculate dose adjustments for clomipramine, oxycodone, propafenone, risperidone, and venlafaxine (Table 2).

Please review the complete therapeutic recommendations that are located here: (2, 3).

Table 2. *CYP2D6* phenotypes and the therapeutic recommendations for propafenone therapy, from The Dutch Pharmacogenetics Working Group (2016)

CYP2D6 Phenotype	Recommendations for propafenone therapy
Ultrarapid metabolizer	Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).
Intermediate metabolizer	Insufficient data to allow calculation of dose adjustment. Adjust dose in response to plasma concentration and record ECG or select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).
Poor metabolizer	Reduce dose by 70%, record ECG, monitor plasma concentration

The level of evidence for the therapeutic (dose) recommendations is 4/4 ("good quality") for poor metabolizers, and 3/4 ("moderate quality") for intermediate and ultrarapid metabolizer types. The Table is adapted from Swen J.J., Nijenhuis M., de Boer A., Grandia L. et al. Pharmacogenetics: from bench to byte - an update of guidelines. Clinical pharmacology and therapeutics. 2011;89(5):662–73 (2, 3).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

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Rasburicase Therapy and *G6PD* and *CYB5R* Genotype

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Introduction

Rasburicase (brand name Elitek) is a uric oxidase used to treat the high levels of uric acid that are associated with tumor lysis syndrome (TLS).

Tumor lysis syndrome is a potentially life-threatening condition caused by rapid break down of tumor cells during chemotherapy. Tumor lysis syndrome is associated with the treatment of aggressive lymphoma and leukemia, but it may also occur with other tumors including solid tumors. Massive cell breakdown results in the release of potassium, phosphate, and uric acid into the circulation. Urate crystals can precipitate in the kidneys, causing acute kidney damage.

Prophylaxis and treatment of TLS involve aggressive intravenous (IV) hydration and the use of drugs to lower uric acid levels. Rasburicase breaks down uric acid to a more soluble metabolite (allantoin), which is then eliminated by the kidneys. A by-product of this reaction is hydrogen peroxide, an oxidizing agent.

Red blood cells that lack the enzyme glucose-6-phosphate dehydrogenase (G6PD) are sensitive to oxidative damage caused by agents like hydrogen peroxide due to a deficiency in nicotinamide adenine dinucleotide phosphate (NADPH). Once exposed, the red blood cells become rigid, trapped, and are rapidly broken down (hemolysis). This can lead to a deficiency of mature red blood cells (hemolytic anemia) and the production of red blood cells with abnormally high levels of methemoglobin (methemoglobinemia).

Approximately 400 million people worldwide have G6PD deficiency. Most of these individuals are asymptomatic. However, they are at risk of life-threating hemolytic reactions and methemoglobinemia if given oxidizing drugs such as rasburicase.

Rasburicase is contraindicated in individuals with G6PD deficiency. The FDA-approved drug label states that individuals at higher risk for G6PD deficiency should be screened before starting rasburicase therapy, for example, individuals of African or Mediterranean ancestry (Table 1) (1). Approximately 12% of African-Americans have G6PD deficiency.

A rare cause of methemoglobinemia is a deficiency of antioxidant enzymes such as cytochrome b5 reductase 3 (CYB5R3). The drug label states it is not known whether individuals who have a deficiency of this enzyme, or another enzyme with antioxidant activity, are at increased risk of methemoglobinemia or hemolytic anemia during rasburicase therapy.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published dosing recommendations for rasburicase based on *G6PD* phenotype (Table 2). The CPIC states for individuals with normal *G6PD* phenotype, there is no reason to withhold rasburicase based on G6PD status. For individuals who are G6PD deficient, with or without hemolytic anemia, rasburicase is contraindicated. For individuals who have a variable *G6PD* phenotype, G6PD enzyme activity must be measured to ascertain that G6PD status is normal. For cases where rasburicase is contraindicated, alternative drugs include allopurinol (2).

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Table 1. FDA Drug Label for Rasburicase. Contraindicated in G6PD Deficiency (2019)

Phenotype	Recommendations
G6PD deficiency	Do not administer rasburicase to individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Immediately and permanently discontinue rasburicase in individuals developing hemolysis. Screen individuals at higher risk for G6PD deficiency (for example, individuals of African or Mediterranean ancestry) before starting rasburicase

This table is adapted from (1).

Table 2. CPIC Recommended Therapeutic Use of Rasburicase in relation to G6PD Phenotype (2014)

G6PD phenotype	Implications for phenotypic measures	Dosing recommendations for rasburicase	Classification of recommendations[a]
Normal[b]	Low or reduced risk of hemolytic anemia	No reason to withhold rasburicase based on G6PD status[b]	Strong
Deficient or deficient with CNSHA	At risk of acute hemolytic anemia	Rasburicase is contraindicated; alternatives include allopurinol[c]	Strong
Variable[b]	Unknown risk of hemolytic anemia	To ascertain that G6PD status is normal, enzyme activity must be measured; alternatives include allopurinol[c]	Moderate

CNSHA, chronic nonspherocytic hemolytic anemia
[a] Rating scheme described in Supplementary Material online (see Strength of Recommendations, (2)).
[b] A negative or inconclusive genetic test cannot be assumed to indicate normal G6PD phenotype; an enzyme activity test is needed to assign G6PD phenotype in such cases.
[c] Allopurinol is associated with severe cutaneous reactions in the rare carriers of the HLA-B*58:01 allele.
This table is adapted from (2).

Drug: Rasburicase

Rasburicase is a urate-lowering drug used to prevent and manage TLS. In individuals with cancers such as acute leukemia and lymphoma, chemotherapy can lead to massive break down of cells, which releases large amounts of potassium, phosphate, and nucleic acids into the circulation. The purines found in nucleic acids are broken down to uric acid, which leads to hyperuricemia (uric acid levels above 6.8 mg/dL).

Tumor lysis syndrome is potentially life threatening – hemorrhage may occur, and acute kidney damage can be caused by the precipitation of urate crystals within the kidney tubules. The management of TLS is centered on prevention, with aggressive IV hydration and the use of hypouricemic agents such as allopurinol and rasburicase.

The tumors that have the highest risk of TLS are acute lymphoblastic leukemia and aggressive lymphomas, especially Burkitt's lymphoma. But TLS can also occur in other tumors including solid tumors –– risk factors include a high tumor burden, a high proliferative rate, and a high sensitivity to chemotherapy (3).

Rasburicase is a uric acid oxidase (uricase). It converts uric acid to allantoin – a metabolite that is 5–10 times more soluble than uric acid, which can be excreted by the kidneys. The uricase enzyme is present in many mammals, but absent in humans. Rasburicase is a recombinant protein derived from a genetically modified strain of yeast (*Saccharomyces cerevisiae*). Pegloticase is another uricase that is used to manage gout, and is derived from the uricase found in pigs.

Rasburicase is given by an IV infusion, daily, for up to 5 days. Dosing beyond 5 days, or giving more than one course, is not recommended. Recent studies have found that conservative treatment with rasburicase is as beneficial, with a single dose of rasburicase being sufficient to normalize the uric acid levels in most individuals with TLS (4-6).

Rasburicase may cause fetal harm and should only be used during pregnancy if the potential benefit to the mother justifies the potential risk to the fetus. There are no adequate studies in pregnant women, but in animal studies, rasburicase was shown to be teratogenic (caused abnormal development in rabbit embryos). (1)

The use of rasburicase is contraindicated in individuals known to have G6PD deficiency, and individuals at risk of G6PD deficiency should be screened before starting rasburicase therapy. This is because an oxidizing agent, hydrogen peroxide, is produced during the conversion of uric acid to allantoin.

Individuals who have G6PD deficiency have red blood cells that are susceptible to oxidative damage. If exposed to agents such as hydrogen peroxide, the red blood cells become rigid, get trapped, and are subsequently destroyed by macrophages in the spleen, bone marrow, and liver. The rapid destruction of red blood cells is called hemolysis, and it may result in hemolytic anemia (a deficiency of red blood cells or hemoglobin, caused by hemolysis).

In addition, rasburicase can result in methemoglobinemia –– the production of red blood cells with abnormally high levels of oxidised hemoglobin, namely, methemoglobin. Individuals who may be at increased risk of methemoglobinemia include individuals with a rare hereditary form of methemoglobinemia. This condition may be asymptomatic, and only become apparent after an adverse reaction to an oxidizing drug such as rasburicase (1).

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on chromosome Xq28. As such, males are hemizygous for one *G6PD* allele, making them more susceptible to this X-linked disorder. Females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide (7), with a worldwide prevalence of approximately 5%. This deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once was, endemic, for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean (8-10). In the US, G6PD deficiency is more common among African-Americans, affecting approximately 12% (11).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulphydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. Red blood cells that lack G6PD also have a deficiency of NADPH(12).

Red blood cells that are G6PD and NADPH deficient are more susceptible to oxidative stress (for example, by oxygen free radicals and hydrogen peroxide). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the uric acid lowering drugs pegloticase and rasburicase, the antimalarial drug primaquine, the antibiotic sulfamethoxazole, and the skin cancer drug dabrafenib.

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. But at birth, they are predisposed to neonatal jaundice, and throughout life, they will

be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid oxidizing agents when possible, including drugs such as rasburicase.

Symptomatic individuals with G6PD deficiency may suffer from episodes of acute hemolytic anemia or chronic non-spherocytic hemolytic anemia. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency and to induce formation of new red blood cells.

More than 180 genetic variants of the *G6PD* gene have been identified so far, with approximately 400 biochemical and enzyme variants (13). Most genetic variations are missense point variants (14). Large deletions are rare, and a complete lack of G6PD activity is thought to be fatal in utero.

The normal (wild-type) copy of the *G6PD* gene is known as *G6PD* B, and is found in most Caucasians, Asians, and Blacks. Common *G6PD* variants include:

- *G6PD* A+ (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of Blacks from Africa (15)
- *G6PD* A- (p.Asn126Asp and p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (16). Additional *A*-haplotypes have also been identified, both with the A+ variant with a second SNP (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (17)
- *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is the most common abnormal variant in Caucasians (18)
- *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in Asians (19)

The World Health Organization categorized G6PD variants into 5 classes according to the level of enzyme activity and severity of hemolysis. Class 1 variants are the most severe, but rare. These variants have less than 10% of normal GP6D enzyme activity and are associated with chronic hemolytic anemia.

Most individuals with G6PD deficiency have variants that belong to class II (enzyme activity less than 10% but no chronic hemolytic anemia) and class III (enzyme activity between 10% and 60%). Class II and III variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but for most of the time, affected individuals have no symptoms. Class IV and V variants are not considered to be clinically significant, class IV variants are associated with normal enzyme activity, and class V variants with increased enzyme activity (2).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) assign G6PD phenotype based on *G6PD* genotype (Table 3) (2).

Table 3. Assignment of likely G6PD Phenotype based on Genotype/Diplotype (CPIC 2014)

Likely phenotype	Definition	Genotype	WHO class for G6PD variants[a]	Example of diplotype[b]
Normal	Very mild or no enzyme deficiency (less than 60% of normal enzyme levels)	A male who has a nondeficient (class IV) allele	IV	B, Sao Boria
		A female who has 2 nondeficient (class IV) alleles	IV/IV	B/B, B/Sao Boria

Table 3. continued from previous page.

Likely phenotype	Definition	Genotype	WHO class for G6PD variants[a]	Example of diplotype[b]
Deficient	Less than 10–60% of normal enzyme activity	A male who has a deficient (class II–III) allele	II, III	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		A female who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA	A male who has a class I allele	I	Bangkok, Villeurbanne
		A female who has 2 deficient (class I variants) alleles	I/I	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[c]	Normal or deficient enzyme activity[c]	A female who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III	B/A–, B/Mediterranean, B/Bangkok

CNSHA, chronic nonspherocytic hemolytic anemia
WHO, World Health Organization
[a] WHO classifications (from ref. 14, other details from ref. 17, from (2)). Class I variants are extremely rare; the distinction between class II and III variants is not clear, and the "class V" very high activity variant has been reported in only a single case. Therefore, almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.
[b] Due to the large number of G6PD variants, many other diplotypes may be possible besides those given as examples here; see Supplementary Table S1 online for a more comprehensive list of variant alleles with their assigned WHO class (2).
[c] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online (G6PD heterozygotes)) (2).
This table is adapted from (2).

Gene: *CYB5R3*

The cytochrome b5 reductases (CYB5R family, formerly known as methemoglobin reductases) are a family of flavoproteins that catalyze reduction reactions by using NADH. In humans, there are 4 members of this family (CYB5R1-4), and they are involved in several metabolic reactions, including the formation and breakdown of fatty acids, cholesterol synthesis, and the metabolism of drugs (20).

One important reaction primarily catalyzed by CYB5R3 is the reduction of methemoglobin to hemoglobin. Hemoglobin binds and delivers oxygen to the body's tissues, while methemoglobin does not.

Methemoglobin is produced when heme ferrous iron molecules (Fe2+) are oxidized to ferric iron (Fe3+), which are unable to bind oxygen. In addition, the remaining ferrous iron molecules have increased affinity for oxygen, and are less likely to release oxygen to the peripheral tissues (21).

Normally, the oxidation of hemoglobin occurs at a slow rate –– approximately 1% of red blood cells contain methemoglobin (3). An increase in methemoglobin (methemoglobinemia) is caused either by an increased production (for example, triggered by the ingestion of poison, street drugs, and certain medications) or be decreased removal (for example, kidney failure). Symptoms include a bluish skin color (cyanosis), headache,

fatigue, and shortness of breath. When levels of methemoglobin approach 10%, cyanosis occurs. Higher levels may cause seizures, and levels above 30% are life threatening.

Most cases of methemoglobinemia are acquired. The antibiotics dapsone and chloroquine, anesthetics such as benzocaine, and rasburicase, have all been associated with methemoglobinemia.

Much less commonly, methemoglobinemia is inherited. There are 2 main causes of hereditary methemglobinemia –– Hemoglobin M disease and CYB5R3 deficiency. The former is an autosomal dominant disorder in which a variant occurs in one of the globin chains and results in defective hemoglobin. The latter, CYB5R3 deficiency, is a rare autosomal recessive disorder that is more common in specific populations, for example, Athabascan Alaskans, and Navajo Indians. A variant in the *CYB5R3* gene results in 2 forms of CYB5R deficiency: in type 1, the mutated enzyme is unstable with reduced activity; type 2 is lethal in early pregnancy because the mutated enzyme has no activity (3, 22, 23).

In type 1 CYB5R3 deficiency, the mutated enzyme is expressed in all tissues – but the enzyme is only deficient in red blood cells. This is because unlike other cell types, mature red blood cells lack the ability to synthesize new proteins. Therefore the mutated enzyme, which is easily degraded, is not replaced (22).

Individuals who have 2 copies of the *CYB5R3* variant (homozygous) have high levels of methemoglobin (up to 40%) and may appear cyanotic. However, their symptoms are typically mild ("blue but well") and lifespan is normal – this is because they are adapted to accommodate cyanosis, for example, they have an increased red blood cell mass (24).

In contrast, individuals who have one copy of the *CYB5R3* variant (heterozygous) have lower levels of methemoglobin (approximately 10%) and do not have cyanosis. However, because these individuals are not adapted, they are at high risk of developing life-threatening methemogolobinemia if they are treated with medications that can increase methemoglobin levels (3).

The drug label for rasburicase warns that rasburicase can result in methemoglobinemia in some individuals, and warms to immediately and permanently discontinue rasburicase in individuals developing methemoglobinemia (1).

Linking Gene Variation with Treatment Response

G6PD

Although evidence that links the G6PD status of individuals taking rasburicase with an increased risk of hemolytic anemia is limited to case reports, it is well known that hydrogen peroxide, which is produced during rasburicase therapy, can cause acute hemolysis in individuals with G6PD deficiency. Therefore, many countries, including the US, have warned that the use of rasburicase is contraindicated in individuals with G6PD deficiency (2, 21, 25-33).

CYB5R3

There are no published reports of methemoglobinemia attributed to the combination of rasburicase therapy with an underlying CYB5R3 deficiency. According to the drug label, rasburicase-induced methemoglobinemia occurs in less than 1% of individuals, and their CYB5R3 status is not stated. The drug label states that "it is not known whether individuals with deficiency of cytochrome b5 reductase (formerly known as methemoglobin reductase) or of other enzymes with antioxidant activity are at increased risk for methemoglobinemia or hemolytic anemia" (1).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for rasburicase response, and the genes *G6PD* and *CYB5R3*. Molecular genetic testing can be used to confirm the diagnosis of G6PD or CYB5R3 deficiency. Testing may also be used to screen females with a family history of G6PD deficiency to see if they are carriers.

G6PD deficiency is inherited in an X-linked recessive pattern and most individuals are asymptomatic throughout life.

X-linked disorders affect males at a much higher rate than females because males only have one copy of the X chromosome (hemizygous, XY). Since females have 2 copies of the X chromosome (XX) they tend to be less affected. However, female carriers can present with a range of phenotypes from no symptoms through a severe deficiency due to the high frequency of *G6PD* variants. Females randomly inactivate one X chromosome in somatic cells during development, resulting in a mixed population of somatic cells expressing one *G6PD* allele or the other.

Glucose-6-phosphate dehydrogenase deficiency occurs in homozygous and compound heterozygous females (who have inherited 2 copies of *G6PD* deficiency alleles) and in heterozygous females (one normal *G6PD* allele and one deficiency *G6PD* allele) with skewed X-chromosome inactivation of the functional allele (9). Genetic testing alone is insufficient for heterozygous females with one normal function *G6PD* allele, as the expression of the 2 alleles will vary between blood cells and over time (2).

A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* gene to their daughters, but not to their sons (12, 34).

The FDA recommends that individuals at risk of G6PD deficiency be screened for G6PD deficiency before starting rasburicase therapy. However, individuals of all ancestries may be G6PD deficient (worldwide prevalence of 5%). Therefore, caution must be taken in all individuals when initiating rasburicase therapy.

In routine clinical practice, G6PD deficiency is diagnosed by measuring G6PD activity in red blood cells. Two different types of enzyme activity tests are used: qualitative and quantitative. Often, qualitative tests do not accurately detect individuals with intermediate G6PD activity. False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, screening for G6PD levels should be performed 23 months after a blood transfusion or hemolytic episode. Note, false negatives have been reported (2, 12, 35).

In men, if genetic testing was used to determine that an individual was positive for G6PD deficiency, the use of rasburicase would be contraindicated. However, a negative result cannot be entirely relied upon because only a small subset of G6PD variants are routinely tested for via targeted assays (2, 4, 12, 34, 36). In addition, G6PD phenotype may be unpredictable in heterozygous females because of X-chromosome inactivation, which can happen in a variable percentage of somatic cells.

Universal neonatal screening programs for G6PD deficiency are employed in some countries with a high incidence of G6PD deficiency (more than 3–5% of males) (37). These populations are primarily in Asia, Africa, along the Mediterranean and in the Middle East. Screening either uses quantitative enzyme activity assays, or the fluorescent spot test that visually identifies NADPH, which is produced by G6PD (if the blood spot does not fluoresce, the test is positive for G6PD deficiency) (2).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

Hemolysis

Rasburicase is contraindicated in patients with G6PD deficiency because hydrogen peroxide is one of the major by-products of the conversion of uric acid to allantoin. In clinical studies, hemolysis occurs in <1% patients receiving rasburicase; severe hemolytic reactions occurred within 2–4 days of the start of rasburicase. Immediately and permanently discontinue rasburicase administration in any patient developing hemolysis. Institute appropriate patient monitoring and support measures (e.g., transfusion support). Screen patients at higher risk for G6PD deficiency (e.g., patients of African or Mediterranean ancestry) prior to starting rasburicase.

Methemoglobinemia

In clinical studies, methemoglobinemia occurred in <1% patients receiving rasburicase. These included cases of serious hypoxemia requiring intervention with medical support measures. It is not known whether patients with deficiency of cytochrome b5 reductase (formerly known as methemoglobin reductase) or of other enzymes with antioxidant activity are at increased risk for methemoglobinemia or hemolytic anemia. Immediately and permanently discontinue rasburicase administration in any patient identified as having developed methemoglobinemia. Institute appropriate monitoring and support measures (e.g., transfusion support, methylene-blue administration).

Please review the complete therapeutic recommendations that are located here: (1).

2014 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

[…]

As stated above, rasburicase use is contraindicated by the FDA, the European Medicines Agency, and the Pharmaceuticals and Medical Devices Agency in those with G6PD deficiency. If, on the basis of genotyping, a deficient status can be unambiguously assigned to a patient, that would be a sufficient contraindication to the use of rasburicase. However, due to the limitations of genetic testing (discussed above), in most cases it is necessary to perform G6PD enzyme testing to assign G6PD status.

The FDA recommends that patients at higher risk of G6PD deficiency, such as those with African or Mediterranean ancestry, be tested for G6PD deficiency before initiation of rasburicase. However, it should be noted that patients of all ancestries may be G6PD deficient. The drug labels do not specifically mention genetic testing, but with the increased availability of genetic test results some patients may be diagnosed with G6PD deficiency preemptively; if so, such definitive results could be used to preclude prescribing of rasburicase and potentially other oxidative drugs even in the absence of G6PD enzyme activity results.

Pediatrics. Much of the evidence relating G6PD deficiency to rasburicase-induced hemolysis and methemoglobinemia was generated in neonates or children (Supplementary Table S7 online), and thus these guidelines apply to neonates, children, and adults.

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.*

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature for Selected *G6PD* Variants

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Boria	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3: c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3: c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
* WHO classifications based on (38).

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Risperidone Therapy and *CYP2D6* Genotype

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Introduction

Risperidone is the most commonly prescribed antipsychotic medication in the US. It is an atypical (second generation) antipsychotic used in the treatment of schizophrenia, bipolar disorder, severe dementia, and irritability associated with autism.

Risperidone is metabolized to the active metabolite 9-hydroxyrisperidone by the enzyme CYP2D6 and to a lesser extent by CYP3A4. Individuals who carry two inactive copies of the *CYP2D6* gene are termed "poor metabolizers" and may have a decreased capacity to metabolize risperidone. These individuals may be at a higher risk of adverse effects because of increased exposure to plasma risperidone, compared to normal metabolizers, who carry two active copies of *CYP2D6*. Individuals who are CYP2D6 ultrarapid metabolizers (who carry more than two functional copies of *CYP2D6*) may have a decreased response to therapy, resulting from lower steady-state risperidone concentrations.

The FDA-approved drug label states that analysis of clinical studies involving a modest number of poor metabolizers (n=70) does not suggest that poor and extensive (normal) metabolizers have different rates of adverse effects (1). In addition, the Dutch Pharmacogenetics Working Group (DPWG) recently changed its dosing recommendations to "no action is needed" for CYP2D6 poor metabolizers taking risperidone (2).

Drug: Risperidone

Risperidone is an atypical antipsychotic primarily used in the treatment of schizophrenia and manic or mixed episodes in bipolar disorder. Risperidone may also be used as part of the management of aggression and/or psychosis in severe dementia and irritability associated with autistic disorder in children and adolescents (1).

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine. Known as "first-generation" or "typical" antipsychotics, these drugs are used to treat psychosis (regardless of the underlying cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions.

All antipsychotics, with the exception of aripiprazole, are dopamine receptor antagonists. Blockade of the D2 dopamine receptor in the brain's limbic system is thought to improve the "positive" symptoms of schizophrenia, such as delusions and hallucinations, which are signs of psychosis.

However, typical antipsychotics also block dopamine receptors in the nigrostriatal pathway. This can cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects. Risperidone is an atypical antipsychotic. The most common side effects of risperidone therapy are sedation and dry mouth, but the rates of both appear to be low, at around 5% (3). Other atypical antipsychotics approved by the FDA include aripiprazole, asenapine, brexpiprazole, cariprazine, clozapine, lurasidone, olanzapine, quetiapine, and ziprasidone.

Atypical antipsychotics, such as risperidone, are thought to transiently occupy D2 receptors and then rapidly dissociate, to allow for normal dopamine neurotransmission (4). Because risperidone has high affinity for the D2

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receptor but binds it "loosely", it does not block dopamine receptors in the nigrostriatal pathway and extrapyramidal side effects are less likely (5).

Risperidone also blocks serotonin receptors, alpha 1 adrenergic receptors, and, to a lesser extent, histamine H1 and alpha 2 adrenergic receptors.

The main route of risperidone metabolism is in the liver by the enzyme CYP2D6. The major active metabolite, 9-hydroxyrisperidone, contributes to the pharmacological effects of this drug (5). While risperidone and 9-hydroxyrisperidone are often regarded as equipotent, they display different affinities towards the two target receptors (D2 and 5HT2A), where risperidone appears to be approximately 2-fold more potent than 9-hydroxyrisperidone. There is also a difference in brain distribution; risperidone is distributed more to the CNS (6).

Genetic variations in the *CYP2D6* gene may contribute to an increased risk of adverse events associated with risperidone therapy (7). Individuals who are "CYP2D6 poor metabolizers" carry two no function copies of the *CYP2D6* gene. In these individuals, standard doses of risperidone may lead to increased plasma levels of risperidone and decreased levels of 9-hydoxyrisperidone.

However, it is unclear to the extent to which *CYP2D6* genotype influences the efficacy and safety of risperidone therapy. One small study of 76 patients with schizophrenia reported that CYP2D6 poor metabolism was associated with greater clinical improvement in the total Positive and Negative Syndrome Scale (PANSS) (8). Other studies have reported a higher rate of adverse reactions and drug discontinuations in CYP2D6 poor metabolizers compared to normal metabolizers (5, 9, 10).

The ratio of risperidone to 9-hdroxyrisperidone, which largely reflects CYP2D6 phenotype, may be a risk factor for different side effects (11). Because prolactin levels mainly correlate with 9-hydroxyrisperidone levels, CYP2D6 ultrarapid metabolizers may experience different side effects than normal metabolizers (12). In addition, because elderly patients accumulate 9-hydroxyrisperidone due to reduced renal function, older patients who are CYP2D6 poor metabolizers (and others with reduced renal function) are at particular risk of side effects during risperidone treatment (5).

Individuals who are "CYP2D6 ultrarapid metabolizers" may have decreased plasma levels of risperidone, due to increased CYP2D6 activity—these individuals carry more than two functional copies of the *CYP2D6* gene. A small study of 85 patients taking long-lasting risperidone showed that the plasma concentrations of risperidone and its active metabolite were subtherapeutic in three individuals who were CYP2D6 ultrarapid metabolizers. The study, however, did not report whether these changes affected the effectiveness or tolerability of the drug in these patients (13).

Overall, it remains unclear whether the accurate determination of an individual's *CYP2D6* genotype, together with therapeutic drug monitoring, has the potential to optimize the response of CYP2D6 poor metabolizers and ultrarapid metabolizers to antipsychotic therapy (9, 14).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic, with over 100 star (*) alleles described (15). *CYP2D6*1* is the reference (or wild-type) allele encoding an enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal enzyme activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *36, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (15).

Individuals who have more than two normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," whereas individuals who carry two normal or one normal and one decreased function allele are classified as "normal metabolizers" (also referred to as "extensive metabolizers").

Individuals with one normal and one no function allele or two decreased function alleles are also categorized as "normal metabolizers" by recent nomenclature guidelines (16), but have also been categorized as "intermediate metabolizers" elsewhere in the literature. Subjects with one decreased and one no function allele are predicted to be "intermediate metabolizers" and those with two no function alleles are considered to be "poor metabolizers" (Table 2).

Table 2: 2016 Assignment of CYP2D6 phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 ultrarapid metabolizer (approximately 1–20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN* [b]
CYP2D6 normal metabolizer (approximately 72–88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 intermediate metabolizer (approximately 1–13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 2 continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 poor metabolizer (approximately 1–10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see (17).
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (17).

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (18, 19, 20, 21), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (19, 21, 22, 23, 24). There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (25).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (26, 27).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are currently available for risperidone response and for the *CYP2D6* gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results (28). However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (29).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- A normal (previously referred to as "extensive") metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score greater than 2 (17, 30)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Risperidone is extensively metabolized in the liver. The main metabolic pathway is through hydroxylation of risperidone to 9-hydroxyrisperidone by the enzyme, CYP 2D6. A minor metabolic pathway is through N-dealkylation. The main metabolite, 9-hydroxyrisperidone, has similar pharmacological activity as risperidone. Consequently, the clinical effect of the drug results from the combined concentrations of risperidone plus 9-hydroxyrisperidone.

CYP 2D6, also called debrisoquin hydroxylase, is the enzyme responsible for metabolism of many neuroleptics, antidepressants, antiarrhythmics, and other drugs. CYP 2D6 is subject to genetic polymorphism (about 6%–8% of Caucasians, and a very low percentage of Asians, have little or no activity and are "poor metabolizers") and to inhibition by a variety of substrates and some non-substrates, notably quinidine. Extensive[2] CYP 2D6 metabolizers convert risperidone rapidly into 9-hydroxyrisperidone, whereas poor CYP 2D6 metabolizers convert it much more slowly. Although extensive metabolizers have lower risperidone and higher 9-hydroxyrisperidone concentrations than poor metabolizers, the pharmacokinetics of risperidone and 9-hydroxyrisperidone combined, after single and multiple doses, are similar in extensive and poor metabolizers.

Please review the complete therapeutic recommendations that are located here: (1).

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

CYP2D6 Poor metabolizers:

No action is needed for this gene-drug interaction.

The genetic variation can result in both an increase in side effects and a stronger decrease in schizophrenia symptoms. In addition to this, the genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

CYP2D6 intermediate metabolizers:

No action is needed for this gene-drug interaction.

There is little evidence to support an increase in side effects caused by the genetic variation. The genetic variation may lead to a decrease in the required maintenance dose. However, as the effect on the dose is smaller than that of the normal biological variation, action is not useful.

CYP2D6 ultrarapid metabolizers:

No action is needed for this gene-drug interaction.

Genetic variation may lead to an increase in the required maintenance dose. However, as the effect is smaller than that of the normal biological variation, action is not useful.

Please review the complete therapeutic recommendations that are located here: (2).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] The FDA statement uses the term "extensive metabolizer." CPIC recently introduced standardized nomenclature for pharmacogenetic terms, which included replacing the term "extensive metabolizer" with the term "normal metabolizer." More details can be found in the 2016 paper, "Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC)" 16. Caudle, K.E., H.M. Dunnenberger, R.R. Freimuth, J.F. Peterson, et al., *Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC).* Genet Med, 2016.

Nomenclature

Nomenclature for selected *CYP2D6* alleles

Common allele name	Alternative names / Major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	Includes at least two functional variants*: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.5:c.886T>C	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region	rs28371725

SNP= Single Nucleotide Polymorphism
*In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

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Simeprevir Therapy and *IFNL3* Genotype

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Introduction

NOTE: ARCHIVED ON 15 JULY 2020 BECAUSE SIMEPREVIR IS NO LONGER LICENSED FOR USE IN THE USA. THIS SUMMARY IS FOR HISTORIAL REFERENCE ONLY AND WILL NOT BE UPDATED.

Simeprevir is a hepatitis C virus (HCV) protease inhibitor used in combination with other drugs to treat chronic hepatitis genotype 1 or 4 infection (1).

Previously, the standard care of patients with HCV infection was peginterferon alfa and ribavirin, but ~40-50% of patients with HCV genotype 1 infection had a suboptimal sustained virological response (SVR) (2).

A SVR is defined as undetectable HCV RNA by the end of treatment and at a specific number of weeks after the end of treatment. The addition of simeprevir increased the SVR in patients with HCV genotype 1 infection who were previously untreated. However, there were reports of treatment failure, most commonly in adults, who failed to respond to previous peginterferon and ribavirin treatment (3).

The FDA-approved drug label for simeprevir contains information regarding a genetic variant near the *IFNL3* gene (a C to T change; rs12979860), which is a strong predictor of response to peginterferon alfa and ribavirin treatment. The label states that in phase 3 clinical trials, SVR rates were lower in patients with CT and TT genotypes, compared to patients with the CC genotype. However, patients of all *IFNL3* genotypes had highest SVR rates when being treated with regimens that included simeprevir.

In addition, the label strongly recommends patients with HCV genotype 1a infection should be screened for the presence of virus with the S3 Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (4).

Drug class: HCV Protease Inhibitors

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon (PEG-IFN), and more recently, the addition of antiviral protease inhibitors such as simeprevir.

Protease inhibitors are the first direct-acting antivirals to be approved for the treatment of HCV, and simeprevir is the first second-generation agent to become available. Simeprevir has largely replaced the use of the first-generation protease inhibitors, boceprevir and telaprevir, which have less favorable side effect profiles.

Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

The addition of simeprevir to a PEG-IFN and ribavirin treatment regimen increases the SVR in patients with chronic hepatitis caused by genotype type 1 or 4 hepatitis C virus, and the response to treatment is influenced by the patient's *IFNL3* genotype.

The FDA-approved drug label for simeprevir states that simeprevir should only be used in combination with other antiviral drugs, such as in combination with PEG-IFN and ribavirin; or in combination with sofosbuvir

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(HCV nucleotide-analogue NS5B polymerase inhibitor) (1). However, because IFN-free regimes are fast becoming the current standard of care for hepatitis C, simeprevir tends to be prescribed with sofosbuvir rather than IFNs.

Drug: Simeprevir

Acute infection with HCV is usually asymptomatic, and about 15-45% of people who are infected clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years. It is thought that over 180 million people are infected with HCV worldwide (5).

The HCV is classified by genotype, based on the RNA viral strands. There are 6 classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (6). Genotype 1 is the most difficult to treat, as it is less likely than genotypes 2 and 3 to respond to therapy.

Simeprevir has been FDA-approved for use in combination with other drugs, for the treatment of adults with chronic hepatitis C, caused by an infection with genotype 1 or 4 HCV.

During the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cancer (hepatocellular carcinoma). Approximately 45% of patients with chronic hepatitis C will develop liver cancer within 20 years from the initial infection.

Until recently, the standard of care for hepatitis C infection was based on therapy with peginterferon and ribavirin. Approximately half of the patients cleared the HCV infection, as shown by a SVR, but adverse effects were common and sometimes life-threatening (2). Treatment was expensive and inconvenient, lasting up to 48 weeks.

Protease inhibitors such as simeprevir were specifically developed to improve the effectiveness of peginterferon and ribavirin therapy. Teleprevir was the first drug to be developed, but severe dermatological adverse effects and liver toxicity limited its use. Simeprevir belongs to the second generation of drugs, and has an improved therapeutic index.

Simeprevir prevents maturation of the HCV by blocking viral protein synthesis. Specifically, simeprevir inhibits the viral protease NS3/4A which is responsible for cleaving and processing the HCV polyprotein precursor (7). Several mutations in this viral NS3/4A protease are associated with a reduced susceptibility to simeprevir. One of the most common and clinically significant mutations is the Q80K polymorphism. The FDA-approved drug label states that patients with HCV genotype 1a infection should be screened for the presence of virus with the Q80K polymorphism. If Q80K is detected, the label strongly recommends that alternative therapy be considered (1).

The combination of protease inhibitors such as simeprevir with peginterferon and ribavirin therapy has led to a much more effective treatment of hepatitis C in patients who were “treatment naïve” (no history of HCV treatment) and among “relapsers” (patients who had relapsed after previous HCV therapy). This was evidenced by improvement in the SVR and reduction of treatment from 48 to 24 weeks, without any increase in peginterferon and ribavirin adverse effects (3, 8).

The treatment options for hepatitis C continue to evolve. Currently, IFN-free treatment regimes for hepatitis C are considered to be the standard of care. The IFN-free combination of simeprevir plus sofosbuvir has been found to be a highly effective treatment, with studies reporting high SVR12 rates for the majority of patients with chronic HCV infection (from about 84% to 94%) (9-11).

Genetic variants in the *IFNL3* gene have been shown to strongly influence treatment response to PEG interferon-alpha-based regimens (including regimens with simeprevir) in previously untreated patients with HCV genotype 1 infection (4). However, data are currently lacking on how *IFNL3* variants influence an individual's response to simeprevir when used with sofosbuvir in an IFN-free regimen.

Gene: *IFNL3*

The *IFNL3* gene, previously known as *IL28B*, encodes interferon lambda-3 (IFN-λ3) and is involved in the immune response to hepatitis C.

When a person is infected by a virus, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block viral replication and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

There are three classes of IFNs: type I (IFN-α/β), type II (IFN-γ) and type III (IFN-λ). The IFNL3 is a type III interferon, and as such, induces a strong antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (12).

IFNL3 is only highly expressed in hepatocytes and epithelial cells, in contrast to other similar interferons, such as IFN-α, which are expressed in most cell types. IFNL3 exerts its actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IL28RA receptor chains (4).

The first two *IFNL3* variants to be commonly tested for are rs12979860 and rs8099917. These variants are in close proximity to each other near the *IFNL3* gene, and are in strong linkage disequilibrium. HCV genotype 1 patients with the "favorable" genotypes (CC for rs12979860 and TT for rs8099917) respond better to treatment as they are associated with an approximate 2-fold increase in SVR. However, the exact mechanism how these variants influence treatment outcome is not yet known (4).

In a US cohort of mixed ethnicity, variants in rs12979860 predicted treatment response in HCV genotype 1 infection patients: CC genotype individuals were more likely to spontaneously clear acute HCV infection and TT genotype individuals had the poorest response to treatment. Accordingly, CT genotype individuals had an intermediate response that was between those of the CC and TT genotype patients (4).

The response to HCV treatment varies across different populations, which can be largely explained by differences in allele frequencies. The rs12979860 'C' allele is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (4).

Among Asians and individuals of European descent, the rs8099917 variant best predicts treatment response (13-15). Moreover, recently a variant in the *IFNL4* gene (rs368234815), was found to be superior to rs12979860 in predicting treatment outcome in individuals of African ancestry. Together with another *IFNL4* variant (rs117648444), the combination of these two variants was found to have greater treatment response prediction compared to testing for single variants (12).

Genetic Testing

Genetic testing for *IFNL3* is available, and is used to predict response to peg-IFN and RBV in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to best manage their HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

rs12979860 CC, favorable genotype

rs12979860 CT, unfavorable genotype

rs12979860 TT, unfavorable genotype (4).

Before starting a treatment regimen with simeprevir in patients with HCV genotype 1a infection, the FDA strongly recommends screening patients for the presence of virus with the "NS3 Q80K" polymorphism. The FDA states that an alternative therapy to simeprevir should be considered if Q80K is detected (1).

Therapeutic Recommendations based on Genotype

PLEASE NOTE: SIMEPREVIR IS NO LONGER LICENSED FOR USE IN THE USA. THE MEDICAL GENETICS SUMMARY WAS ARCHIVED ON 15 JULY 2020. THIS INFORMATION IS PROVIDED FOR HISTORAL REFERENCE ONLY.

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): A genetic variant near the gene encoding interferon-lambda-3 (IL28B rs12979860, a C [cytosine] to T [thymine] substitution) is a strong predictor of response to Peg-IFN-alfa and RBV (PR). In the Phase 3 trials, IL28B genotype was a stratification factor.

Overall, SVR rates were lower in subjects with the CT and TT genotypes compared to those with the CC genotype. Among both treatment-naïve subjects and those who experienced previous treatment failures, subjects of all IL28B genotypes had the highest SVR rates with simeprevir-containing regimens.
Please review the complete therapeutic recommendations that are located here: (1)

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs12979860	/	NM_001276254.2:c.151-152G>A	N/A	rs12979860
rs8099917	/	N/A	N/A	rs8099917

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Simvastatin Therapy and *SLCO1B1* Genotype

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Introduction

Simvastatin (brand name Zocor) is a member of the statin class of drugs, used to regulate low-density lipoprotein (LDL) cholesterol levels in various conditions, including familial hypercholesterolemia (FH), primary hyperlipidemia, hypertriglyceridemia, and primary dysbetalipoproteinemia. Statins are also used to reduce total mortality risk associated with coronary heart disease, non-fatal myocardial infarction, and revascularization procedures in adults at high risk of coronary heart disease events, including those with established vascular disease or diabetes. Approved by the US FDA for use primarily in adults, simvastatin is also approved for children aged 10 and older to manage FH (1). Administered as a pro-drug, simvastatin must be metabolized to simvastatin acid, which then acts in the liver and other tissues to reduce cholesterol production by competitively inhibiting HMG-CoA reductase (3-hydroxy-methylglutaryl-coenzyme). Simvastatin acid also promotes an increase in the uptake of LDL from the bloodstream, resulting in a reduction in cardiovascular risk and improved health outcomes for most individuals. However, individuals can experience adverse reactions; the most common are statin-associated musculoskeletal symptoms (SAMS) or other serious reactions.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy indicate that individuals with decreased function in the organic anion transporting polypeptides 1B1 (OATP1B1) hepatic transport enzyme (encoded by the *SLCO1B1* gene) have an increased risk of SAMS (2, 3). The CPIC guidelines provide dosing recommendations based on an individual's predicted phenotype, stating that individuals with decreased or poor metabolizer phenotypes should be prescribed an alternative statin or a lower dose of simvastatin (Table 1) (2). Criteria for choosing the relative potency of an alternative statin or the dose of simvastatin are also outlined by CPIC (Figure 1) (2). The DPWG guidelines focus on the most common functional variant, a single nucleotide variation (SNV) at rs4149056, NM_006446.5:c.521T>C, recommending that individuals heterozygous or homozygous for the variant allele, resulting in decreased or poor function phenotype, choose an alternative statin (Table 2) (3, 4). The US FDA does not specifically address *SLCO1B1* genetic variation in the simvastatin drug label, but it does discuss various medications that are either contraindicated (strong cytochrome P450 enzyme 3A4 [CYP3A4] inhibitors, gemfibrozil, cyclosporin and danazol) with simvastatin or may increase the risk of myopathy (1). The drug label for simvastatin in Switzerland, however, describes the increased risk of SAMS for individuals who have at least one variant allele at rs4149056, and recommends considering genotyping results indicating a CC genotype at this SNV during risk-benefit assessment before prescribing 80 mg doses of simvastatin due to higher myopathy risks (5). The interplay of genetics, co-medications, comorbidities, and simvastatin dose highlights the complex factors that contribute to an individual's risk of developing SAMS.

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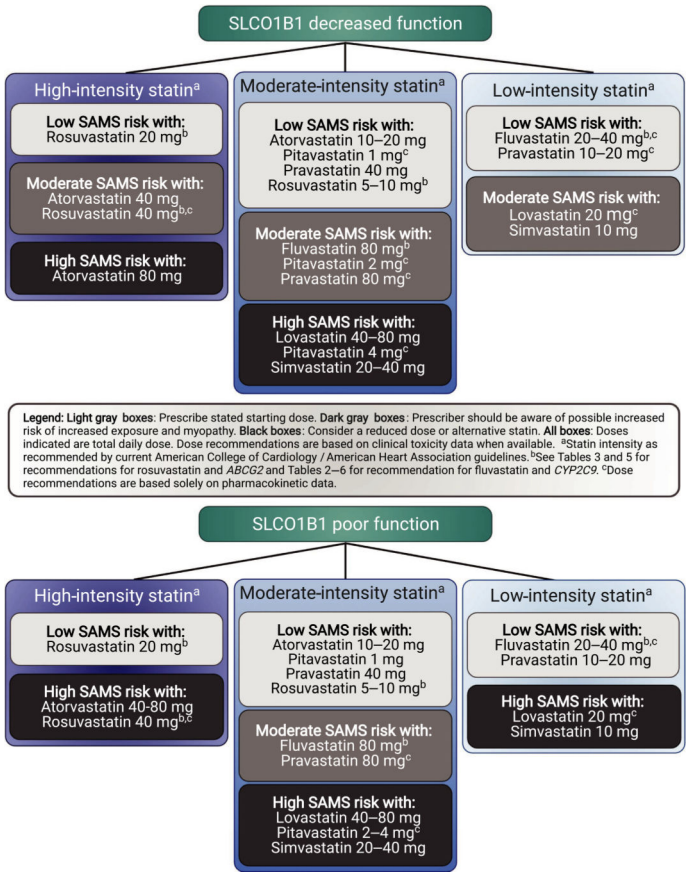

Figure 1: The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1, ABCG2, and CYP2C9 genotypes and Statin-Associated Musculoskeletal Symptoms.

SLCO1B1 recommendations with intensity and statin dose stratified by SLCO1B1 phenotype; all doses assume adult dosing. SAMS, statin-associated musculoskeletal symptoms.

Reproduced from Clin Pharma and Therapeutics, Volume: 111, Issue: 5, Pages: 1007-1021, First published: 12 February 2022, DOI: (10.1002/cpt.2557) © 2022, The Authors. (2)

Table 1: The Clinical Pharmacogenetics Implementation Consortium (CPIC) Recommendations for Simvastatin Based on *SLCO1B1* Phenotype in Adults (2022)

Phenotype	Implications	Dosing recommendation	Classification of recommendation	Considerations
SLCO1B1 increased function	Typical myopathy risk and statin exposure	Prescribe desired starting dose and adjust doses based on disease-specific guidelines.	Strong	The potential for drug–drug interactions and dose limits based on renal and hepatic function and ancestry should be evaluated before initiating a statin.
SLCO1B1 normal function	Typical myopathy risk and statin exposure	Prescribe desired starting dose and adjust doses based on disease-specific guidelines.	Strong	The potential for drug–drug interactions and dose limits based on renal and hepatic function and ancestry should be evaluated before initiating a statin.

Table 1 continued from previous page.

Phenotype	Implications	Dosing recommendation	Classification of recommendation	Considerations
SLCO1B1 decreased (or possible decreased) function	Increased simvastatin acid exposure as compared with normal function; increased risk of myopathy	Prescribe an alternative statin depending on the desired potency[a]. If simvastatin therapy is warranted, limit dose to <20 mg/day.	Strong	The potential for drug–drug interactions and dose limits based on renal and hepatic function should be evaluated before initiating a statin. The effects of drug–drug interactions may be more pronounced, resulting in a higher risk of myopathy.
SLCO1B1 poor function	Increased simvastatin acid exposure as compared with normal function; highly increased myopathy risk	Prescribe an alternative statin depending on the desired potency[a].	Strong	The potential for drug–drug Interactions and dose limits based on renal and hepatic function should be evaluated before initiating a statin. The effects of drug–drug interactions may be more pronounced, resulting in a higher risk of myopathy.

[a] Statin potency and genotype recommendations are summarized in Figure 1.
Table adapted from (2).

Table 2: The Dutch Pharmacogenetics Working Group (DPWG) Recommendations for Simvastatin Based on *SLCO1B1* Genotype (2020)

SLCO1B1 genotype[a]	Risk	Recommendation
521CC	When using simvastatin 80 mg/day, the risk of myopathy is increased 30-fold to 18% and the risk of severe myopathy is increased 48-fold to 12%. When using 40 mg/day, this risk is increased 7-fold to 1% and 11-fold to 0.68%. The gene variation leads to reduced simvastatin transport to the liver, which increases the simvastatin plasma concentration and therefore the risk of side effects.	Choose an alternative. Consider any additional risk factors for statin-induced myopathy. Atorvastatin is affected less severely by the *SLCO1B1* gene variation but is also affected by CYP3A4 inhibitors such as amiodarone, verapamil, and diltiazem. Use of atorvastatin is not recommended for individuals with additional risk factors for statin-induced myopathy. Rosuvastatin and pravastatin are influenced to a lesser extent by the *SLCO1B1* gene variation. They are also not influenced by CYP3A4 inhibitors such as amiodarone, verapamil, and diltiazem. Fluvastatin is not significantly influenced by the *SLCO1B1* gene variation or CYP3A4 inhibitors.

Table 2 continued from previous page.

SLCO1B1 genotype[a]	Risk	Recommendation
521TC	When using simvastatin 80 mg/day, the risk of myopathy is increased 5-fold to 3% for moderately severe to severe myopathy and 1.3% for severe myopathy. When using 40 mg/day, this risk is increased 2.6-fold to 0.39% and 0.17%. The gene variation leads to reduced simvastatin transport to the liver, which increases the simvastatin plasma concentration and therefore the risk of side effects.	Choose an alternative. Consider any additional risk factors for statin-induced myopathy. Atorvastatin is affected less severely by the *SLCO1B1* gene variation but is also affected by CYP3A4 inhibitors such as amiodarone, verapamil, and diltiazem. Use of atorvastatin is not recommended for individuals with additional risk factors for statin-induced myopathy. Rosuvastatin and pravastatin are influenced to a lesser extent by the *SLCO1B1* gene variation. They are also not influenced by CYP3A4 inhibitors such as amiodarone, verapamil, and diltiazem. Fluvastatin is not significantly influenced by the *SLCO1B1* gene variation or CYP3A4 inhibitors. If an alternative is not an option: 1. Avoid simvastatin doses exceeding 40 mg/day (for example, by adding ezetimibe) 2. Advise the individual to report muscle symptoms.

[a] DPWG literature review found association with only the T>C variation at rs4149056 (NM_006446.5:c.521T>C) and simvastatin side effects, though other gene variations were assessed (4).
This table adapted from (3) and personal communication from M. Nijenhuis (see Acknowledgements).

Drug: Simvastatin

Simvastatin, an HMG-CoA reductase inhibitor, is used with diet to manage FH, hypertriglyceridemia, primary dysbetalipoproteinemia, and to lower the risk of fatal cardiovascular events in individuals with coronary heart disease, cerebrovascular disease, peripheral vascular disease, diabetes, or a combination of these conditions. Simvastatin is primarily indicated for adults but is also approved for pediatric use in individuals aged 10 and older with FH. The recommended pediatric dose ranges from 10 mg to 40 mg daily, while adults are recommended 40 mg daily, with a rare exception of 80 mg daily only for those who have been taking this higher dose for more than 12 months without evidence of muscle toxicity. (1)

Statins are recommended as a first-line treatment for elevated LDL cholesterol levels or for diabetic individuals by the American College of Cardiology and American Heart Association (6) as well as by the American Association of Clinical Endocrinologists and American College of Endocrinology (7). Statins are contraindicated in individuals with acute liver failure or decompensated cirrhosis (1). Simvastatin should not be used with medications that strongly inhibit CYP3A4 (which includes macrolide antibiotics, some azole anti-fungal medications, antiviral medications, and nefazodone), nor with cyclosporine, danazol, or gemfibrozil (1). Consuming grapefruit juice while taking simvastatin can also increase the plasma levels of simvastatin due to inhibition of CYP3A4 (1, 5)

Statins, including simvastatin, work by inhibiting the HMG-CoA reductase enzyme, leading to decrease cholesterol production (8). This inhibition also reduces levels of mevalonate, which leads to upregulation of other enzymes involved in cholesterol biosynthesis, including the LDL receptor, further decreasing plasma LDL cholesterol levels (9). For individuals with FH due to LDL receptor loss of function, statins reduce cholesterol levels by decreasing the production of apolipoprotein-B containing lipoproteins in the liver (9). Decreased circulating lipid levels are associated with decreased risk of cardiovascular disease (CVD) and atherosclerotic CVD (ASCVD) (10). Simvastatin is absorbed by cells expressing the OATP1B1 drug transporter; in the liver, it is metabolized by CYP3A enzymes to simvastatin acid, which inhibits HMG-CoA reductase (11, 12). Simvastatin can be excreted from the liver via ABCB1 and ABCC2 transporters (11).

Higher doses of simvastatin and higher plasma levels of simvastatin are associated with higher rates of adverse effects, (12). including SAMS, a leading cause of drug discontinuation. The frequency of SAMS was found to directly correlate with simvastatin dose in clinical studies, ranging between 0.61–0.9% of individuals taking an 80 mg dose to 0.02–0.03% frequency with a 20 mg dose (1). Risk factors for SAMS include age of 65 or older, uncontrolled hypothyroidism, renal impairment, certain drug–drug interactions (discussed below), and Chinese ancestry (1). Individuals with reduced function in the drug-transporting enzyme OATP1B1 may be more likely to experience this toxicity (2, 3, 13) due to increased plasma concentration of simvastatin and its metabolites (14, 15). The CPIC guidelines stratify the relative risk of SAMS for statin use by decreased or poor OATP1B1 function (Figure 1) and are intended to be used with cardiovascular expert guidelines when selecting which statin and dose to use (see Therapeutic Recommendations Based on Genotype below for more information). The clinical presentation of SAMS can range from no muscle symptoms with serum creatine kinase (CK) elevations less than 4 times the upper normal limit (severity rating scale [SRM] 0) through the most severe presentation of immune-mediated necrotizing myositis (IMNM) (SRM 6). This standardized scale aids clinicians and researchers in understanding and managing SAMS (16).

Reports of muscle pain while taking statins may be a result of the nocebo effect, as suggested by a study of individuals with a history of SAMS. Out of 200 participants, 151 individuals reported similar frequencies of muscle pain during alternating periods of statin and placebo use, with two-thirds of the cohort resuming long-term statin use at the conclusion of the study (17). Balancing the concerns of SAMS with the therapeutic aim of statin therapy is important, as suboptimal statin use can increase cardiovascular event risk. Suboptimal use included discontinuation, nonadherence, dose reduction, and statin switching leading to reduced efficacy. One study in the UK reported that out of 1005 study participants, 156 individuals had suboptimal statin use and these individuals were at an increased risk of major adverse cardiovascular events (hazard ratio of 2.1) and all-cause mortality (hazard ratio 2.46) (18). Subgroup analysis determined that statin discontinuation or nonadherence were the major contributors to this risk (18).

Serious complications with simvastatin therapy include IMNM and hepatic dysfunction, and therapy should be discontinued if IMNM is suspected or serious hepatic injury (with clinical symptoms, jaundice, or both) occurs (1). Signs of IMNM include proximal muscle weakness, elevated serum CK persisting after statin discontinuation, and necrotizing myopathy on muscle biopsy (1). Two myositis-specific autoantibodies can also be detected during IMNM: anti-signal recognition particle and anti-HMG-CoA Reductase antibodies (19, 20). Individuals with IMNM may require treatment with immunosuppressive agents (1). More common but less severe side effects include upper respiratory infection, headache, abdominal pain, constipation, and nausea (1). There have also been reports of elevated hemoglobin A1c (HbA1C) and fasting glucose levels with statin therapy (1). Simvastatin is not recommended during pregnancy due to decreased synthesis of cholesterol and other biologically active substances derived from cholesterol (1). The specific therapeutic needs of the individual should be considered, though the FDA-approved label states that hyperlipidemia treatment during pregnancy is generally not necessary, given the chronic nature of the atherosclerotic process (1). Clinical information does not indicate a drug-associated risk of major congenital malformations if used during pregnancy, but there is insufficient evidence to evaluate the risk of miscarriage associated with simvastatin therapy (1). There is also a lack of data on the safety of simvastatin use during breastfeeding, and the consensus for clinical practice is to avoid its use by breastfeeding mothers (21). Some advocate for the continued use of statins by expectant mothers and women attempting to conceive with FH due to the increase in lipid levels during pre-conception and pregnancy (12–15 months per pregnancy) (22). Additionally, adverse pregnancy outcomes (including gestational hypertension or diabetes, preeclampsia, or pre-term birth) can increase ASCVD risk, and the natural lipid fluctuations during pregnancy may put individuals with elevated baseline cholesterol levels at greater risk (23). More clinical research is needed to assess the risk of various statins with adverse maternal and fetal or neonatal outcomes (23).

The use of statins in pediatric individuals has been shown to be safe and effective for individuals with heterozygous FH aged 10 and older. A controlled clinical study showed no significant effect on growth or sexual maturation in male (n=99) or female (n=76, all one-year post-menarche) participants (1). Additional studies have examined the efficacy and side effects of varying dosing of simvastatin in pediatric individuals with FH, and reported no clinically relevant side effects (24). Statin therapy in a pediatric population appears safe, though continuity of care remains important as these individuals transition from pediatric to adult clinical care (25).

Individuals aged 65 and over are at increased risk of adverse effects of simvastatin (1). Pharmacokinetic studies suggest that individuals aged 70–78 have a 45% higher plasma level of simvastatin compared with those aged 18–30. Individuals aged 65 and older taking 80 mg simvastatin are at increased risk of myopathy compared with those under 65 years of age taking the same high dose (1). A separate retrospective study reported that high-dose statin use (including simvastatin) in individuals aged 65 and older was associated with an increased burden of myalgia and elevated liver enzymes compared with a control cohort taking low-dose statins (26). Hepatic and renal impairment, often observed in geriatric individuals, are also risk factors for SAMS regardless of age (1).

Gene: *SLCO1B1*

The *SLCO1B1* gene, located on the short arm of chromosome 12, encodes OATP1B1, one of the major hepatic influx transport proteins. Primarily expressed on the basolateral surface of hepatocytes, OATP1B1 facilitates the uptake of various endogenous and exogenous compounds including bile acids, thyroid hormones, bilirubin, methotrexate, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and statins. Decreased expression or function of OATP1B1 can lead to increased plasma concentrations of these substrates, potentially resulting in systemic drug toxicity or adverse effects. Genetic variations at the *SLCO1B1* locus, as well as drug–drug interactions, can change OATP1B1 function and increase an individual's risk for adverse effects from these substrate medications. (27, 28)

The Pharmacogene Variation Consortium (PharmVar) maintains a standardized nomenclature of *SLCO1B1* variants, commonly called star (*) alleles (27). Each star allele is defined by a core variant or variants inherited together on a single allele, or haplotype, with the pair of alleles present in any individual called their diplotype or genotype. Star alleles are assigned a clinical function level by CPIC. Of the 44 star alleles defined by PharmVar, only 13 *SLCO1B1* star alleles have been assigned a clinical functional status by CPIC (Table 3) (29, 30). The *SLCO1B1*1* and *37 alleles are classified as normal function, while multiple alleles such as *SLCO1B1*5, *9*, and *15, are classified as having no function. The *SLCO1B1*14* and *20 alleles are classified as increased-function alleles (29). It is common in pharmacogene nomenclature to assign the *1 allele as the reference allele and the baseline for "normal" allele function. Also, *1 is the presumed allele when no variants are identified by testing.

Table 3: Functional Classification of *SLCO1B1* Allele (CPIC, 2021)

Allele clinical functional status	*SLCO1B1* star alleles
Increased function	*14, *20
Normal function	*1, *37
No function	*5, *9, *15, *23, *31, *46, *47, *48, *49
Unknown function	*43, *44, *45

Table adapted from (29). CPIC - Clinical Pharmacogenetics Implementation Consortium

The normal-function *SLCO1B1*37* allele is defined by a SNV that substitutes an aspartic acid (D) for asparagine (N) at position 130 of the protein (rs2306283, NM_006446.5:c.388A>G) with no other functionally significant variants (29, 31). This variant is present in 18 other star alleles with varying clinical functions, underscoring the importance of testing the breadth of known star alleles for accurate genotyping (27). Another commonly studied

SNV is rs4149056 (NM_006446.5:c.521T>C), found in the *SLCO1B1*5*, *SLCO1B1*15, SLCO1B1*40*, *SLCO1B1*46*, and *SLCO1B1*47* alleles. All but one of these alleles are classified as no function by CPIC—the one outlier allele, *SLCO1B1*40*, has uncertain function due to the presence of other variants with unknown functional impact (27, 30). The rs4149056 variant is found in 4 out of the 9 no-function alleles. The remaining 5 no-function alleles are defined by other SNVs or structural variants resulting in either a full or partial gene deletion (31).

Historic star allele designations have been reviewed and standardized by PharmVar, leading to some alleles being reclassified as sub-alleles based on shared core variants and biochemical functional status (27). The biochemical activity of a protein does not automatically translate to the same level of clinical activity; thus, in vitro pharmacokinetic/pharmacodynamic studies identifying hypo-active forms of OATP1B1 may ultimately result in a clinical phenotype no different than a complete loss-of-function allele (27).

An individual's *SLCO1B1* genotype (pair of inherited alleles) can predict their phenotype (Table 4) (32). Individuals with normal or increased function OATP1B1 transporter phenotype are at a lower risk of adverse drug reactions, while those with poor function or decreased function phenotypes are at higher risk of adverse reactions due to higher plasma concentrations of the substrate medication and its metabolites. An individual is assigned a phenotype of "possible decreased function" when one known no-function allele is present, and the second allele has unknown or uncertain function (2).

Table 4: Selected *SLCO1B1* Phenotype-Genotype Predictions (CPIC, 2022)

Clinical phenotype	Example *SLCO1B1* genotype	Clinical alert category[a]
Normal function	*1/*1 *1/*14 *1/*37	Low risk
Increased function	*14/*14 *14/*20 *20/*20	Low risk
Decreased function	*1/*5 *1/*9 *15/*37	High risk
Possible decreased function	*2/*5 *2/*15 *3/*9	High risk
Poor function	*5/*5 *5/*9 *9/*23 *15/*15	High risk

[a] These genotypes and phenotypes are recommended by CPIC to be flagged as low or high risk when implemented in electronic health records notifications. CPIC - Clinical Pharmacogenetics Implementation Consortium
This table is adapted from (32).

The frequency of *SLCO1B1* alleles can vary significantly based on population-specific genetic ancestry. However, allele frequencies cited reported in the literature may not fully represent allelic variation within a population. Owing to the recent reclassification of some historic star alleles and the underrepresentation of many populations in genetic and genomic research, these frequencies should be considered cautiously (27). For example, studies of populations living in the United Arab Emirates or Qatar examined only one or 2 SNVs and assigned the most common genotype based on those variants, excluding other star alleles (33, 34). Additional studies using current PharmVar allele definitions are needed across multiple populations to understand allele

and phenotype frequencies for *SLCO1B1*. The following information should be interpreted as approximate frequencies based on existing publications.

The *SLCO1B1*1* allele is clinically determined to be a normal-function allele, with a frequency of 50% or less for all CPIC biogeographical groups (35). However, the *SLCO1B1*37* allele—previously called *1B and also a normal-function allele—is the most frequently reported haplotype in African-American and Sub-Saharan-African populations, with a frequency of 76–80%, and 60% in East Asian populations (35).

Reduced- and increased-function alleles are less common globally. The no-function allele *SLCO1B1*15* is more common in many biogeographical groups compared with *SLCO1B1*5*. The combined frequency of both can range from 24% in American, 20% in Near Eastern, 17% in European, 12% in East Asian, 7% in Central/South Asian, 1–2% in African-American/Afro-Caribbean and Sub-Saharan populations, to not observed in Oceanian populations (35). The SNV that is shared between *SLCO1B1*5* and *SLCO1B1*15* was observed at a frequency of 24% in a Qatari population of mixed genetic ancestry, while the SNV associated with *SLCO1B1*37* was found in approximately 50% of study participants (33). Other no-function alleles are observed far less frequently, such as *SLCO1B1*9* due to variation at rs59502379, which has an allele frequency of 0–4.6% of the global populations in the Allele Frequency Aggregate (ALFA) data (36). The increased-function *SLCO1B1*14* allele has been reported almost exclusively in European genetic backgrounds at a frequency of 12% (35).

Variations in *SLCO1B1* causes altered transportation of endogenous substances such as bilirubin. The rare, inherited disorder Rotor syndrome is caused by bi-allelic loss of function variants in both *SLCO1B1* and *SLCO1B3*, which encodes another organic anion transporting polypeptide (37). Rotor syndrome presents with a benign form of hyperbilirubinemia and jaundice, clinically indistinguishable from Dubin-Johnson syndrome, though the elevated bilirubin seen in Rotor syndrome is a mix of both conjugated and unconjugated forms (38). Inherited in an autosomal recessive pattern, Rotor syndrome does not require therapy, but diagnosis is important to ensure other, more serious hepatobiliary disorders are not the cause of the jaundice (38).

Phenoconversion

Phenoconversion occurs when certain medications inhibit OATP1B1 activity, reducing transport of various substrates into hepatocytes and leading to a lower activity phenotype than predicted by genotype alone. Several drugs have been identified by in vitro assays to inhibit OATP1B1 including atorvastatin, cyclosporin, digoxin, gemfibrozil, ketoconazole, and rifampin among others (28). In vivo administration of cyclosporine with rosuvastatin or fluvastatin resulted in increased statin exposure, presumably due to decreased function of OATP and CYP3A4 (39, 40, 41). The US FDA's approved label for simvastatin states that concomitant use of simvastatin with cyclosporine, danazol, or gemfibrozil is contraindicated due to increased risk of myopathy (1).

Wojtyniak and colleagues have made available their drug–drug gene interaction data in the form of a clinical decision support tool at https://nemos.shinyapps.io/simvastatin_simulator/. However, the website states that "decisions on therapeutics and dosing recommendations should not exclusively be build[sic] based on the results of the simvastatin exposure simulator and do not replace clinical judgement" (42).

Linking *SLCO1B1* Genetic Variation with Treatment Response

The risk of SAMS for simvastatin correlates with *SLCO1B1* variants that reduce OATP1B1 function, thereby increasing an individual's exposure to simvastatin and its metabolites. Increased and normal function *SLCO1B1* haplotypes do not have a clinically significant impact on simvastatin metabolism or increase the risk of adverse effects above an individual's baseline risk (2). The variant at rs4149056 (c.521T>C; the defining SNV for *SLCO1B1*5* and also a part of *SLCO1B1*15*) was found to increase simvastatin acid exposure (as measured by total plasma concentration over time, or area under the curve) by approximately 40% in healthy volunteers, increasing the risk of SAMS (14). This variant also increased exposure to simvastatin acid in a pediatric population (43), though the CPIC found insufficient data to make pediatric-specific recommendations (2). A

pooled analysis of 11 studies on the association of rs4149056 variation and SAMS risk in Caucasians with the CC or TC genotype indicated a higher risk of myopathy, with the CC genotype carriers having a 2.81 odds ratio (OR) for myopathy compared with the wildtype (TT) genotype, and heterozygotes (CT genotype) having an OR of 1.78 (13). The pooled analysis of studies in Asian populations, though including only 2 publications, reported a 1.8 times higher risk of SAMS when the C allele was present (13). Increased plasma levels of simvastatin acid were also reported to be associated with *SLCO1B1* variants at rs11045819, (c.463C>A, which is the defining SNV for *SLCO1B1*4*) and rs34671512 (c.1929A>C, one of 2 SNVs in *SLCO1B1*20*) (12).

O'Brien and colleagues, in a retrospective analysis of more than 11,000 medical records over 5 years, found that, after correcting for covariates, individuals who self-reported as "Black/African-American" or "other/multiple race" were less likely to experience adverse reactions to simvastatin (44). This study also found that lower age and comorbid hypertension were significant covariates for increased probability of adverse reaction to simvastatin. However, the authors noted a relatively small number of adverse events and acknowledged the limitations off often incomplete electronic health record data (44). A study of nearly 300 individuals with FH found that variation at rs4149056 (c.521T>C) was not associated with SAMS; instead, increased age was the most significant risk factor (45). The US FDA's approved label also advises that advanced age (65 years and older) is a risk factor for SAMS (1).

Increased risk of developing type-2 diabetes is another side effect of statin use (46, 47, 48), and variation at rs4149056 has been associated with an increased risk, as indicated by elevated hemoglobin A1C levels (34). However, a large study of over 7,500 individuals (1,373 individuals treated with statins and 6,415 not treated with statins) found no association between rs4149056 T>C variation and incidence of diabetes or changes in blood glucose levels (15).

Studies examining the link between *SLCO1B1* genetic variation and the efficacy of statins reported that decreased OATP1B1 transport (most often studied in the context of rs4149056, c.521T>C genotype) correlates with an attenuated effect on the lipid-lowering capability of statins, including simvastatin. However, the effect is small (<10 mg/dl) and unlikely to impact the frequency of vascular events, leading CPIC to base their recommendations primarily on the risk of SAMS and pharmacokinetic data (see Supplement of (2)) rather than on efficacy concerns. Many studies reported a milder reduction in cholesterol in individuals with one or 2 copies of the variant rs4149056 c.521T>C allele, though it is unclear if this is due to medication nonadherence (specifically due to myopathy or other adverse effects), differences in genetic background, reduced OATP1B1 function, or a combination of these factors (49, 50, 51, 52, 53, 54, 55).

In contrast, no association was found between *SLCO1B1* variants at rs4149056 (c.521T>C), rs2306283 (c.388A>G), or rs4363657 (g.89595T>C) and lipid levels following simvastatin therapy in a cohort of nearly 400 Thai individuals with hypercholesterolemia (56). Similarly, a review of several studies in Brazil found no association between *SLCO1B1* variants (namely, the *SLCO1B1*5*, **15*, **4*, and **14* alleles) and efficacy of atorvastatin or simvastatin (57).

Additional Genes of Interest

Given the role of CYP3A enzymes in metabolizing simvastatin to simvastatin acid, it is not surprising that some studies have examined the association between *CYP3A4* variation and simvastatin pharmacokinetics. Like other cytochrome P450 family members, genetic variation at the *CYP3A* loci can result in decreased or no function of the encoded enzyme. While CPIC has not assigned a clinical function to *CYP3A4* haplotypes (58), the biochemical function has been used to predict metabolism phenotypes of intermediate or poor CYP3A4 metabolizers (12). One study reported a genome wide association with *CYP3A4*2* (rs55785340, c.664T>C) genotype, assigned as an intermediate metabolizer phenotype, and increased simvastatin acid exposure (12). Individuals heterozygous for the *CYP3A4*22* or *CYP3A5*3* alleles (classified as intermediate metabolizers) had significantly higher plasma simvastatin concentrations and lower total and plasma LDL cholesterol levels (59).

However, Kitzmiller and colleagues reported no association between *CYP3A4*22* (a decreased-function allele) or *CYP3A5*3* (clinically categorized by CPIC as a no-function allele (60)) and the cholesterol-lowering response to simvastatin (50).

Further research found a connection between statin response and variations in the hepatic efflux transporter *ABCB1* and a leukocyte immunoglobin receptor locus, *LILRB5* (61). The SNV at rs1045642 in the *ABCB1* gene, resulting in a synonymous protein change, was linked to a significant reduction in non-high-density lipoprotein (HDL) levels in a recessive inheritance model. The *LILRB5* variant rs12975366 showed effects in a dominant fashion, leading to a more pronounced decrease in non-HDL levels. These 2 loci appear to have a synergistic effect, although further studies are needed for confirmation.

An analysis of the UK Biobank data indicated a potential association between *NAT2* genetic variation and statin use. The *NAT2* locus encodes N-acetyltransferase 2 and has been liked to abnormal sensitivity to amifampridine. Wendt and colleagues reported an association between the *NAT2*5* allele and LDL cholesterol levels, with diplotypes including this allele being more common among statin users than non-users. They proposed a model where the NAT2 enzyme might acetylate LDL cholesterol, affecting its binding to LDL receptors and indirectly impacting statin efficacy (62).

An association has been observed between variants in HLA loci and IMNM in a Japanese cohort. Several variant alleles were found to be present at a higher rate in individuals with IMNM, although the study did not specifically assess statins as a trigger for IMNM. Candidate *HLA*-risk alleles for IMNM include *A*02:07, B*46, C*01:02, DRB1*08:03, DRB1*11:01, DQB1*06:01, DPB1*02:02, DPB1*05:01*. (20)

Genetic Testing

The NIH Genetic Testing Registry (GTR) offers tests for simvastatin response and *SLCO1B1* genetic variation. Considering the recent reclassification of *SLCO1B1* alleles by PharmVar, it is important to consider the specific testing methodology and review the genotype-phenotype assignments. Targeted SNV genotyping may only examine the most common functional variants without distinguishing between known haplotypes of differing functional status, such as *SLCO1B1*5* versus *SLCO1B1*40*. Gene sequencing may not detect structural variations or changes in copy number. Resources such as the Genotype Selection Interface (GSI) from PharmGKB, the Pharmacogenomics Clinical Annotation Tool (PharmCAT), and the PharmVar *SLCO1B1* allele definitions assist in report interpretation. The decision to test for *SLCO1B1* variation before or during standard -dose simvastatin therapy depends on the managing clinician. The FDA has no requirement to assay SLCO1B1 before therapy nor does CPIC issue guidance regarding testing. However, the DPWG recommends genotyping before starting an 80 mg/day dose of simvastatin for drug tolerance, and considers testing beneficial at a 40 mg/day dose, advising testing before or directly after initiating treatment (4). The data from PharmGKB for the Swissmedic drug labeling recommends *SLCO1B1* genotype testing with simvastatin therapy, both alone and with ezetimibe (11).

The clinical validity of *SLCO1B1* genotyping in predicting SAMS has been assessed. The SEARCH study, which focused on individuals with a history of myocardial infarction taking 80 mg simvastatin, found that individuals with at least one C allele at rs4149056, had a significantly increased risk of myopathy over 5 years, with an OR of 4.5 per C allele (63). An analysis of this study showed that at least one C allele found by genotyping has a positive predictive value (PPV) for myopathy risk of 4.1%, a negative predictive value (NPV) of 99.4%, a specificity of 73.7% and sensitivity of 70.4%; biallelic C genotype at this SNV has a PPV of 18.6%, NPV of 99.8%, specificity of 98.3%, and sensitivity of 25.1% (64). No additional risk loci for SAMS have been identified (65).

One study reported that utilization of *SLCO1B1* pharmacogenetic testing within a clinical decision support tool led to fewer prescriptions of medications that increase the risks of SAMS for individuals with *SLCO1B1* at-risk genotypes. This information is more likely to result in the cancellation of a simvastatin prescription if the genotype is known before prescribing rather than afterward (66). A similar study found that clinical testing and

reporting of the *SLCO1B1* genotype to guide simvastatin therapy resulted in noninferior outcomes for the genotype-guided group, with no instances of simvastatin prescribing for known OATP1B1 decreased or poor function phenotypes, suggesting that, given the information in advance, physicians avoid prescribing simvastatin to individuals with an at-risk genotype (67).

The *SLCO1B1* Gene Interactions with Medications Used for Additional Indications

The FDA has included the *SLCO1B1* gene on very few drug labels to date; including rosuvastatin (another statin), elagolix, and viloxazine (68). Elagolix is a gonadotropin-releasing hormone antagonist medication used to manage pain associated with endometriosis (69). Viloxazine is a selective norepinephrine reuptake inhibitor used for attention deficit hyperactivity disorder (70). Guidelines are available from CPIC on *SLCO1B1* and multiple statins,(2) and PharmGKB, CPIC, and the FDA also provide additional information on gene-drug interactions involving *SLCO1B1* (search for "SLCO1B1").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

Warnings and Precautions- Myopathy and Rhabdomyolysis

Simvastatin may cause myopathy and rhabdomyolysis… Risk factors for myopathy include age 65 years or greater, uncontrolled hypothyroidism, renal impairment, concomitant use with certain other drugs (including other lipid lowering therapies), and higher simvastatin dosage; Chinese patients on simvastatin may be at higher risk for myopathy…The risk of myopathy is increased by elevated plasma levels of simvastatin and simvastatin acid. The risk is also greater in patients taking an 80 mg daily dosage of simvastatin compared with patients taking lower simvastatin tablets dosages and compared with patients using other statins with similar or greater LDL-C lowering efficacy.

Steps to Prevent or Reduce the Risk of Myopathy and Rhabdomyolysis

The concomitant use of strong CYP3A4 inhibitors with simvastatin is contraindicated. If short-term treatment with strong CYP3A4 inhibitors is required, temporarily suspend simvastatin during the duration of strong CYP3A4 inhibitor treatment. The concomitant use of simvastatin with gemfibrozil, cyclosporine, or danazol is also contraindicated … Simvastatin dosage modifications are recommended for patients taking lomitapide, verapamil, diltiazem, dronedarone, amiodarone, amlodipine or ranolazine.

Please review the complete therapeutic recommendations that are located here: (1)

2022 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC):

Phenotype: SLCO1B1 decreased function or SLCO1B1 possible decreased function

[1] The FDA has distinct labels for specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Implications: Increased simvastatin acid exposure as compared with normal function; increased risk of myopathy.

Dosing recommendation: Prescribe an alternative statin depending on the desired potency (see Figure 1 for recommendations for alternative statins). If simvastatin therapy is warranted, limit dose to <20 mg/day.

Phenotype: SLCO1B1 poor function

Implications: Increased simvastatin acid exposure compared with normal and decreased function; highly increased myopathy risk.

Dosing recommendation: Prescribe an alternative statin depending on the desired potency (see Figure 1 for recommendations for alternative statins)

Please review the complete therapeutic recommendations that are located here: (2)

2020 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

SLCO1B1 521CC: [simvastatin]

When using simvastatin 80 mg/day, the risk of myopathy is increased 30-fold to 18% and the risk of severe myopathy is increased 48-fold to 12%. When using 40 mg/day, this risk is increased 7-fold to 1% and 11-fold to 0.68% respectively. The gene variation leads to reduced simvastatin transport to the liver, which increases the simvastatin plasma concentration and therefore the risk of side effects.

1. Choose an alternative

Consider any additional risk factors for statin-induced myopathy.

Atorvastatin is affected less severely by the SLCO1B1 gene variation, but is also affected by CYP3A4 inhibitors such as amiodarone, verapamil and diltiazem. Use of atorvastatin is not recommended for patients with additional risk factors for statin-induced myopathy.

Rosuvastatin and pravastatin are influenced to a lesser extent by the SLCO1B1 gene variation. They are also not influenced by CYP3A4 inhibitors such as amiodarone, verapamil and diltiazem.

Fluvastatin is not significantly influenced by the SLCO1B1 gene variation or CYP3A4 inhibitors.

SLCO1B1 521TC: [simvastatin]

When using simvastatin 80 mg/day, the risk of myopathy is increased 5-fold to 3% for moderately severe to severe myopathy and 1.3% for severe myopathy. When using 40 mg/day, this risk is increased 2.6-fold to 0.39% and 0.17% respectively. The gene variation may lead to reduced simvastatin transport to the liver, which may increase simvastatin plasma concentrations and therefore the risk of side effects.

1. Choose an alternative

Consider any additional risk factors for statin-induced myopathy.

Atorvastatin is affected less severely by the SLCO1B1 gene variation, but is also affected by CYP3A4 inhibitors such as amiodarone, verapamil and diltiazem. Use of atorvastatin is not recommended for patients with additional risk factors for statin-induced myopathy.

Rosuvastatin and pravastatin are influenced to a lesser extent by the SLCO1B1 gene variation. They are also not influenced by CYP3A4 inhibitors such as amiodarone, verapamil and diltiazem.

Fluvastatin is not significantly influenced by the SLCO1B1 gene variation or CYP3A4 inhibitors.

2. If an alternative is not an option:

1. Avoid simvastatin doses exceeding 40 mg/day (for example, by adding ezetimibe)[a]

2. Advise the patient to report muscle symptoms. [a]

Please review the complete therapeutic recommendations that are located here: (3) [a] Note that minor variations in wording versus the cited guidelines are included here based on personal communication from DPWG.

Nomenclature for Selected *SLCO1B1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*SLCO1B1*1*	SLCO1B1*1A	NM_006446.5	NP_006437.3	
*SLCO1B1*4*	35305C>A (P155T)	NM_006446.5:c.463C>A	NP_006437.3:p.Pro155Thr	rs11045819
*SLCO1B1*5*	37041T>C (V174A)	NM_006446.5:c.521T>C	NP_006437.3:p.Val174Ala	rs4149056
*SLCO1B1*9*	64425G>C (G488A)	NM_006446.5:c.1463G>C	NP_006437.3:p.Gly488Ala	rs59502379
*SLCO1B1*14*[a]	35230A>G (N130D)	NM_006446.5:c.388A>G	NP_006437.3:p.Asn130Asp	rs2306283
	35305C>A (P155T)	NM_006446.5:c.463C>A	NP_006437.3:p.Pro155Thr	rs11045819
*SLCO1B1*15*[b]	35230A>G (N130D)	NM_006446.5:c.388A>G	NP_006437.3:p.Asn130Asp	rs2306283
	37041T>C (V174A)	NM_006446.5:c.521T>C	NP_006437.3:p.Val174Ala	rs4149056
*SLCO1B1*20*[c]	35230A>G (N130D)	NM_006446.5:c.388A>G	NP_006437.3:p.Asn130Asp	rs2306283
	97468A>C (L643F)	NM_006446.5:c.1929A>C	NP_006437.3:p.Leu643Phe	rs34671512
*SLCO1B1*37*	SLCO1B1-*1B, *1F, *1G, *1H 35230A>G (N130D)	NM_006446.5:c.388A>G	NP_006437.3:p.Asn130Asp	rs2306283

[a] *SLCO1B1*14* represents a consolidated core haplotype encompassing the previously named *SLCO1B1*14* and *SLCO1B1*18* alleles.
[b] *SLCO1B1*15* represents a consolidated core haplotype encompassing the previously named *SLCO1B1*15A, SLCO1B1*15B,* and *SLCO1B1*17* alleles.
[c] *SLCO1B1*20* represents a consolidated core haplotype encompassing the previously named *SLCO1B1*20, SLCO1B1*21,* and *SLCO1B1*35* alleles.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (71).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature is from the Pharmacogene Variation (PharmVar) Consortium (27).

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Siponimod Therapy and *CYP2C9* Genotype

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Introduction

Siponimod (brand name Mayzent) is a sphingosine-1-phosphate (S1P) receptor modulator used in the treatment and management of relapsing forms of multiple sclerosis (MS) in adults. It works by targeting lymphocytes to decrease the number of circulating cells that are associated with MS symptomatic attacks and disease progression and may also have a direct neuroprotective impact. Siponimod strongly binds to the S1P type 1 and type 5 receptors that are abundantly expressed on lymphocytes and multiple other cell types in the central nervous system (CNS). Off-target interactions and effects on cardiac cells may occur, also. The use of a dose titration schedule is recommended to decrease the risk of bradycardia (see Table 1, Table 2) (1, 2). This medication is approved for multiple forms of relapsing MS (RMS) in the United States (1) and for active, secondary progressive disease in Europe and Canada (2, 3).

Siponimod is metabolized by members of the cytochrome P450 family, specifically CYP2C9 and, to a lesser extent CYP3A4. The *CYP2C9* gene is polymorphic and activity scores are used to categorize diplotype into phenotype. Decreased CYP2C9 metabolic activity is associated with increased exposure to siponimod and increased risk of adverse effects. Therefore, individuals with the *CYP2C9**3/*3 diplotype (activity score = 0) are contraindicated from taking siponimod (1, 2). Individuals with one copy of the no-function **3* allele (diplotype with activity scores of 0.5 or 1.0) are advised to take half the standard maintenance dose (1, 2). Consideration of genotype and activity score is essential for CYP2C9-based siponimod dosing because labeled dose recommendations are not categorized by phenotype. In the US, there is a modified titration schedule for individuals with a *CYP2C9**3 allele (Table 1)(1); however, the European prescribing guidelines do not modify the titration schedule for individuals with a single copy of the *CYP2C9*3* allele (heterozygous for *CYP2C9*3*) (Table 2) (2). The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy similarly recommends a 50% reduced maintenance dosage for intermediate metabolizers (IM) (Table 3) (4). It should be noted that dose recommendations in the Siponimod package label are limited to diplotypes consisting of only *CYP2C9 *1*,**2*, and **3* alleles due to lack of clinical data on the impact of other decreased or no-function alleles(1), while other medication and testing guidelines also consider**5, *6, *8,* and **11* (5, 6).

Table 1: The FDA Recommended Titration Schedule and Dosage based on *CYP2C9* Genotype (2023)

Titration day	Standard dose[a]	Reduced dose[b]
Day 1	0.25 mg	0.25 mg
Day 2	0.25 mg	0.25 mg
Day 3	0.5 mg	0.5 mg
Day 4	0.75 mg	0.75 mg
Day 5	1.25 mg	1 mg

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Table 1 continued from previous page.

Titration day	Standard dose[a]	Reduced dose[b]
Day 6 (maintenance)	2 mg	1 mg

[a] The standard dose of 2 mg maintenance dose and titration schedule is indicated for those with the following *CYP2C9* genotype: **1/*1*, **1/*2*, **2/*2*
[b] The reduced dose of 1 mg maintenance dose and modified titration schedule is indicated for those with the following *CYP2C9* genotype: **1/*3*, **2/*3*
This table is adapted from (1).

Table 2: The EMA Recommended Titration Schedule and Dosage based on *CYP2C9* Genotype (2023)

Titration day	Standard dose[a]	Reduced dose[b]
Day 1	0.25 mg	0.25 mg
Day 2	0.25 mg	0.25 mg
Day 3	0.5 mg	0.5 mg
Day 4	0.75 mg	0.75 mg
Day 5	1.25 mg	1.25 mg[c]
Day 6 (maintenance)	2 mg	1 mg[c]

[a] The standard dose is recommended for those individuals who do not have a *CYP2C9*2* or **3* allele.
[b] The reduced maintenance dose of 1 mg is recommended for individuals with *CYP2C9*1/*3* or **2/*3* genotype.
[c] Note that the EMA recommended titration schedule is the same regardless of *CYP2C9* genotype. The additional exposure of 0.25 mg on day 5 for CYP2C9 IM individuals does not compromise safety. EMA – European Medicines Agency, IM – intermediate metabolizers
This table is adapted from (2).

Table 3: The DPWG Recommended Dosage based on *CYP2C9* Genotype (2020)

CYP2C9 genotype	Recommended action	Rationale and risk summary
*CYP2C9*1/*3, CYP2C9*2/*3, or comparable genotype classified as IM*	Use 50% of the normal maintenance dose Reconsider the choice and potential benefit of siponimod if the individual is also using a moderate CYP3A4 inducer, such as modafinil.	Theoretically, the risk of adverse effects is increased, as the genetic variation results in higher plasma concentrations of siponimod. For the comparable genetic variation *1/*3, the moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model
*CYP2C9*3/*3* *or comparable genotype classified as PM*	Avoid siponimod	Siponimod is contraindicated in individuals with the comparable genetic variation *3/*3. Theoretically, the risk of adverse effects is greatly increased, as the genetic variation results in much higher plasma concentrations of siponimod
*CYP2C9*1/*2* *CYP2C9*2/*2*	No action is required for this drug-gene interaction	The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects

DPWG - The Dutch Pharmacogenetics Working Group, IM - Intermediate metabolizer, PM - Poor metabolizer
This table is adapted from (4).

Drug: Siponimod

Siponimod is a modulator of S1P receptors, specifically type 1 and type 5, used in the treatment of some forms of RMS. It is approved for use in the United States by the FDA, in the European Union by the European Medicines Agency (EMA), and in Canada by Health Canada. In the US, siponimod is authorized for all forms of RMS, including isolated syndrome, relapsing-remitting disease, and active secondary progressive MS (SPMS)(1). In contrast, the EMA and Health Canada have authorized it only for use in active SPMS (progressive MS with evidence of active disease based on relapses or imaging features of inflammatory activity) (2, 3). In all cases, the

medication is approved for adults and is contraindicated in individuals with the *CYP2C9*3/*3* genotype. (1, 2, 7) Due to its nonselective binding and adverse effects in cardiac cells, siponimod is also contraindicated in individuals with a recent history (previous 6 months) of myocardial infarction, unstable angina, stroke, transient ischemic attack, heart failure requiring hospitalization, or class III/IV heart failure (1). Cardiac conduction defects, such as Mobitz type II second- or third-degree atrioventricular (AV) block, or sick sinus syndrome without a functioning pacemaker, are also contraindications for siponimod therapy (1).

Siponimod is extensively absorbed (≥70%) with high oral bioavailability (84%), reaching maximum plasma concentration approximately 4 hours following dose administration (1). Following the prescribed dose titration period, siponimod usually reaches steady-state levels in the circulation after 6 days of the maintenance dosage. Although siponimod can cross the blood brain barrier, it is estimated that 68% of the medication remains in plasma (1). Siponimod is primarily metabolized via oxidation by the cytochrome P450 family members 2C9 (CYP2C9, accounting for 79% of the metabolism) and 3A4 (CYP3A4, accounting for 18.5%) (1, 8). The elimination half-life is 30 hours, and no unchanged siponimod is detected in urine, indicating it is fully metabolized before excretion. The main metabolites, M3 and M17, are not thought to contribute to the efficacy or safety of siponimod. Following discontinuation of therapy, complete elimination from the body may take up to 10 days, and the residual effect of reduced peripheral lymphocyte count may persist for 3 to 4 weeks. (1, 8, 9)

Differences in CYP2C9 activity, either due to genetic variation or inhibition from the dual CYP2C9/3A4 inhibitor fluconazole, are associated with reduced metabolism of siponimod (9) and increased overall exposure to the medication (1, 10). The most common CYP2C9 loss-of-function alleles are the *CYP2C9*3* no-function allele and the **2* reduced reduced-function allele. Individuals with 2 copies of the *CYP2C9*3* no-function allele are contraindicated for siponimod treatment, and those with reduced CYP2C9 enzyme activity due to a single **3* allele, specifically, the *CYP2C9*1/*3* or **2/*3* diplotype, are advised to take a lower daily maintenance dose (1 mg) compared to individuals without these genotypes (2 mg daily dose) (1). Although the FDA-approved label mentions other *CYP2C9* alleles with reduced or no functional activity that might have a similar effect on metabolism, there is inadequate evidence to justify a recommendation for altered dosing based on the presence of those alleles. In contrast, the DPWG guideline for siponimod recommends a similar dose adjustment for those with a genotype comparable to *CYP2C9*3* heterozygous individuals, and avoidance of siponimod for those with a genotype comparable to homozygous *CYP2C9*3*. (4)

Multiple sclerosis is an immune-mediated disorder of the CNS, characterized by neuroinflammation, focal demyelination, and axonal damage (11). It affects more than 2 million people worldwide, with approximately 85% of these individuals having the relapsing-remitting form of MS, which is characterized by periodic attacks followed by partial or complete remission, at disease onset (12). The relapsing-remitting phenotype is usually followed by a secondary progressive phase (SPMS) consisting of gradual disability accrual, with or without overlapping relapses (13). Typically, MS affects individuals between the ages of 20 to 40 and can lead to significant disability (14). The specific symptoms and severity of the disease vary depending on the site and severity of lesions in the CNS. Common symptoms include fatigue, spasticity, weakness, tremor, visual impairment, pain, motor paralysis, cognitive impairment, and bladder and bowel problems (12). The clinical presentation of MS forms a spectrum, ranging from less severe forms of radiologically or clinically isolated syndrome to more aggressive primary progressive MS in which there is little to no relapse from symptoms, with persistent disease progression (15). Both genetics and environmental exposures are believed to play a role in determining the risk of developing MS, similar to other autoimmune disorders (11).

Receptors for S1P are expressed by a variety of cell types including astrocytes, oligodendrocytes, erythrocytes, myocytes, lymphocytes, and cells of the eyes and spleen (16). Prolonged activation of S1P1 receptors on lymphocytes leads to their internalization and prevents cellular exit from lymph nodes. This reduces migration of these immune cells from the peripheral bloodstream into the CNS, preventing inflammatory relapses (17) . Siponimod can also readily cross the blood brain barrier. Preclinical and clinical data suggest that binding of

siponimod to S1P1 and S1P5 receptors in the CNS may also provide a direct protective and regenerative effect on neuronal cells, and this may be achieved independent of effects on peripheral lymphocyte counts (18, 19, 20).

Unlike a previous generation nonselective S1P modulator, fingolimod, siponimod targets only the type 1 and 5 receptors and does not require phosphorylation. This increased specificity reduces, but does not eliminate, the off-target adverse effects of siponimod on cardiac tissue (21). Specifically, siponimod can cause a transient and dose-dependent decrease in heart rate, which can be largely improved by following a specific dose titration schedule at the initiation of therapy (or following daily treatment interruption for more than 4 days) (22). Additionally, for individuals with preexisting cardiac conditions such as sinus bradycardia and first- or second-degree AV block, the drug labeling recommends a first dose monitoring period of 6 hours to address potential symptomatic bradycardia. (1, 2)

Compared to placebo, siponimod improves imaging-based metrics for disease progression (for example, lesion volume by MRI) and reduces the risk of confirmed disability progression, relapses, and the annual relapse rate (23). The benefits of siponimod are more substantial in individuals with active MS, as observed in the EXPAND extension study (24). Secondary analysis of the EXPAND trial reported a clinically meaningful improvement in cognitive function for individuals in the siponimod arm versus placebo (25). A systemic review of the BOLD (phase II) and core EXPAND (phase III) trials found low-certainty evidence supporting the benefit of 2 mg daily siponimod for the primary and secondary measures reported in those studies. However, the review called for additional trials to examine longer treatment duration for potential adverse effects and inclusion of other active controls(12). In contrast, a review of multiple disease-modifying therapies for RMS and active SPMS concluded that the EXPAND trial showed a significant reduction in disability progression and relapse rates (26). The neuroprotective effect of siponimod has led some groups to begin preclinical studies to assess its efficacy in autoimmune neuritis and optic nerve damage due to glaucoma (27, 28). Additional, ongoing clinical studies of siponimod can be found in the online database ClinicalTrials.gov, including a study for pediatric individuals (ages 10-17) with MS (NCT04926818) (29).

As siponimod specifically targets the immune system, it is essential to assess individuals before treatment for signs of active infection and to test for immunity to varicella zoster virus (either history of chicken pox or vaccination). Effective therapy with siponimod can lead to a 20–30% decrease in peripheral lymphocyte counts, increasing the individual's risk for infection. A recent (within prior 6 months) complete blood count should be taken before initiating treatment (1). Similarly, individuals without immunity to varicella zoster infection should complete a vaccination course 4 weeks before starting siponimod (1). Studies have found that individuals on a S1P receptor modulator therapy (including siponimod and fingolimod) also have impaired responses to vaccination to SARS-CoV-2 and may be at an increased risk of break-through infection following vaccination (30, 31, 32). Siponimod may have a limited effect on the immune response following other vaccinations such as influenza or pneumococcal polysaccharide (33).

Other side effects including macular edema, elevated liver enzymes, and hypertension were reported in a higher proportion of individuals on siponimod compared to placebo (23). Thus, the FDA recommends an ophthalmic evaluation, as well as measurement of transaminase and bilirubin levels before starting treatment (1). Treatment with S1P receptor modulating therapies is also associated with a higher risk of cutaneous malignancies, such as basal cell carcinoma. Therefore, individuals should be monitored for suspicious skin lesions before starting treatment and periodically during treatment with siponimod (1). The extension of the EXPAND trial observed an increase in basal cell carcinoma incidence with increased time on siponimod, specifically for 3–5 years, with an incidence rate of 0.9 per "100 patient-years" compared to 0.7 for the placebo arm (24).

The use of siponimod in pediatric individuals, nursing mothers, pregnant women, or individuals aged 65 and over has not been sufficiently studied to decide if this medication is safe or if modified administration would be needed for these specific groups. Safety and efficacy in a pediatric population have not been established. Animal studies have suggested that siponimod may be able to cause fetal harm when taken by a pregnant woman. There

is a pregnancy exposure registry available for enrollment: MotherToBaby (www.mothertobaby.org/join-study). Rat studies suggest siponimod, its metabolites, or both are detectable in milk (1). However, there are no data on the presence of siponimod in human breastmilk, its effects on breastfed infants, or the impact on milk production in lactating females. While it is unlikely that siponimod would be present in breast milk in significant amounts, it is potentially toxic to a breastfed infant. Based on the related drug fingolimod, experts recommend avoiding its use during breastfeeding (34). Individuals aged 65 or older should be managed with care due to the greater frequency of renal, cardiac, or hepatic dysfunction in this population. However, there is no specific recommendation in the drug labeling to suggest altered dosing for these individuals. (1)

Gene: *CYP2C9*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP450 genes are highly polymorphic, and genetic variants can lead to reduced, absent, or increased enzyme activity.

The CYP2C9 enzyme metabolizes approximately 15–20% of clinically used drugs, and atypical metabolic activity caused by genetic variants in the *CYP2C9* gene can play a significant role in adverse drug reactions (35, 36). Among CYP2C isoforms expressed in the liver, CYP2C9 is the most abundant (37, 38).

The *CYP2C9* gene is polymorphic, with more than 80 known alleles (39). The wild-type allele is designated *CYP2C9*1.* In a test of multiple CYP2C9 variants, when none of the tested variants are detected, the genotype is assigned as *CYP2C9*1,* and the gene is predicted to produce a fully functional enzyme with normal enzyme activity (40). Individuals who have 2 normal-function alleles, for example, *CYP2C9*1/*1*, are classified as "normal metabolizers" (Table 4). Each allele is assigned an activity score of 0 (no-function), 0.5 (reduced-function), or 1 (normal-function), and the combined score for both alleles is used to determine the phenotype. This approach was initially described for standardization of phenotype for the gene *CYP2D6* and is used for multiple CYP loci by the Clinical Pharmacogenetics Implementation Consortium (CPIC) (37, 41). A combination of alleles resulting in an activity score of 1.5 or 1 is classified as an IM phenotype, while a combined activity score of 0 or 0.5 is classified as a poor metabolizer (PM) phenotype (Table 4) (40). However, the effect of the reduced-function allele yielding a higher activity score than the no-function allele may result in different recommendations for IMs with activity scores of 1.5 and 1. For example, the CPIC guideline for the CYP2C9-phenytoin interaction recommends no adjustments are needed from typical dosing strategies for IMs with an activity score of 1.5, but recommends subsequent doses are reduced by 25% for IMs with an activity score of 1 (40).

Allelic variants associated with reduced, or no enzyme activity include *CYP2C9*2* (p.Arg144Cys), *CYP2C9*3* (p.Ile359Leu), *CYP2C9*5* (p.Asp360Glu), *CYP2C9*8* (p.Arg150His) and *CYP2C9*11* (p.Arg335Trp). The **2* reduced-function allele is more common in European (13%), Near Eastern (13%), and Central/South Asian (11%) populations than in Latino (8%), Oceanian (3%), East Asian (0.2%), or African (1–2%) descent populations. The **3* no-function allele is less common (<10% in most populations, except for Central/South Asian, at approximately 11%), and it is extremely rare in African populations. In African descent populations, the *CYP2C9*5* no-function (~1%), and **8* (6–8%), and **11* (1–3%) reduced-function alleles are also common. (42, 43, 44, 45)

Table 4. The CPIC Assignment of likely *CYP2C9* Phenotype based on Genotype

CYP2C9 phenotype[a,b]	Genotype	Activity score	Examples of diplotype
Normal metabolizer	An individual with 2 normal-function alleles	2	*1/*1, *1/*9

Table 4. continued from previous page.

CYP2C9 phenotype[a,b]	Genotype	Activity score	Examples of diplotype
Intermediate metabolizer	An individual with one normal-function allele plus one decreased-function allele OR one normal-function allele plus one no-function allele OR 2 decreased-function alleles	1.5	*1/*2, *1/*8
		1	*1/*3, *2/*2, *8/*11
Poor metabolizer	An individual with one no-function allele plus one decreased-function allele; OR 2 no-function alleles	0.5	*2/*3, *2/*6
		0	*3/*3, *3/*6, *3/*5

[a] Assignment of allele function and associated citations can be found at the CPIC website, also see (46)
[b] See the *CYP2C9* Frequency Table (45) for population-specific allele and phenotype frequencies.
Note: There are no known cases of CYP2C9 ultrarapid metabolizers. CPIC - Clinical Pharmacogenetics Implementation Consortium
This CPIC table has been adapted from (5)

Phenoconversion

Medications can alter CYP2C9 enzymatic activity, resulting in conversion to a different metabolizer phenotype than would be predicted from an individuals' genotype. This phenomenon is referred to as phenoconversion. Any medication that is a substrate for CYP2C9 may compete with other concomitant CYP2C9 substrate medications, leading to a reduced effective enzyme activity level for all CYP2C9-metabolized medications. Other non-substrate medications that inhibit the CYP2C9 enzyme can alter the pharmacokinetic parameters of any drug that is a substrate of CYP2C9 through mechanisms other than competition for metabolism. An individual who is predicted from their genotype to be an IM and is taking more than one CYP2C9 substrate or inhibitor, such as amiodarone, fluconazole, metronidazole, sulfaphenazole, or voriconazole (37, 38), may present phenotypically like a PM. Conversely, CYP2C9 can be induced by other medications, leading to increased enzyme activity compared to the activity predicted by the genotype. Administration of rifampin and carbamazepine can induce CYP2C9 and the related enzyme CYP3A4 (1). Additional CYP2C9 inducers include nifedipine, hyperforin (found in St. John's wort herbal supplement), phenobarbital, phenytoin, dicloxacillin, flucloxacillin, and tamoxifen (37). If an individual who is predicted to be an IM is taking a CYP2C9 substrate and an inducer, the individual may present phenotypically more like a normal metabolizer because the increased activity produced by the inducer may counteract some of the decreased activity produced by the reduced-function allele.

Linking *CYP2C9* Genetic Variation with Treatment Response

The manufacturer provided data for EMA and FDA authorization indicates that individuals with *CYP2C9*3/*3* genotype (activity score 0) should not take siponimod due to substantially elevated plasma levels of the medication, increased by 285% compared to *CYP2C9*1/*1* exposure (1, 2). The DPWG also recommends avoiding siponimod in individuals with *CYP2C9*3/*3* genotype (4). However, this contraindication is not extended to other genotypes with a PM phenotype, as the total exposure of *CYP2C9*2/*3* (activity score 0.5) was only 91% higher (1). Citing a lack of experimental or clinical data on the impact of other no-function alleles (including alleles like *CYP2C9*6*), the FDA-approved label states it is likely that other no-function alleles "will have similar effects on siponimod pharmacokinetics" though clinical guidance is not provided(1).

Individuals with the reduced-function genotype of *CYP2C9*1/*3* (activity score of 1) or *CYP2C9*2/*3* (activity score of 0.5) are recommended to take half of the standard maintenance dose, as stated by the FDA, EMA drug labels, and the DPWG (1, 2, 4). Again, other alleles that confer an IM phenotype are not specifically addressed by the FDA drug labeling, and the reduced dosage recommendation is specific to these genotypes. It should be noted that the versions of the drug label approved in the US (1) and Canada specifically recommend that individuals with *CYP2C9*2/*2* genotype be given the standard dose (2 mg daily maintenance dose).The

European approved version of the drug label does not explicitly address dosing for individuals with *CYP2C9*2/*2* genotype (2).

Currently, the dose recommended for *CYP2C9*3* heterozygotes cannot be extrapolated to all IM-associated genotypes. Much of the available literature for siponimod pharmacokinetics was published before the use of activity scoring and phenotype assignments were standardized (5). *In vitro* metabolism studies with human liver microsomes reported a 2.7-fold decrease in siponimod metabolism in samples with *CYP2C9*2/*2* genotype and simulations of plasma concentration of siponimod were elevated in both the **2/*2* and **3/*3* genotype (9). However, *in vivo* studies described in the drug label show a 25% increase in plasma concentration (measured as area under the curve) with the *CYP2C9*2/*2* genotype compared to **1/*1* (1).The clearance of siponimod reported for *CYP2C9*2/*2* was higher than *CYP2C9*1/*3*, and the *CYP2C9*2/*3* clearance was well above *CYP2C9*3/*3*(47). The difference in *in vitro* and *in vivo* metabolism of siponimod raises a question as to whether the *CYP2C9*2* variant may not confer as significant of a decrease in enzyme activity for siponimod as compared to other CYP2C9 substrates. Contrary to other medications, siponimod pharmacogenetic dosing recommendations are not made based on metabolizer phenotype but rather specific allele identification based on known effects of those alleles.

This model for pharmacogenetic recommendations highlights the need for more studies to assess the impact of CYP2C9 alleles found in populations with non-European genetic ancestry. Some authors have observed that the incidence of MS is higher in blacks (racial assignment extracted from health and military records, most likely reflecting self-reported identification (48, 49); incidence rates averaged to 10.2 per 100,000 as compared to 6.9 in whites, 2.9 in Hispanics and 1.4 in Asians) and the EXPAND and BOLD clinical trials for siponimod did not report race or ethnicity in their studies (50). The FDA review documentation for siponimod approval indicated that approximately 95% of the participants in EXPAND (also referred to as CBAF312A2304) were white (51). Additional decreased or no-function *CYP2C9* alleles (for example, **5, *6, *8* and **11)* are highly relevant across a broad range of racial and ethnic backgrounds, indicating the need for additional analysis and consideration for dosing adjustment (50). Based on altered pharmacokinetics of phenytoin and warfarin in individuals with a *CYP2C9*11* allele, analysis of this allele is highly recommended before prescribing medications like siponimod, especially due to their narrow therapeutic window and CYP2C9 substrate status (52).

Due to the more rapid metabolism of siponimod, co-medication with drugs that strongly induce CYP2C9 and CYP3A4 is not recommended in all individuals. Specifically, co-medication with moderate or strong CYP3A4 inducers such as modafinil or efavirenz, is not recommended in individuals with *CYP2C9*1/*3* or **2/*3* IM genotype. Conversely, co-medication with drugs that inhibit CYP2C9 and CYP3A4, such as fluconazole is also not recommended for all individuals due to the risk of increased exposure and potential adverse effects. (1)

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests available for siponimod drug response and the *CYP2C9* gene.

For *CYP2C9*, the Association for Molecular Pathology has recommended alleles that should be included in clinical genotyping assays (6). Testing results are typically reported as a diplotype, such as *CYP2C9*1/*2,* and may include an interpretation of the individual's predicted metabolizer phenotype (normal, intermediate, or poor) and an activity score (Table 5). Testing laboratories will report an allele assignment of *CYP2C9*1/*1* if no tested variant is detected in the sample. However, if an allele is not covered by the test, the *1/*1 diplotype does not mean that the individual does not have that variant; it only indicates the person does not have any of the variants included in the test. Therefore, reviewing the testing methods and alleles covered by the specific test used is crucial to understanding what a **1/*1* report truly means for the individual tested.

The *CYP2C9* Gene Interactions with Medications Used for Additional Indications

The CYP2C9 enzyme is involved in the metabolism of a wide range of medications, making genotyping results informative for many drugs. Medications that may be affected by *CYP2C9* genetic variation include:

- NSAIDs like celecoxib, flurbiprofen, piroxicam, all of which are metabolized by CYP2C9; thus individuals with reduced enzyme activity will experience increased exposure and have a higher risk of side effects.
- Cannabinoids like dronabinol, a synthetic form of delta-9-tetrahydrocannabinol, are metabolized by CYP2C9 and decreased enzyme activity increases an individual's exposure to the active compound
- Anti-convulsant medications like phenytoin, which are metabolized by CYP2C9, and a loss of function in this enzyme increases exposure to the medication and the risk of adverse effects.
- Uric acid lowering medications like lesinurad; individuals who lack CYP2C9 activity have an increased exposure to lesinurad, and an increased risk of side effects.
- Anticoagulants like warfarin, individuals with reduced or no function *CYP2C9* alleles require lower doses of this medication.

Additional information on gene-drug interactions for *CYP2C9* are available from PharmGKB, CPIC and the FDA (search for "CYP2C9").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2023 Statement from the US Food and Drug Administration (FDA):

CYP2C9 Genotype Determination

Test patients for CYP2C9 variants to determine CYP2C9 genotype.

[…]

Recommended Dosage in Patients With CYP2C9 Genotypes *1/*1, *1/*2, or *2/*2

Maintenance Dosage

After treatment titration (see Treatment Initiation), the recommended maintenance dosage of [siponimod] is 2 mg taken orally once daily starting on Day 6.

[…]

Recommended Dosage in Patients With CYP2C9 Genotypes *1/*3 or *2/*3

Maintenance Dosage

In patients with a CYP2C9*1/*3 or *2/*3 genotype, after treatment titration (*see Treatment Initiation*), the recommended maintenance dosage of [siponimod] is 1 mg taken

orally once daily starting on Day 5.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Administer tablets whole; do not split, crush, or chew [siponimod] tablets.

Treatment Initiation

Initiate [siponimod] with a 4-day titration, as shown in Table 2... A 7-tablet starter pack should be used for patients who will be titrated to the 1-mg maintenance dosage.

[…]

[Siponimod] is contraindicated in patients who have:

A CYP2C9*3/*3 genotype

Please review the complete therapeutic recommendations that are located here: (1).

2023 Statement from the European Medicines Agency (EMA):

Before initiation of treatment, patients must be genotyped for CYP2C9 to determine their CYP2C9 metaboliser status …

In patients with a CYP2C9*3*3 genotype, siponimod should not be used …

Treatment initiation

Treatment has to be started with a titration pack that lasts for 5 days. Treatment starts with 0.25 mg once daily on days 1 and 2, followed by once-daily doses of 0.5 mg on day 3, 0.75 mg on day 4, and 1.25 mg on day 5, to reach the patient's prescribed maintenance dose of siponimod starting on day 6 (see Table 1).

[…]

Treatment maintenance

In patients with a CYP2C9*2*3 or *1*3 genotype, the recommended maintenance dose is 1 mg …

The recommended maintenance dose of siponimod in all other CYP2C9 genotype patients is 2 mg.

Please review the complete therapeutic recommendations that are located here: (2).

2023 Statement from Health Canada:

Contraindications:

Siponimod is contraindicated in: […]

• Patients with a CYP2C9*3*3 genotype

[…]

Dosing Considerations:

Prior to initiating treatment with MAYZENT the following assessments should be done to guide patient selection and treatment:

CYP2C9 genotype

The CYP2C9 genotype has a significant impact on siponimod metabolism.

• Determine the CYP2C9 genotype of the patient to establish CYP2C9 metabolizer status. CYP2C9 genotyping prior to initiating treatment with siponimod will be offered by the manufacturer through its Patient Support Program.

• MAYZENT is contraindicated in patients with a CYP2C9*3*3 genotype […]

• Dose adjustments are recommended for patients with CYP2C9*1*3 or a CYP2C9*2*3 genotype

[…]

Recommended Dose and Dosage Adjustment

Treatment has to be initiated in all patients with a starter pack that lasts for 5 days […] The dose titration starts with 0.25 mg once daily on day 1 and day 2, followed by once daily doses of 0.5 mg on day 3 (two tablets of 0.25 mg), 0.75 mg on day 4 (three tablets of 0.25 mg), and 1.25 mg on day 5 (five tablets of 0.25 mg), to reach the maintenance dose of 2 mg [siponimod] starting on day 6.

CYP2C9 Genotypes

In patients with a CYP2C9*2*3 or *1*3 genotype, the same starter pack should be used and treatment should be initiated as described above (see Table 1). On Day 6 the maintenance dose should be adjusted to 1 mg

Please review the complete therapeutic recommendations that are located here: (7)

2020 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP):

CYP2C9 IM ANDERS[other]: siponimod

Theoretically, the risk of adverse effects in increased, as the genetic variation results in higher plasma concentrations of siponimod.

- use 50% of the normal maintenance dose - reconsider the choice and the potential benefit of siponimod if the patient is also using a moderate CYP3A4 inducer, such as modafinil

For the comparable genetic variation *1/*3, the moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model.

CYP2C9 PM ANDERS[other]: siponimod

Siponimod is contraindicated in patients with the comparable genetic variation *3/*3. Theoretically, the risk of adverse effects is greatly increased, as the genetic variation results in much higher plasma concentrations of siponimod.

- avoid siponimod

CYP2C9*1/*2: siponimod

NO action is required for this gene-drug interaction.

The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects.

CYP2C9*2/*2: siponimod

NO action is required for this gene-drug interaction.

The genetic variation can slightly increase the exposure to siponimod. However, the effect is too small to expect any impact on efficacy or adverse effects.

CYP2C9*2/*3: siponimod

Theoretically, the risk of adverse effects in increased, as the genetic variation results in higher plasma concentrations of siponimod.

- use 50% of the normal maintenance dose - reconsider the choice and the potential benefit of siponimod if the patient is also using a moderate CYP3A4 inducer, such as modafinil

For this genetic variation, a moderate CYP3A4 inducer results in a reduction in the exposure of siponimod by 49%, according to a pharmacokinetic model.

Please review the complete therapeutic recommendations that are located here: (4)

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.4:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.4:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.4:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.4:c.818del	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.4:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*9*	752A>G His251Arg	NM_000771.4:c.752A>G	NP_000762.2:p.His251Arg	rs2256871
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.4:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (53).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Sofosbuvir Therapy and *IFNL4* Genotype

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Created: January 25, 2017.

Introduction

Sofosbuvir is an antiviral agent used in the treatment of chronic hepatitis C virus (HCV) infection. Sofosbuvir is FDA-approved to treat patients infected with HCV genotypes 1, 2, 3, and 4, as part of a combination antiviral treatment regimen (1). HCV genotype 1 is the most prevalent worldwide and HCV genotype 3 is the next most prevalent (2). Sofosbuvir may also be used as part of the treatment regimen of HCV genotypes 5 or 6 (3).

About 180 million people worldwide are infected with chronic hepatitis C, which is a major cause of chronic liver disease, cirrhosis, and liver cancer. Viral eradication is suboptimal with peginterferon plus ribavirin-based therapy, with only about half of patients with HCV genotype 1 infection achieving a sustained virological response (SVR) after 24 weeks (4). A SVR is defined as undetectable HCV RNA by the end of treatment or at a specific number of weeks after the initiation of treatment, e.g., undetectable HCV RNA at 12 weeks is annotated (SVR12).

Direct-acting antivirals (DAAs), such as sofosbuvir, were developed to improve viral eradication rates. They target HCV-encoded proteins involved in viral replication and infection. Sofosbuvir, the first and thus far only DAA, targets NS5B polymerase, the viral enzyme required for HCV RNA replication.

Sofosbuvir may be used in combination with peginterferon. The genetic variant rs12979860, located in the *INFL4* gene, is a strong predictor of response to peginterferon-based therapies. The variant is a C to T change—individuals with the favorable "C/C" genotype have about a 2-fold higher likelihood of achieving SVR compared to individuals with CT or TT genotypes (5). (Note, because the association of rs12979860 with treatment response was reported several years before the discovery of *IFNL4*, the variant is commonly, but mistakenly, referred to as *IL28B*, which is the previous name for the *IFNL3* gene.)

For specific treatment regimens that include sofosbuvir, although the *IFNL4* variant still influences treatment outcomes, the SVR remains relatively high for all *IFNL4* genotypes. For example in the NEUTRINO study, which is referred to in the FDA-approved drug label for sofosbuvir, the SVR12 rate was 99% in individuals with baseline C/C alleles and 87% in individuals with baseline non-C/C alleles. The individuals in this study had HCV genotype 1 or 4 infection, and were receiving sofosbuvir plus peginterferon plus ribavirin therapy (1, 6).

The drug label for sofosbuvir also discusses viral resistance. In cell culture, the amino acid substitution S282T in the viral NS5B polymerase is associated with reduced susceptibility to sofosbuvir (7). During the ELECTRON trial, this substitution was transiently detected in one individual who relapsed during sofosbuvir monotherapy. However, the clinical significance of such substitutions remains unknown (1).

Drug Class: Direct Acting Antivirals for HCV

The treatment of hepatitis C virus (HCV) has evolved over the years. Initially, interferon (IFN) was used as monotherapy. This was followed by the addition of the antiviral agent ribavirin (a nucleoside analogue) to peginterferon. However, only about half of the HCV genotype 1-infected patients cleared their infection, and adverse effects were common and sometimes life-threatening (4). Treatment was also expensive and inconvenient, lasting up to 48 weeks.

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Direct-acting antivirals (DDAs) improved the effectiveness of peginterferon and ribavirin therapy. These agents target specific viral proteins required for viral replication and infection.

HCV is a single-stranded RNA virus that encodes structural proteins (to encode the viral capsid and envelope) and non-structural proteins (required for viral replication). The DDAs target several of the non-structural proteins, the viral protease (NS3/NS4A), the viral RNA polymerase (NS5B), and a viral protein thought to regulate replication and viral assembly (NS5A).

Currently, there are four classes of drugs in clinical use or in development, which are classified by their therapeutic target:

- Protease inhibitors e.g., simeprevir, grazoprevir, paritaprevir
- Nucleoside polymerase inhibitors e.g., sofosbuvir
- Non-nucleoside polymerase inhibitors
- NS5A inhibitors e.g., ledipasvir

Successful treatment of hepatitis C is confirmed when no trace of HCV can be found after treatment has finished. This is referred to as the SVR, which is defined as undetectable HCV RNA by a quantification assay at the end of treatment, and typically 12 (SVR12) or 24 weeks (SVR24) after the end of treatment.

Drug: Sofosbuvir

Sofosbuvir is a nucleotide analogue used in the treatment of chronic HCV infection as part of a combination antiviral treatment regimen.

The early stages of infection with HCV are usually asymptomatic—about 15-45% of people spontaneously clear the virus within 6 months of infection without any treatment. The remaining 55-85% of people will develop chronic HCV infection, which may also be asymptomatic for many years (8).

However, during the natural course of HCV infection, patients develop liver fibrosis, which, without treatment, can progress to liver cirrhosis and liver cancer (hepatocellular carcinoma). The risk of developing liver cancer for a patient with HCV-related cirrhosis is approximately 2-6% per year (9).

HCV is classified by genotype, based on the nucleotide sequence of the viral RNA. There are six major classes of genotype, numbered 1-6, with multiple subtypes e.g., 1a, 1b, 2a, 2b. In the US, approximately 70% of people with HCV infection have genotype 1, with genotype 1a more common than 1b (8). Genotype 1 was formerly the most difficult to cure with interferon-based therapies, as it was less likely than genotypes 2 and 3 to respond to therapy. With the introduction of DAA-based, interferon-free treatments, this is no longer the case.

Sofosbuvir is indicated for the treatment of genotype 1, 2, 3 or 4 chronic HCV infection and is generally considered to have moderate to high efficacy for all six genotypes (10). For the treatment of genotype 1 or 4 infections, the drug label recommends a combination therapy of sofosbuvir plus peginterferon alfa plus ribavirin. For the treatment of genotype 2 or 3 infections, the combination therapy of sofosbuvir plus ribavirin is recommended (1).

Sofosbuvir is a NS5B nucleotide analogue and a prodrug. Once inside a liver cell, sofosbuvir is activated by phosphorylation to a nucleoside triphosphate that competes with nucleotides during viral replication. Binding of the analogue to the viral NS5B polymerase results in RNA chain termination, thus inhibiting the virus from replicating its genome (11).

The safety and efficacy of sofosbuvir has been established in several clinical trials. The usual dose of sofosbuvir is a 400mg tablet, taken once a day for 12 weeks, in combination with other antiviral agents. Sofosbuvir is generally well tolerated, with no side effects beyond those associated with placebo therapy (10, 12).

Sofosbuvir forms the backbone of a several treatment regimens including DAA such as sofosbuvir/velpatasvir and sofosbuvir/velpatasvir/voxilaprevir. The regimen sofosbuvir/ ledipasvir has been found to result in high SVR rates in shorter periods of time, but costs may be prohibitive (7).

Genetic variants in the *IFNL4* gene have been shown to strongly influence treatment response to peginterferon-based regimens in previously untreated patients with HCV genotype 1 infection (5, 13). Such variants also appear to influence the outcomes of treatment regimens that include sofosbuvir. For example, the rs12979860 genotype predicts the response to 8 weeks of treatment with sofosbuvir/ledipasvir (14).

In addition, several substitutions that occur with the viral NS5B polymerase have been reported. Most notably, a S282T polymorphism has been associated with sofosbuvir resistance (15). In cell cultures, the S282T substitution is associated with a reduced susceptibility to sofosbuvir. However, the clinical significance of such substitutions is not yet known, as they appear to be detrimental to viral fitness. So far, the S282T substitution has only been detected in one patient who experienced a relapse while being treated with sofosbuvir monotherapy in a trial, and the substitution was no longer detectable at week 12 post-treatment (1).

Gene: *IFNL4*

The *IFNL4* gene encodes interferon lambda-4 (IFN-λ4) and is involved in the immune response to hepatitis C.

When a person is infected by viruses, including HCV, their immune response includes the production of interferons. These signaling proteins induce changes in infected and uninfected cells to block the viral replication cycle and stop the spread of virus. Interferons are given as part of treatment for HCV to strengthen this innate response.

Three classes of IFNs exist: type I (IFN-α/β), type II (IFN-γ), and type III (IFN-λ). The most recent interferon to be discovered, *IFNL4*, belongs to the type III class. It is located upstream of *IFNL3* and is a functional gene in the majority (>95%) of the African population. But in about 50% of the European population and in most of the east Asian population, IFNL4 is a pseudogene, created by a frameshift-causing deletion polymorphism (rs368234815) (16-18).

As a type III interferon, IFNL4, induces an antiviral state in responsive cells with a higher risk of viral infection, such as mucosal cells (17). IFNL4 exerts its actions by interacting with a cytokine receptor complex, which is composed of the IL10RB and IFNLR1 receptor chains (5). Expression of IFNLR1 is largely restricted to cells of epithelial origin, which includes hepatocytes. In contrast, receptors for type I interferons, such as IFN-α, are expressed in most cell types.

The first two variants to be commonly tested for are rs12979860 (located in *IFNL4*) and rs8099917, which lies proximate to *IFNL4*. These variants are in close proximity to each other and are in strong linkage disequilibrium (5). Linkage disequilibrium means that the variants are linked to treatment response more than would be expected in the general population.

HCV genotype 1 patients with the "favorable" genotypes (CC for rs12979860 and TT for rs8099917) respond better to interferon-based treatment—favorable genotypes are associated with an approximate 2-fold increase in SVR (5). However, for specific treatment regimens which include sofosbuvir, although an individual's *IFNL4* genotype still influences treatment outcomes, the SVR for non-favorable genotypes remains relatively high (1).

In the NETURINO study, patients with HCV genotype 1 or 4 who had not received previous treatments for HCV infection were treated with a regimen of sofosbuvir plus peginterferon plus ribavirin for 12 weeks. The SVR12 rate was 99% (89/90) in subjects with baseline rs12979860 C/C alleles and 87% (200/230) in subjects with baseline rs12979860 non-C/C alleles (6).

Similarly, in the PHOTON trial, patients with HCV genotype 1 infection and co-infection with HIV were treated with a combination of sofosbuvir and ribavarin. The SVR12 rates were 80% (24/30) in subjects with baseline rs12979860 C/C allele and 75% (62/83) in subjects with baseline rs12979860 non-C/C alleles (1).

The frequency of the rs12979860 'C' allele varies globally across different populations—it is commonly found in East Asians (allele frequency nearly 0.9), followed by Caucasians (0.63) and Hispanics (0.55), and is the least common among individuals of African origin (0.39) (5).

In individuals of African ancestry, the rs368234815 variant is superior to rs12979860, and together with another *IFNL4* variant (rs117648444), the combination of testing these two variants gives a greater treatment response prediction compared to testing for single variants (16, 17).

Genetic Testing

Genetic testing for *IFNL4* is used to predict response to peginterferon and ribavarin in HCV genotype 1 patients. The results can help clinicians and patients make informed decisions on how to manage HCV infection.

The rs12979860 variant is most commonly tested, and the results are typically reported in the following format:

rs12979860 CC, favorable genotype

rs12979860 CT, unfavorable genotype

rs12979860 TT, unfavorable genotype (5).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): NEUTRINO was an open-label, single-arm trial that evaluated 12 weeks of treatment with sofosbuvir in combination with peginterferon alfa 2a and ribavirin in treatment-naïve subjects with genotype 1, 4, 5 or 6 HCV infection compared to pre-specified historical control. [...] SVR12 rates were 99% (89/90) in subjects with genotype 1 or 4 HCV and baseline IL28B C/C allele and 87% (200/230) in subjects with genotype 1 or 4 HCV and baseline IL28B non-C/C alleles[2].

It is estimated that the SVR12 in patients who previously failed pegylated interferon and ribavirin therapy will approximate the observed SVR12 in NEUTRINO subjects with multiple baseline factors traditionally associated with a lower response to interferon-based treatment. The SVR12 rate in the NEUTRINO trial in genotype 1 subjects with IL28B non-C/C alleles, HCV RNA greater than 800,000 IU/mL and Metavir F3/F4 fibrosis was 71% (37/52).

[…]

In a pooled analysis of 982 subjects who received sofosbuvir in Phase 3 trials, 224 subjects had post- baseline NS5B genotypic data from next generation nucleotide sequencing (assay cutoff of 1%).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

[2] Note: Recent studies report that the rs12979860 variant is in the IFNL4 gene, and not the IFNL3 gene (previously called IL28B). Therefore, a more accurate term for describing an individual's genotype would be "rs12979860 C/C", instead of "IL28B C/C".

Treatment-emergent substitutions L159F (n=6) and V321A (n=5) were detected in post-baseline samples from GT3a-infected subjects across the Phase 3 trials. No detectable shift in the phenotypic susceptibility to sofosbuvir of subject isolates with L159F or V321A substitutions was seen. The sofosbuvir-associated resistance substitution S282T was not detected at baseline or in the failure isolates from Phase 3 trials. However, an S282T substitution was detected in one genotype 2b subject who relapsed at Week 4 post-treatment after 12 weeks of sofosbuvir monotherapy in the Phase 2 trial P7977-0523 [ELECTRON]. The isolate from this subject displayed a mean 13.5-fold reduced susceptibility to sofosbuvir. For this subject, the S282T substitution was no longer detectable at Week 12 post-treatment by next generation sequencing with an assay cutoff of 1%.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
rs12979860	/	NM_001276254.2:c.151-152G>A	N/A	rs12979860
rs8099917	/	N/A	N/A	rs8099917

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Tafenoquine Therapy and *G6PD* Genotype

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Introduction

Tafenoquine is an antimalarial agent that was approved by the FDA in 2018 for (1) preventing malaria (brand name Arakoda, 100 mg tablets), and for (2) the radical cure of malaria (brand name Krintafel, 150 mg tablets) caused by Plasmodium vivax (P. vivax) (1, 2).

Malaria is caused by the Plasmodium parasite, which infects mosquitos and is spread to humans when an infected mosquito bites a person. In 2018 the World Health Organization (WHO) estimated 228 million cases of malaria occurred worldwide (3).

There are several clinical patterns of malaria that are caused by different species of the parasite. In P. vivax malaria, the parasite can lie dormant in the liver as hypnozoites, until it emerges weeks or months later, to cause a relapse of malaria. In combination with an antimalarial active against the blood stage parasites, tafenoquine provides a radical cure of P. vivax by targeting its dormant liver stage, thus preventing malaria relapse.

Tafenoquine is the second drug of its kind (with hypnozoiticidal activity) to be approved by the FDA. The first was primaquine, approved in 1952. Because of its longer half-life, tafenoquine can be dosed less frequently than primaquine, which may improve compliance. For example, when used for the radical cure of P. vivax malaria, tafenoquine is taken as a single 300 mg dose (in uncomplicated cases, in persons aged 16 years and older). In contrast, primaquine radical cure is recommended to be given daily over 14 days (4), or higher doses over 7 days (5).

Tafenoquine, like primaquine, should not be used in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In the case of tafenoquine, an individual with <70% of normal G6PD activity is considered deficient and should not take the drug (6).Worldwide, approximately 400 million people have a deficiency of the G6PD enzyme, but most are asymptomatic and do not know they are at risk (7). A lack of G6PD in red blood cells makes the cells susceptible to damage by oxidative stress. Usually, only low levels of oxidative stress occur naturally, and so the condition is undetected.

However, certain drugs, which include tafenoquine and primaquine, are oxidizing agents. In people with G6PD deficiency, these drugs cause irreparable oxidative damage to the red blood cells, which are then rapidly destroyed (hemolysis). This can lead to a potentially life-threatening deficiency of mature red blood cells (hemolytic anemia).

The FDA-approved drug label for tafenoquine states that testing for G6PD must be performed before starting tafenoquine therapy, and that all individuals should be monitored for signs of hemolysis (Table 1). In addition, because of the risk of tafenoquine causing fetal harm in a woman pregnant with a fetus with G6PD deficiency, pregnancy testing is highly recommended in women of reproductive age. Consequently, tafenoquine therapy is contraindicated in adults when the G6PD status is either unknown, intermediate or deficient, namely, enzyme activity lower than 70%, in pregnancy, and in breastfeeding mothers when the infant's G6PD status is either unknown or deficient (1). To date, no safety studies have been reported in children.

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Table 1. The FDA Drug Label for Tafenoquine (Arakoda). Contraindicated in G6PD Deficiency. (2019)

Phenotype	Warnings and precautions
G6PD deficiency	Hemolytic Anemia: G6PD testing must be performed before prescribing tafenoquine due to the risk of hemolytic anemia. Monitor individuals for signs or symptoms of hemolysis. G6PD Deficiency in Pregnancy or Lactation: tafenoquine may cause fetal harm when administered to a pregnant woman with a G6PD-deficient fetus. Tafenoquine is not recommended during pregnancy. A G6PD-deficient infant may be at risk for hemolytic anemia from exposure to tafenoquine through breast milk. Check infant's G6PD status before breastfeeding begins.

G6PD: Glucose-6-phosphate dehydrogenase
This FDA table is adapted from (1).

Disease: Malaria

Malaria is a serious tropical disease caused by a parasite (*Plasmodium*) that spreads to humans by infected mosquitos. The only available vaccine is moderately effective and acts only against *Plasmodium falciparum (P. falciparum)* species (8). Widely recommended antimalarial drugs such as tafenoquine can be used for prevention –– this is known as chemoprophylaxis. The type of chemoprophylaxis recommended depends upon the individual taking the prophylaxis (namely, age, pregnancy status, and medical comorbidities) and the nature of travel –– specifically, the countries travelled to, the length of stay, the species of Plasmodium that are most prevalent, and the level of drug resistance.

Despite chemoprophylaxis, travel to malaria-endemic areas is not without risk. Individuals at elevated risk for malaria complications include pregnant women (9) and adults who have had their spleen removed (10). If travel cannot be avoided, chemoprophylaxis should be combined with additional precautions to avoid mosquito bites, such as bed nets and repellents. In 2018, the WHO estimated 228 million cases of malaria occurred worldwide, and malaria was responsible for 405,000 deaths. (3)

Malaria is found in over 100 countries and occurs throughout most tropical regions in the world. These regions include large parts of Africa, Asia, Central and South America, and parts of the Middle East and Pacific islands (3, 11). Individuals who are heterozygous carriers for sickle cell disease and G6PD deficiency have a protective advantage against malaria, and as a result, the frequency of such genetic conditions is higher in countries where malaria is endemic (12).

Malaria is transmitted to humans by the bite of an infected *Anopheles* mosquito. Only female mosquitos spread the infection (females feed on human blood, males feed on nectar). Although malaria can also be spread by sharing contaminated needles or via a contaminated blood transfusion, these are rare means of transmission.

There are several different Plasmodium species, but only a few species cause the most malaria cases:

- *P. falciparum*
  - The most common cause of malaria, and death from malaria
  - Predominates in sub-Saharan Africa
  - Also found in regions of Australasia (Papua New Guinea, Southeast Asia), and the Caribbean (Haiti and the Dominican Republic)
- *P. vivax*
  - A common cause of malaria outside of Africa
  - Most frequent species found in Central and South America
  - Parasite has a dormant, hypnozoite stage
  - Early gametocytes that infect mosquitos
- *P. malariae*
  - Less common

    - Found in most areas where malaria is endemic
- *P. ovale*
    - Less common
    - Parasite has a dormant, hypnozoite stage
- *P. knowlesi*
    - Less common
    - Found in some Southeast Asia areas

The first stage of malaria infection begins when an infected mosquito bites the human host. Typically, mosquitos bite at dusk, or during the night. As the mosquito feeds, infective parasite sporozoites (the motile spore-like stage in the life cycle of this parasitic sporozoan, which is the infective agent) are inoculated into humans. The sporozoites travel to the liver, where they invade liver cells and asexually reproduce to form schizonts. The liver schizonts contain daughter merozoites. This process is asymptomatic, and because it occurs outside of the red blood cell (erythrocyte), it is known as the exoerythrocytic stage.

Some species of the parasite (*P. vivax* and *P. ovale*) have an additional dormant stage in the liver. The parasite exists as hypnozoites, which can stay in the liver for weeks or months without causing any clinical symptoms.

The second stage of malaria infection is the erythrocytic stage. It begins when the liver schizonts rupture and release the daughter merozoites into the bloodstream. The merozoites invade red blood cells, digest hemoglobin, produce a toxic metabolite (hemozoin), and damage red blood cell membranes. Infected, brittle red blood cells are rapidly broken down (hemolysis) and if too many damaged red blood cells get trapped in the spleen, the spleen can rapidly enlarge (splenic sequestration).

Some of the daughter merozoites differentiate into male or female gametocytes (sexual forms). When they are ingested by a mosquito, they mature, fertilize and reproduce, and develop into sporozoites. When the mosquito feeds again, the sporozoites are inoculated into another human host and the cycle of malaria transmission is complete.

The erythrocytic stage of malaria is usually associated with fever, and malaria should always be suspected in anyone with a fever who has recently returned from a malaria-endemic region, even if antimalarial chemoprophylaxis was correctly followed. Other symptoms and signs include nausea, vomiting, abdominal pain, tachycardia (fast heart rate), diaphoresis (sweating), chills, and myalgia (muscle pain).

The complications of malaria infection include severe anemia, cerebral malaria, and multi-organ failure. Without correct diagnosis and prompt treatment, malaria can be fatal.

Drug: Tafenoquine

Malaria drugs can be used to prevent malaria as primary prophylaxis, to prevent infection (started before travel to a country where malaria is endemic) or as terminal prophylaxis to prevent a relapse of malaria (started after returning home from prolonged travels in malaria-endemic regions) (13).

Tafenoquine is one of 2 antimalarials, the other is primaquine, that is used both for primary prophylaxis and radical cure of malaria. Specifically, tafenoquine is approved by the FDA for 2 indications:

- Prophylaxis of malaria, in adults (Arakoda, 100 mg tablets) (11, 14).
- Radical cure of *P. vivax* malaria, in persons aged 16 years and older (Krintafel, 150 mg tablets) (2).

The "radical cure" of *P. vivax* malaria refers to the complete elimination of the malaria parasite from the body. Specifically, the elimination of both parasites in the blood and parasites that are lying dormant in the liver, known as hypnozoites, which can cause malaria relapse weeks or months after travel. This occurs in malaria caused by *P. vivax* (a common cause of malaria) and *P. ovale* (a less common cause).

Tafenoquine is the most recent addition to the drug family of 8-aminoquinoline antimalarials, which only includes one other actively prescribed drug: primaquine. Both drugs provide malaria prophylaxis, and both drugs can prevent relapse of *P. vivax* malaria. However, tafenoquine has a longer half-life, allowing for less frequent dosing (13, 15-17).

For prophylaxis against all species of malaria, treatment with tafenoquine (Arakoda) is started just 3 days before travel (loading regimen of 200 mg once daily for 3 days). Tafenoquine is then taken once a week while traveling in the malaria-endemic area (maintenance regimen of 200 mg once weekly, starting 7 days after the last loading dose), with one final dose taken after returning home, away from the malaria-endemic area (terminal prophylaxis regimen of 200 mg once, 7 days after the last maintenance dose) (1).

For the radical cure of *P. vivax* malaria, tafenoquine (Krintafel) is taken as a single 300 mg dose in conjunction with a blood schizontocidal antimalarial. Previously, the best available treatment for radical cure of *P.vivax* was a 14-day course of primaquine, (2) although 7-day courses are used in some countries (5).

Tafenoquine is active against the different forms of the malaria parasite: the pre-erythrocytic (liver stage), the erythrocytic (red blood cell, asexual form) stage, and the gametocytes (sexual form). By targeting the pre-erythrocytic stage, tafenoquine prevents the parasite from developing erythrocytic forms and halts progression of the disease. Although the molecular target of tafenoquine is not known, in vitro studies suggest that the drug may inhibit hematin polymerization, which kills the parasite, and also causes red blood cells to shrink (13, 17). It is also thought that tafenoquine has many different metabolites to target the different stages of the parasites (18).

Before starting tafenoquine therapy, all adults must be tested for G6PD deficiency. Individuals with G6PD deficiency have red blood cells that are susceptible to oxidative damage. If exposed to oxidizing agents such as tafenoquine or primaquine, the red blood cells become rigid, get trapped, and are subsequently destroyed by macrophages in the spleen, bone marrow, and liver. The rapid destruction of red blood cells is called hemolysis, and it may result in hemolytic anemia (low number of red blood cells due to increased hemolysis without sufficient production of new cells).

The degree of G6PD activity can vary based on the allele(s) present in the individual. Individuals with a partial decrease in G6PD function are still susceptible to hemolysis while taking 8-aminoquinoline antimalarial drugs. Primaquine can be prescribed for individuals with at least 30% of the normal levels of G6PD enzyme activity. Individuals with intermediate (30–70%) levels of activity should be monitored for hemolysis. Tafenoquine, however, should not be given to individuals with less than 70% of the normal G6PD enzymatic activity. (6)

Pregnancy testing is also recommended for women of reproductive age, because tafenoquine may cause fetal harm when given to a woman who is pregnant with a G6PD-deficient fetus. Therefore, the FDA does not recommend tafenoquine therapy during pregnancy. Tafenoquine is also contraindicated during breastfeeding when the infant is G6PD deficient or if the G6PD status of the infant is unknown.

One study found that in women with normal (>80%) G6PD enzymatic activity, tafenoquine and primaquine resulted in a similar decline in hemoglobin (19, 20). The impact of such 8-aminoquinolines is not clear in individuals with intermediate enzyme activity (30–80%). Usually primaquine is not recommended for those presenting with activity under 30%. Tafenoquine clinical studies were safe in individuals with more than 70% of activity.

Adverse reactions to tafenoquine include hemolytic anemia, as discussed above, in addition to methemoglobinemia (elevations in methemoglobin that require careful monitoring of individuals with NADH-dependent methemoglobin reductase deficiency); uncommon psychiatric effects (including anxiety, abnormal dreams, and insomnia); and serious hypersensitivity reactions (including angioedema and urticaria –– tafenoquine therapy should stop and not be re-administered). Due to the long half-life of tafenoquine (approximately 13–19 days), signs or symptoms of psychiatric and hypersensitivity adverse reactions that may

occur could be delayed in onset, duration, or both, so individuals should be advised to seek medical attention if symptoms occur.

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on chromosome Xq28. As such, males are hemizygous for one *G6PD* allele, making them more susceptible to this X-linked disorder. Females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45,X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide (7), with a worldwide prevalence of approximately 5%. Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once was, endemic for example,, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean (21-23). In the US, G6PD deficiency is more common among African-Americans, affecting approximately 12% (24).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. Red blood cells that lack G6PD also have a deficiency of NADPH. (25)

Red blood cells that are G6PD and NADPH deficient are more susceptible to oxidative stress (for example, by reactive oxygen species and hydrogen peroxide). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the antimalarial drugs primaquine and tafenoquine, the antibacterials dapsone and sulfamethoxazole, the skin cancer drug dabrafenib, and the uric acid lowering drugs pegloticase and rasburicase.

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. However, at birth, they maybe predisposed to neonatal jaundice, and throughout life, they will be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid exposure to oxidizing agents when possible, including drugs such as tafenoquine.

Symptomatic individuals with G6PD deficiency may suffer from episodes of acute hemolytic anemia or chronic non-spherocytic hemolytic anemia. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions. Folic acid may be given to prevent the worsening of anemia in individuals with folate deficiency and to induce formation of new red blood cells.

More than 180 genetic variants of the *G6PD* gene have been identified so far, with approximately 400 biochemical and enzyme variants (26). Most known *G6PD* variants are missense, which can also be inherited as haplotypes that are comprised of more than one variant allele (27). Large deletions are rare, and a complete lack of G6PD activity is thought to be fatal in utero.

The normal (wild-type) copy of the *G6PD* gene is known as *G6PD* B, and is found in most Caucasians, Asians, and most Africans. Common *G6PD* variants include:

- *G6PD* A+ (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of Blacks from Africa (28)

- *G6PD* A- (p.Asn126Asp with p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (29). Additional *A-* haplotypes have also been identified, both with the A+ variant with a second SNP (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (30)
- *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is a common pathogenic variant in Caucasians (31)
- *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in Asians (32)

The WHO categorized *G6PD* variants into 5 classes according to the level of enzyme activity and severity of hemolysis. Class 1 variants are the most severe, but rare. These variants have less than 10% of normal GP6D enzyme activity—often as low as 1% or less—and are associated with chronic hemolytic anemia.

Most individuals with G6PD deficiency have variants that belong to class II (enzyme activity less than 10% but no chronic hemolytic anemia) and class III (enzyme activity between 10% and 60%). Class II and III variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but most of the time, affected individuals are asymptomatic. Class IV and V variants are not considered to be clinically significant, class IV variants are associated with normal enzyme activity, and class V variants with increased enzyme activity (33).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has assigned *G6PD* phenotypes based on *G6PD* genotypes (Table 2) (33).

Table 2. Assignment of likely *G6PD* Phenotype based on Genotype/Diplotype (CPIC 2014)

Likely phenotype	Definition	Genotype	WHO class for *G6PD* variants[a]	Example of diplotype[b]
Normal	Very mild or no enzyme deficiency (less than 60% of normal enzyme levels)	A male who has a nondeficient (class IV) allele	IV	B, Sao Boria
		A female who has 2 nondeficient (class IV) alleles	IV/IV	B/B, B/Sao Boria
Deficient	Less than 10–60% of normal enzyme activity	A male who has a deficient (class II–III) allele	II, III	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		A female who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA	A male who has a class I allele	I	Bangkok, Villeurbanne
		A female who has 2 deficient (class I variants) alleles	I/I	Bangkok/Bangkok, Bangkok/Villeurbanne

Table 2. continued from previous page.

Likely phenotype	Definition	Genotype	WHO class for *G6PD* variants[a]	Example of diplotype[b]
Variable[c]	Normal or deficient enzyme activity[c]	A female who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III	B/A–, B/Mediterranean, B/Bangkok

CNSHA, chronic nonspherocytic hemolytic anemia
WHO, World Health Organization
[a] WHO classifications (from ref. 14, other details from ref. 17, from (33)). Class I variants are extremely rare; the distinction between class II and III variants is not clear, and the "class V" very high activity variant has been reported in only a single case. Therefore, almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.
[b] Due to the large number of G6PD variants, many other diplotypes may be possible besides those given as examples here; see Supplementary Table S1 online for a more comprehensive list of variant alleles with their assigned WHO class (33). For HGVS terms, please see the Nomenclature table below.
[c] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online (G6PD heterozygotes)) (33).
This CPIC table is adapted from (33).

Linking Gene Variation with Treatment Response

Tafenoquine is contraindicated in individuals with G6PD deficiency, and published drug trials have only included adults with normal (>70%) G6PD activity. However, one study reported that 2 females with G6PD deficiency were mistakenly diagnosed as having normal G6PD activity and given tafenoquine. Both females were positive for the (A-) *G6PD* variant. While the female who was homozygous for this variant remained asymptomatic, the female who was heterozygous for this variant required a blood transfusion, emphasizing the importance of accurate G6PD testing (34, 35).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for tafenoquine response and the *G6PD* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD deficiency and testing may also be used to screen females with a family history of G6PD to see if they are carriers.

Glucose-6-phosphate dehydrogenase deficiency is inherited in an X-linked recessive pattern and most individuals are asymptomatic throughout life.

X-linked disorders affect males at a much higher rate than females because males only have one copy of the X chromosome (hemizygous, XY). Since females have 2 copies of the X chromosome (XX) they tend to be less affected. However, female carriers can present with a range of phenotypes from no symptoms through a severe deficiency due to the high frequency of *G6PD* variants. Females randomly inactivate one X chromosome in somatic cells during development, resulting in a mixed population of somatic cells expressing one *G6PD* allele or the other.

Glucose-6-phosphate dehydrogenase deficiency occurs in homozygous and compound heterozygous females (who have inherited 2 copies of *G6PD* deficiency alleles) and in heterozygous females (one normal *G6PD* allele and one deficiency *G6PD* allele) with skewed X-chromosome inactivation of the functional allele (22). Genetic testing alone is insufficient for heterozygous females with one normal function *G6PD* allele, as the expression of the 2 alleles will vary between blood cells and over time (33).

A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons.

The FDA recommends that all adults be tested for G6PD deficiency before starting tafenoquine therapy. In routine clinical practice, G6PD deficiency is diagnosed by measuring G6PD activity in red blood cells. Two different types of enzyme activity tests are used: qualitative and quantitative. Often, qualitative tests do not accurately detect individuals with intermediate G6PD activity, hence the importance of the quantitative enzyme assay before initiating tafenoquine therapy (6). False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, screening for G6PD should be performed 2–3 months after a blood transfusion or hemolytic episode. Note, false negatives have been reported (25, 33, 36).

In men, if genetic testing determined that an individual was G6PD deficient, the use of tafenoquine would be contraindicated. However, a negative genetic testing result cannot be entirely relied upon because only a small subset of *G6PD* variants are routinely tested for. In addition, the *G6PD* phenotype may be unpredictable in heterozygous females because of random X-chromosome inactivation.

The WHO recommends that neonatal screening be performed in areas where G6PD deficiency occurs in more than 3–5% of males (22). These populations are primarily in Asia, Africa, along the Mediterranean and in the Middle East. Screening either uses quantitative enzyme activity assays, or the WHO-approved fluorescent spot test –– a qualitative test that visually identifies NADPH, which is produced by G6PD (if the blood spot does not fluoresce, the test is positive for G6PD deficiency) (33, 37). However, a negative fluorescent spot test does not mean the G6PD activity level is within safe limits for tafenoquine therapy, this must be ascertained with a quantitative test.

Therapeutic Recommendations based on Genotype

This section contains excerpted [1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA) for tafenoquine (Krintafel):

Hemolytic Anemia

Due to the risk of hemolytic anemia in patients with G6PD deficiency, G6PD testing must be performed before prescribing tafenoquine. Due to the limitations with G6PD tests, physicians need to be aware of residual risk of hemolysis and adequate medical support and follow-up to manage hemolytic risk should be available. Treatment with tafenoquine is contraindicated in patients with G6PD deficiency or unknown G6PD status. In clinical trials, declines in hemoglobin levels were reported in some G6PD-normal patients. Monitor patients for clinical signs or symptoms of hemolysis. Advise patients to discontinue tafenoquine and seek medical attention if signs of hemolysis occur.

G6PD Deficiency in Pregnancy and Lactation

Potential Harm to the Fetus

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

The use of tafenoquine during pregnancy may cause hemolytic anemia in a G6PD-deficient fetus. Even if a pregnant woman has normal levels of G6PD, the fetus could be G6PD deficient. Advise females of reproductive potential that treatment with tafenoquine during pregnancy is not recommended and to avoid pregnancy or use effective contraception during treatment and for 3 months after the last dose of tafenoquine. If a pregnancy is detected during tafenoquine use, discontinue tafenoquine as soon as possible and switch to an alternative prophylactic drug for malaria during pregnancy.

Potential Harm to the Breastfeeding Infant

A G6PD-deficient infant may be at risk for hemolytic anemia from exposure to tafenoquine through breast milk. Infant G6PD status should be checked before breastfeeding begins. Tafenoquine is contraindicated in breastfeeding women when the infant is found to be G6PD deficient or the G6PD status of the infant is unknown. Advise the woman with a G6PD-deficient infant or if the G6PD status of the infant is unknown not to breastfeed during treatment with tafenoquine and for 3 months after the final dose.

Methemoglobinemia

Asymptomatic elevations in methemoglobin have been observed in the clinical trials of tafenoquine. Institute appropriate therapy if signs or symptoms of methemoglobinemia occur. Carefully monitor individuals with nicotinamide adenine dinucleotide (NADH)-dependent methemoglobin reductase deficiency. Advise patients to discontinue tafenoquine and seek medical attention if signs of methemoglobinemia occur.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature for Selected *G6PD* Variants

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Boria	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3: c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3 :c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3: c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient	rs5030869

Table continued from previous page.

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
* WHO classifications based on (38)

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Tamoxifen Therapy and *CYP2D6* Genotype

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Introduction

Tamoxifen (brand name Nolvadex) is a selective estrogen receptor modulator (SERM) that is commonly used in both the treatment and prevention of breast cancer. When taken for 5 years, tamoxifen almost halves the rate of breast cancer recurrence in individuals who have had surgery for estrogen-receptor–positive (ER+) breast cancer.

Tamoxifen is the endocrine therapy of choice for treatment of premenopausal women with ER+ breast cancer, and an important alternative, or sequential treatment for postmenopausal women with ER+ breast cancer. In addition, tamoxifen is the only hormonal agent approved by the FDA for the prevention of premenopausal breast cancer in women who are at high risk, and the treatment of premenopausal invasive breast cancer and ductal carcinoma *in situ* (DCIS).

The CYP2D6 enzyme metabolizes a quarter of all prescribed drugs and is one of the main enzymes involved in converting tamoxifen into its major active metabolite, endoxifen. Genetic variation in the *CYP2D6* gene may lead to increased ("ultrarapid metabolizer"), decreased ("intermediate metabolizer"), or absent ("poor metabolizer") enzyme activity. Individuals who are intermediate or poor metabolizers may have reduced plasma concentrations of endoxifen and benefit less from tamoxifen therapy.

At this time, the FDA-approved drug label for tamoxifen does not discuss genetic testing for *CYP2D6* (Table 1) (1). The National Comprehensive Cancer Network (NCCN) Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy (Table 2), and this recommendation is consistent with the 2010 update of the American Society of Clinical Oncology (ASCO) Guidelines (the most recent update, 2014, does not discuss pharmacogenetic testing) (2, 3).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recently published updated guidelines for the dosing of tamoxifen based on CYP2D6 phenotype, with therapeutic recommendations for each metabolizer phenotype (Table 3). For CYP2D6 poor metabolizers, CPIC recommends using an alternative hormonal therapy, such as an aromatase inhibitor for postmenopausal women; or an aromatase inhibitor along with ovarian function suppression in premenopausal women. This recommendation is based on these approaches being superior to tamoxifen regardless of *CYP2D6* genotype, and the knowledge that CYP2D6 poor metabolizers who switched from tamoxifen to anastrozole do not have an increased risk of recurrence. The CPIC recommendation also states that higher dose tamoxifen (40 mg/day) can be considered if there are contraindications to aromatase inhibitor therapy; however, the increased endoxifen concentration among CYP2D6 poor metabolizers treated with a higher tamoxifen dose does not typically reach the level as in normal metabolizers (4).

Recommendations from the Dutch Pharmacogenetics Working Group (DWPG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) also discuss using an alternative drug to tamoxifen in CYP2D6 poor metabolizers (Table 4) (5).

Table 1. The FDA (2018) Drug Label for Tamoxifen: Metabolism

Recommendations
Tamoxifen is a substrate of CYP3A, CYP2C9 and CYP2D6, and an inhibitor of P-glycoprotein.

This FDA table is adapted from (1)

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Table 2. NCCN (2018). *CYP2D6* Phenotypes and Therapeutic Recommendations for Tamoxifen

Genetic test	Recommendation
CYP2D6	Given the limited and conflicting evidence at this time, the NCCN Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy. This recommendation is consistent with the ASCO Guidelines. When prescribing a selective serotonin reuptake inhibitor (SSRI), it is reasonable to avoid potent and intermediate CYP2D6 inhibiting agents, particularly paroxetine and fluoxetine, if an appropriate alternative exists.

This National Comprehensive Cancer Network (NCCN) table is adapted from (2). ASCO - American Society of Clinical Oncology

Table 3. CPIC (2018). Dosing Recommendations for Tamoxifen based on CYP2D6 Phenotype

Phenotype		Implications	Therapeutic recommendation[b]	Classification of recommendation[a]
Metabolizer status	Activity score			
CYP2D6 ultrarapid metabolizer	>2.0	Therapeutic endoxifen concentrations	Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong
CYP2D6 normal metabolizer	1.5–2.0	Therapeutic endoxifen concentrations	Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0 (no *10 allele present)[b]	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Optional[b]
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0 (*10 allele present)[b]	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Moderate[b]

Table 3. continued from previous page.

Phenotype		Implications	Therapeutic recommendation[b]	Classification of recommendation[a]
Metabolizer status	Activity score			
CYP2D6 intermediate metabolizer	0.5	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Consider hormonal therapy such as an aromatase inhibitor for post-menopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid CYP2D6 strong to weak inhibitors.	Moderate
CYP2D6 poor metabolizer	0	Lower endoxifen concentrations compared with normal metabolizers; higher risk of breast cancer recurrence, event-free and recurrence-free survival compared with normal metabolizers.	Recommend alternative hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype and based on knowledge that CYP2D6 poor metabolizers switched from tamoxifen to anastrozole do not have an increased risk of recurrence. Note, higher dose tamoxifen (40 mg/day) increases but does not normalize endoxifen concentrations and can be considered if there are contraindications to aromatase inhibitor therapy.	Strong

Activity score – for a description of how scores are calculated, please see the "Genetic Testing" section below.
[a]Rating scheme described in the CPIC Supplement (4).
[b] CPIC has generally classified individuals with an activity score of 1 as a "normal metabolizer." However, in the case of tamoxifen, prescribing recommendations for those with an activity score (AS) of 1.0 are allele dependent, based on the presence of the *10 allele. Those individuals with an AS of 1.0 on the basis of a *10 allele are provided a "moderate" recommendation. In contrast, prescribing recommendations for those with an activity score of one based on the presence of CYP2D6 alleles other than *10 are graded as "optional" because the recommendations are primarily extrapolated from evidence generated from *10 individuals (i.e., limited data for clinical outcomes and pharmacokinetics for this group).
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (4)

Table 4. DPWG (2015). *CYP2D6* Phenotypes and Therapeutic Recommendations for Tamoxifen

CYP2D6 phenotype	Recommendation
Ultrarapid metabolizer	No action is needed for this gene-drug interaction.
Intermediate metabolizer	1 Select an alternative or measure the endoxifen concentration and increase the dose if necessary by a factor of 1.5–2. Aromatase inhibitors are a possible alternative for post-menopausal women. 2. If tamoxifen is selected: avoid co-medication with CYP2D6 inhibitors such as paroxetine and fluoxetine.

Table 4. continued from previous page.

CYP2D6 phenotype	Recommendation
Poor metabolizer	Select an alternative or increase the dose to 40 mg/day and monitor the endoxifen concentration. Studies have demonstrated that poor metabolizers can achieve an adequate endoxifen concentration when the dose is increased to 40-60 mg/day. Aromatase inhibitors are a possible alternative for post-menopausal women.

This Dutch Pharmacogenetics Working Group (DWPG) table is adapted from (5).

Drug: Tamoxifen

Tamoxifen is a SERM that is used in both the treatment and prevention of breast cancer.

For treatment, tamoxifen is used in both men and women with metastatic breast cancer, particularly among individuals with ER+ tumors. Tamoxifen is also used as adjuvant treatment among women who have undergone surgery and radiation, as this almost halves the rate of reoccurrence of breast cancer in woman with ER+ tumors. Tamoxifen reduces the risk of progression to invasive breast cancer in women with DCIS.

For prevention, tamoxifen has been shown to reduce the occurrence of contralateral breast cancer. And in women who do not have breast cancer, tamoxifen has been shown to reduce the incidence of breast cancer in women at high risk. Risk factors for breast cancer include increasing age, Caucasian race, the number of first-degree relatives with breast cancer, obesity (for postmenopausal women), and an increased exposure to estrogen (e.g., early menarche, later age of first pregnancy or no children, absence of breastfeeding, later menopause) (1, 4).

Tamoxifen acts on the estrogen receptor and has both estrogenic and anti-estrogenic actions, depending on the target tissue. In the breast tissue, it acts as an anti-estrogen (inhibitory effect) and competitively inhibits cancerous ER+ cells from receiving the estrogen they need to proliferate.

In other tissues, such as the endometrium, tamoxifen acts as an estrogen agonist (stimulatory effect) leading to some of the adverse effects associated with tamoxifen therapy. These include endometrial hyperplasia, endometrial polyps, and around a 2.5 times higher risk of developing endometrial cancer. Hot flashes are the most common side effect associated with tamoxifen use, which affect up to 80% of women, and there is also an increased risk of depression (6-8).

The antiestrogenic properties of tamoxifen are expected to affect fetal reproductive functions and increase the risk of fetal harm. Therefore, women may be advised not to become pregnant while taking tamoxifen or within 2 months of discontinuing tamoxifen, and to use barrier or nonhormonal contraception (1).

Tamoxifen also increases the risk of thromboembolic events, such as deep vein thrombosis and pulmonary embolism. The risk of tamoxifen-associated thromboembolic events is further increased when tamoxifen is coadministered with chemotherapy. The drug label for tamoxifen states that the risks and benefits of tamoxifen therapy should be carefully considered in women with a history of thromboembolic events (1).

Some studies suggest that clinicians should consider screening breast cancer individuals before prescribing adjuvant tamoxifen to identify women who are at risk of thrombotic embolic disease as a result of having the Factor V Leiden (p.R506Q) variant, or a variant in the estrogen receptor gene (*ESR1*) (9-12). However, a small substudy (N=81) of the national surgical adjuvant breast and bowel project breast cancer prevention (NSABP P-1) trial found no benefit in screening women for Factor V Leiden or *F2* prothrombin (c.*97G>A) thrombophilia to identify women who may not be appropriate for tamoxifen therapy due to an increased risk for thromboembolic side effects (13).

Tamoxifen is inactive, and its active metabolite endoxifen (4-hydroxy-N-desmethyl tamoxifen) is thought to mediate most of its therapeutic effects. Both endoxifen and another metabolite, 4-hydroxytamoxifen, have around a 100-fold higher affinity for the ER compared with tamoxifen, but endoxifen is thought to be the major metabolite because plasma levels of endoxifen tend to be several-fold higher.

The mechanism of action of tamoxifen involves binding to the ER and inducing a conformational change that blocks or changes the expression of estrogen-dependent genes. It is also likely that tamoxifen interacts with other protein cofactors (both activators and repressors) and binds with different estrogen receptors (ER-alpha or ER-beta) to produce estrogenic and anti-estrogenic effects in different tissues (14).

The tamoxifen metabolite, norendoxifen, has also been found to act as an aromatase inhibitor *in vitro* (albeit at high concentrations). Aromatase inhibitors are a class of drug used to treat breast cancer and gynecomastia. They decrease the amount of estrogen available by inhibiting the conversion of steroids such as androgen into estradiol (15).

The pharmacokinetics of tamoxifen are complex, involving many enzymes (including several cytochrome P450 enzymes) and transporter proteins (including ATP-binding cassette transporters (ABC) transporters). However, CYP2D6 is thought to be important because it mediates the formation of endoxifen via the conversion of the inactive primary metabolite N-desmethyl tamoxifen.

The response to tamoxifen therapy (i.e., clinical efficacy and side effects) varies widely between individuals. This is due to a number of variables, including drug interactions (e.g., coadministration of a drug that inhibits or induces *CYP2D6*) and interindividual differences in drug metabolism driven by polymorphic germline *CYP2D6* variant alleles (16-18).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse superfamily of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are often very polymorphic and can result in reduced, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. And, CYP2D6 is the main enzyme that catalyzes the rate-limiting step in the metabolism of tamoxifen to its potent metabolite, endoxifen. Other CYP enzymes involved in tamoxifen metabolism include CYP2C9, CYP2C19, CYP2B6, CYP3A4, and CYP3A5.

CYP2D6 Alleles

The CYP2D6 enzyme catalyzes the main pathway for converting tamoxifen into its most potent metabolite, endoxifen, and together with other CYP enzymes, catalyzes the formation of 4-hydroxytamoxifen. Therefore, genetic variations in the *CYP2D6* gene can influence tamoxifen metabolism (19).

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 5).

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., CYP2D6 *4/*4). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (e.g., CYP2D6 poor metabolizer). However, the activity score system is not standardized across clinical laboratories or *CYP2D6* genotyping platforms.

Table 5. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *14B, *17, *29, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *15, *19, *20, *21, *36, *38, *40, *42*

For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

*CYP2D6*1* is assigned when no variant is detected and is assumed to have normal enzyme activity (CYP2D6 normal metabolizer phenotype). The *CYP2D6 *2*, **33*, and **35* alleles are also considered to have near-normal activity.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a nonfunctioning enzyme include **3*, **4*, **5,* and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **6,* and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (20).

Additional variant alleles and their multi-ethnic population frequencies have previously been reported (21). Moreover, given the structural variability of the *CYP2D6* region at chromosome 22q13.2, full gene deletion and duplication alleles, as well as complex tandem alleles with *CYP2D6*'s pseudogene, *CYP2D7*, also occur in some individuals and populations (22).

CYP2D6 Phenotypes

In the US and globally, most individuals, around 70-80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41).

Individuals who have one normal function and one no function allele (e.g., *1/*4) or 2 decreased function alleles (e.g., *41/*41) are also categorized as "normal metabolizers" by recent nomenclature guidelines (23), but have also been categorized as "intermediate metabolizers" (24).

Individuals who have more than 2 normal function copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1–10% of Caucasian individuals. For individuals of North African, Ethiopian and Saudi ancestry, the frequency is 16–28% (Table 6) (4).

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one no function allele, e.g., *4/*41) or poor metabolizers (2 no function alleles e.g., *4/*4).

Approximately 6–10% of European Caucasians are poor metabolizers, mainly due to the prevalent nonfunctional **3*, **4* and **5* alleles. Compared with Europeans, individuals of Asian descent are likelier to be intermediate metabolizers due to high population frequencies of the *CYP2D6*10* decreased function allele. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are likelier than Europeans to be intermediate metabolizers because of the prevalence of a wide range of decreased function variants. (20, 25-27)

Table 6. CPIC (2018). Assignment of likely CYP2D6 Phenotype based on Genotype

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 ultrarapid metabolizer	>2.0	An individual with duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]

Table 6. continued from previous page.

Phenotype[a]		Genotype	Examples of *CYP2D6* diplotypes[b]
Metabolizer status	Activity score		
CYP2D6 normal metabolizer	1.5–2.0	An individual with 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2
CYP2D6 normal metabolizer or intermediate metabolizer (controversy remains)[b]	1.0	An individual with 2 decreased function alleles or one normal function and one no function allele. *An activity score (AS) of 1.0 is associated with decreased tamoxifen metabolism to endoxifen compared with an AS of 1.5 or 2.*	*1/*4, *1/*5, *41/*41
CYP2D6 intermediate metabolizer	0.5	An individual with one decreased function and one no function allele	*4/*10, *4/*41, *5/*9
CYP2D6 poor metabolizer	0	An individual with only no functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] See the CYP2D6 frequency table 1 in (4) for race-specific allele and phenotype frequencies.
[b]For a complete list of CYP2D6 diplotypes and resulting phenotypes, see the CYP2D6 genotype to phenotype table in (4). Note that genotypes with an activity score of 1 are classified as normal metabolizers in the online CPIC CYP2D6 genotype to phenotype table (4).
[c]Where xN represents the number of CYP2D6 gene copies. For individuals with CYP2D6 duplications or multiplications, see supplemental data for additional information on how to translate diplotypes into phenotypes.
[d]Individuals with an activity score of 1.0 may be classified as intermediate metabolizers by some reference laboratories. A group of CYP2D6 experts are currently working to standardize the CYP2D6 genotype to phenotype translation system. CPIC will update the CPIC website accordingly (CYP2D6 genotype to phenotype table).
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (4).

Linking Gene Variation with Treatment Response

Genetic variation in the *CYP2D6* gene is associated with variation in plasma concentrations of endoxifen and is thought to account for up to approximately 50% of the variability in endoxifen concentrations (28).

- Individuals who are CYP2D6 poor metabolizers (activity score 0) have lower plasma endoxifen concentrations compared with normal metabolizers (with an activity score of 1.5-2.0).
- Individuals with reduced CYP2D6 activity (activity score 0.5-1) have lower plasma endoxifen concentrations compared with normal metabolizers (with an activity score of 1.5-2.0) (4).

However, while it is clear that tamoxifen biotransformation to endoxifen is highly dependent on CYP2D6 activity, the association between tamoxifen efficacy and *CYP2D6* genotype or endoxifen concentration is less clear. Because the role of *CYP2D6* in tamoxifen response has yet to be fully determined, *CYP2D6* testing remains controversial (29-40).

Some studies conclude that the *CYP2D6* genotype has minimal or no effect on tamoxifen therapy outcomes (41-45). A 2019 prospective clinical study (n=667) found no association between *CYP2D6* genotype or endoxifen concentration and clinical outcome in individuals with early-stage breast cancer receiving adjuvant tamoxifen (46).

In contrast, other studies suggest that *CYP2D6* variant alleles may be important predictors of tamoxifen clinical outcomes (28, 40, 47-52). In particular, in Asians, studies of populations with a high frequency of the decreased function *CYP2D6*10* allele (e.g., Han Chinese), found that individuals with *CYP2D6**10/*10 received less benefit from tamoxifen and poorer disease-free survival (53-55).

However, the high degree of inter-individual variability of tamoxifen metabolism and treatment outcomes is not fully accounted for by *CYP2D6* variation. Additional contributors may include genetic variation in other metabolic pathways and the sequestration of lipophilic tamoxifen metabolites into fat tissues (17, 30, 48, 56).

Genetic Testing

The NIH Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for tamoxifen response and for the *CYP2D6* gene.

The *CYP2D6* gene is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, pseudogenes, and tandem alleles. The complexity of genetic variation complicates the correct determination of *CYP2D6* diplotype.

Genetic testing is currently available for approximately 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (4).

A result for copy number, if available, is also important when interpreting CYP2D6 genotyping results. Gene duplications and multiplications are denoted by "xN" e.g., *CYP2D6*1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6* *1/*1, normal metabolizer", this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 6).

The CYP2D6 phenotype can be defined by the sum of the 2 activity scores, which is usually in the range of 0–3.0:

- An ultrarapid metabolizer has an activity score greater than 2
- A normal metabolizer phenotype has an activity score of 1.5–2.0
- A normal metabolizer or intermediate metabolizer has a score of 1.0
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0 (4)

A standardized *CYP2D6* genotype to phenotype assignment logic is currently being developed by an international working group of CYP2D6 experts and both the CPIC and DPWG.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2018 Statement from the US Food and Drug Administration (FDA)

Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite found in patients' plasma. The biological activity of N-desmethyl tamoxifen appears to be similar to that of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.

minor metabolites in plasma. Tamoxifen is a substrate of cytochrome P-450 3A, 2C9 and 2D6, and an inhibitor of P-glycoprotein.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Statement from the National Comprehensive Cancer Network (NCCN)

The cytochrome P-450 (CYP450) enzyme, CYP2D6, is involved in the conversion of tamoxifen to endoxifen. Over 100 allelic variants of *CYP2D6* have been reported in the literature. Individuals with wild-type *CYP2D6* alleles are classified as extensive metabolizers of tamoxifen. Those with one or two variant alleles with either reduced or no activity are designated as intermediate metabolizers and poor metabolizers, respectively. A large retrospective study of 1325 patients found that time to disease recurrence was significantly shortened in poor metabolizers of tamoxifen. However, the Breast International Group (BIG) 1-98 trial reported on the outcome based on *CYP2D6* genotype in a subset of postmenopausal patients with endocrine-responsive, early invasive breast cancer. The study found no correlation between *CYP2D6* allelic status and disease outcome or between *CYP2D6* allelic status and tamoxifen-related adverse effects. A genetic analysis of the ATAC trial found no association between *CYP2D6* genotype and clinical outcomes. Given the limited and conflicting evidence at this time, the NCCN Breast Cancer Panel does not recommend CYP2D6 testing as a tool to determine the optimal adjuvant endocrine strategy. This recommendation is consistent with the ASCO Guidelines. When prescribing a selective serotonin reuptake inhibitor (SSRI), it is reasonable to avoid potent and intermediate CYP2D6 inhibiting agents, particularly paroxetine and fluoxetine, if an appropriate alternative exists.

Please review the complete therapeutic recommendations that are located here: (2).

2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Table 3 summarizes the therapeutic recommendations for tamoxifen prescribing based on the CYP2D6 phenotype. Based on current evidence, CYP2D6 UMs and NMs are expected to achieve therapeutic endoxifen concentrations after administration of tamoxifen and should receive the recommended standard of care doses of tamoxifen. CYP2D6 PMs and IMs (including patients with an AS of 1.0, see Supplement) are expected to have lower endoxifen concentrations compared to NMs and have a higher risk of breast cancer recurrence, and worse event-free survival compared to NMs. For CYP2D6 PMs, a "strong" therapeutic recommendation was provided to recommend alternative hormonal therapy such as an aromatase inhibitor (AI) for postmenopausal women or AI along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype and based on knowledge that CYP2D6 PMs patients who switch from tamoxifen to anastrozole do not exhibit an increased risk of recurrence. Given that escalation of tamoxifen dose from 20–40 mg/day in CYP2D6 PM significantly increases endoxifen concentrations (but not to concentrations achieved in CYP2D6 NMs), the use of an AI (± ovarian function suppression) is recommended in this setting. Tamoxifen 40 mg/day can be considered for CYP2D6 PM if there are contraindications to AI use. There are no clinical data that toremifene, another selective estrogen receptor modulator that also undergoes bioactivation, should be substituted for tamoxifen based on *CYP2D6* genotype.

For CYP2D6 IMs and *CYP2D6*10/*10* or *CYP2D6*10*/decreased function allele, a "moderate" recommendation was made to consider use of an alternative hormonal therapy (i.e., aromatase inhibitor) for postmenopausal women or AI plus ovarian function suppression in premenopausal women is recommended. In CYP2D6 IMs, if AIs are contraindicated, consideration can be given to the use of a higher FDA-approved dose of tamoxifen (40 mg/day), which is known to result in significantly higher endoxifen concentrations without an increase in toxicity. Based on extrapolation from evidence in *10 individuals, a similar recommendation applies to

individuals who carry other decreased function alleles resulting in an AS of 1.0 but with an "optional" recommendation, given the paucity of data for this group.

In general, prolonged overlap of tamoxifen with strong and moderate CYP2D6 inhibitors should be avoided in tamoxifen-treated patients, whereas weak inhibitors are also contraindicated in CYP2D6 IMs.

Please review the complete therapeutic recommendations that are located here: (4)

2015 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 IM: TAMOXIFEN

This gene variation reduces the conversion of tamoxifen to the active metabolite endoxifen. This can result in reduced effectiveness.

Recommendation:

1 select an alternative or measure the endoxifen concentration and increase the dose if

necessary by a factor of 1.5-2

Aromatase inhibitors are a possible alternative for post-menopausal women.

2. if TAMOXIFEN is selected: avoid co-medication with CYP2D6 inhibitors such as paroxetine

and fluoxetine

CYP2D6 PM: TAMOXIFEN

This gene variation reduces the conversion of tamoxifen to the active metabolite endoxifen. This can result in reduced effectiveness.

Recommendation:

1 select an alternative or increase the dose to 40 mg/day and monitor the endoxifen

concentration

Studies have demonstrated that PM can achieve an adequate endoxifen concentration when the dose is increased to 40-60 mg/day.

Aromatase inhibitors are a possible alternative for post-menopausal women.

CYP2D6 UM: TAMOXIFEN

No action is needed for this gene-drug interaction.

As a result of the genetic variation, the plasma concentration of the active metabolites 4- hydroxytamoxifen and endoxifen can increase. However, there is no evidence that this results in an increase in the side effects.

Background information

Mechanism: The main conversion route of tamoxifen is by CYP3A4/5 to the relatively inactive N-desmethyltamoxifen. This is converted by CYP2D6 to endoxifen (4-hydroxy-N-desmethyltamoxifen), which has an anti-oestrogenic effect that is 30-100x stronger than tamoxifen. Tamoxifen is further converted by CYP2D6 to

the active metabolite 4-hydroxytamoxifen. This metabolite is as potent as endoxifen, but occurs at much lower concentrations. CYP3A4/5 converts 4-hydroxytamoxifen further to endoxifen.

Please review the complete therapeutic recommendations that are located here: (5).

2010 Excerpt from the American Society of Clinical Oncology (ASCO) guideline[2]

"Are There Specific Patient Populations That Derive Differing Degrees of Benefit from an AI Compared With Tamoxifen?"

Recommendation: Direct evidence from randomized trials does not identify a specific marker or clinical subset that predicted which adjuvant treatment strategy—tamoxifen, AI monotherapy, or sequential therapy—would maximally improve outcomes for a given patient. Among men with breast cancer, tamoxifen remains the standard adjuvant endocrine treatment. The Update Committee recommends against using *CYP2D6* genotype to select adjuvant endocrine therapy. The Committee encouraged caution with concurrent use of CYP2D6 inhibitors (such as bupropion, paroxetine, fluoxetine; see Table 11 in the full guideline for a complete list of inhibitors) and tamoxifen because of the known drug-drug interactions.

Comment: The adjuvant endocrine therapy recommendations in this update are for all women, irrespective of any specific clinical subset or prognostic marker. AI therapy has not been evaluated in men, thus the continued recommendation that men with breast cancer receive adjuvant tamoxifen.

Data suggest that variability in tamoxifen metabolism affects the likelihood of cancer recurrence in patients treated with tamoxifen. Factors that contribute to this variability include concurrent use of other drugs that inhibit the CYP2D6 isoenzyme and pharmacogenetic variation (polymorphisms) in *CYP2D6* alleles. It is not yet known whether these variations account for differences in outcomes among patients treated with tamoxifen.

Available data on CYP2D6 pharmacogenetics are insufficient to recommend testing as a tool to determine an adjuvant endocrine strategy. Patients who clearly benefit from known CYP2D6 inhibitors might consider avoiding tamoxifen because of potential pharmacologic interactions. Conversely, patients who receive tamoxifen may prefer to avoid concurrent use of known CYP2D6 inhibitors if suitable alternatives are available."

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Not applicable - variant occurs in a non-coding region and results in a splicing defect	rs3892097
*CYP2D6*5*	Not applicable - variant results in a whole gene deletion			
*CYP2D6*6*	1707delT Trp152Gly	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T Pro34Ser	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852

[2] The 2014 ASCO practice guideline focused update does not address pharmacogenetic testing (JCO July 20, 2014 vol. 32 no. 212255-2269).

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names / major SNP	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*17*	Includes at least two functional variants: 1023C>T (Thr107Ile) 2850C>T (Cys296Arg)	NM_000106.5:c.320C>T NM_000106.6:c.886C>T	NP_000097.3:p.Thr107Ile NP_000097.3:p.Arg296Cys	rs28371706 rs16947
*CYP2D6*41*	2988G>A	NM_000106.5:c.985+39G>A	Not applicable – variant occurs in a non-coding region and is linked to aberrant splicing	rs28371725

SNP= Single Nucleotide Polymorphism
Note: In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and 2988G>A occurs with 2850C>T.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (57).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Version History

To view an earlier version of this summary, please see 2014 and 2016 editions.

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Thioguanine Therapy and *TPMT* and *NUDT15* Genotype

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Introduction

Thioguanine (brand name Tabloid) is used to treat acute nonlymphocytic leukemias, such as acute myeloid leukemia (AML). Thioguanine is an analogue of the nucleic acid guanine and belongs to the drug class of thiopurines.

Thioguanine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. The active metabolites are metabolized and inactivated by the enzyme thiopurine methyltransferase (TPMT) and the enzyme nudix hydrolase 15 (NUDT15). Individuals with reduced activity of either enzyme are exposed to higher levels of thioguanine and have a higher risk of toxicity side effects, including severe bone marrow suppression (myelosuppression).

The FDA-approved drug label states that testing for TPMT and NUDT15 deficiency should be considered in individuals who experience severe bone marrow toxicities or repeated episodes of myelosuppression. The label also includes dosing recommendations for when *TPMT* or *NUDT15*, or both, genotypes are known. For individuals with a pharmacogenetic deficiency in either enzyme, the initial dose of thioguanine should be reduced, and individuals who have a deficiency in both enzymes may require more substantial dose reductions. The label notes that individuals with a complete deficiency of either enzyme often continue to require a lower dose, which is 10% or less than the standard thioguanine dose (Table 1) (1).

Dosing recommendations for thioguanine based on *TPMT* and *NUDT15* genotype have also been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG). Both the CPIC and DPWG guidelines recommend substantial dose reductions for individuals who have low or deficient enzyme activity, including considering an alternative drug to thioguanine, particularly when treating a non-malignant condition (Table 2, Table 3) (2-4).

Table 1. FDA Drug Label Dosage and Administration of Thioguanine (2020)

Enzyme	Dosage and administration
TPMT NUDT15	• Individuals with homozygous deficiency of either thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) enzyme typically require 10% or less of the standard thioguanine dosage. • Reduce initial dosage in individuals who are known to have homozygous* TPMT or NUDT15 deficiency. • Most individuals with heterozygous TPMT or NUDT15 deficiency tolerate recommended thioguanine doses, but some require dose reduction based on toxicities. • Individuals who are heterozygous for both TPMT and NUDT15 deficiency may require more substantial dosage reductions. • Reduce the dosage based on tolerability.

* This also applies to compound heterozygous TPMT or NUDT15 deficiency, as multiple no function alleles exist. See Tables 4 and 5. This FDA table is adapted (1).

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Table 2. CPIC Recommended Dosing of Thioguanine by *TPMT* Phenotype (2018 Update)

Phenotype	Implications for thioguanine phenotypic measures	Dosing recommendations for thioguanine	Classification of recommendations[b]
TPMT normal metabolizer	Lower concentrations of TGN metabolites; but note that TGN after thioguanine are 5–10 × higher than TGN after mercaptopurine or azathioprine. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with the normal starting dose[a] (e.g., 40–60 mg/m^2/day) and adjust doses of thioguanine and of other myelosuppressive therapy without any special emphasis on thioguanine. Allow 2 weeks to reach steady state after each dose adjustment.	Strong
TPMT intermediate metabolizer OR TPMT possible intermediate metabolizer	Moderate to high concentrations of TGN metabolites; but note that TGN after thioguanine are 5–10 × higher than TGN after mercaptopurine or azathioprine. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with reduced doses (50–80% of normal dose) if normal starting dose[a] is ≥ 40–60 mg/m^2/day (e.g., 20–48 mg/ m^2/day) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.	Moderate
TPMT poor metabolizer	Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with drastically reduced doses (reduce daily dose[a] by 10-fold and dose 3 times weekly instead of daily) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing thioguanine over other agents. For non-malignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong

TGN, thioguanine nucleotides; TPMT, thiopurine methyltransferase.
[a]Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, a dose reduction might not be recommended for intermediate metabolizers.
b Rating scheme described in Supplemental Material (2).
This CPIC table is adapted from (2).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both TPMT and NUDT15 is known. Please see (2).

Table 3. CPIC Recommended Dosing of Thioguanine by *NUDT15* Phenotype (2018 Upate)

Phenotype	Implications for thiopurine phenotypic measures	Dosing recommendations for thioguanine	Classification of recommendations[b]
NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with the normal starting dose[a] (40–60 mg/m^2/day). Adjust doses of thioguanine and of other myelosuppressive therapy without any special emphasis on thioguanine. Allow 2 weeks to reach steady state after each dose adjustment.	Strong

Table 3. continued from previous page.

Phenotype	Implications for thiopurine phenotypic measures	Dosing recommendations for thioguanine	Classification of recommendations[b]
NUDT15 intermediate metabolizer OR NUDT15 possible intermediate metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with reduced doses (50–80% of normal dose) if normal starting dose[a] is ≥40–60 mg/m^2/day (e.g., 20–48 mg/m^2/day) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.	Strong
NUDT15 poor metabolizer	Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Reduce doses to 25% of normal dose[a] and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For non-malignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong

[a] Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.
[b] Rating scheme described in Supplemental Material.
This CPIC table is adapted from (2).
Note, CPIC have also published recommendations for thiopurine dosing when the status of both thiopurine methyltransferase (TPMT) and nudix hydrolase 15 (NUDT15) is known. Please see (2).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease (IBD), rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine).

All 3 agents have similar effects but are typically used for different indications. Thioguanine is most commonly used to treat myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

There is increasing evidence that DNA testing for *NUDT15* and *TPMT* before initiating thiopurine therapy is clinically useful. In Europeans and Africans, inherited TPMT deficiency is the primary genetic cause of thiopurine intolerance, whereas for Asians, risk alleles in *NUDT15* explains most thiopurine-related myelosuppression (1, 5).

Drug: Thioguanine

Thioguanine is an anti-neoplastic agent used to treat AML. Acute myeloid leukemia is the most common acute leukemia in adults, accounting for approximately 80% of cases, and the incidence increases with age. It is a less common cause of acute leukemia in children, accounting for less than 10% of cases.

Acute myeloid leukemia is characterized by a proliferation of the myeloid lineage of blood cells, causing an accumulation of abnormal and immature cells in the blood, bone marrow, and sometimes other tissues. This

causes a disruption in the production of normal red blood cells, platelets, and mature granulocytes, leading to anemia, bleeding, and an increased risk of infection.

When thioguanine is used as part of a combination chemotherapy for AML, treatment more frequently induces remission and a longer duration of remission compared with using thioguanine alone. However, because of the high risk of liver toxicity, thioguanine is not recommended for long-term use. Younger individuals with AML tend to have a better response to thioguanine than older individuals (1).

Like all thiopurines, thioguanine is a purine analogue, and acts as an antimetabolite. Thioguanine is metabolized by 2 main pathways: bioactivation by hypoxanthine phosphoribosyltransferase and metabolized to form the major active metabolite (TGNs) or metabolized to an inactive metabolite by TPMT-mediated methylation or by NUDT15-mediated dephosphorylation of deoxythioguanine nucleotides.

The cytotoxicity of thioguanine is due, in part, to the incorporation of TGNs into DNA. In addition to inhibiting de novo purine synthesis, thioguanine also inhibits purine nucleotide interconversions (1).

The most frequent adverse reaction to thioguanine is myelosuppression, which typically can be reversed by decreasing the dose of thioguanine. However, individuals who have 2 nonfunctional *TPMT* alleles experience life-threatening myelosuppression after starting treatment with conventional doses of thioguanine. Similarly, individuals that have nonfunctional *NUDT15* alleles are at risk of thioguanine-induced myelosuppression.

Another adverse effect of thioguanine treatment when used in treating AML is hyperuricemia, which frequently occurs because of the rapid lysis of tumor cells. Liver toxicity associated with vascular endothelial damage has been reported when thioguanine is used for maintenance therapy in acute lymphoblastic leukemia (ALL) as an alternative to mercaptopurine, or for long-term continuous therapy as an immunomodulator. Liver toxicity usually presents as the clinical syndrome of hepatic veno-occlusive disease (hyperbilirubinemia, tender hepatomegaly, weight gain due to fluid retention, and ascites) or with signs of portal hypertension (splenomegaly, thrombocytopenia, and esophageal varices). For this reason, the long-term use of thioguanine is not recommended (1).

Gene: *TPMT*

The *TPMT* gene encodes thiopurine S-methyltransferase, which is historically classified as a phase II metabolism enzyme. Importantly, TPMT is one of the main enzymes involved in the metabolism of thiopurines, including thioguanine.

The *TPMT* gene is highly polymorphic, with over 40 reported variant star (*) alleles (6-8). The *TPMT*1* allele is associated with normal enzyme activity (wild type).

The *TPMT*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. Individuals who are normal metabolizers are more likely to have a typical response to thioguanine and a low risk of myelosuppression; however, all individuals receiving thioguanine require close monitoring (9-12).

Most individuals are TPMT normal metabolizers (~86–97%). Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (9, 10, 13):

- *TPMT*2* (c.238G>C)
- *TPMT*3A* (c.460G>A and c.719A>G in *cis*)
- *TPMT*3C* (c.719A>G)

Individuals who are TPMT poor metabolizers (~0.3% of individuals of European or African ancestry) have 2 no function *TPMT* alleles (Table 4). When treated with thioguanine, these individuals will universally experience life-threatening bone marrow suppression because of high levels of TGNs (1).

Individuals who are TPMT intermediate metabolizers (approximately 3–14% of the general population) are heterozygous for one no function *TPMT* allele. When treated with thioguanine, these individuals may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs and are at an increased risk of moderate to severe bone marrow suppression.

Table 4. Assignment of likely *TPMT* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*8, *3A/*7
Poor metabolizer	An individual with 2 no function alleles	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4,*2/*3C, *3A/*4
Indeterminate	An individual with 2 uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele	*6/*8 *1/*8

TPMT, thiopurine methyltransferase; NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15
[a] See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (2). for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (2).

The frequency of *TPMT* variant alleles vary among different ethnic populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (6).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely (6, 14).

Gene: *NUDT15*

The *NUDT15* gene encodes an enzyme that belongs to the nudix hydrolase superfamily. Members of this superfamily catalyze the hydrolysis of nucleoside diphosphates, which are created as a result of oxidative damage (e.g., from treatment with drugs such as thiopurines).

Nudix hydrolase 15 is directly involved in the metabolism of thiopurines, as it catalyzes the conversion of active metabolites (TdGTP) to less toxic metabolites (TdGMP) and in doing so, prevents the incorporation of toxic metabolites into DNA (15).

In individuals with reduced or absent NUDT15 activity (intermediate or poor metabolizers, Table 5), the reduction in NUDT15-mediated degradation of TdGTP results in more TdGTP available for incorporation into DNA, leading to increased DNA damage and cell death. These individuals subsequently have increased sensitivity to thiopurines at standard doses, including an increased risk of severe myelosuppression (16).

Similar to *TPMT*, the *NUDT15* gene is polymorphic, as the PharmVar Consortium has catalogued 21 variant alleles. However, most variants are rare, and the clinical significance of many *NUDT15* star (*) alleles is unclear.

The first *NUDT15* variant associated with thiopurine toxicity is p.R139C (rs116855232), which is present in both the *NUDT15*2* and *NUDT15*3* haplotypes. This amino acid change results in an unstable protein with almost no enzymatic activity. (16)

The FDA drug label for thioguanine cites one study of 1028 children with ALL, wherein the tolerated maintenance dose of the related drug, mercaptopurine, varied greatly, depending on the degree of TPMT, or NUDT15, or both, deficiency. Individuals who were heterozygous deficient for only one gene tolerated between 50-90% of the planned dosage. However, the tolerated dosage dropped to 30-50% of the planned dosage for individuals who were heterozygous deficient for both TPMT and NUDT15. Individuals who had bi-allelic deficiency of either TPMT or NUDT15 only tolerated 5–10% of the planned mercaptopurine dosage. (1, 15)

Deficiency of NUDT15 is rare among individuals with European or African ancestry (found in less than 1%); however, NUDT15 deficiency is more common among individuals with East Asian ancestry (e.g., Korea, China, Japan, Vietnam) (~2%) (2, 17, 18).

Table 5. Assignment of likely *NUDT15* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype[a]	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*5, *3/*6
Poor metabolizer	An individual with 2 no function alleles	*2/*2, *2/*3, *3/*3
Indeterminate	An individual with 2 uncertain function alleles OR one normal function allele plus one uncertain function allele	*1/*4, *1/*5 *4/*5, *5/*6

TPMT, thiopurine methyltransferase; NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15
[a] See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (2) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.
This CPIC table is adapted from (2).

Linking Gene Variation with Treatment Response

Genetic variation in the *TPMT* and *NUDT15* genes strongly influences the safety of thiopurine therapy, specifically, influencing the risk of treatment-related bone marrow suppression (19).

Thiopurine methyltransferase deficiency is the primary genetic cause of thiopurine intolerance in Europeans and Africans, and NUDT15 deficiency is a more common cause in Asians and Hispanics.

The clinical impact of variant *NUDT15* alleles was discovered more recently than for *TPMT*, and there is less evidence available to guide dose adjustments. However, there is one clinical trial in progress that addresses azathioprine (a thiopurine) dosing guided by the status of both TPMT and NUDT15, for the treatment of IBD (17, 20, 21).

Currently, *TPMT* and *NUDT15* testing is not required by the FDA before starting treatment with any thiopurine (azathioprine, mercaptopurine, or thioguanine); however, both genes were listed in the recently published FDA Association tables as pharmacogenetic associations with data supporting therapeutic management

recommendations (22). Consequently, routine genotyping for *TPMT* and *NUDT15* polymorphisms has not been universally adopted (23).

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are available for the thioguanine drug response, and the genes *TPMT* and *NUDT15*. The genes may be tested separately, or together, as part of a test panel that evaluates the drug response to thiopurines.

As with many commercial tests, only the most common variants are usually tested (e.g., for *TPMT*, the **2*, **3A*, and **3C* allele, which accounts for more than 90% of known inactivating alleles). This means that rare, or previously undiscovered variants, or both, will not be detected by variant-specific genotyping methods (9, 10, 24-27).

It is important to note that for *TPMT*3A*, 2 variants, c.460G>A and c.719A>G, are in *cis*. The variant, c.460G>A by itself is *TPMT*3B* and c.719A>G by itself is *TPMT*3C*. Most clinical laboratories are unable to phase the 2 variants. In most cases, especially if the individual is of European ancestry, the laboratory will assume the 2 variants are in *cis*, though the possibility of the variants being in *trans* cannot be ruled out.

Phenotype testing is also available for *TPMT*. Phenotype tests directly measure TPMT enzyme activity in red blood cells. In adult individuals taking thioguanine as an immunosuppressive agent, there is strong evidence of a near 100% concordance between phenotype and genotype testing. Inflammatory disease processes do not interfere with the accuracy of TPMT activity measurements if the blood sample is taken under standard conditions (e.g., not within 2 months of a blood transfusion) (11).

However, the FDA recommends considering all clinical information when interpreting results from phenotypic testing used to determine the level of thiopurine nucleotides or TPMT activity in erythrocytes. This is because some co-administered drugs can influence measurement of TPMT activity in blood, and blood from recent transfusions will misrepresent an individual's actual TPMT activity.

In individuals with leukemia, the concordance between *TPMT* phenotype and genotype is poor. By the time of diagnosis, red cell TPMT activity is typically greatly reduced because of atypical hematopoiesis. Therefore, phenotype testing may wrongly identify an individual as having a TPMT deficiency, e.g., an individual who has 2 functional copies of the *TPMT* gene (homozygous wild-type) may be determined as having only one functional copy and one nonfunctional variant (*TPMT* heterozygous); and an individual who is *TPMT* heterozygous may be wrongly determined to be *TPMT* homozygous (2 copies of nonfunctional *TPMT* variants) (28).

In addition, during the course of chemotherapy, *TPMT* phenotype testing may reveal excessively high TPMT activity. This is thought to be due to an excess of young red blood cells with their associated higher level of TPMT enzyme activity. Therefore, to avoid an incorrect TPMT status, genotype testing is recommended for individuals with leukemia (28).

Finally, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying individuals at risk of adverse reactions from thiopurine treatment, and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (12, 29-31).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants

2020 Statement from the US Food and Drug Administration (FDA):

Thioguanine, known chemically as 2-amino-1,7-dihydro-6H-purine-6-thione, is an analogue of the nucleic acid constituent guanine, and is closely related structurally and functionally to mercaptopurine.

[…]

Metabolism and Genetic Polymorphism

Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of mercaptopurine, accumulate excessive cellular concentrations of active 6-TGNs, and are at higher risk for severe myelosuppression. In a study of 1028 children with ALL, the approximate tolerated mercaptopurine dosage range for patients with TPMT and/or NUDT15 deficiency on mercaptopurine maintenance therapy (as a percentage of the planned dosage) was as follows: heterozygous for either TPMT or NUDT15, 50-90%; heterozygous for both TPMT and NUDT15, 30- 50%; homozygous for either TPMT or NUDT15, 5-10%.

Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the *TPMT* gene and have little or no TPMT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The *TPMT*2*, *TPMT*3A*, and *TPMT*3C* alleles account for about 95% of individuals with reduced levels of TPMT activity. NUDT15 deficiency is detected in <1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the *NUDT15* gene, and approximately 21% have one loss-of-function allele. The p.R139C variant of *NUDT15* (present on the *2 and *3 alleles) is the most commonly observed, but other less common loss-of-function *NUDT15* alleles have been observed.

Consider all clinical information when interpreting results from phenotypic testing used to determine the level of thiopurine nucleotides or TPMT activity in erythrocytes, since some coadministered drugs can influence measurement of TPMT activity in blood, and blood from recent transfusions will misrepresent a patient's actual TPMT activity.

[…]

Warnings

[…]

Evaluate patients with repeated severe myelosuppression for thiopurine S-methyltransferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency. TPMT genotyping or phenotyping (red blood cell TPMT activity) and *NUDT15* genotyping can identify patients who have reduced activity of these enzymes. Patients with homozygous TPMT or NUDT15 deficiency require substantial dosage reductions. Bone marrow suppression could be exacerbated by coadministration with drugs that inhibit TPMT, such as olsalazine, mesalazine, or sulphasalazine.

[…]

Laboratory Tests

Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities or repeated episodes of myelosuppression.

[…]

may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Drug Interactions

[…]

As there is in vitro evidence that aminosalicylate derivatives (e.g., olsalazine, mesalazine, or sulphasalazine) inhibit the TPMT enzyme, they should be administered with caution to patients receiving concurrent thioguanine therapy.

Dosage and Administration

[…]

Patients with homozygous deficiency of either TPMT or NUDT15 enzyme typically require 10% or less of the standard thioguanine dosage. Reduce initial dosage in patients who are known to have homozygous TPMT or NUDT15 deficiency. Most patients with heterozygous TPMT or NUDT15 deficiency tolerate recommended thioguanine doses, but some require dose reduction based on toxicities. Patients who are heterozygous for both TPMT and NUDT15 may require more substantial dosage reductions. Reduce the dosage based on tolerability.

Please review the complete therapeutic recommendations that are located here: (1).

2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

TPMT recommendation

If starting doses are already high (e.g., 75 mg/m^2 of mercaptopurine), as is true in some ALL treatment regimens, lower than normal starting doses should be considered in TPMT intermediate metabolizers and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers. This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL. Even at these markedly reduced dosages, erythrocyte TGN concentrations in TPMT poor metabolizers remain well above those tolerated and achieved by the majority of patients (who are TPMT normal metabolizers).

In some nonmalignant conditions, alternative agents may be chosen for TPMT intermediate or poor metabolizers rather than reduced doses of thiopurines; if thiopurines are used, full starting doses are recommended for TPMT normal metabolizers, reduced doses (30–80% of target dose) in TPMT intermediate metabolizers, and substantially reduced doses (or use of an alternative agent) in TPMT poor metabolizers.

Some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype, these recommendations apply regardless of the method used to assess TPMT status.

NUDT15 recommendation

Similar to TPMT, tolerated mercaptopurine dosage is also correlated with the number of nonfunctional alleles of the NUDT15 gene. In fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of TPMT vs. NUDT15 decreased function alleles, there remains a paucity of multi-ethnic studies examining both TPMT and NUDT15 variants.

Therefore, our NUDT15 recommendations parallel those for TPMT. For NUDT15 normal metabolizers (NUDT15*1/*1), starting doses do not need to be altered. For NUDT15 intermediate metabolizers (e.g., NUDT15*1/*3), reduced starting doses should be considered to minimize toxicity, particularly if the starting doses are high (e.g., 75 mg/m^2/day for mercaptopurine). For NUDT15 poor metabolizers (e.g., NUDT15*3/*3), substantially reduced doses (e.g.,10 mg/m^2/day of mercaptopurine) or the use of an alternative agent should be considered.

As for TPMT, there is substantial variability in the tolerated thiopurine dosages within NUDT15 intermediate metabolizers, with a minority of individuals who do not seem to require significant dose reduction. Therefore, genotype-guided prescribing recommendations apply primarily to starting doses; subsequent dosing adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines). In contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in NUDT15 poor metabolizers and pre-emptive dose reductions are strongly recommended.

The NUDT15 poor metabolizer phenotype is observed at a frequency of about 1 in every 50 patients of East Asian descent, which is more common than the TPMT poor metabolizer phenotype in Europeans, and, thus, genotyping NUDT15 in the Asian populations may be of particular clinical importance. NUDT15 deficiency is also more prevalent in individuals of Hispanic ethnicity, particularly those with high levels of Native American genetic ancestry.

Please review the complete therapeutic recommendations, which include CPIC's recommended course of action if both TPMT and NUDT15 genotypes are known, located here: (2).

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

TPMT Intermediate Metabolizer

The risk of serious adverse events such as myelosuppression is increased. The genetic variation increases the concentration of the active metabolites of thioguanine.

IMMUNOSUPPRESSION

- Start with 75% of the standard dose
- Adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and efficacy.

LEUKEMIA

- start with 75% of the standard thioguanine dose, or start with the standard dose and reduce to 75% if side effects necessitate a dose reduction
- It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.
  The initial dose should be adjusted based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also NUDT15 IM or NUDT15 PM.

TPMT Poor Metabolizer

The risk of serious, life-threatening adverse events such as myelosuppression is strongly increased. The genetic variation increases the concentration of the active metabolites of thioguanine.

- Choose an alternative or use 6-7% of the standard dose
  Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.
- If the dose is decreased: advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) develop.

NUDT15 Intermediate Metabolizer

Grade ≥ 2 leukopenia occurs in an estimated 40% of these patients with standard therapy. The genetic variation increases the concentration of the fully activated metabolite of thioguanine.

IMMUNOSUPPRESSION

- start with 75% of the standard dose
- Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.
- Monitoring should be performed at an increased frequency.

LEUKEMIA

- start with 75% of the standard thioguanine dose or start with the standard dose and reduce to 75% if side effects necessitate a dose reduction
- It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.
- Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.
- Monitoring should be performed at an increased frequency.

Note: more stringent dose reductions are necessary if the patient is also TPMT IM.

NUDT15 Poor Metabolizer

Grade ≥ 2 leukopenia occurs in an estimated 95% of these patients with standard therapy. The genetic variation increases the concentration of the fully activated metabolite of thioguanine.

- avoid thioguanine
- if it is not possible to avoid thioguanine: use 10% of the standard dose and advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur
- Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and efficacy.
- Monitoring should be performed at an increased frequency.

For more information about TPMT and NUDT15 phenotypes: see the general background information about TPMT and NUDT15 on the KNMP Knowledge Bank or on www.knmp.nl (search for TPMT or NUDT15).

Please review the complete therapeutic recommendations that are located here: (3, 4).

Nomenclature for Selected *TPMT* and *NUDT15* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains 2 variants in cis: c.460G>A and c.719A>G			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*NUDT15*3*	p.R139C c.415C>T	NM_018283.4:c.415C>T	NP_060753.1:p.Arg139Cys	rs116855232

Note: the p.R139C variant of nudix hydrolase 15 (*NUDT15)* is present on the *NUDT15*2* and *NUDT15*3* alleles.
The TPMT Nomenclature Committee defines the nomenclature and numbering of novel thiopurine methyltransferase *(TPMT)* variants.
Nomenclature for *NUDT15* is available from the Pharmacogene Variation (PharmVar) Consortium.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS)

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Thioridazine Therapy and *CYP2D6* Genotypes

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Introduction

Thioridazine is an antipsychotic used in the treatment of schizophrenia and psychosis. Its use is reserved for patients who have failed to respond to or cannot tolerate other antipsychotics.

Thioridazine has been shown to prolong the QT interval (the time taken for the heart ventricles to depolarize and repolarize) in a dose related manner. Drugs with this potential have been associated with the life-threatening ventricular tachycardia, "torsades de pointes".

The CYP2D6 enzyme is involved in metabolizing thioridazine. About 7% of the population has reduced enzyme activity because of variants in the *CYP2D6* gene. In individuals with low CYP2D6 activity, standard doses of thioridazine may lead to higher drug levels in the plasma, and increase the risk of cardiac arrhythmias.

The FDA-approved drug label for thioridazine states that thioridazine is contraindicated in individuals who are known to have reduced levels of CYP2D6 activity. The label also states it is contraindicated to coadminister thioridazine with drugs that inhibit CYP2D6 (e.g., fluoxetine, paroxetine) or inhibit the metabolism of thioridazine (e.g., fluvoxamine, propranolol, and pindolol) (1).

Drug Class: Antipsychotics

The first antipsychotics to be discovered in the 1950s were haloperidol and chlorpromazine, followed by other agents, including fluphenazine, loxapine, pherphenazine, pimozide, thioridazine, thiothixene, and trifluoperazine.

Known as "first generation" or "typical" antipsychotics, these drugs were used to treat psychosis (regardless of the cause), chronic psychotic disorders (e.g., schizophrenia), and other psychiatric conditions. However, prominent adverse effects included extrapyramidal side effects such as tardive dyskinesia, muscle rigidity, and tremors, i.e., Parkinsonian-like symptoms.

Newer antipsychotics, known as "second generation" or "atypical" antipsychotics, have a lower risk of extrapyramidal side effects. However, many have serious metabolic effects. These antipsychotics include aripiprazole, clozapine, iloperidone, olanzapine, and risperidone.

Drug: Thioridazine

Thioridazine is a first generation "typical" antipsychotic used in the treatment of schizophrenia. Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3–0.7% (2). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. Before the discovery of the first antipsychotics in the 1950s, the management of schizophrenia relied heavily upon sedation, electroconvulsive therapy, and institutionalization.

The symptoms of schizophrenia fall into three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

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Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

The use of thioridazine is reserved for patients who have failed to respond to or cannot tolerate the side effects of other antipsychotics. The FDA-approved drug label for thioridazine strongly recommends that prior to starting thioridazine, a patient should be given at least two trials, each with a different antipsychotic drug product, at an adequate dose, for an adequate duration of time. The label also states that for patients who do require chronic treatment with thioridazine, the smallest dose and the shortest duration of treatment should be sought and the need for continued treatment should be reassessed periodically; and cautions that the efficacy of thioridazine in treating patients with refractory schizophrenia is unknown (1).

The main action of both first-generation and second-generation antipsychotics appears to be the post-synaptic blockade of D2 dopamine receptors in the brain. (An exception is aripiprazole, which is a D2 partial agonist.) Blockade of the D2 receptor in the brain's limbic system is thought to improve the "positive" symptoms of schizophrenia (3).

However, because the first-generation antipsychotics also block dopamine receptors in the nigrostriatal pathway, they cause movement disorders known as extrapyramidal side effects. These disorders include akathisia (motor restlessness), dystonia (abnormal muscle tone), and tardive dyskinesia (involuntary and repetitive movements).

Compared to other first generation antipsychotics, thioridazine shares a similar efficacy, but has a lower risk of extrapyramidal side effects (4, 5, 6). However, a higher level of EKG changes is associated with thioridazine therapy (6).

Antipsychotics, and thioridazine in particular, can inhibit cardiac ion channels. Most first generation antipsychotics block the cardiac potassium channel KCNH2, previously known as the human ether-a-go-go-related gene (hERG) (7, 8). Blockade of this channel reduces inward potassium current, resulting in longer cardiac repolarization times. On the EKG, this manifests as a prolonged QT interval. In extreme cases, this can lead to a life-threatening ventricular tachycardia known as torsades de pointes ("twisting of the points") (9, 10, 11).

At one point, thioridazine was one of the most commonly used medications for major mental health disorders. However, numerous case reports of sudden, unexpected death led to label changes in 2000, which recommended that thioridazine be used as a last resort (12). In 2005, the manufacturer Novartis discontinued the branded form of thioridazine because of its association with QT prolongation, but generic forms are still available in the US (13).

Thioridazine is metabolized by CYP2D6 to the active metabolite mesoridazine, which is further metabolized to sulforidazine, both of which are more potent than thioridazine. In addition, both thioridazine and mesoridazine have similar effects on the QT interval (14, 15).

Recent research has found that thioridazine is active against multidrug resistant tuberculosis, when used in combination with other antituberculosis drugs. Thioridazine increases the permeability of the cell-envelope, enabling the enhanced uptake of antibiotics (16).

The FDA drug label states that no teratogenic effect has been shown with thioridazine to date. However, all drugs should be kept to a minimum during pregnancy, so thioridazine should be given only when the benefits

exceed the possible risks to mother and fetus. Of note, neonates exposed to antipsychotic drugs during the third trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery which vary in severity; while in some cases symptoms have been self-limited, in other cases neonates have required intensive care unit support and prolonged hospitalization.

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is highly polymorphic; over 100 star (*) alleles are described and currently catalogued at the Pharmacogene Variation (PharmVar) database (17). *CYP2D6*1* is the reference (or wild-type) allele encoding enzyme with normal activity. The *CYP2D6*2*, **33*, and **35* alleles are also considered to confer normal activity (Table 1).

Table 1. Activity status of selected *CYP2D6* alleles

Allele type	*CYP2D6* Alleles
Normal function	**1, *2, *33, *35*
Decreased function	**9, *10, *17, *29, *41*
No function	**3-*8, *11-*16, *19-*21, *38, *40, *42*

For a detailed list of *CYP2D6* alleles, please see (5).

An activity score can be assigned to each *CYP2D6* allele, e.g., 1 for each functional allele, 0.5 for a decreased function allele, and 0 for a no function allele. Individuals who carry more than two normal function copies (e.g., multiple copies) of the *CYP2D6* gene are "ultrarapid metabolizers", whereas individuals who are "normal metabolizers" either carry two normal function copies of *CYP2D6*, or a combination of normal/decreased/no function alleles that result in an activity score between 1.0 and 2.0. Individuals who are intermediate or poor metabolizers carry copies of decreased or no function *CYP2D6* alleles, respectively (Table 2).

Table 2. 2016 Assignment of *CYP2D6* phenotypes by CPIC

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 Ultrarapid metabolizer (approximately 1-20% of patients)[a]	Greater than 2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN* *(*1/*2)xN* *(*2/*2)xN* [b]
CYP2D6 Normal metabolizer (approximately 72-88% of patients)	1.0 – 2.0[c]	An individual carrying two normal function alleles or two decreased function alleles or one normal and no function allele or one normal function and decreased function allele or combinations of duplicated alleles that result in an activity score of 1.0 to 2.0	**1/*1* **1/*2* **2/*2* **1/*9* **1/*41* **41/*41* **1/*5* **1/*4*
CYP2D6 Intermediate metabolizer (approximately 1-13% of patients)	0.5	An individual carrying one decreased function and one no function allele	**4/*41* **5/*9* **4/*10*

Table 2. continued from previous page.

Phenotype	Activity Score	Genotypes	Examples of diplotypes
CYP2D6 Poor metabolizer (approximately 1-10% of patients)	0	An individual carrying two no function alleles	**4/*4* **4/*4xN* **3/*4* **5/*5* **5/*6*

[a] For population-specific allele and phenotype frequencies, please see
[b] Where *xN* represents the number of *CYP2D6* gene copies (N is 2 or more).
[c] Patients with an activity core of 1.0 may be classified as intermediate metabolizers by some reference laboratories.
For more information about activity scores, please see the Genetic Testing section.
This table has been adapted from Hicks J.K., Sangkuhl K., Swen J.J., Ellingrod V.L., Müller D.J., Shimoda K., Bishop J.R., Kharasch E.D., Skaar T.C., Gaedigk A., Dunnenberger H.M., Klein T.E., Caudle K.E. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC®) for *CYP2D6* and *CYP2C19* Genotypes and Dosing of Tricyclic Antidepressants: 2016 Update. Clinical pharmacology and therapeutics. 2016 Dec 20 [Epub ahead of print] (18).

The most common no function alleles include *CYP2D6*3*, **4*, **5*, and **6* (19, 20, 21, 22), and the most common decreased function alleles include *CYP2D6*9, *10, *17, *29* and **41* (23, 24, 25, 26, 27) .There are large inter-ethnic differences in the frequency of these alleles. For example, *CYP2D6*4* is the most common no function allele in Caucasians, but is less abundant in subjects with African ancestry, and is rare in Asians. In contrast, the decreased function allele *CYP2D6*10* is the most common allele in Asians, and *CYP2D6*17* is almost exclusively found in individuals with African ancestry (28).

Consequently, the phenotype frequencies also vary substantially among the major ethnicities and may vary among populations. Approximately 6-10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent no function *CYP2D6*4* and **5* alleles (29, 30).

In individuals who are *CYP2D6* poor metabolizers, standard doses of thioridazine may lead to the drug accumulating in the plasma. Since a dose-related side effect of thioridazine is prolongation of the QTc interval, which is a potentially life threatening event, the FDA has stated that the use of thioridazine is contraindicated in individuals who are known to have reduced CYP2D6 activity (1, 31). In addition, the label also states it is contraindicated to coadminister thioridazine with other drugs that inhibit CYP2D6 activity (e.g., the antidepressants fluoxetine and paroxetine) or inhibit the metabolism of thioridazine (e.g., the beta-blockers propranolol and pindolol, and the antidepressant fluvoxamine) (1).

Genetic Testing

The NIH's Genetic Testing Registry, GTR, provides examples of the genetic tests that are currently available for the thioridazine response and the CYP2D6 gene.

Results are typically reported as a diplotype, such as *CYP2D6 *1/*1*. A result for copy number, if available, is also important when interpreting *CYP2D6* results (32). However, it needs to be noted that the number of variants tested varies substantially among laboratories and there is no standardized way to report results (33).

If the test results include an interpretation of the patient's predicted metabolizer phenotype, this should be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1 for each copy of a normal function allele). The phenotype is defined by the sum of the two scores:

- An extensive (normal) metabolizer phenotype has an activity score of 1 to 2
- An intermediate metabolizer has an activity score of 0.5
- A poor metabolizer has an activity score of 0
- An ultrarapid metabolizer has an activity score of greater than 2 (18, 34)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA): Reduced cytochrome P450 2D6 isozyme activity drugs that inhibit this isozyme (e.g., fluoxetine and paroxetine) and certain other drugs (e.g., fluvoxamine, propranolol, and pindolol) appear to appreciably inhibit the metabolism of thioridazine. The resulting elevated levels of thioridazine would be expected to augment the prolongation of the QTc interval associated with thioridazine and may increase the risk of serious, potentially fatal, cardiac arrhythmias, such as Torsades de pointes type arrhythmias. Such an increased risk may result also from the additive effect of coadministering thioridazine with other agents that prolong the QTc interval. Therefore, thioridazine is contraindicated with these drugs as well as in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of P450 2D6.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature

Nomenclature of selected *CYP2D6* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.5:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Variant results in a whole gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly • CYP2D6T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.5:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
*CYP2D6*41*	2850C>T[2] (Cys296Arg)	NM_000106.5:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.5:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725

[1] In the literature, 1023C>T is also referred to as 1111C>T, and 2850C>T is also referred to 2938C>T.
[2] In the literature, 2850C>T is also referred to as 2938C>T.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation Consortium (PharmVar) https://www.pharmvar.org/.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

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Tramadol Therapy and *CYP2D6* Genotype

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Introduction

Tramadol (brand names ConZip, Ultram, UltramER, Odolo) is an analgesic used to treat moderate to severe pain. It is used for a variety of pain conditions, including post-operative pain, cancer pain, and musculoskeletal pain. Tramadol is a centrally acting opioid analgesic with mu-opioid binding activity as well as weak inhibition of reuptake of norepinephrine and serotonin.

The CYP2D6 enzyme converts tramadol to the active metabolite, O-desmethyltramadol (M1), which has a significantly higher affinity for the mu-opioid receptor than tramadol. The M1 metabolite is up to 6 times more potent than tramadol in producing analgesia.

Individuals who have reduced CYP2D6 activity are known as "intermediate metabolizers" and those with absent CYP2D6 activity are known as "poor metabolizers." The standard recommended doses of tramadol may not provide adequate pain relief in these individuals because of reduced levels of M1. Whereas in individuals who have increased CYP2D6 activity ("ultrarapid metabolizers"), standard doses of tramadol may result in a higher risk of adverse events because of increased exposure to M1.

The 2021 FDA-approved drug label warns that individuals who are ultrarapid metabolizers (UMs) should not use tramadol because of the risk of life-threatening respiratory depression and signs of opiate overdose (for example, extreme sleepiness, confusion, or shallow breathing) (Table 1) (1).

The prevalence of CYP2D6 UM varies but is thought to be present in approximately 1–10% of Caucasians (European, North American), 3–4% of Blacks (African Americans), and 1–2% of East Asians (Chinese, Japanese, Korean). The frequency of UM phenotype has been reported to be even higher in some groups, including Ashkenazi Jews and regional populations in the Middle East.

Furthermore, tramadol is not recommended in nursing mothers due to the potential exposure to high levels of M1 causing life-threatening respiratory depression, if the mother is a UM. At least one death was reported in a nursing infant who was exposed to high levels of morphine in breast milk because the mother was an UM of codeine, which—similar to tramadol—is activated by CYP2D6 metabolism.

Tramadol is contraindicated for all children younger than age 12 and for all individuals under the age of 18 when being used for post-operative analgesia following tonsillectomy or adenoidectomy, or both. The label warns that life-threatening respiratory depression and death have occurred in children who received tramadol, and in at least one case, the child was an UM of tramadol.

The Clinical Pharmacogenetics Implementation Consortium (CPIC) recommends that for an individual identified as a CYP2D6 UM, a different non-CYP2D6 dependent analgesic should be used to avoid the risk of severe toxicity with standard dosing of tramadol. The CPIC also recommends avoiding tramadol in individuals identified as CYP2D6 poor metabolizers (PMs) due to the possibility of lack of effect (Table 2) (2).

The Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) provides dosing recommendations for tramadol based on CYP2D6 genotype (Table 3). The DPWG states it is not possible to calculate a dose adjustment for tramadol, because when the ratio of tramadol

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and M1 is altered, the nature and total analgesic effect of tramadol also changes. For CYP2D6 UM, DPWG recommends selecting an alternative drug to tramadol - but not codeine, which is also metabolized by CYP2D6. Alternative drugs include morphine (not metabolized by CYP2D6) and oxycodone (which is metabolized by CYP2D6 to a limited extent, but this does not result in differences in side effects in clinical practice). For CYP2D6 poor (PM) and intermediate metabolizers (IM), DPWG recommends increasing the dose of tramadol, and if this does not have the desired effect, selecting an alternative drug (not codeine) (Table 3) (3).

Table 1. The FDA Tramadol Dosing Recommendation based on CYP2D6 Genotype (2021)

	CYP2D6 ultrarapid metabolizers
All ultrarapid metabolizer individuals	Some individuals may be ultrarapid metabolizers because of a specific CYP2D6 genotype. These individuals convert tramadol into its active metabolite, O-desmethyltramadol (M1), more rapidly and completely than other people. This rapid conversion results in higher than expected serum M1 levels. Even at labeled dosage regimens, individuals who are ultrarapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose (such as extreme sleepiness, confusion, or shallow breathing). Therefore, individuals who are ultrarapid metabolizers should not use tramadol hydrochloride tablets.
Ultrarapid metabolizer children	Life-threatening respiratory depression and death have occurred in children who received tramadol. Some of the reported cases followed tonsillectomy or adenoidectomy, or both; in at least one case, the child had evidence of being an ultrarapid metabolizer of tramadol due to a CYP2D6 polymorphism. Tramadol hydrochloride is contraindicated in children younger than 12 years of age and in children younger than 18 years of age following tonsillectomy or adenoidectomy, or both. Avoid the use of tramadol hydrochloride tablets in adolescents 12–18 years of age who have other risk factors that may increase their sensitivity to the respiratory depressant effects of tramadol
Infants nursing from ultrarapid metabolizer mothers	Tramadol is subject to the same polymorphic metabolism as codeine, with ultrarapid metabolizers of CYP2D6 substrates being potentially exposed to life-threatening levels of the active metabolite O-desmethyltramadol (M1). At least one death was reported in a nursing infant who was exposed to high levels of morphine in breast milk because the mother was an ultrarapid metabolizer of codeine. A baby nursing from an ultrarapid metabolizer mother taking tramadol hydrochloride tablets could potentially be exposed to high levels of M1, and experience life-threatening respiratory depression. For this reason, breastfeeding is not recommended during treatment with tramadol hydrochloride tablets.

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

Table 2. The CPIC Tramadol Therapy Recommendations Based on CYP2D6 Phenotype (2021)

Phenotype[a]	Activity score[b]	Implications	Genotype	Examples of diplotypes[b]	Recommendations for tramadol therapy[d]
Ultrarapid metabolizer	> 2.25	Increased formation of O-desmethyltramadol (active metabolite) leading to higher risk of toxicity	More than 2 copies of normal-function alleles	**1/*1xN*[c] **1/*2xN*	Avoid tramadol use because of potential for toxicity. If opioid use is warranted, consider a non-codeine opioid.
Normal metabolizer	1.25–2.25*	Expected O-desmethyltramadol (active metabolite) formation	2 normal-function alleles, or one normal-function allele and one decreased-function allele, or combinations of duplicated alleles that result in an activity score of 1.25–2.25	**1/*10* **1/*41* **1/*9* **10/*41x3* **1/*1* **1/*2* **2x2/*10*	Use tramadol label-recommended age- or weight-specific dosing.

Table 2. continued from previous page.

Phenotype[a]	Activity score[b]	Implications	Genotype	Examples of diplotypes[b]	Recommendations for tramadol therapy[d]
Intermediate metabolizer	0.25–1*	Reduced O-desmethyltramadol (active metabolite) formation.	One decreased-function allele and one no-function allele, or 2 decreased-function alleles	**4/*10* **4/*41* **10/*10* **10/*41* **41/*41* **1/*5*	Use tramadol label-recommended age or weight-specific dosing. If no response and opioid use is warranted, consider a non-codeine opioid
Poor metabolizer	0	Greatly reduced O-desmethyltramadol (active metabolite) formation leading to diminished analgesia.	2 no-function alleles	**3/*4* **4/*4* **5/*5* **5/*6*	Avoid tramadol use because of possibility of diminished analgesia. If opioid use is warranted, consider a non-codeine option.
Indeterminant metabolizer	n/a	n/a	An individual having one or 2 uncertain-function alleles	**1/*22* **1/*25* **22/*25*	No recommendation

[a] See the *CYP2D6* Frequency Table for race-specific allele and phenotype frequencies from PharmGKB and CPIC.
[b] Assignment of allele function and allele activity values including citations for allele function can be found at PharmGKB (*CYP2D6* Allele Definition Table and *CYP2D6* Allele Functionality Table) and CPIC.
For a complete list of *CYP2D6* diplotypes and resulting phenotypes, see the *CYP2D6* Genotype to Phenotype Table at PharmGKB and CPIC.
[C] Where xN represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see supplemental data in (2) for additional information on how to translate diplotypes into phenotypes.
[d] The strength of therapeutic recommendations is "moderate" for intermediate metabolizers, and "strong" for all other metabolizers.
Table is adapted from (2).

Table 3. The DPWG Recommendations for Tramadol Dosing based on CYP2D6 Phenotype (2017)

CYP2D6 phenotype	Dosing recommendations
Poor metabolizer (gene dose 0, absent enzyme activity)	It is not possible to provide a recommendation for dose adjustment, because the total analgesic effect changes when the ratio between the mother compound and the active metabolite changes. 1. be alert to a reduced effectiveness 2. in the case of inadequate effectiveness: - try a dose increase. - if this does not work, choose an alternative* 3. if no alternative is selected: advise the individual to report inadequate analgesia
Intermediate metabolizer (gene dose 0.5-1, decreased enzyme activity)	
Ultrarapid metabolizer (gene dose ≥ 3) (enhanced enzyme activity)	As the total analgesic effect changes when the ratio between the mother compound and the active metabolite changes, the effect of a dose reduction cannot be predicted with certainty. 1. select an alternative* 2. if an alternative is not possible: - use 40% of the standard dose - advise the individual to report side effects (such as drowsiness, confusion, constipation, nausea and vomiting, respiratory depression or urine retention).

* Do not select codeine, as this is also metabolized by CYP2D6. Morphine is not metabolized by CYP2D6. Oxycodone is metabolized by CYP2D6 to a limited extent, but this does not result in differences in analgesia and side effects in clinical practice.
This table is adapted from (3).

Drug: Tramadol

Tramadol is a synthetic opioid used to treat moderate to severe pain. Tramadol is used for both acute and chronic pain, and is commonly prescribed for post-operative pain, pain caused by cancer, and musculoskeletal

pain. In the USA, tramadol is classified as a Schedule IV controlled substance (1, 4). Drugs and other substances that are considered controlled substances under the Controlled Substances Act (CSA) are divided into 5 schedules based on whether they have an accepted medical indication and the potential for abuse or addiction. Schedule II drugs have a high potential for abuse that may lead to severe psychological or physical dependence, and schedule III have a lower potential for abuse. Schedule IV drugs have even further reduced potential for abuse relative to schedule III. (5)

Tramadol is structurally similar to codeine and morphine. Opioid prescriptions are common in the USA. One study estimated that between 2011 and 2012, nearly 7% of the adult population had taken an opioid in the 30 days before participation in the study (6). Opioid prescribing rates peaked in the USA in 2012, with over 255 million prescriptions annually and a prescribing rate of 81.3 prescriptions per 100 persons. Prescribing rates have consistently declined and in 2018, 51.4 prescriptions were written per 100 persons. (7)

As an opioid, tramadol is relatively weak. However, it is thought to have a unique mechanism of action involving different targets. Tramadol is administered as a racemic mixture of 2 enantiomers, (+) tramadol and (-) tramadol, which inhibit pain transmission at the spinal cord by inhibiting serotonin reuptake (+ tramadol) and noradrenaline reuptake (- tramadol). (2)

Both tramadol and its major active metabolite, M1, bind to the mu-opioid receptor. However, M1 has a significantly higher affinity (approximately 200 times greater) for the opioid receptor than tramadol and is thus more potent at providing analgesia. However, tramadol and its metabolites are proposed to exert their antinociceptive effect via a multimodal mechanism that includes serotonin and norepinephrine reuptake inhibition, as well as binding to the mu-opioid receptor. (8, 9, 10, 11, 12, 13)

Tramadol requires bioactivation in the liver to be converted to M1 and exert its full analgesic effect. This process is primarily mediated by the CYP2D6 enzyme, and the level of activity of the CYP2D6 enzyme influences the plasma concentration of M1. Other CYP enzymes (CYP2B6 and CYP3A4) catalyze the production of N-desmethyl tramadol (M2), an inactive metabolite (14). Other enzymes are involved in further metabolism, pharmacokinetics and pharmacodynamics of tramadol, but the clinical relevance of variants in those genes has yet to be determined (15).

Adverse effects of tramadol therapy include dizziness, nausea, constipation, and headache. Owing to its effects on serotonin and norepinephrine reuptake, additional risks of tramadol therapy include the risk of seizures, suicidal tendencies, and serotonin syndrome. Seizure risk is especially notable in individuals who are already taking antidepressants or other drugs that decrease the seizure threshold. There is also an increased risk of suicide, therefore tramadol should not be prescribed for individuals who are suicidal or prone to addictions—the use of non-narcotic analgesics should be considered instead (1). Serotonin syndrome is a potentially life-threatening syndrome that may occur with the use of tramadol, especially if other serotonergic medications such as antidepressants (selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, tricyclic antidepressants) or other drugs that impair the metabolism of tramadol are used concurrently (for example, CYP2D6 and CYP3A4 inhibitors such as amiodarone, quinidine, erythromycin, or ritonavir). Symptoms of serotonin syndrome can variably include changes in mental status (for example, agitation, hallucinations, coma), autonomic instability (for example, tachycardia, labile blood pressure, hyperthermia), neuromuscular aberrations (such as hyperreflexia, incoordination) and gastrointestinal symptoms (for example, nausea, vomiting, diarrhea) (1, 16).

Because tramadol has mu-opioid agonist activity, there is a risk of abuse and addiction, even under appropriate medical use. Therefore, as for all individuals treated with opioids, there should be careful monitoring of individuals taking tramadol. The longer an individual is on continuous tramadol therapy, the greater the risk of tolerance (the need to increase the dose of drug to maintain a defined level of analgesia in the absence of disease progression). Physical dependence upon tramadol is manifested by withdrawal symptoms after the use of

tramadol is stopped abruptly. Symptoms include restlessness, rhinorrhea, lacrimation, and chills (1, 12). Concomitant medication with benzodiazepines or other CNS depressants is also discouraged due to the heightened risk of profound sedation, respiratory depression, coma, and death. Consider naloxone or nalmefene —opioid antagonists—prescription for emergency treatment of opioid overdose causing respiratory depression, but be aware that naloxone administration may increase the risk of seizure (1).

The drug label warns that women of reproductive age should be informed that tramadol can cause fetal harm, and women should inform their healthcare provider if they suspect or know they are pregnant. The prolonged use of tramadol during pregnancy can result in neonatal opioid withdrawal syndrome, which may be life threatening if not recognized and treated. The signs are diverse, and include feeding difficulties, breathing problems, and seizures. Additionally, chronic use of opioids can reduce fertility in both females and males of reproductive potential; these effects may or may not be reversible (1). Breastfeeding is not recommended during tramadol treatment (1, 17).

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in decreased, absent, or increased enzyme activity.

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers (18).

The *CYP2D6* Alleles

The *CYP2D6* gene is highly polymorphic, as over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, or absent enzyme function (Table 4). (19)

The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (for example, *CYP2D6 *4/*4*). Based on function, each allele can be assigned an activity score from 0 to 1, which in turn is often used to assign a phenotype (for example, CYP2D6 PM). However, the activity score system is not standardized across all clinical laboratories or *CYP2D6* genotyping platforms. The CPIC revised their activity scoring guidelines in October 2019 to promote harmonization. The CYP2D6 phenotype is defined by the sum of the 2 activity scores, which is usually in the range of 0 to 3.0: (20)

- An ultrarapid metabolizer (UM) has an activity score greater than 2.25
- A normal metabolizer phenotype (NM) has an activity score of 1.25–2.25
- An intermediate metabolizer (IM) has an activity score of >0–<1.25
- A poor metabolizer (PM) has an activity score of 0

Table 4. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See PharmVar. Activity scores from (2).

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. The *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity.

Other *CYP2D6* alleles include variants that produce a non-functioning enzyme (for example, **3*, **4*, **5*, and **6*) (21, 22, 23, 24, 25) or an enzyme with decreased activity (for example, **10*, **17*, and **41*) PharmGKB [Internet]. Palo Alto (CA): Stanford University. Haplotype CYP2D6*10} (see Table 4). There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in individuals with European ancestry, **17* more common in Africans, and **10* more common in Asians. (26)

Larger structural variants at the *CYP2D6* locus have also been described, including gene duplications, deletions, tandem alleles, and gene conversions. As one might expect, deletions result in a no-function allele (for example, the **5* allele is a deletion). Duplications have been reported for alleles with normal function and decreased function, as well. In the case of allele duplications, the activity scores for the full complement of *CYP2D6* alleles are summed to determine the predicted metabolizer phenotype. Additional details on structural variants are available from PharmVar (see the Structural Variation for CYP2D6 document).

Allele Frequencies Vary between Populations

Among Asians and in individuals of Asian descent, only approximately 50% of *CYPD6* alleles are of normal function, and the frequency of *CYP2D6* duplications is as high as 45%, although this may have been overestimated by not accounting for tandem hybrid alleles (for example, **36+*10*). (27) Other studies on a USA population suggested less than 50% of alleles detected within Asian-descent individuals are normal-function alleles in a single copy, with 30% of alleles arising from structural variants (duplications or deletions). (28) Common no-function variants are *CYP2D6*36* and *CYP2D6*4.* (28) Both these alleles contain the variant "c.100C>T", which if present alone, results in *CYP2D6*10* (see Nomenclature table). (26, 27, 29, 30) The *CYP2D6*36* allele is the result of a gene conversion event with the pseudogene *CYP2D7* (19). This no-function allele is most commonly found in individuals of Asian ancestry (28).

Among Africans and African Americans, only approximately 50% of *CYP2D6* alleles are normal function. (21, 26, 31, 32) African Americans also have been found to have a higher frequency of no-function structural variants or decreased-function single-copy variant alleles versus Caucasian or Hispanic Americans. (28)

Middle-Eastern countries show a great diversity in phenotypic and allelic distribution for *CYP2D6* (33), though on average, these individuals show a lower frequency of PM phenotypes (0.91%) and higher ultrarapid phenotypes (11.2%) than other ethnicities (Note: Oceania and Middle-Eastern ethnicities are combined in this study). (19)

Among European countries, there is diversity of allelic distribution. Gene duplications were more common in the south-eastern countries (Greece, Turkey: 6%) and less common in northern countries (Sweden and Denmark, <1%). Meanwhile, *CYP2D6*4* and *CYP2D6*5* alleles were more common in the north and less common in the south. (34) Worldwide *CYP2D6* genotype and phenotype frequencies have been catalogued and recently published (35).

CYP2D6 Phenotype

***CYP2D6* Phenotype Frequencies Vary between Populations**

Normal metabolizers: Between 43–67% of individuals have 2 normal-function alleles (**1* or **2*), or one normal-function allele and one decreased-function allele, resulting in a "normal metabolizer" phenotype based on the CPIC/PharmGKB activity scores (18). These individuals are most likely to have a phenotypically normal response to tramadol. However, there is a large amount of variability in tramadol response within individuals

genotyped as normal metabolizers (NMs), and the causes of this variation, among individuals with the same diplotype, are unknown. (36)

Intermediate metabolizers: Between 10–44% of individuals are IMs—they have either 2 decreased-function alleles or one normal- or decreased-function and one no-function allele. (35, 37) These individuals may not respond as well to tramadol because the metabolism of tramadol to M1 is reduced. A study of a diverse USA urban population of children found that roughly 8% of subjects were IMs, though this may be higher due to the broader range for IM activity scores. (38) Within the USA, it has been observed that individuals of African or Asian descent were most likely to be classified as IMs (20–28% of population by ethnicity). (28) Similarly, PharmGKB reports that the highest frequency of IM activity scores are found in Sub-Saharan-African and East-Asian populations (37).

Poor metabolizers: Between 0.4–6.5% of individuals are PMs—they have 2 no-function alleles. (18, 32) In these individuals, tramadol will provide little or no pain relief. Poor metabolizers are more commonly found in European Caucasians and their descendants. The no-function *CYP2D6*4* and **5* alleles largely account for the PM phenotype in these populations (23, 24, 39). It should be noted that the frequency of PMs can be much lower in certain populations including East Asian, Oceania and Middle-Eastern (19). Studies of USA multi-ethnic populations have estimated the prevalence of PMs to be between 1.5–5.7% (28, 38).

Ultrarapid metabolizers: Individuals who are UMs have an enzyme activity score greater than 2.25, often due to an increased copy number of the *CYP2D6* gene. The UM phenotype has been estimated to be present in 1–2% of individuals, but the prevalence varies widely in different populations. It is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (32, 40). PharmGKB reports that the Oceanian population has the highest prevalence of UM phenotype (18). Ultrarapid metabolizers made up 9% of subjects in an urban multi-ethnic population with a large portion of Hispanic/Latino subjects (38). A larger study of USA individuals predicted an UM phenotype in only 2.2% of individuals, regardless of ethnicity (28).

Pharmacologic Conversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and thus the metabolizer phenotype of any individual. Administration of multiple drugs, sometimes called polypharmacy or co-medication, can lead to a phenomenon called phenoconversion whereby an individual with one metabolizer genotype can have enzymatic activity of a different metabolizer group (higher or lower, depending on the medications). Enzymatic activity of CYP2D6 can be inhibited or reduced by medications including duloxetine, paroxetine, fluoxetine, bupropion, amiodarone (note: this is a weak inhibitor), and quinidine (40, 41, 42, 43). This can result in NMs or IMs responding to medications as if they were PMs. Thus, co-medication with multiple CYP2D6 substrates may result in reduced metabolism of these drugs. In the case of tramadol, this may present as reduced analgesic effect. In contrast, discontinuing a co-medication can increase the rate of CYP2D6 metabolism to the genotype predicted activity level.

Other Genes of Note

OPRM1

The mu-opioid receptor is encoded by the *OPRM1* gene. The mu-opioid receptor is a G-coupled protein receptor and is a key signal transducer for the desired analgesic effect of opioids such as tramadol and codeine. There are more than 200 known variant alleles of *OPRM1*, and some variants have been suggested to have a role in opioid response or predisposition to opioid use disorders (44, 45). However, CPIC's expert review found inconsistent evidence linking any of these alleles to post-operative dose requirements for some opioids and the effect on morphine dose adjustment was deemed not to be clinically actionable (2).

COMT

Catechol-o-methyltransferase (COMT) is an enzyme involved in the methylation and degradation of adrenaline, noradrenaline, and dopamine. This enzyme regulates the concentration of catecholamines and thus is a key regulator of the pain perception pathways (46). The variant rs4680 (p.Val158Met) in *COMT* has been suggested to result in decreased levels of methylation activity (2, 46). However, CPIC's review found variable evidence associating this variant with analgesia response or opioid dose requirements and thus makes no recommendations based on *COMT* genotype (2).

CYP2B6 and *CYP3A4/5*

Other cytochrome P450 enzymes are involved in the metabolism of tramadol. The formation of the inactive metabolite M2 is mediated by CYP3A4 and CYP2B6. Variants leading to reduced function of these enzymes may lead to increased levels of tramadol, though there are no specific prescribing recommendations based on the presence of these alleles. (11, 47)

Linking Gene Variation with Treatment Response

It has been established that genetic variation in the *CYP2D6* gene can be responsible for the variability in CYP2D6 enzyme expression, and consequently, for variability in an individual's analgesic response to tramadol. Individuals with normal levels of CYP2D6 activity ("CYP2D6 normal metabolizers") are mostly likely to benefit from tramadol therapy (48).

The standard recommended doses of tramadol may lead to severe adverse effects in individuals who have increased CYP2D6 activity. In these individuals, tramadol is converted to M1 more rapidly and completely, leading to higher than expected levels of M1 and a higher risk of adverse events. In contrast, individuals who have absent CYP2D6 enzyme activity may have little analgesic effect from standard doses of tramadol and may request a stronger opioid. This is not drug seeking; this is an inability to benefit from tramadol therapy because of an inability to produce adequate levels of M1.

Therefore, clinicians should consider CYP2D6 testing in individuals who either have a minimal response to standard doses of tramadol (possible CYP2D6 PMs), or who have unexpected adverse effects (possible CYP2D6 UMs) (45, 49, 50). Several studies have investigated the feasibility and impact of pharmacogenetic testing for *CYP2D6* variation and have concluded that—in multiple care settings—this is both feasible and has the potential to improve pain management while decreasing adverse reactions (42, 51, 52, 53).

Tramadol should not be used in individuals with increased CYP2D6 activity. Even at labeled dosage regimens, individuals (especially children) may have life-threatening or fatal respiratory depression, or experience the signs of opiate overdose, such as extreme sleepiness, confusion, and shallow breathing.

There have been several cases of respiratory depression and death of children who have received tramadol. A case report describes a child with the *CYP2D6* ultrarapid genotype, who had severe respiratory depression after taking tramadol for pain relief following a tonsillectomy, a day case procedure (54, 55, 56).

Because of the risks of respiratory depression, tramadol is contraindicated for all children younger than age 12 years of age and is contraindicated in children of any age undergoing tonsillectomy or adenoidectomy, or both. Tramadol should also be avoided in children who are obese, have obstructive sleep apnea, have severe lung disease, or any other risk factors that may increase their sensitivity to the respiratory depressant effects of tramadol. This warning is similar to the drug label warning for codeine - a structurally similar opioid that is also bioactivated by CYP2D6.

For CYP2D6 intermediate and PMs, an increased dose of tramadol may be required. Alternatively, a different analgesic (a non-CYP2D6-dependent opioid or a non opioid) may be more appropriate. However, codeine as well as hydrocodone are not suitable alternatives to tramadol because they are also metabolized by CYP2D6. Oxycodone is to a lesser degree metabolized by CYP2D6 (it is primarily metabolized by CYP3A4), but neither the CPIC nor the DPWG find sufficient evidence to support a *CYP2D6* genotype correlation with altered analgesia or side effects, thus oxycodone can be considered as an alternate opioid. Opioids that are not primarily metabolized by CYP2D6 include morphine, oxymorphone, buprenorphine, fentanyl, methadone, and hydromorphone. (6, 12, 31, 45, 57, 58, 59, 60, 61, 62, 63, 64)

The enzymatic activity of CYP2D6 can also be affected by co-medications. The FDA-approved drug label does not specifically recommend adjusting dosage, but encourages close monitoring of individuals taking CYP2D6 inhibitors. (1) One recent study reported a higher incidence of breakthrough pain requiring additional medication in the hospital setting for tramadol individuals also taking fluoxetine and bupropion (65).

The *CYP2D6* Gene Interactions with Medications Used for Additional Indications

The CYP family of enzymes is involved in metabolism of many substances and CYP2D6 especially has been implicated in altered pharmacologic responses for many compounds. The drugs can be categorized into many different classes:

- Antipsychotics—for example, aripiprazole, risperidone, thioridazine and—to a lesser extent—clozapine is metabolized by CYP2D6. According to the FDA, aripiprazole dosage should be reduced for PMs and thioridazine is contraindicated for individuals who are known to have reduced CYP2D6 activity due to increased risk of potentially fatal side effects. Ultrarapid metabolizers may have a decreased plasma concentration of risperidone.
- Tricyclic antidepressants—for example, amitriptyline, and imipramine may require dosage adjustments, potentially guided by therapeutic drug monitoring, to achieve the desired therapeutic range in UMs or PMs. Ultimately, tricyclic antidepressants may be ineffective in CYP2D6 UMs
- Serotonin and norepinephrine reuptake inhibitors—for example atomoxetine and venlafaxine may have reduced efficacy in UMs at standard doses while PMs are at risk of elevated plasma concentrations for both medications. The DPWG advises against use of venlafaxine in CYP2D6 PMs and IMs.
- Cardiovascular dysfunction—for example, carvedilol, metoprolol, and propafenone are all metabolized by CYP2D6 and PMs will have higher plasma concentrations of these medications compared with NMs resulting in potentially undesired side effects or (in the case of metoprolol) extensive slowing of the heart rate.
- Anti-cancer medications—for example, tamoxifen is activated by CYP2D6 and IMs or PMs may have reduced benefit from tamoxifen therapy.
- Various therapies for genetic disorders—for example eliglustat used in the treatment of Gaucher disease, and deutetrabenazine used in the treatment of Huntington disease—have reduced dose recommendations for CYP2D6 PMs. The CYP2D6 UMs may not achieve adequate concentrations of eliglustat and therefore CYP2D6 genotyping is required before initiation of eliglustat therapy.

It is important to note that *CYP2D6* is the most common biomarker in drug responses for FDA drug labels, the list provided here is by no means exhaustive. Additional information on gene-drug interactions for *CYP2D6* are available from PharmGKB, CPIC and the FDA (search for “CYP2D6”).

Genetic Testing

Genetic testing is available for many (~30) of the variant *CYP2D6* alleles. Usually, an individual's result is reported as a diplotype, which includes one maternal and one paternal allele, for example, *CYP2D6 *1/*2*. When individuals have more than 2 copies of the *CYP2D6*, the copies of the allele are denoted by an "xN", for example, *CYP2D6*1/*2x2*. Some laboratories also use the notation of DUP to indicate an increase in copy number, but the report does not specify the number of duplications nor the allele that has been duplicated.

Genetic tests for tramadol response and the *CYP2D6* gene can be found on the NIH Genetic Testing Registry. The available CYP2D6 tests include targeted single-gene tests as well as multi-gene panels or genome-wide sequencing tests. In addition, variant *CYP2D6* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (66).

The test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be confirmed by checking the diplotype and calculating the CYP2D6 activity score, as described in the "*CYP2D6* Alleles" section above.

Variants in other genes, such as *COMT*, *ABCB1*, *UGT2B7* and *OPRM1*, may also influence an individual's response to tramadol. However, evidence is lacking on whether genetic testing for these variants will aid optimum dosing. (67, 68, 69, 70)

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2021 Statement from the US Food and Drug Administration (FDA):

Ultra-Rapid Metabolism of Tramadol and Other Risk Factors for Life-threatening Respiratory Depression in Children

Life-threatening respiratory depression and death have occurred in children who received tramadol. Tramadol and codeine are subject to variability in metabolism based upon CYP2D6 genotype (described below), which can lead to increased exposure to an active metabolite. Based upon post-marketing reports with tramadol or with codeine, children younger than 12 years of age may be more susceptible to the respiratory depressant effects of tramadol. Furthermore, children with obstructive sleep apnea who are treated with opioids for post-tonsillectomy and/or adenoidectomy pain may be particularly sensitive to their respiratory depressant effect. Because of the risk of life-threatening respiratory depression and death:

- Tramadol hydrochloride extended-release tablets are contraindicated for all children younger than 12 years of age.
- Tramadol hydrochloride extended-release tablets are contraindicated for post-operative management in pediatric patients younger than 18 years of age following tonsillectomy and/or adenoidectomy.
- Avoid the use of tramadol hydrochloride extended-release tablets in adolescents 12 to 18 years of age who have other risk factors that may increase their sensitivity to the respiratory depressant effects of tramadol unless the benefits outweigh the risks. Risk factors include conditions associated with hypoventilation,

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

such as postoperative status, obstructive sleep apnea, obesity, severe pulmonary disease, neuromuscular disease, and concomitant use of other medications that cause respiratory depression.
- As with adults, when prescribing opioids for adolescents, healthcare providers should choose the lowest effective dose for the shortest period of time and inform patients and caregivers about these risks and the signs of opioid overdose.

Nursing Mothers

Tramadol is subject to the same polymorphic metabolism as codeine, with ultra-rapid metabolizers of CYP2D6 substrates being potentially exposed to life-threatening levels of the active metabolite O-desmethyltramadol (M1). At least one death was reported in a nursing infant who was exposed to high levels of morphine in breast milk because the mother was an ultra-rapid metabolizer of codeine. A baby nursing from an ultra-rapid metabolizer mother taking tramadol hydrochloride [extended-release] tablets could potentially be exposed to high levels of M1, and experience life-threatening respiratory depression. For this reason, breastfeeding is not recommended during treatment with tramadol hydrochloride extended-release tablets.

***CYP2D6* Genetic Variability: Ultra-rapid metabolizer**

Some individuals may be ultra-rapid metabolizers because of a specific *CYP2D6* genotype (e.g., gene duplications denoted as *1/*1xN or *1/*2xN). The prevalence of this CYP2D6 phenotype varies widely and has been estimated at 1 to 10% for Whites (European, North American), 3 to 4% for Blacks (African Americans), 1 to 2% for East Asians (Chinese, Japanese, Korean), and may be greater than 10% in certain racial/ethnic groups (i.e., Oceanian, Northern African, Middle Eastern, Ashkenazi Jews, Puerto Rican). These individuals convert tramadol into its active metabolite, O-desmethyltramadol (M1), more rapidly and completely than other people. This rapid conversion results in higher than expected serum M1 levels. Even at labeled dosage regimens, individuals who are ultra-rapid metabolizers may have life-threatening or fatal respiratory depression or experience signs of overdose (such as extreme sleepiness, confusion, or shallow breathing). Therefore, individuals who are ultra-rapid metabolizers should not use tramadol hydrochloride tablets.

[…]

Drug Interactions

Inhibitors of CYP2D6:

Clinical Impact: The concomitant use of tramadol hydrochloride tablets and CYP2D6 inhibitors may result in an increase in the plasma concentration of tramadol and a decrease in the plasma concentration of M1, particularly when an inhibitor is added after a stable dose of tramadol hydrochloride tablets is achieved. Since M1 is a more potent μ-opioid agonist, decreased M1 exposure could result in decreased therapeutic effects, and may result in signs and symptoms of opioid withdrawal in patients who had developed physical dependence to tramadol. Increased tramadol exposure can result in increased or prolonged therapeutic effects and increased risk for serious adverse events including seizures and serotonin syndrome. After stopping a CYP2D6 inhibitor, as the effects of the inhibitor decline, the tramadol plasma concentration will decrease and the M1 plasma concentration will increase. This could increase or prolong therapeutic effects but also increase adverse reactions related to opioid toxicity, such as potentially fatal respiratory depression.

Intervention: If concomitant use of a CYP2D6 inhibitor is necessary, follow patients closely for adverse reactions including opioid withdrawal, seizures and serotonin syndrome. If a CYP2D6 inhibitor is discontinued, consider lowering tramadol hydrochloride tablets dosage until stable drug effects are achieved. Follow patients closely for adverse events including respiratory depression and sedation.

Examples: Quinidine, fluoxetine, paroxetine and bupropion

Inhibitors of CYP3A4:

Clinical Impact: The concomitant use of tramadol hydrochloride tablets and CYP3A4 inhibitors can increase the plasma concentration of tramadol and may result in a greater amount of metabolism via CYP2D6 and greater levels of M1. Follow patients closely for increased risk of serious adverse events including seizures and serotonin syndrome, and adverse reactions related to opioid toxicity including potentially fatal respiratory depression, particularly when an inhibitor is added after a stable dose of tramadol hydrochloride tablets is achieved. After stopping a CYP3A4 inhibitor, as the effects of the inhibitor decline, the tramadol plasma concentration will decrease, resulting in decreased opioid efficacy or a withdrawal syndrome in patients who had developed physical dependence to tramadol.

Intervention: If concomitant use is necessary, consider dosage reduction of tramadol hydrochloride tablets until stable drug effects are achieved. Follow patients closely for seizures and serotonin syndrome, and signs of respiratory depression and sedation at frequent intervals. If a CYP3A4 inhibitor is discontinued, consider increasing the tramadol hydrochloride tablets dosage until stable drug effects are achieved and follow patients for signs and symptoms of opioid withdrawal.

Examples: Macrolide antibiotics (e.g., erythromycin), azole-antifungal agents (e.g. ketoconazole), protease inhibitors (e.g., ritonavir)

CYP3A4 Inducers:

Clinical Impact: The concomitant use of tramadol hydrochloride tablets and CYP3A4 inducers can decrease the plasma concentration of tramadol, resulting in decreased efficacy or onset of a withdrawal syndrome in patients who have developed physical dependence to tramadol. After stopping a CYP3A4 inducer, as the effects of the inducer decline, the tramadol plasma concentration will increase, which could increase or prolong both the therapeutic effects and adverse reactions, and may cause seizures, serotonin syndrome, and/or potentially fatal respiratory depression.

Intervention: If concomitant use is necessary, consider increasing the tramadol hydrochloride tablets dosage until stable drug effects are achieved. Follow patients for signs of opioid withdrawal. If a CYP3A4 inducer is discontinued, consider tramadol hydrochloride tablets dosage reduction and monitor for seizures and serotonin syndrome, and signs of sedation and respiratory depression. Patients taking carbamazepine, a CYP3A4 inducer, may have a significantly reduced analgesic effect of tramadol. Because carbamazepine increases tramadol metabolism and because of the seizure risk associated with tramadol, concomitant administration of tramadol hydrochloride tablets and carbamazepine is not recommended.

Examples: Rifampin, carbamazepine, phenytoin

[….]

Special populations: Poor/Extensive Metabolizers, CYP2D6

The formation of the active metabolite of tramadol, M1, is mediated by CYP2D6, a polymorphic enzyme. Approximately 7% of the population has reduced activity of the CYP2D6 isoenzyme of cytochrome P450 metabolizing enzyme system. These individuals are “poor metabolizers” of debrisoquine, dextromethorphan and tricyclic antidepressants, among other drugs. Based on a population PK analysis of Phase 1 studies with IR tablets in healthy subjects, concentrations of tramadol were approximately 20% higher in “poor metabolizers” versus “extensive metabolizers,” while M1 concentrations were 40% lower.

Please review the complete therapeutic recommendations that are located here: (1).

2020 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

For CYP2D6 normal metabolizers (i.e. CYP2D6 activity score 1.25 to 2.25), a label recommended age- or weight-specific starting dose of codeine or tramadol, as recommended in the product label, is warranted. A label recommended starting dosing is also recommended for intermediate metabolizers (i.e. activity score of 0.25 to 1); these patients should be monitored closely for less than optimal response and should be offered an alternative analgesic if warranted. For CYP2D6 poor metabolizers (i.e. activity score of 0), current evidence supports the avoidance of codeine and tramadol and the use of an alternative analgesics due to the likelihood of suboptimal or lack of effect. There is insufficient evidence in the literature to recommend a higher dose of codeine or tramadol in poor metabolizers, especially considering the evidence that some adverse events do not differ between poor and normal metabolizers (19). For CYP2D6 ultrarapid metabolizers (namely, activity score of >2.25), codeine or tramadol should not be used, in order to avoid the risk of severe toxicity with label-recommended dosing. Non-opioid analgesics and if needed, other opioids that are not affected by CYP2D6 phenotype, are potential alternatives for use in CYP2D6 poor and ultrarapid metabolizers based on the type, severity and chronicity of the pain being treated.

Please review the complete therapeutic recommendations that are located here: (2, 36).

2017 Summary of recommendations from the Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

Ultra-rapid metabolizer (gene dose ≥ 3) (enhanced CYP2D6 enzyme activity)

The genetic variation increases the conversion of tramadol to a metabolite with a stronger opioid effect. This can result in an increase in potentially life-threatening side effects.

Recommendation:
As the total analgesic effect changes when the ratio between the mother compound and the active metabolite changes, the effect of a dose reduction cannot be predicted with certainty.

1. select an alternative

   Do not choose codeine, as it is contra-indicated for CYP2D6 UM.
   Morphine is not metabolized by CYP2D6.
   Oxycodone is metabolized by CYP2D6 to a limited extent, but this does not result in differences in side effects in patients.

2. if an alternative is not possible:
   - use 40% of the standard dose
   - advise the patient to report side effects (such as drowsiness, confusion, constipation, nausea and vomiting, respiratory depression or urine retention).

Intermediate metabolizer (gene dose 0.5-1) (decreased CYP2D6 enzyme activity)

The genetic variation reduces the conversion of tramadol to a metabolite with a higher activity. This can result in reduced analgesia.

Recommendation:
It is not possible to provide a recommendation for dose adjustment, because the total analgesic effect changes when the ratio between the mother compound and the active metabolite changes.

1. be alert to a reduced effectiveness
2. in the case of inadequate effectiveness:
   - try a dose increase
   - if this does not work: choose an alternative

   Do not select codeine, as this is also metabolized by CYP2D6.

   Morphine is not metabolized by CYP2D6.
   Oxycodone is metabolized by CYP2D6 to a limited extent, but this does not result in differences in analgesia in patients.
3. if no alternative is selected: advise the patient to report inadequate analgesia

Poor metabolizer (gene dose 0) (absent CYP2D6 enzyme activity)

The genetic variation reduces the conversion of tramadol to a metabolite with a higher activity. This can result in reduced analgesia.

Recommendation:
It is not possible to provide a recommendation for dose adjustment, because the total analgesic effect changes when the ratio between the mother compound and the active metabolite changes.

1. be alert to a reduced effectiveness
2. in the case of inadequate effectiveness:
   - try a dose increase.
   - if this does not work: choose an alternative
     Do not select codeine, as this is also metabolized by CYP2D6.
     Morphine is not metabolized by CYP2D6.
     Oxycodone is metabolized by CYP2D6 to a limited extent, but this does not result in differences in analgesia in patients.
3. if no alternative is selected: advise the patient to report inadequate analgesia

Please review the complete therapeutic recommendations that are located here: (3).

Nomenclature

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
*CYP2D6*3*	4181G>C (Ser486Thr)	NM_000106.6:c.886C>T	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Gene deletion			
*CYP2D6*6*	1707 del T Trp152Gly CYP2D6T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852

Nomenclature of Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*36*[3]	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] In the literature, 1023C>T is also referred to as 1111C>T
[2] In the literature, 2851C>T is also referred to as 2938C>T
[3] *CYP2D6*36* is a gene conversion with *CYP2D7;* variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (71).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Trastuzumab Therapy and *ERBB2* Genotype

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Introduction

Trastuzumab (brand name, Herceptin) is a monoclonal antibody used in the treatment of breast and gastric/gastroesophageal cancer. It targets an epidermal growth factor receptor encoded by the *ERBB2* gene, which is commonly referred to as the *HER2* gene. Multiple biosimilar products to Herceptin are now available: Kanjinti, Trazimera, Ontruzant, Herzuma and Ogivri.

The *ERBB2* gene is overexpressed in 15–20% of breast cancers and 15–20% of gastric and esophageal cancers. Overall, "HER2 positive" tumors are associated with a faster rate of growth and—in some cases—a poorer prognosis in absence of anti-HER2 therapy. The use of trastuzumab in treatment regimens improves outcomes, with limited adverse effects that include cardiac toxicity.

The FDA-approved drug label states that trastuzumab should only be used to treat individuals with tumors that have either HER2 protein overexpression or *ERBB2* gene amplification, as determined by an accurate and validated FDA-approved assay, specific for the type of tumor tested (breast or gastric) (Table 1)(1). The FDA-approved drug label for all trastuzumab biosimilars describes only the use of trastuzumab in adjuvant treatment of breast cancer, though its efficacy in neoadjuvant care for breast cancer (reviewed in part by (2)) and esophageal adenocarcinoma (3) has also been documented.

The most recent update (2018) of the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines continues to state that all newly diagnosed individuals with breast cancer must have an HER2 test performed. Individuals who then develop metastatic disease must have an HER2 test performed in a metastatic site, if tissue sample is available (4).

Table 1. The FDA Indications and Usage of Trastuzumab (2020)

Individual selection*	Breast cancer (adjuvant treatment)	Metastatic breast cancer	Metastatic gastric cancer
Tumor HER2 status	HER2-positive	HER2-positive	HER2-positive
Indications and usage	Indicated for adjuvant treatment of HER2 overexpressing node positive or node negative breast cancer. -As part of a treatment regimen consisting of doxorubicin, cyclophosphamide, and either paclitaxel or docetaxel # -As part of a treatment regimen with docetaxel and carboplatin # -As a single agent following multi-modality anthracycline based therapy #	Indicated in combination with paclitaxel for first-line treatment of HER2-overexpressing metastatic breast cancer, or as a single agent for treatment of HER2-overexpressing breast cancer	Indicated for the treatment of individuals with HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma who have not received prior treatment for metastatic disease. (in combination with cisplatin and capecitabine or 5-fluorouracil)#

* Select individuals based on HER2 protein overexpression or *ERBB2* gene amplification in tumor specimens.
# Combination therapy administration notes are specific to FDA labels for Trazimera and Ogivri (5, 6)
This FDA table was adapted from (1).

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Drug Class: HER2 Inhibitors

Human epidermal growth factor receptor 2 (commonly referred to as HER2 or HER-2/neu) is encoded by the gene *ERBB2*, which is a transmembrane receptor tyrosine kinase. Overexpression of *ERBB2* leads to rapid cell growth in multiple types of solid tumors. The HER2 can be inactivated by a class of chemicals known as tyrosine kinase inhibitors or via targeted monoclonal antibodies. An increasing number of HER2-targeted therapies have been approved to treat HER2-positive breast cancer, including:

- Pertuzumab––monoclonal antibody (brand name Perjeta)
- Trastuzumab––monoclonal antibody (brand name Herceptin)
- Ado-trastuzumab emtansine––antibody-drug-conjugate (monoclonal antibody attached to a chemotherapy drug (brand name Kadcyla, also called TDM-1)
- Fam-trastuzumab deruxtecan––antibody-drug-conjugate (brand name Enhertu)
- Neratinib––a kinase inhibitor (brand name Nerlynx)
- Lapatinib––a kinase inhibitor (brand name Tykerb)
- Tucatinib––a kinase inhibitor (brand name Tykysa)
- Dacomitinib––a kinase inhibitor (brand name Vizimpro)

There are several more anti-HER2 drugs progressing through clinical trials, and some trials are looking at whether HER2-targeted therapies could be used to treat other tumors that overexpress HER2, such as colorectal and non-small-cell lung cancer. However, early results are not replicating the success of HER2-targeted therapies in breast and gastric cancer (7, 8, 9).

Drug: Trastuzumab

Trastuzumab (brand name, Herceptin) is a monoclonal antibody that targets HER2 (a tyrosine kinase receptor, encoded by the gene *ERBB2*). Multiple biosimilar products to trastuzumab are now available under the brand names Kanjinti, Trazimera, Ontruzant, Herzuma and Ogivri. Trastuzumab is only used to treat specific tumors that overexpress ERBB2, which are known as "HER2-positive" tumors.

Trastuzumab is the first molecular targeted agent approved as standard therapy for gastric cancer, and is the only HER2 targeted therapy approved for the treatment of advanced gastric cancer. The 2015 NCCN guidelines recommend the first-line treatment of trastuzumab combined with chemotherapy in individuals overexpressing HER2 (10).

Trastuzumab was also the first targeted anti-HER2 therapy available for metastatic HER2-positive breast cancer, and led to significant improvement in prognosis over the previous standard of care chemotherapy regimens, and was the first monoclonal antibody to be approved for non-metastatic HER2-positive breast cancer (11).

Trastuzumab is typically used with chemotherapy as neoadjuvant or adjuvant treatment of early-stage HER2-positive breast cancer. Neoadjuvant therapy is given before primary therapy to shrink a tumor to an operable size, help make decisions for further therapy after surgery, allow for breast-conserving surgery, and increase the chance of long-term, disease-free survival. Adjuvant therapies are used after surgery to increase the chance of long-term disease-free survival. Trastuzumab can be used with chemotherapy with or without another HER2 targeted agent pertuzumab (12). Trastuzumab is also used in the treatment of HER2-positive metastatic breast cancer and HER2-positive metastatic gastric cancer (1, 13).

Pertuzumab, another targeted anti-HER2 therapy, can also be added to multiple treatment regimens, including trastuzumab/chemotherapy combination therapy, in the neoadjuvant, adjuvant, or metastatic setting (14, 15, 16, 17, 18). Regardless of whether initial treatment was given before or after surgery, adjuvant trastuzumab should be continued to complete one year of therapy.

Recently, another HER2-targeted therapy ado-trastuzumab emtansine (Kadcyla) has been approved by the FDA for adjuvant treatment of HER2-positive early breast cancer (EBC) in individuals who have residual invasive disease after receiving taxane and trastuzumab-based treatment.

Before treatment with trastuzumab begins, overexpression of the ERBB2 protein or amplification of the *ERBB2* gene must first be determined. The FDA recommends that testing be performed using an FDA-approved test for the specific tumor type (breast or gastric tumor), in a laboratory with demonstrated proficiency with the technology being used. This is because the benefits of trastuzumab have only been proven in individuals with tumors that overexpress *ERBB2*. In addition, although trastuzumab is generally well tolerated, the risks of treatment include infusion reactions, pulmonary toxicity, and cardiomyopathy that can result in cardiac failure (1, 19).

Trastuzumab targets the ERBB2 receptor protein by binding to the juxtamembrane portion of the extracellular domain. This binding limits the receptor's ability to activate its intrinsic tyrosine kinase, which in turn, limits the activation of numerous signaling pathways that can promote the growth of cancerous cells.

A number of proposed mechanisms may underlie the anti-tumor effects of trastuzumab. Of note, when HER2 is overexpressed, HER2 receptors exist on the cell surface as homodimers and heterodimers with other HER family receptors. It has been suggested that trastuzumab has preferential activity against breast cancers driven by HER2 homodimers (20). Additionally, it has been proposed that trastuzumab disrupts signaling activation by the ligand-independent HER2/HER3 complex (21). The HER2-HER3 dimerized receptor is thought to be highly active, triggering many signaling cascades in the absence of a "true" ligand (22).

Another proposed mechanism is antibody-dependent cellular cytotoxicity (ADCC). Once trastuzumab has bound to a cancer cell, immune cells (typically activated natural killer cells) bind to trastuzumab and initiate lysis of the cancer cell (23). Trastuzumab may also mediate the enhanced internalization and degradation of the HER2 receptor, inhibit angiogenesis, and inhibit HER2 shedding by preventing the cleavage of HER2 and the subsequent release of its extracellular domain (24, 25).

Unfortunately, cancer is a complex disease involving many different genes and pathways. Breast cancer or gastric cancer may start to progress again during trastuzumab therapy. Possible mechanisms that may facilitate disease progression during treatment include increased signaling from the HER family of receptors, an upregulation of downstream signaling pathways, and an increased level of insulin growth factor-1 receptor (26, 27, 28, 29, 30, 31).

Gene: *ERBB2 (HER2)*

The HER protein family consists of 4 members: the epidermal growth factor receptor (EGFR), *ERBB2* (HER2), *ERBB3* (HER3), and *ERBB4* (HER4) (see Nomenclature for Selected Genes below). All 4 members are transmembrane tyrosine kinase receptors, and they regulate a number of important cellular processes, such as cell growth, survival, and differentiation (32).

The genes *ERBB2* and *EGFR* are proto-oncogenes. Proto-oncogenes are a group of genes that, when mutated or expressed at abnormally high levels, can contribute to normal cells becoming cancerous cells. The mutated version of the proto-oncogene is called an oncogene. Proto-oncogenes typically encode proteins that stimulate cell division, regulate cell differentiation, and halt cell death. All these are important biological processes. However, the increased production of these proteins, caused by oncogenes, can lead to the proliferation of poorly differentiated cancer cells (33).

The official gene symbol for HER2 is *ERBB2*, which is derived from a viral oncogene with which the receptor shares homology; "v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2." However, clinicians commonly refer to the *ERBB2* gene as "*HER2*" or "HER2/neu" (neu was the name given to the gene that caused

cancer derived from a rodent neuro/glioblastoma). The *HER2* is the legacy gene symbol for *ERBB2* and may be more commonly used by the community; HER2 is also commonly used to describe the protein encoded by the *ERBB2* gene.

One unique feature of HER2 compared with the other receptors in the HER family is the absence of a known ligand. It is therefore thought that this receptor may permanently be in an activated state, or it may become activated during heterodimerization with one of the other members of the HER family (25, 30). One unique feature of HER3 is that it has very little enzymatic activity compared with the other tyrosine kinase receptors in the HER family. It is therefore thought that an important role of HER3 is to act as a heterodimerization partner for HER2 (34, 35) and other HER family members.

When a partner such as HER3 binds to HER2, the heterodimer undergoes activation, which stimulates the intrinsic tyrosine kinase activity of the receptor. Autophosphorylation of several key residues of the receptor triggers the downstream activation of many commonly used growth factor signaling pathways, such as the PI3K/AKT/mTOR pathway and the RAS/RAF/MEK/ERK pathway (36, 37). Impaired ERBB2 signaling is associated with the development of neurodegenerative diseases (38), such as Alzheimer disease (39), whereas excessive ERBB2 signaling is associated with the development of cancers (40).

The *ERBB2* is overexpressed in approximately 15–20% of breast tumors as a result of amplification of the *ERBB2* gene, and tumors with increased HER2 typically have a higher growth rate and more aggressive clinical behavior, with the exception of esophagogastric adenocarcinoma (41, 42, 43, 44, 45). Although gene amplification is frequently seen in cancer and other degenerative disorders, the underlying basis for amplification remains largely unknown (46). And in the case of *ERBB2*, although sequence variants have been identified, it is most commonly a wildtype *ERBB2* gene copy that is overexpressed in tumors (47). In approximately 1% of breast cancers, activating mutations in *ERBB2* can be identified that are likely to drive tumorigenesis without *ERBB2* amplification (48). Importantly, it has recently been shown that these variants are further acquired in estrogen receptor-positive metastatic breast cancer, conferring resistance to endocrine therapy (49). Early results from one study (NCT02564900) suggest that trastuzumab deruxtecan may be effective as an anti-HER2 therapy in HER2-low expressing (HER2 "negative" scores of 1+ or 2+ by standard IHC testing) tumors (50).

Linking Gene Overexpression with Treatment Response

Overexpression of HER2 is strongly linked to a beneficial treatment response to trastuzumab. As a consequence, current guidelines for breast cancer treatment limit the use of HER2-blocking agents to tumors with HER2 gene amplification or with HER2 protein overexpression (namely, IHC 3+) (4, 43, 44).

Genetic Testing

The NIH Genetic Testing Registry (GTR) displays tests that are available for the *ERBB2* gene and the trastuzumab drug response. Increased awareness of *ERBB2* testing and trastuzumab administration may improve quality of care and individual outcomes (51). In addition, because HER2 expression can change over time in breast cancer, retesting the HER2 status upon change in disease status is helpful in determining the appropriate treatment. Somatic alterations in *ERBB2* have been observed in multiple solid tumor types, though the frequency of variants and the specific variants are highly variable (9). Testing of the tumor tissue itself for *ERBB2* overexpression is recommended before initiating anti-HER2 therapies.

Tumor Testing for *ERBB2* (HER2) Gene and Protein

There are 2 main methods used for *ERBB2*/HER2 testing: testing for overexpression of the HER2 protein using immunohistochemistry (IHC) or testing for gene amplification using *in situ* hybridization (ISH). Each assay type

has diagnostic pitfalls that must be avoided, and so the pathologist who reviews the histologic findings should determine the optimal assay (IHC or ISH) for the determination of HER2 status (43, 44).

In an IHC assay, a slice of tumor tissue is stained, along with a control sample that contains high levels of HER2. The tumor sample is then examined by light microscopy to assess the intensity of membrane staining—the amount of staining correlates with the quantity of HER2 protein and is typically graded from 0 to 3+ in breast tumor biopsies (4):

- IHC 0 means no visible staining or membrane staining that is incomplete and is faint/barely perceptible and in ≤ 10% of tumor cells
- IHC 1+ is also an "HER2 negative" result—there is a staining pattern of incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells
- IHC 2+ is an "HER2 equivocal result"—invasive breast cancer with "weak to moderate complete membrane staining observed in >10% of tumor cells."
- IHC 3+ is an "HER2 positive result"—there is a staining pattern with circumferential membrane staining that is complete, intense and in >10% of tumor cells. This should be readily appreciated using a low-power objective and observed within a homogenous and continuous invasive cell population.

For gastroesophageal adenocarcinoma, a similar scoring metric is used for HER2 testing (52):

- IHC 0 is a negative result, Surgical specimens have no reactivity or membranous activity in <10% of tumor cells; biopsy specimens have no reactivity in any tumor cell
- IHC 1+ is also negative. Surgical specimens have faint/barely perceptible membranous reactivity in ≥10% of tumor cells, cells are reactive in only part of their membrane; biopsy specimens show that tumor cells cluster with faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained.
- IHC2+ is "HER2 equivocal". Surgical specimens have weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells. Biopsy specimens have tumor cells that cluster with weak to moderate complete, basolateral or lateral membranous activity irrespective of percentage of tumor cells stained.
- IHC3+ is HER2 positive. Surgical specimens have Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumor cells. Biopsy specimens show tumor cell cluster* with strong complete, basolateral or lateral membranous activity irrespective of percentage of tumor cells stained

For an equivocal (IHC 2+) result in either breast or gastric cancer, either a reflex test must be ordered (same specimen using ISH), or a new test must be ordered (using a new specimen, if available, using ISH or FISH) to confirm the results.

The ISH assay, or FISH/CISH assay (fluorescence or chromogenic in situ hybridization), measures *ERBB2* gene amplification by measuring *ERBB2* DNA—the actual number of copies of the *ERBB2* genes are counted. Using the FISH assay, under the microscope, the genes appear as red signals or dots, in a blue-stained cancer cell nucleus. The result is usually either FISH negative (normal level of *ERBB2* gene) or FISH positive (at least twice as much as normal level of *ERBB2* gene), but in a small number of cases the FISH result will be equivocal due to a low level of *ERBB2* amplification. The use of a control helps distinguish between a negative result and a non-informative result caused by an error. Approximately 25% of individuals who have an IHC 2+ result will have a FISH positive result (53).

For the complete algorithms for evaluation of HER2 protein expression using IHC or ISH, please see the American Society of Clinical Oncology (ASCO) / College of American Pathologists (CAP) clinical practice guideline update, located here (4 , 44 , 52).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2020 Statement from the US Food and Drug Administration (FDA)

Select individuals based on HER2 protein overexpression or *HER2* gene amplification in tumor specimens. Assessment of HER2 protein overexpression and *HER2* gene amplification should be performed using FDA-approved tests specific for breast or gastric cancers by laboratories with demonstrated proficiency. Information on the FDA-approved tests for the detection of HER2 protein overexpression and *HER2* gene amplification is available at: http://www.fda.gov/CompanionDiagnostics.

Assessment of HER2 protein overexpression and *HER2* gene amplification in metastatic gastric cancer should be performed using FDA-approved tests specifically for gastric cancers due to differences in gastric vs. breast histopathology, including incomplete membrane staining and more frequent heterogeneous expression of *HER2* seen in gastric cancers.

Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Please review the complete therapeutic recommendations that are located here: (1)

FDA-approved medical devices for HER2 can be searched for here.

2018 Update: American Society of Clinical Oncology (ASCO) /College of American Pathologists (CAP) Recommendations for Breast Cancer

First released in 2007 and updated in 2013 and 2018, the recommendations by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) human epidermal growth factor receptor 2 (HER2) testing Expert Panel are aimed at improving the analytic validity of HER2 testing and the clinical utility of HER2 as a predictive biomarker for potential responsiveness to therapies targeting the HER2 protein.

2013: ASCO/CAP Key Recommendations for Oncologists

- Must request HER2 testing on every primary invasive breast cancer (and on metastatic site, if stage IV and if specimen available) from an individual with breast cancer to guide decision to pursue HER2-targeted therapy. This should be especially considered for an individual who previously tested HER2 negative in a primary tumor and presents with disease recurrence with clinical behavior suggestive of HER2-positive or triple-negative disease.
- Should recommend HER2-targeted therapy if HER2 test result is positive, if there is no apparent histopathologic discordance with HER2 testing and if clinically appropriate.
- Must delay decision to recommend HER2-targeted therapy if initial HER2 test result is equivocal. Reflex testing should be performed on the same specimen using the alternative test if initial HER2 test result is equivocal or on an alternative specimen.
- Must not recommend HER2-targeted therapy if HER2 test result is negative and if there is no apparent histopathologic discordance with HER2 testing.

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug.

- Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results equivocal). The oncologist should confer with the pathologist regarding the need for additional HER2 testing on the same or another tumor specimen.
- If the HER2 test result is ultimately deemed to be equivocal, even after reflex testing with an alternative assay (i.e., if neither test is unequivocally positive), the oncologist may consider HER2-targeted therapy. The oncologist should also consider the feasibility of testing another tumor specimen to attempt to definitely establish the tumor HER2 status and guide therapeutic decisions. A clinical decision to ultimately consider HER2-targeted therapy in such cases should be individualized on the basis of individual status (comorbidities, prognosis, and so on) and individual preferences after discussing available clinical evidence.

2018: ASCO/CAP Updated Key Recommendations for HER2 testing

[…]

Two recommendations addressed via correspondence in 2015 are included. First, immunohistochemistry (IHC) 2+ is defined as invasive breast cancer with weak to moderate complete membrane staining observed in >10% of tumor cells. Second, if the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may (not "must") be ordered on the excision specimen based on specific clinical criteria.

Please review the complete ASCO/CAP recommendations in the 2013 update (22) and 2018 update (4).

2016 American Society of Clinical Oncology (ASCO) /College of American Pathologists (CAP) Recommendations for Gastroesophageal Adenocarcinoma (GEA)

Key Points and Recommendations for Clinicians

- Recommendation 1.1: In individuals with advanced GEA who are potential candidates for HER2-targeted therapy, the treating clinician should request HER2 testing on tumor tissue (Type: evidence based; Quality of evidence: high; Strength of recommendation: strong).
- Recommendation 1.2: Treating clinicians or pathologists should request HER2 testing on tumor tissue in the biopsy or resection specimens (primary or metastasis) preferably prior to the initiation of trastuzumab therapy if such specimens are available and adequate. HER2 testing on FNA specimens (cell blocks) is an acceptable alternative (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: recommendation/moderate).
- Recommendation 1.3: Treating clinicians should offer combination chemotherapy and HER2-targeted therapy as the initial treatment for appropriate individuals with HER2 positive tumors who have advanced GEA (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).

Key Points and Recommendations for Pathologists

- Recommendation 2.1: Laboratories/pathologists must specify the antibodies and probes used for the test and ensure that assays are appropriately validated for HER2 IHC and ISH on GEA specimens (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).
- Recommendation 2.2: When GEA HER2 status is being evaluated, laboratories/pathologists should perform/order IHC testing first, followed by ISH when IHC result is 2+ (equivocal). Positive (3+) or negative (0 or 1+) HER2 IHC results do not require further ISH testing (Type: evidence based; Quality of evidence: high; Strength of recommendation: strong).

- Recommendation 2.3: Pathologists should use the Ruschoff/Hofmann method in scoring HER2 IHC and ISH results for GEA (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).
- Recommendation 2.4: Pathologists should select the tissue block with the areas of lowest grade tumor morphology in biopsy and resection specimens. More than one tissue block may be selected if different morphologic patterns are present (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: recommendation/moderate).
- Recommendation 2.5: Laboratories should report HER2 test results in GEA specimens in accordance with the CAP "Template for Reporting Results of HER2 (ERBB2) Biomarker Testing of Specimens From Individuals With Adenocarcinoma of the Stomach or Esophagogastric Junction" (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).
- Recommendation 2.6: Pathologists should identify areas of invasive adenocarcinoma and also mark areas with strongest intensity of HER2 expression by IHC in GEA specimen for subsequent ISH scoring when required (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).
- Recommendation 2.7: Laboratories must incorporate GEA HER2 testing methods into their overall laboratory quality improvement program, establishing appropriate quality improvement monitors as needed to ensure consistent performance in all steps of the testing and reporting process. In particular, laboratories performing GEA HER2 testing should participate in a formal proficiency testing program, if available, or an alternative proficiency assurance activity (Type: evidence based; Quality of evidence: moderate/intermediate; Strength of recommendation: strong).
- Recommendation 2.8: There is insufficient evidence to recommend for or against genomic testing in GEA individuals at this time.

Please review the complete ASCO/CAP recommendations here (52).

Nomenclature for Selected Genes Associated with Trastuzumab Response

Official gene symbol	Alternative gene symbols
EGFR	*ERBB1* *ERBB* *HER1*
ERBB2	*HER2* *HER-2* *HER-2/neu* *NEU*
ERBB3	*HER3*
ERBB4	*HER4*

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Valbenazine Therapy and *CYP2D6* Genotype

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Introduction

Valbenazine (marketed as Ingrezza in the US and Dysval in Japan) is a vesicular monoamine transporter 2 (VMAT2) inhibitor used in the treatment of tardive dyskinesia (TD) or Huntington disease (HD) chorea (1, 2). Valbenazine and its active metabolite inhibit the transporter protein that packages neurotransmitters into synaptic vesicles, reducing uncontrollable movements attributed to dopamine receptor hypersensitivity and other synaptic dysfunction. The active valbenazine metabolite is further metabolized into inactive compounds by cytochrome P450 (CYP450) family enzymes, including the CYP2D6 protein.

Individuals with no CYP2D6 enzymatic activity (poor metabolizers [PMs]) will have a higher exposure to valbenazine and its active metabolite, which can increase the risk of exposure-related adverse reactions (1). Therefore, the US FDA-approved label recommends that known CYP2D6 PMs take no more than 40 mg valbenazine daily. Individuals with other metabolizer phenotypes may take up to 80 mg daily, with dosing based on individual symptoms and tolerability (Table 1) (1). Notably, the FDA also advises the same lower dose for individuals taking a strong CYP2D6 inhibitor (1).

The FDA-approved label further cautions that individuals with HD are at increased risk for depression and suicidal ideation or behavior, particularly with VMAT2 inhibitor therapy (1). The recommended dosing in this population follows a slower titration time scale than for TD, with dose increases of 20 mg every 2 weeks until either efficacy is achieved, or the maximum tolerated dose is reached.

Table 1: Statement on CYP2D6-based Dosing of Valbenazine from the Food and Drug Administration (FDA, 2024)

CYP2D6 status	Effect	Recommendation
PM	Increased exposure to valbenazine and active metabolite	40 mg daily maximum dose
Non-PM, no inhibitory medications	Normal metabolism	Initial dose of 40 mg daily Increase to 80 mg after one week for TD; increase in 20 mg increments every 2 weeks for HD up to 80 mg (consider 40 mg or 60 mg based on response and tolerability)
Non-PM, strong CYP2D6 inhibitor co-medication	Increased exposure to valbenazine and active metabolite	40 mg daily maximum dose

PM: Poor metabolizer; TD: Tardive dyskinesia; HD: Huntington disease
Table adapted from (1).

Drug: Valbenazine

Valbenazine, also known as Ingrezza, is a reversible inhibitor of VMAT2 indicated for the treatment of adults with TD or chorea associated with HD (1). For TD, valbenazine is initially dosed at 40 mg per day, which may be increased to 80 mg daily after one week, with an option to dose at 40 or 60 mg based on response and tolerability (1). For HD chorea, dosing also begins at 40 mg once daily, but should be titrated in 20 mg increments every 2

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weeks up to 80 mg, with lower doses of 40 or 60 mg considered based on response and tolerability (1). Valbenazine was also investigated as a treatment for tics associated with Tourette syndrome; however, it did not meet primary efficacy outcome measures (3). In Japan, valbenazine is approved to treat TD as valbenazine tosilate (brand name Dysval) and is dosed at 40–80 mg daily, adjusted based on individual symptoms (2).

Valbenazine belongs to the broader class of VMAT2 inhibitors, which facilitate the packaging of neurotransmitters and other monoamines into vesicles, enabling controlled release through vesicle fusion with the plasma membrane. The VMAT2 protein is expressed in neurons, neuroendocrine cells, platelets, and mast cells, and has high affinity for histamine, serotonin, norepinephrine, and dopamine (4). Inhibition of VMAT2 activity reduces dopaminergic signaling in the brain by decreasing dopamine availability for synaptic vesicle release.

Both valbenazine and deutetrabenazine are structurally related to tetrabenazine, differing only in chemical modifications that enhance stability. The active metabolite of all 3 medications is [+]-alpha-dihydrotetrabenazine ([+]-alpha-HTBZ). Compared with other VMAT2 inhibitors, valbenazine is metabolized more slowly via hydrolysis of a valine ester, reaching peak concentration within 1–1.5 hours after dosing, with a systemic half-life of approximately 20 hours (5). The formation of [+]-alpha-HTBZ is delayed, with peak concentrations reached 4–9 hours after dosing and a half-life similar to the parent compound (15–20 hours) (1, 5). Another metabolite, NBI-136110, is also formed from valbenazine but has much lower affinity for VMAT2 than [+]-alpha-HTBZ (6). Studies indicate that valbenazine's pharmacokinetic parameters are similar in individuals from North America (European or African) and East Asian (Korean) descent (7). Beyond hydrolysis, CYP450 enzymes contribute to valbenazine metabolism, CYP3A4/5 enzymes form mono-oxidized valbenazine and [+]-alpha-HTBZ is metabolized by CYP2D6 (1). Higher exposure to valbenazine and [+]-alpha-HTBZ may increase the risk of exposure-related adverse reactions (1).

Significant adverse effects of valbenazine include depression, suicidal ideation in individuals with HD, hypersensitivity reactions, somnolence, QT prolongation, neuroleptic malignant syndrome (NMS) and Parkinsonism (1). One analysis of adverse events reported to the FDA suggests that females may have a moderately higher likelihood of serious adverse reactions than males in response to valbenazine therapy (8). This analysis also found a high reporting odds ratio for neurological adverse events, including drooling, dyskinesia, bradykinesia, and slow speech (8).

Depression and suicidal ideation have been reported in clinical trials and real-world data for VMAT2 inhibitors. The FDA-approved drug label includes a black box warning for depression and suicidal ideation in HD treatment, recommending extra caution when treating individuals with a history of depression, suicidal behavior (1). Suicidal ideation was significantly associated with this VMAT2 inhibitors in an analysis of data from the FDA Adverse Event Reporting System (FAERS), likely due to underlying psychiatric conditions in this population. There was no significant association between valbenazine use and suicidal behavior (9). Another analysis of FAERS data confirmed an association of VMAT2 inhibitors with suicidal ideation, but increased odds of suicidal behavior were not observed with valbenazine or deutetrabenazine (9).

Hypersensitivity reactions, including angioedema affecting the larynx, glottis, lips and eyelids, or urticaria and rash may occur; treatment should be discontinued if these reactions occur (1). It is unlikely that the reported angioedema event from the KINECT-HD clinical trial was caused by the medication, but rather a food allergy unrelated to the medication or its ingredients (10). Somnolence, sedation, or excessive sleepiness were common adverse reactions observed during clinical trials. Individuals should avoid activities requiring alertness until they know their response to valbenazine (1, 11, 12).

Elongation of the QT interval of the cardiac rhythm may occur though it is unlikely to be clinically significant at recommended doses. Individuals at higher risk for clinically significant QT elongation include those on strong CYP2D6 or CYP3A4 inhibitors or those with reduced CYP2D6 activity due to genetic variation (PMs).

Individuals with genetic or pharmacologically reduced CYP2D6 or CYP3A4 activity may require a reduced dose of 40 mg daily (1). Those with underlying long QT syndrome or arrhythmia should avoid valbenazine (1).

The symptoms of NMS include hyperpyrexia, muscle rigidity, altered mental status, and autonomic instability such as tachycardia (1). This complex of symptoms is potentially fatal and can be difficult to diagnose. If NMS is suspected, immediately discontinue valbenazine, and symptomatic treatment and medical monitoring for NMS as well as any concomitant serious medical problems should begin. Recurrent NMS has been reported after resumption of valbenazine therapy; individuals should be monitored carefully. (1)

Parkinsonism may also develop with VMAT2 inhibitor therapy (13), observed in 4.7% of HD trials (compared to 0% with placebo) and in post-marketing reports for TD use (1, 8, 14, 15). The Parkinson-like symptoms may be more disruptive daily routines and functional abilities than untreated HD or TD, and dose reduction or discontinuation of valbenazine is recommended in cases of clinically significant symptoms. (1)

There is limited data on drug-associated risks for valbenazine use in pregnant or lactating women. Animal studies showed increased stillbirths in rat pups when the mother received valbenazine during pregnancy, though no malformations were observed in the live littermates; a risk to human viability during pregnancy is possible (1). No data exists regarding valbenazine or metabolites in human breast milk, or the impact on breastfed infants. Based on animal studies, valbenazine compounds may be transferred into breast milk, potentially at concentrations higher than in plasma (1). Women are advised not to breastfeed during valbenazine treatment or for 5 days after the final dose (1, 16). Valbenazine has not been established as safe or effective in a pediatric population (1). However, there is off-label use of valbenazine and other VMAT2 inhibitors to manage *NKX2-1* related disorders with chorea in both children and adults (17).

No dose adjustment is required for elderly individuals (1). A pooled analysis of 6-week randomized, placebo-controlled trials (KINECT, KINECT 2, and KINECT 3) and long-term clinical trials (KINECT 3 extension and KINECT 4) showed no difference in response rates or adverse events between study participants under 55 and those aged 55 and older (18). One analysis of the FDA adverse event reporting data for valbenazine showed a higher probability for neurological adverse events in middle-aged adults (18–64) compared to elderly adults (65+), possibly attributed to the higher incidence of schizophrenia in this age group (8). A post-hoc analysis of the J-KINECT study found that treatment emergent adverse events led to a higher discontinuation rate in elderly participants compared to younger participants, though overall adverse event types and incidence were similar between groups (19).

No dosage adjustment is needed for individuals with renal impairment (1). However, for individuals with moderate or severe hepatic impairment, a dose reduction is recommended to prevent increased exposure to valbenazine [+]-alpha-HTBZ (1).

Tardive Dyskinesia

Tardive dyskinesia is a medication-induced movement disorder. These movements are involuntary and repetitive, most commonly affecting the tongue, mouth, jaw, and face, but also potentially affecting the limbs and trunk. Severe cases are associated with difficulty speaking and swallowing, and the condition can be disfiguring and stigmatizing, significantly impacting quality of life (20).

Tardive dyskinesia is caused by medicines that block dopamine receptors, including antipsychotics (for example, aripiprazole, clozapine, risperidone, thioridazine) and antiemetic drugs used to treat nausea and vomiting (such as metoclopramide and prochlorperazine). Tardive dyskinesia can be irreversible and lifelong, persisting even after the causative medicine has been stopped (21). Studies indicate that PM or ultra-rapid metabolizer (UM) CYP2D6 phenotype individuals on antipsychotic therapy are more likely to have an Abnormal Involuntary Movement Scale (AIMS) score indicative of TD, with PMs experiencing more severe TD (22). The increased TD severity may be due to altered drug metabolism.

Approximately 25% of individuals treated with antipsychotics develop TD, though it is more common with first-generation than second-generation antipsychotics (23). Tardive syndromes, including TD, affect up to one-third of individuals with schizophrenia treated with antipsychotics (24). Initially, the prevalence of TD was expected to decrease as newer antipsychotics were developed that are less likely to cause TD. However, TD remains prevalent, partly because the newer antipsychotics have expanded indications, including depression, bipolar disorder, personality disorder, irritability in autism spectrum disorder, as well as off-label uses including insomnia and anxiety. Consequently, the population at risk for TD has increased (25, 26, 27, 28). This population may also include children or adolescents on antipsychotics, with a TD prevalence between 5–20% based on a review of thirteen studies (29).

Because TD is irreversible, prevention is crucial, requiring both limited use of drugs that may cause TD and early diagnosis (30, 31). While not a cure, valbenazine and related VMAT2 inhibitors have been shown to reduce the abnormal movements associated with TD and are generally well tolerated. However, there are no head-to-head comparisons between VMAT2 inhibitors, and TD symptoms return approximately 4 weeks after treatment is discontinued (23, 24, 26, 30, 32, 33, 34, 35).

Several genetic loci have been linked to TD risk in response to antipsychotic therapy (36). In addition to *CYP2D6*, the *CYP1A2* locus variation is associated with more frequent limb-truncal TD, and environmental factors such as smoking may additively impact CYP1A2 protein expression and further influence TD presentation (37). Variants in dopamine signaling pathways (specifically *DRD2* and *DRD3*) and serotonin systems (*HTR2A)* have also been associated with TD development (38, 39, 40, 41). Notably, the association with *COMT* (encoding catechol-O-methyltransferase, a dopamine metabolizing enzyme) and TD risk appears specific to females (42). Variants in *HSPG2* have been associated with TD in both Japanese and European ancestry cohorts (43, 44). Additionally, variation in *SLC18A2*, which encodes VMAT2, has been associated with TD risk or protection (discussed further below).

Gene: *CYP2D6*

The CYP450 family is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are highly polymorphic, resulting in decreased, absent, or increased enzyme activity. One prominent CYP450 member, CYP2D6, is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers.

The *CYP2D6* Alleles

The *CYP2D6* gene is highly polymorphic, with over 170 star (*) alleles described and cataloged by the Pharmacogene Variation (PharmVar) Consortium, each associated with either normal, decreased, absent, or uncertain enzyme function (Table 2) (45, 46). Star alleles are defined by the variants detected on a single chromosome (haplotype).

An individual's combination of *CYP2D6* haplotypes determines their diplotype (for example, *CYP2D6 *4/*4*). Based on their impact on enzyme function, each allele is assigned an activity score from 0 to 1, which is then used to assign a phenotype (for example, CYP2D6 PM). However, the activity score system is not standardized across all clinical laboratories or *CYP2D6* genotyping platforms, resulting in some variability. To promote consistency, the Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG) standardized their *CYP2D6* genotype-to-phenotype methods in 2019, creating a consensus activity scoring guideline. The CYP2D6 phenotype is predicted from the diplotype activity score, defined by the sum of the allele score values, which typically ranges from 0 to 3.0: (47)

- A UM has an activity score greater than 2.25

- A normal metabolizer (NM) has an activity score of 1.25–2.25
- An intermediate metabolizer (IM) has an activity score of >0–<1.25
- A PM has an activity score of 0

Table 2. Activity Status of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Activity score
Normal function	**1, *2, *27, *33*	1
Decreased function	**17, *41, *49*	0.5
Strongly decreased function	**10*	0.25
No function	**3, *4, *5, *6, *36*	0

For a comprehensive list of *CYP2D6* alleles, please See the Pharmacogene Variation Consortium. Activity scores from (48).

The *CYP2D6*1* allele is the wild-type allele when no variants are detected and is associated with normal enzyme activity and the NM phenotype. The *CYP2D6*2*, **27*, and **33* alleles are also considered to have near-normal activity. Other *CYP2D6* alleles include variants that produce non-functioning enzymes (for example, **3*, **4*, and **6*) (49, 50, 51) or enzymes with decreased activity (for example, **10*, **17*, and **41*) (46, 52) (see Table 2). There are notable interethnic differences in allele frequency, with **3*, **4*, **6,* and **41* being more common in individuals of European ancestry, **17* more common in African ancestry, and **10* more common in Asian ancestry. (53)

Larger structural variants at the *CYP2D6* locus, including gene duplications, deletions, tandem alleles, gene hybrids, and gene conversions have also been described (54). Deletions result in no-function alleles (for example, the **5* allele). Duplications have been reported for alleles with normal, decreased, and no function. In cases of allele duplications, the activity scores of all *CYP2D6* alleles are summed to predict the metabolizer phenotype. Additional details on structural variants are available from PharmVar (see the document Structural Variation for CYP2D6) (55).

The frequency of the *CYP2D6* star alleles with altered function varies among global populations, resulting in different metabolizer phenotype frequencies. Given CYP2D6's role in the drug metabolism, the literature on allele and phenotype frequency is expansive. Most populations have a high frequency of normal-function star alleles, resulting in a high proportion of NMs. However, reduced-function alleles like *CYP2D6*10* are highly prevalent in East Asian populations, leading to a higher proportion of IMs. Many groups in sub-Saharan Africa have higher frequencies of decreased-function alleles like *CYP2D6*17* and **29*, correlating with lower CYP2D6 activity scores. Additional information on allele and phenotype frequencies is available in the CYP2D6 supplemental chapter.

Pharmacologic Conversion of CYP2D6 Phenotype

Factors other than genotype can affect CYP2D6 enzyme activity and, thus the metabolizer phenotype of any individual. Administration of an interacting medication can lead to a phenomenon called phenoconversion, in which an individual with one metabolizer genotype exhibits the enzymatic activity of a different metabolizer group (either higher or lower, depending on the medications). Studies indicate that 17–25% of individuals are impacted by phenoconversion (56).

The enzymatic activity of CYP2D6 can be inhibited by medications including strong inhibitors such as paroxetine, fluoxetine, bupropion, and quinidine, as well as moderate inhibitors like duloxetine (57, 58, 59, 60). This can potentially result in NMs or IMs responding to medications as if they were PMs, depending on the inhibitor's strength. Strong inhibitors can completely inhibit CYP2D6, while moderate inhibitors can reduce its activity by 50%. Therefore, co-medication of multiple CYP2D6 strong or moderate inhibitors may reduce the metabolism of drug substrates, as observed in psychiatric pharmacotherapy (61, 62). In contrast, discontinuing a

concomitant CYP2D6 inhibitor may allow an individual's CYP2D6 activity to revert to their genetically predicted baseline phenotype.

Integration of CYP2D6 phenoconversion into clinical practice requires knowledge of multiple clinical factors, and tools have been developed to support clinicians in this integration (63).

Other Genes of Interest: *SLC18A2*

The gene *SLC18A2* is a member of the solute carrier family and encodes VMAT2. Bi-allelic loss-of-function alleles in *SLC18A2* cause brain vesicular monoamine transport disease, also known as Parkinsonism-dystonia 2, infantile-onset, a movement disorder that presents similarly to cerebral palsy (64, 65, 66, 67, 68, 69, 70). Symptoms of brain vesicular monoamine transport disease include global developmental delay, intellectual disability, truncal hypotonia, dystonia, and oculogyric crises, among other findings (70).

The *SLC18A2* locus has also been linked with TD, with variants either increasing risk for (rs2015586) or protecting against (rs363224) TD (71, 72). Variation in *SLC18A2* has been reported in healthy individuals, though variants leading to a change in the amino acid sequence (2 missense variants in *SLC18A2*) are notably rarer than *SLC6A4* (SERT, 9 missense variants) (73). Analysis of the 4 variants identified in one study showed only one variant had notable difference (though it was non-significant) on valbenazine interaction with the VMAT2 protein, a substitution of threonine (Thr) at amino acid 249 for methionine (Met) (rs534529508; NM_003054.6:c.746C>T; NP_003045.2:p.Thr249Met). This variant was also identified in a cohort of individuals with Parkinson disease (73). Additional studies may be warranted to determine if *SLC18A2* variants are associated with response to VMAT2 inhibitors, including efficacy or adverse effects such as parkinsonism.

Linking *CYP2D6* Genetic Variation with Treatment Response

The role of CYP2D6 in valbenazine metabolism is largely based on the established metabolism of [+]-alpha-HTBZ by CYP2D6. A small clinical study demonstrated that CYP2D6 PMs (n=25) had approximately a 2-fold higher exposure to [+]-alpha-HTBZ than NM (1, 5). Analysis of IMs (n=7) indicated only a 22% increase in exposure compared to NMs and was deemed clinically insignificant (1). Additionally, inhibition of CYP2D6 enzymatic activity by paroxetine co-medication also led to increased [+]-alpha-HTBZ exposure in otherwise healthy individuals (74).

The frequency of CYP2D6 PMs is lower in East Asian populations than in the North American population included in the KINECT clinical trials, prompting a separate study of CYP2D6 IM phenotype and valbenazine pharmacokinetics in a Korean population (7). This study reported similar plasma concentrations of [+]-alpha-HTBZ (also referred to as NBI-98782) between NM and IM participants (7). Another study based in Japan (J-KINECT) examined treatment response in a cohort of Japanese ancestry and found similar rates of improvement (measured as a decrease in AIMS score) and adverse reactions as compared to the KINECT studies (12, 75). Based on pharmacokinetics and metabolism data, CYP2D6 PMs are advised to take no more than 40 mg daily per the Pharmaceuticals and Medical Devices Agency (Japan) review (2).

Genetic Testing

The NIH Genetic Testing Registry (GTR) includes tests for *CYP2D6* and valbenazine response. The available *CYP2D6* tests include targeted single-gene tests to multi-gene panels. In addition, the Association for Molecular Pathology (AMP) has recommended a panel of variant *CYP2D6* alleles to include in clinical genotyping assays (76). For *CYP2D6*, the AMP recommends that the minimum panel of variant alleles should include *2, *3, *4, *5, *6, *9, *10, *17, *29, *41, and copy number interrogation.

Results are reported as a diplotype, such as *CYP2D6 *1/*1*, and copy number testing is very important when interpreting *CYP2D6* results (77). When individuals have more than 2 copies of *CYP2D6*, the copies of the allele

are denoted by an "xN", where the "N" can either be quantified or unquantified (for example, *CYP2D6*1/*2x2* or *CYP2D6 *1/*2xN*). Some laboratories also use "DUP" to indicate an increase in copy number, though reports may not always specify the number of duplications or the allele that has been duplicated due to technical limitations. Test results may include an interpretation of the individual's predicted metabolizer phenotype, which can be verified by checking the diplotype and calculating the *CYP2D6* activity score as described in the "*CYP2D6* Alleles" section. Consideration of the detected alleles is important when interpreting test reports that do not specify which allele is duplicated or how many copies are present, as the activity score can vary if the DUP allele has decreased or no function.

Studies have reported successful implementation of pharmacogenetic testing to guide medication selection and dosing in a clinical setting (78, 79, 80). Of particular concern are cases where individuals are on multiple medications for chronic conditions, where gene–drug or gene–drug–drug interactions can significantly affect medication response (81).

The *CYP2D6* Gene Interactions with Medications Used for Additional Indications

The CYP enzyme family is involved in the metabolism of many substances, with CYP2D6 being implicated in altered pharmacologic responses for many compounds. The drugs can be categorized into many different classes:

- Antipsychotics—for example, aripiprazole, risperidone, thioridazine and—to a lesser extent—clozapine is metabolized by CYP2D6. The FDA recommends the aripiprazole dosage should be reduced in PMs and contraindicates thioridazine in individuals with reduced CYP2D6 activity due to increased risk of potentially fatal side effects. The UMs may have a decreased plasma concentration of risperidone, per DPWG recommendations.

- Tricyclic antidepressants—for example, amitriptyline, and imipramine may require dosage adjustments, potentially guided by therapeutic drug monitoring, to achieve therapeutic range in UMs or PMs. Tricyclic antidepressants may be ineffective in CYP2D6 UMs, depending on the indication and dosage used.

- Serotonin and norepinephrine reuptake inhibitors—for example atomoxetine and venlafaxine may have reduced efficacy in UMs at standard doses, while PMs are at risk of elevated plasma concentrations. The Dutch Pharmacogenetics Working Group advises against venlafaxine in CYP2D6 PMs and IMs.

- Cardiovascular dysfunction—for example, carvedilol, metoprolol, and propafenone are metabolized by CYP2D6. The PMs may have higher plasma concentrations compared with NMs, resulting in potentially undesired side effects or (in the case of metoprolol) extensive slowing of the heart rate.

- Anticancer medications—for example, tamoxifen is activated by CYP2D6, and IMs or PMs may have reduced benefit from tamoxifen therapy.

- Pain management—for example, codeine and tramadol are prodrugs that require activation by CYP2D6 to achieve the desired analgesic effect.

- Various therapies for genetic disorders—for example eliglustat used to treat Gaucher disease, and deutetrabenazine, used in HD, have reduced dose recommendations for CYP2D6 PMs. The CYP2D6 UMs may not achieve adequate concentrations of eliglustat, making *CYP2D6* genotyping necessary before initiating eliglustat therapy.

It is important to note that *CYP2D6* is the most referenced biomarker in FDA drug labels; thus, the list provided here is not exhaustive. Additional information on *CYP2D6* gene–drug interactions are available from PharmGKB, CPIC and the FDA (search for "CYP2D6").

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2024 Statement from the US Food and Drug Administration (FDA):

The recommended dosage for known CYP2D6 poor metabolizers is [valbenazine] 40 mg once daily.

[…]

The recommended dosage for patients receiving strong CYP2D6 inhibitors is [valbenazine] 40 mg once daily.

[…]

Dosage reduction of [valbenazine] is recommended for known CYP2D6 poor metabolizers. Increased exposure (C_{max} and AUC) to valbenazine's active metabolite was observed in CYP2D6 poor metabolizers. Increased exposure of active metabolite may increase the risk of exposure-related adverse reactions.

Please review the complete therapeutic recommendations that are located here: (1).

Nomenclature of Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*2	2851C>T	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*3	2550delA	NM_000106.6:c.775del	NP_000097.3:p.Arg259fs	rs35742686
CYP2D6*4	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
CYP2D6*5	Gene deletion			
CYP2D6*6	1707 del T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
CYP2D6*10	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*17	1022C>T	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*27	3854G>A	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
CYP2D6*31	2851C>T	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance with nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*36[1]	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C	NM_000106.6:c.1432C>T+ NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735+ rs766507177
	4159G>C	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G	NM_000106.6:c.1444G>A+ NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221+ rs75467367
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*41	2851C>T	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2989G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
	4181G>C	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
CYP2D6*49	100C>T	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

[1] CYP2D6*36 is a gene conversion with CYP2D7; variants provided here are from the Pharmacogene Variation Consortium.
Alleles described in this table are selected based on discussion in the text above. This is not intended to be an exhaustive description of known alleles.
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from the Pharmacogene Variation (PharmVar) Consortium.

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Vemurafenib Therapy and *BRAF* and *NRAS* Genotype

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Introduction

Vemurafenib is a kinase inhibitor used in the treatment of patients with unresectable or metastatic melanoma with the *BRAF* V600E variant.

BRAF is an intracellular kinase in the mitogen-activated protein kinases (MAPK) pathway. BRAF is involved in regulating important cell functions such as cell growth, division, differentiation, and apoptosis. BRAF is also a proto-oncogene—when mutated it has the ability to transform normal cells into cancerous cells.

Variation in the kinase domain of BRAF have been associated with various cancers. The most common *BRAF* variant, V600E, constitutively activates the kinase, and causes cell proliferation in the absence of growth factors that would normally be required. The V600E variant is detected in approximately 50% of melanomas (1, 2).

The FDA-approved drug label for vemurafenib states that the presence of *BRAF* V600E mutation in tumor specimens should be confirmed, using an FDA-approved test, before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma (3).

Variations in *NRAS*, also an oncogene, are found in up to 30% of all malignancies and in approximately 15-20% of melanomas. *NRAS* variants activate MAPK and have been implicated in in acquired resistance to BRAF inhibitors. Vemurafenib's label warns that one adverse effect associated with therapy may be the progression of pre-existing chronic myelomonocytic leukemia with *NRAS* mutation (3). Other adverse effects include arthralgia, rash, alopecia, photosensitivity reaction, pruritus, and skin papilloma.

Drug: Vemurafenib

Vemurafenib is a BRAF kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with the *BRAF* V600E variant, as detected by an FDA-approved test. It was one of the first molecularly targeted agents to receive FDA approval for advanced melanoma (3). Off-label uses of vemurafenib include the treatment of other BRAF V600E positive tumors that are not responding to traditional treatments, e.g., refractory hairy cell leukemia (4).

Skin cancer is the most common of all cancers. Although melanoma is the least common type of skin cancer, accounting for approximately 1% of cases, it is responsible for the majority of deaths from skin cancer. In the US, the lifetime risk of melanoma is approximately 2.5% for whites, 0.5% for Hispanics, and 0.1% for blacks (5).

Most cases of malignant melanoma are diagnosed at an early stage, when the tumor is localized and surgical excision can be curative. However, the 5-year survival rate drops from 98% for localized disease, to only 16% for patients with metastatic disease.

For patients with advanced metastatic or unresectable malignant melanoma, treatment options typically include immunotherapy and targeted therapy. Although chemotherapy was once widely used, it does not increase survival and therefore its use is now limited to patients who are not candidates for further treatment with either immunotherapy or targeted therapy, and for whom there is no appropriate clinical trial.

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High-dose interleukin2 (IL2) therapy may be successful in a minority of cases, but can only be used in select patients with good organ function because of the risk of severe toxicity. Immunotherapy drugs include antibodies that target programmed cell death protein 1 (PD1), e.g., nivolumab and pembrolizumab (6); and ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated protein 4 (CTLA4). Oncolytic virus therapy with T-VEC (talimogene laherparepvec) is one of the newer immunotherapy drugs approved for melanoma.

Targeted therapies are designed to inhibit components of the MAPK signaling pathway, primarily when it is constitutively activated in melanomas with the activating BRAF mutation, V600E. Drugs in this category include vemurafenib and dabrafenib, which inhibit BRAF, and trametinib and cobimetinib, which target downstream kinases MEK1 and MEK2, respectively.

Vemurafenib is a potent inhibitor of the kinase domain of the variant *BRAF* V600E. It acts by decreasing signaling through the MAPK pathway, leading to the reduced transcription of genes involved in various cellular responses. Combining vemurafenib with MEK inhibitors may potentiate these effects and has been shown to extend survival (7, 8).

Both targeted therapy with vemurafenib and immunotherapy regimens (e.g., nivolumab plus ipilimumab) have been shown to improve overall survival in patients with metastatic melanoma compared with chemotherapy (9, 10). However, at this time there are no randomized trials that compare targeted therapy with immunotherapy, and there are little data regarding the appropriate combinations and sequencing of these therapies for patients with a *BRAF* V600E variant.

In the BRIM3 trial, vemurafenib improved overall survival (13.6 versus 9.7 months) and progression-free survival (6.9 versus 1.6 months) when compared to cytotoxic chemotherapy (dacarbazine)(11). However, virtually every patient treated with a BRAF inhibitor eventually demonstrated disease progression (12). Most patients developed mechanisms of acquired resistance, which is sometimes associated with NRAS variants, and approximately15% of patients did not achieve tumor regression at all (11, 13-17).

The most common adverse events associated with vemurafenib are skin lesions (benign and malignant), fever, arthralgiab, and fatigue. Skin lesions, such as cutaneous squamous cell carcinoma, tend to occur during the first 8 weeks of treatment. Regular evaluation of the skin is recommended, with excision of suspicious lesions (18). Liver enzymes (transaminases, alkaline phosphatase, and bilirubin) should also be monitored because of the risk of liver injury. Combining BRAF with MEK inhibitors helps reduce the odds of these side effects.

Approximately 50% of cases of metastatic melanoma are found to have the *BRAF* V600E activating variant (1, 2). Because vemurafenib targets the kinase with this variant, patients without *BRAF* variants or with a different type of *BRAF* variant (e.g., V600K) should not be treated with vemurafenib; they will not benefit from vemurafenib therapy and will be needlessly exposed to adverse events. In addition, the FDA drug label warns that BRAF inhibitors have been shown to increase cell proliferation in *BRAF* wild-type cells *in vitro*.

Gene: *BRAF*

RAF is a family of intracellular kinases within the MAPK signaling pathway. The RAF family has three members, ARAF, BRAF, and CRAF (19). RAF, along with RAS (see below), are proto-oncogenes.

Proto-oncogenes are genes that, when mutated or expressed at abnormally high levels, can transform normal cells into cancerous cells. Proto-oncogenes typically encode proteins that stimulate cell division, inhibit cell differentiation, and halt cell death. The increased production of oncogenic proteins can lead to the proliferation of poorly differentiated cancer cells (20).

Germline mutations in BRAF, as well as other components of the MAPK signaling pathway, are associated with birth defects, such as cardiofaciocutaneous syndrome, characterized by heart defects, mental retardation, and a

distinctive facial dysmorphology. Somatic *BRAF* mutations are also associated with several malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas, colorectal carcinoma, and malignant melanoma.

Variations in *BRAF* are detectable in approximately 50% of malignant melanomas, and drive progression of the disease (1, 2). The *BRAF* variant V600E accounts for approximately 90% of variants. This variant is a substitution of adenine for thymine at position 1799 and results in the substitution of valine for glutamate at codon 600. The variant BRAF protein kinase is constitutively active and a highly potent oncogene, with an increase in kinase activity by as much as 500-fold compared to the wild-type (21). The second most common *BRAF* variant is V600K. Substitutions at other sites are rarer (22, 23).

Several drugs are being developed to target *BRAF* mutations, and so far, two drugs have been FDA- approved: vemurafenib and dabrafenib. Unfortunately, less progress has been made in developing targeted therapies for melanoma with wild-type *BRAF*. There are fewer treatment options available, but these include immunotherapy and MEK inhibitors (6, 24).

Gene: *NRAS*

The RAS family contains three genes, HRAS, NRAS, and KRAS, which are essential components of a number of signaling pathways. They act as signal transducers, coupling cell surface receptors to intracellular signaling pathways.

RAS proteins have intrinsic GTPase activity, they are activated by a guanine nucleotide-exchange factor, and inactivated by a GTPase activating protein. RAS proteins regulate cell signal transduction by acting as a switch; they cycle between "on" (GTP-bound) or "off" (GDP-bound) conformations. In the "on" position, RAS proteins transmit extracellular growth signals to the nucleus, primarily via the MAPK pathway. Cells are subsequently stimulated to grow, divide, mature, and differentiate.

Variations in *RAS* genes lead to RAS proteins that are resistant to GTPase, so that GTP-remains permanently bound and the receptor remains "on" providing a continual growth stimulus to cells. Such activating RAS variants are common, having been detected in colorectal cancer, lung cancer, pancreatic cancer, and melanoma.

Variations in NRAS are detectable in 15–30% of melanomas, clustering at codons 12, 13, and 61 (25, 26). These NRAS variants are the second most common oncogenic “driver” mutation in malignant melanomas, behind alternations in *BRAF* (26).

NRAS variants are associated with more aggressive melanomas, and generally a poorer prognosis (26). Currently, no therapies that specifically target NRAS have been approved. However, in the near future newer targeted therapies will likely provide effective treatment options for *NRAS*-variant melanoma (26, 27). Off-label, MEK inhibitors, especially in combination with other agents, have exhibited some efficacy in NRAS-variant melanoma.

NRAS variants are also associated with a number of other conditions, including Noonan syndrome (type 6), somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, and juvenile myelomonocytic leukemia.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the genes *BRAF* and *NRAS*.

The FDA-approved label for vemurafenib states that the presence of the *BRAF* V600E mutation should be confirmed in tumor specimens using an FDA-approved test before starting treatment with vemurafenib. The label also states that vemurafenib is not indicated for treatment of patients with wild-type *BRAF* melanoma.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2016 Statement from the US Food and Drug Administration (FDA):

Vemurafenib is indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test.

Limitation of Use: Vemurafenib is not indicated for treatment of patients with wild-type BRAF melanoma.

Patient Selection: Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with Vemurafenib. Information on FDA-approved tests for the detection of BRAF V600 mutations in melanoma is available at http://www.fda.gov/CompanionDiagnostics.

Please review the complete therapeutic recommendations that are located here: (3)

Nomenclature

Selected BRAF variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
V600E	p.Val600Glu	NM_004333.4:c.1799T>A	NP_004324.2:p.Val600Glu	rs113488022
V600K	p.Val600Lys	NM_004333.4:c.1798_1799delGTinsAA	NP_004324.2:p.Val600Lys	rs121913227
V600R	p.Val600Arg	NM_004333.4:c.1798_1799delGTinsAG	NP_004324.2:p.Val600Arg	rs121913227
V600D	p.Val600Asp	NM_004333.4:c.1799_1800delTGinsAT	NP_004324.2:p.Val600Asp	rs121913377

Selected NRAS variants

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
NRAS G12V	p.Gly12Val	NM_002524.4:c.35G>T	NP_002515.1:p.Gly12Val	rs121913237
NRAS G13R	p.Gly13Arg	NM_002524.4:c.37G>C	NP_002515.1:p.Gly13Arg	rs121434595
NRAS Q61R	p.Gln61Arg	NM_002524.4:c.182A>G	NP_002515.1:p.Gln61Arg	rs11554290
NRAS Q61K	p.Gln61Lys	NM_002524.4:c.181C>A	NP_002515.1:p.Gln61Lys	rs121913254

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

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Venlafaxine Therapy and *CYP2D6* Genotype

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Introduction

Venlafaxine (brand name Effexor) is an antidepressant used in the treatment of major depressive disorder, anxiety, and panic disorders. Venlafaxine belongs to the drug class of serotonin and norepinephrine reuptake inhibitors (SNRIs), as does its major metabolite, desvenlafaxine (brand name Pristiq).

The recommended starting dose for venlafaxine is 75 mg/day, divided into 2 or 3 doses. Depending on tolerability and clinical response, the dose may be increased to 150 mg/day, and if needed, further increased up to 225 mg/day. Only the more severely depressed individuals may respond to higher doses, up to a maximum of 375 mg/day.

Venlafaxine is metabolized into its major active metabolite, O-desmethylvenlafaxine (ODV), primarily by the CYP2D6 enzyme. As such, individuals that have high plasma concentrations of venlafaxine and low plasma concentrations of ODV when taking venlafaxine, indicates they have reduced or absent CYP2D6 activity. This can be caused by concomitant use of medications that inhibit the CYP2D6 enzyme or by germline genetic variation in the *CYP2D6* gene. Individuals who have genetic variants associated with no enzyme activity are called "CYP2D6 poor metabolizers" and account for approximately 7% of Caucasians.

The FDA-approved drug label for venlafaxine does not provide dose adjustments for CYP2D6 poor metabolizers, and states that no dose adjustment is required when venlafaxine is coadministered with a CYP2D6 inhibitor (Table 1) (1). The label states that although imipramine (an antidepressant that inhibits CYP2D6) was found to partially inhibit venlafaxine metabolism, the total concentration of active compounds (venlafaxine plus ODV) was not affected. In addition, the label cites a clinical study comparing venlafaxine use in CYP2D6 poor metabolizers and normal metabolizers, which found that the total concentration of active compounds (venlafaxine plus ODV) was similar in both metabolizer groups.

However, the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy has published venlafaxine dosing recommendations based on CYP2D6 genotype. For CYP2D6 poor and intermediate metabolizers, DPWG recommends that an alternative drug is used. If an alternative medication is not an option and side effects occur, DPWG recommends a venlafaxine dose reduction based on clinical response and drug levels. For individuals who are CYP2D6 ultrarapid metabolizers (increased CYP2D6 activity), the DPWG recommends increasing the dose of venlafaxine up to 150% of the standard dose, or using an alternative drug if dose adjustment based on therapeutic drug monitoring is not possible (Table 2) (2).

Table 1. FDA Venlafaxine: Drug Interactions and *CYP2D6* (2019)

Phenotype	Recommendations for venlafaxine therapy
CYP2D6 poor metabolizers	In a clinical study involving CYP2D6-poor and -normal metabolizers, the total concentration of active compounds (venlafaxine plus ODV), was similar in the 2 metabolizer groups
CYP2D6 inhibitors	No dosage adjustment is required when venlafaxine is coadministered with a CYP2D6 inhibitor

This FDA table is adapted from (1)

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Table 2. DPWG Therapeutic Recommendations for *CYP2D6* and Venlafaxine (2019)

Phenotype	Recommendation
CYP2D6 poor metabolizer	1. Avoid venlafaxine* 2. If it is not possible to avoid venlafaxine and side effects occur: a. reduce the dose b. monitor the effect and side effects or check the plasma concentrations of venlafaxine and O-desmethylvenlafaxine
CYP2D6 intermediate metabolizer	1. Avoid venlafaxine* 2. If it is not possible to avoid venlafaxine and side effects occur: a. reduce the dose b. monitor the effect and side effects or check the plasma concentrations of venlafaxine and O-desmethylvenlafaxine
CYP2D6 ultrarapid metabolizer	1. Be alert to a possible decrease in the sum of the plasma concentrations of venlafaxine and the active metabolite O-desmethylvenlafaxine 2. if necessary, increase the dose to 150% of the standard dose 3. if dose adjustment does not result in efficacy without unacceptable side effects or if dose adjustment based on therapeutic drug monitoring is not possible, then venlafaxine should be avoided*

* Antidepressants that are not metabolized by CYP2D6 –– or to a lesser extent –– include, for example, duloxetine, mirtazapine, citalopram, and sertraline
This DPWG table is adapted from (2)

Drug: Venlafaxine

Venlafaxine is an antidepressant used in the treatment of major depressive disorder, generalized anxiety disorder, social anxiety disorder, and panic disorder. An off-label use of venlafaxine is in the management of post-traumatic stress disorder (1, 3).

Venlafaxine is thought to exert its antidepressant effect by blocking the transporter reuptake proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. This is known as the "potentiation of neurotransmission".

Venlafaxine belongs to the drug class of serotonin-norepinephrine reuptake inhibitors (SNRIs). Other drugs with SNRI activity include atomoxetine (used in the treatment of ADHD) and tramadol (an analgesic). However, because venlafaxine also weakly inhibits dopamine reuptake, it is also referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI).

The toxicity of venlafaxine appears to be higher than for other drugs of the same class. Side effects include an increase in anxiety, insomnia, and nervousness; the precipitation of mania or hypomania in individuals with bipolar disorder; as well as weight loss, reduced appetite, hyponatremia, seizures, cardiac conduction abnormalities, and an increased risk of bleeding events.

There is also a risk of discontinuation syndrome, which may occur if venlafaxine therapy is stopped abruptly or if the dose is reduced. Symptoms include agitation, anorexia, anxiety, and confusion. A gradual reduction in the dose of venlafaxine is recommended, whenever possible (4).

Venlafaxine is metabolized in the liver to its major active metabolite, ODV. Venlafaxine and ODV share similar activity, and ODV is also an FDA-licensed antidepressant (desvenlafaxine).

The formation of ODV is catalyzed by the enzyme CYP2D6. Individuals who lack CYP2D6 activity ("CYP2D6 poor metabolizers") have a higher ratio of venlafaxine to ODV compared with normal metabolizers. As such, a venlafaxine:ODV ratio greater than one strongly predicts individuals who are CYP2D6 poor metabolizers (5).

Other hepatic enzymes (CYP3A4, CYP2C19, and CYP2C9) also metabolize venlafaxine and ODV to minor, less active metabolites (6).

The FDA-approved drug label for venlafaxine states that although CYP2D6 poor metabolizers have increased levels of venlafaxine and decreased levels of ODV compared with individuals with normal CYP2D6 activity, the differences between poor and normal metabolizers are not thought to be clinically important because the sum of venlafaxine and ODV is similar in the 2 groups.

However, recommendations from the DPWG state that for poor and intermediate metabolizers, there is insufficient data to calculate the dose adjustment for venlafaxine and an alternative drug should be used (e.g., citalopram, duloxetine, mirtazapine, sertraline). If an alternative medication is not an option and side effects occur, DPWG recommends a venlafaxine dose reduction based on clinical response, and venlafaxine and ODV plasma level monitoring (7).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

Gene: *CYP2D6*

CYP2D6 is responsible for the metabolism of many commonly prescribed drugs, including antidepressants such as venlafaxine, antipsychotics, analgesics, and beta-blockers.

The *CYP2D6* gene on chromosome 22q13.2 is highly polymorphic. Over 100 star (*) alleles have been described and cataloged at the Pharmacogene Variation (PharmVar) Consortium, and each allele is annotated with either normal, decreased or absent enzyme function (when functional status is known) (Table 3). The combination of *CYP2D6* alleles that a person has is used to determine their diplotype (e.g., *CYP2D6* *4/*4), which subsequently is used to assign a phenotype (e.g., CYP2D6 poor metabolizer).

The *CYP2D6*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype. Other *CYP2D6* alleles considered to have normal activity include **2*, **33*, and **35*.

Alleles that encode an enzyme with decreased activity include **10*, **17*, and **41*, and alleles that encode a non-functioning enzyme include **3*, **4*, **5*, and **6*. There are large inter-ethnic differences in the frequency of these alleles, with **3*, **4*, **5*, **6*, and **41* being more common in Caucasians, **10* more common in Asians, and **17* more common in Africans (8).

Table 3. Activity Status of Selected *CYP2D6* Alleles

Effect on enzyme activity	*CYP2D6* alleles
Increased function	**2xN*
Normal function	**1, *2, *33, *35*
Reduced function	**9, *10, *17, *29, *36, *41*
No function	**3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *19, *20, *21, *38, *40, *42*

Note: xN represents the number of *CYP2D6* gene copies.
For a comprehensive list of *CYP2D6* alleles, please see PharmVar.

Table 4. CPIC Assignment of likely *CYP2D6* Phenotype based on Diplotype (2019)

Likely CYP2D6 metabolizer phenotype[b]	Activity score	Genotype[a]	Examples of *CYP2D6* diplotype
Ultrarapid	>2.25	An individual with duplications of functional alleles	*1/*1xN, *1/*2xN, *2/*2xN[c]
Normal	1.25 to 2.25	An individual with 2 normal function alleles or one normal function and one decreased function allele	*1/*1, *1/*2, *1/*9, *1/*41, *2/*2, *1/*10
Intermediate	>0 to <1.25	An individual with one decreased function and one no function allele	*1/*4, *1/*5, *41/*41, *4/*10, *4/*41, *5/*9
Poor	0	An individual with only non functional alleles	*3/*4, *4/*4, *5/*5, *5/*6

[a] Assignment of allele function and citations for allele function can be found on PharmGKB: Gene Reference Materials for *CYP2D6* (*CYP2D6* Allele Definition Table and *CYP2D6* Allele Functionality Table). For a complete list of *CYP2D6* diplotypes and resulting phenotypes, see the *CYP2D6* Genotype to Phenotype Table. Note that genotypes with an activity score of one are classified as NMs in the online *CYP2D6* genotype to phenotype table (9).
[b] See the *CYP2D6* Frequency Table for race-specific allele and phenotype frequencies (9) or see Gaedigk *et al* (10).
[c] Where xN represents the number of *CYP2D6* gene copies. For individuals with *CYP2D6* duplications or multiplications, see supplemental data for additional information on how to translate diplotype into phenotype.
This Clinical Pharmacogenetics Implementation Consortium (CPIC) table is adapted from (11).

CYP2D6 Normal Metabolizers

Most individuals, around 70–80%, are classified as "normal metabolizers" (also referred to as "extensive metabolizers"). They either have 2 normal function alleles (e.g., *1/*1) or one normal and one decreased function allele (e.g., *1/*41). For these individuals, the standard recommended doses of venlafaxine should apply.

Individuals who have one normal function and one no function allele (e.g., *1/*4) or 2 decreased function alleles (e.g., *41/*41) are also categorized as "normal metabolizers" by recent nomenclature guidelines (12), but have also been categorized as "intermediate metabolizers".

CYP2D6 Intermediate and Poor Metabolizers

Individuals who do not have any fully functional alleles are either intermediate metabolizers (one decreased function and one non-functional allele e.g., *4/*41) or poor metabolizers (2 non-functional alleles e.g., *4/*4). In these individuals, the metabolic capacity of CYP2D6 is decreased, resulting in higher levels of venlafaxine and lower levels of ODV.

Approximately 6–10% of European Caucasians and their descendants are poor metabolizers, mainly due to the prevalent non-functional *4 and *5 alleles. Compared with Europeans, individuals of Asian descent are more likely to be intermediate metabolizers because of prevalent decreased function alleles, such as *10. Approximately 30% of Asians and individuals of Asian descent are intermediate metabolizers. Similarly, Africans and African Americans are more likely to be intermediate metabolizers than Europeans because of the prevalence of a wide range of decreased function variants (8, 13-15).

CYP2D6 Ultrarapid Metabolizers

Individuals who have more than 2 normal functional copies of the *CYP2D6* gene are classified as "ultrarapid metabolizers," which accounts for 1–10% of individuals (Table 4). Each allele contributes to the metabolism of venlafaxine to the active metabolite, ODV.

The ultrarapid metabolizer phenotype is estimated to be present in up to 28% of North Africans, Ethiopians, and Arabs; ~10% in Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (16).

Linking *CYP2D6* Genetic Variation with the Risk of Side Effects and Treatment Response

An individual's CYP2D6 status may influence their risk of side effects from venlafaxine therapy. Individuals who are CYP2D6 poor metabolizers have increased levels of venlafaxine and decreased levels of ODV –– this appears to translate into a higher risk of side effects and a reduced response to therapy (17). Older indivduals may be particularly at risk (18-20).

Side effects reported to occur more frequently in poor metabolizers receiving venlafaxine include gastrointestinal side effects, such as vomiting and diarrhea; and cardiovascular side effects, such as hypertension, tachycardia, and prolonged QTc interval (21, 22).

CYP2D6 genotyping prior to starting venlafaxine therapy would enable personalized dosing, which in combination with therapeutic drug monitoring, could reduce the time taken before an adequate maintenance dose is established, and prevent potential side effects (6, 20, 21, 23-25).

However, evidence for the benefits of routine *CYP2D6* genotyping is mixed. Some studies report that the metabolic changes associated with *CYP2D6* variants do not have a sufficient effect on venlafaxine therapeutic levels, and that *CYP2D6* genotyping would not predict the efficacy of venlafaxine in indivduals with depression (26-30).

Genetic Testing

The NIH's Genetic Testing Registry provides examples of the genetic tests that are currently available for venlafaxine response and for the *CYP2D6* gene.

CYP2D6 is a particularly complex gene that is difficult to genotype because of the large number of variants and the presence of gene deletions, duplications, multiplications, and pseudogenes. The complexity of genetic variation complicates making a correct determination of *CYP2D6* genotype.

Targeted genotyping typically includes up to 30 variant *CYP2D6* alleles (over 100 alleles have been identified so far). Test results are reported as a diplotype, such as *CYP2D6 *1/*1*. However, it is important to note that the number of variants tested can vary among laboratories, which can result in diplotype result discrepancies between testing platforms and laboratories (16).

A result for copy number, if available, is also important when interpreting *CYP2D6* genotyping results. Gene duplications and multiplications are denoted by "xN" e.g., *CYP2D6*1xN with xN representing the number of *CYP2D6* gene copies.

If the test results include an interpretation of the individual's predicted metabolizer phenotype, such as "*CYP2D6* *1/*1, normal metabolizer", this may be confirmed by checking the diplotype and assigning an activity score to each allele (e.g., 0 for no function, 0.5 for decreased function, and 1.0 for each copy of a normal function allele, Table 4).

The *CYP2D6* phenotype is defined by the sum of the 2 activity scores, which is typically in the range of 0 to 3.0:

- An ultrarapid metabolizer has an activity score greater than 2.25
- A normal metabolizer phenotype has an activity score of 1.25 to 2.25
- An intermediate metabolizer has an activity score of >0 to 1.25
- A poor metabolizer has an activity score of 0 (16)

The translation of *CYP2D6* diplotype to phenotype based on the activity score system was recently reported by the CPIC and DPWG (PMID: 31647186) (Table 4).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

In vitro and in vivo studies indicate that venlafaxine is metabolized to its active metabolite, ODV, by CYP2D6, the isoenzyme that is responsible for the genetic polymorphism seen in the metabolism of many antidepressants. Therefore, the potential exists for a drug interaction between drugs that inhibit CYP2D6-mediated metabolism and venlafaxine. However, although imipramine partially inhibited the CYP2D6-mediated metabolism of venlafaxine, resulting in higher plasma concentrations of venlafaxine and lower plasma concentrations of ODV, the total concentration of active compounds (venlafaxine plus ODV) was not affected. Additionally, in a clinical study involving CYP2D6-poor and -extensive metabolizers, the total concentration of active compounds (venlafaxine plus ODV), was similar in the two metabolizer groups. Therefore, no dosage adjustment is required when venlafaxine is coadministered with a CYP2D6 inhibitor.

Please review the complete therapeutic recommendations that are located here: (1).

2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2D6 Poor Metaboliser

There are indications of an increased risk of side effects and a reduced chance of efficacy.

The gene variation reduces the conversion of venlafaxine to the active metabolite O- desmethylvenlafaxine, whilst an association between high O-desmethylvenlafaxine/venlafaxine ratios and response without side effects was found.

It is not possible to offer adequately substantiated advice for dose reduction based on the literature.

1 Avoid venlafaxine

Antidepressants that are not metabolised by CYP2D6 - or to a lesser extent - include, for example, duloxetine, mirtazapine, citalopram and sertraline.

2. If it is not possible to avoid venlafaxine and side effects occur:

a. reduce the dose
b. monitor the effect and side effects or check the plasma concentrations of venlafaxine and O-desmethylvenlafaxine

It is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, while the effectiveness is maintained. In general, it is assumed that the effectiveness is determined by the sum of the

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations where necessary, other author insertions are shown in square brackets.

plasma concentrations of venlafaxine and O-desmethylvenlafaxine. However, the side effects do not appear to be related to this sum.

Furthermore, a reduced effectiveness of venlafaxine has been observed in depression patients with this gene variation.

CYP2D6 Intermediate Metaboliser

There are indications of an increased risk of side effects and a reduced chance of efficacy.

The gene variation reduces the conversion of venlafaxine to the active metabolite O- desmethylvenlafaxine, whilst an association between high O-desmethylvenlafaxine/venlafaxine ratios and response without side effects was found.

It is not possible to offer adequately substantiated advice for dose reduction based on the literature.

1 Avoid venlafaxine

Antidepressants that are not metabolised by CYP2D6 - or to a lesser extent - include, for example, duloxetine, mirtazapine, citalopram and sertraline.

2. if it is not possible to avoid venlafaxine and side effects occur:

a. reduce the dose
b. monitor the effect and side effects or check the plasma concentrations of venlafaxine and O-desmethylvenlafaxine

It is not known whether it is possible to reduce the dose to such an extent that the side effects disappear, while the effectiveness is maintained. In general, it is assumed that the effectiveness is determined by the sum of the plasma concentrations of venlafaxine and O-desmethylvenlafaxine. However, the side effects do not appear to be related to this sum.

CYP2D6 Ultrarapid Metaboliser

It may be difficult to adjust the dose for patients due to altered metabolism between venlafaxine and the active metabolite O-desmethylvenlafaxine. The gene variation increases the conversion of venlafaxine to O-desmethylvenlafaxine and reduces the sum of venlafaxine plus O- desmethylvenlafaxine.

1. be alert to a possible decrease in the sum of the plasma concentrations of venlafaxine and the active metabolite O-desmethylvenlafaxine
2. if necessary, increase the dose to 150% of the standard dose
3. if dose adjustment does not result in efficacy without unacceptable side effects or if dose adjustment based on therapeutic drug monitoring is not possible, then venlafaxine should be avoided

Antidepressants that are not metabolised by CYP2D6 - or to a lesser extent - include, for example, duloxetine, mirtazapine, citalopram and sertraline.

Please review the complete therapeutic recommendations that are located here: (2).

Nomenclature

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names / major variant	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
CYP2D6*4	1846G>A 4180G>C	NM_000106.5:c.506-1G>A NM_000106.5:c.1457G>C	Not applicable––variant occurs in a non-coding region	rs3892097
CYP2D6*5	Not applicable––variant results in a whole gene deletion			
CYP2D6*6	1707 del T	NM_000106.5:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
CYP2D6*10	100C>T Pro34Ser	NM_000106.5:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
CYP2D6*17	Includes at least 2 functional variants*: 1023C>T (Thr107Ile) 2850C>T (Arg296Cys)	NM_000106.5:c.320C>T NM_000106.5:c.886C>T	NP_000097.3:p.Thr107Ile NP_000097.3:p.Cys296Arg	rs28371706 rs16947
CYP2D6*41	2988G>A	NM_000106.5:c.985+39G>A	Not applicable––variant occurs in a non-coding region	rs28371725

Note: In the literature, 1023C>T is also referred to as 1111C>T; and 2850C>T is also referred to 2938C>T.
Note: The variant 1846G>A often occurs with both 4180G>C and 100C>T; and the variant 988G>A occurs with 2850C>T. (Cys296Arg).
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (31).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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Voriconazole Therapy and *CYP2C19* Genotype

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Introduction

Voriconazole (brand name VFend) is a broad-spectrum antifungal agent used to treat invasive fungal infections (IFI). Invasive fungal infections are an important cause of morbidity and mortality in critically ill children and immunocompromised individuals.

Voriconazole is a triazole and is the first line treatment of invasive aspergillosis. It is also licensed to treat candidemia (in individuals who do not have neutropenia), disseminated candidiasis, and esophageal candidiasis. For serious fungal infections caused by Scedosporium and Fusarium species, voriconazole may be used in those who are unable to take, or have not responded to, other therapy (1).

Therapeutic drug monitoring of voriconazole has become the standard of care to ensure efficacy and avoid adverse effects (2, 3). Low serum voriconazole concentrations have been associated with treatment failure, which may have devastating consequences in individuals who are seriously ill with an invasive infection. High serum voriconazole concentrations are associated with adverse effects, such as neurotoxicity.

Interindividual drug serum concentrations vary widely among individuals treated with a dose of voriconazole, which is due in part to genetic variation in the *CYP2C19* gene. Voriconazole is primarily metabolized by the CYP2C19 enzyme, with contributions by CYP2C9 and CYP3A4.

Individuals who lack CYP2C19 activity ("CYP2C19 poor metabolizers") have, on average, 4-fold higher voriconazole exposure than normal metabolizers (Table 1). In contrast, individuals who have increased CYP2C19 activity ("rapid" and "ultrarapid metabolizers") have lower serum concentrations of voriconazole (1, 4). Genetic tests are currently available for the voriconazole response and the *CYP2C19* gene.

The FDA-approved drug label for voriconazole discusses the influence of *CYP2C19* on drug levels but does not provide specific dosing recommendations based on the CYP2C19 metabolizer status (Table 1). The label currently only incorporates the type of infection and the individuals weight into the dosing guidelines (1).

However, dosing recommendations for voriconazole based on CYP2C19 metabolizer type are available from the Dutch Pharmacogenetics Working Group (DPWG, Table 2) and the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 3) (4, 5).

Table 1. FDA (2019) Drug Label for Voriconazole Therapeutic Recommendations based on *CYP2C19* Genotype

Phenotype	Voriconazole
CYP2C19 poor metabolizers	Studies conducted in Caucasian and Japanese healthy subjects have shown that poor metabolizers have, on average, 4-fold higher voriconazole exposure (AUCτ) than their homozygous normal metabolizer counterparts. Subjects who are heterozygous normal metabolizers have, on average, 2-fold higher voriconazole exposure than their homozygous normal metabolizer counterparts

Please see Therapeutic Recommendations based on Genotype for more information from the FDA. This table is adapted from (1).

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Table 2. DPWG (2019) Recommendations for Voriconazole and *CYP2C19* Genotype

Phenotype	Recommendations
CYP2C19 poor metabolizers	Use 50% of the standard dose and monitor the plasma concentration.
CYP2C19 intermediate metabolizers	Monitor the plasma concentration.
CYP2C19 ultrarapid metabolizers	Use an initial dose that is 1.5x higher and monitor the plasma concentration.

Please see Therapeutic Recommendations based on Genotype for more information from DPWG. This table is adapted from (5).

Table 3. CPIC (2016) Dosing Recommendations for Voriconazole Treatment based on CYP2C19 Phenotype for Adults

CYP2C19 phenotype	Implications for voriconazole pharmacologic measures	Therapeutic recommendations	Classification of recommendations[a]
CYP2C19 ultrarapid metabolizer (*17/*17)	In individuals for whom an ultrarapid metabolizer genotype (*17/*17) is identified, the probability of attainment of therapeutic voriconazole concentrations is small with standard dosing	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b]	Moderate[c]
CYP2C19 rapid metabolizer (*1/*17)	In individuals for whom a rapid metabolizer genotype (*1/*17) is identified, the probability of attainment of therapeutic concentrations is modest with standard dosing	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b]	Moderate
CYP2C19 normal metabolizer	Normal voriconazole metabolism	Initiate therapy with recommended standard of care dosing.[b]	Strong
CYP2C19 intermediate metabolizer	Higher dose-adjusted trough concentrations of voriconazole compared with normal metabolizers	Initiate therapy with recommended standard of care dosing.[b]	Moderate
CYP2C19 poor metabolizer	Higher dose-adjusted trough concentrations of voriconazole and may increase probability of adverse events	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.[b] In the event that voriconazole is considered to be the most appropriate agent, based on clinical advice, for an individual with poor metabolizer genotype, voriconazole should be administered at a preferably lower than standard dos- age with careful therapeutic drug monitoring.	Moderate

[a] Rating scheme is described in Supplementary Data online (4).
[b] Further dose adjustments or selection of alternative therapy may be necessary due to other clinical factors, such as drug interactions, hepatic function, renal function, species, site of infection, therapeutic drug monitoring, and comorbidities.
[c] Recommendations based upon data extrapolated from individuals with CYP2C19*1/*17 genotype.
Please see Therapeutic Recommendations based on Genotype for more information from CPIC. This table is adapted from (4).

Drug: Voriconazole

Voriconazole is a broad-spectrum antifungal agent that belongs to the drug class of triazole antifungals. There currently are 5 triazole antifungal drugs licensed for use in the United States: fluconazole, isavuconazole,

itraconazole, posaconazole, and voriconazole. These medications vary in how they are administered, the pathogens they target, and their side effects (6).

Compared with other triazole antifungals, voriconazole has enhanced activity against the Aspergillus species, and similar to other triazole antifungals, voriconazole is active against the Candida species. The Infectious Diseases Society of America recommend voriconazole as the first-line therapy for invasive aspergillosis, and as an alternative therapy for candidemia, in individuals who do not have neutropenia (4, 7).

Voriconazole is also used to treat esophageal candidiasis, disseminated candidiasis (in skin, abdomen, kidney, bladder wall, and wounds), and serious infections caused by Scedosporium apiospermum complex and Fusarium species, including *Fusarium solani* in individuals intolerant of, or refractory to, other therapy (1).

A healthy adult has an immune system that can prevent a fungal infection becoming invasive and disseminating. But IFI can be life threatening in adults who have a weakened immune system. Susceptible individuals may be at the extremes of age (very young, or elderly), or be immunocompromised because of a disease or its treatment (e.g., cancer, chemotherapy, immunosuppression following transplant surgery). Genetic conditions may also cause immunodeficiency. For these individuals, early treatment of IFI is associated with increased survival (3, 8, 9).

Triazoles share a similar mechanism of action – they disrupt the synthesis of ergosterol, an important part of the fungal cell membrane. They do this by inhibiting the fungal enzyme that produces ergosterol (lanosterol 14-alpha-demethylase). The damaged fungal cell membrane becomes more permeable, resulting in cell lysis and death.

Triazoles are generally well tolerated but they have a narrow therapeutic index. Gastrointestinal symptoms are most frequently reported, including nausea, abdominal pain, vomiting, and diarrhea. All triazoles have been associated with liver dysfunction and hepatotoxicity. Therefore, careful monitoring of liver enzymes is recommended for everyone receiving triazole therapy (6).

Voriconazole can cause fetal harm and should not be used during pregnancy unless the benefit to the mother outweighs the risk to the fetus. In animal studies, voriconazole was associated with teratogenicity (abnormal development of the embryo), embryo toxicity, and death. If voriconazole is used during pregnancy, or if the individual becomes pregnant while taking voriconazole, they should be informed of the potential hazards to the fetus.

Adverse effects specifically associated with voriconazole therapy include vision changes (e.g., photopsia – flashes of light, and photophobia – increased sensitivity to light), periostitis (inflammation of the periosteum that surrounds bones), and neurological toxicity (e.g., visual hallucinations, encephalopathy, and neuropathy).

Clinically, it is important to distinguish between vision changes, which tend to be minor and reversible, and visual hallucinations, which may be one of the first indications of severe neurotoxicity.

Voriconazole can be administered orally or by IV, and a loading dose is given at the start of therapy. For the treatment of invasive aspergillosis in adults, an IV loading dose of 6 mg/kg every 12 hours for 2 doses is recommended, followed by an IV maintenance dose of 4 mg/kg every 12 hours. Intravenous treatment should be continued for at least 7 days. After the individual has improved clinically, oral voriconazole can be used instead of IV (recommended maintenance dose of 200 mg every 12 hours).

The voriconazole drug label states that dose adjustment may be indicated for cases of non-response (dose increased), for individuals who cannot tolerate the medication, have liver insufficiency, or for adults who weigh less than 40 kg (dose decreased). The dose may also need to be adjusted based on concurrent therapy, as many drugs (particularly those that inhibit or induce CYP3A4, CYP2C9, or CYP2C19) can lead to altered voriconazole levels (1).

The dosing of voriconazole is further complicated by the elimination of the drug being characterized by "non-linear pharmacokinetics". Pharmacokinetics is the study of the movement of drugs in the body, including the processes of absorption, distribution, metabolism, and excretion. The term "linear pharmacokinetics" refers to a graph that shows a straight line when various factors are plotted e.g., the dose of the drug versus the serum concentration of the drug. For voriconazole, the observed "non-linear" pharmacokinetics means that above a certain drug dose, the concentration of the drug in the serum increases disproportionately. This occurs because the enzymes responsible for metabolizing and eliminating voriconazole become saturated (e.g., CYP2C19), (10).

In children, however, voriconazole has been found to show linear pharmacokinetics over a wider range of drug doses. This is thought to be because children have a higher expression of CYP2C19, and therefore an increased capacity to metabolize voriconazole. This means that children will often require higher doses to achieve therapeutic drug concentrations (3, 11).

There is substantial variability in voriconazole serum drug concentrations among individuals receiving standard doses of voriconazole. This is in part due to non-linear kinetics and other factors listed above (liver function, comorbidities, concurrent medications, age of the individual), as well as the presence of inflammation, and interindividual pharmacogenetic variability (12, 13).

Genetic variants in the *CYP2C19* gene play an important role in voriconazole serum concentration variability. Voriconazole is metabolized primarily by CYP2C19, and to a lesser extent by CYP3A4 and CYP2C9. Individuals who lack CYP2C9 activity (up to 20% of individuals of Asian descent and 3-5% in many other populations, Table 4) will have a higher exposure to voriconazole in response to standard doses, and are at a higher risk of adverse effects (3, 4, 9). Genetic variation in the *CYP3A4* gene may also influence voriconazole pharmacokinetics (14-17).

Therapeutic drug monitoring of voriconazole has now become the standard of care in many medical centers to improve treatment efficacy and avoid toxicity. However, if a individuals's *CYP2C19* status is known, sub- and supratherapeutic voriconazole concentrations can potentially be avoided in individuals vulnerable to severe infections. Voriconazole dosing recommendations based on *CYP2C19* genotype and/or phenotype have been published by CPIC and DPWG (see Therapeutic Recommendations based on Genotype). (2, 4, 5, 18-23).

Although the FDA drug label states voriconazole is indicated for individuals aged 12 years and above, voriconazole is used in children with IFI, and the label discusses pediatric use. As such, CPIC have provided therapeutic recommendations for the use of voriconazole based on *CYP2C19* genotype for pediatric individuals (children and adolescents less than 18 years old) (1, 4).

Gene: CYP2C19

The cytochrome P450 (CYP) superfamily is a large and diverse group of hepatic enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs. The CYP genes are very polymorphic and can result in reduced, absent, or increased drug metabolism.

The CYP2C19 enzyme contributes to the metabolism of a range of clinically important drugs, such as antidepressants, benzodiazepines, antiplatelet agents, some proton pump inhibitors, and antifungal agents such as voriconazole.

The *CYP2C19* gene is highly polymorphic, as there are currently 35 variant star (*) alleles cataloged by the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C19*1* is considered the wild-type allele when no variants are detected and is associated with normal enzyme activity and the "normal metabolizer" phenotype.

The *CYP2C19*17* allele is associated with increased enzyme activity and is found among individuals with 'rapid' (**1/*17*) and 'ultrarapid' (**17/*17*) metabolizer phenotypes. Heterozygous carriers of non-functional alleles (e.g.,

*2 and *3) are classified as 'intermediate metabolizers' (e.g., **1/*2*), and individuals who have 2 non-functional alleles are classified as "poor metabolizers" (e.g., **2/*2*, **2/*3*) (Table 4).

Table 4. CPIC (2016). Assignment of CYP2C19 Phenotype based on Genotype.

Phenotype	Genotype	Examples of diplotypes
CYP2C19 ultrarapid metabolizer (approximately 2–5% of individuals)[a]	An individual with 2 increased function alleles	**17/*17*
CYP2C19 rapid metabolizer (approximately 2–30% of individuals)	An individual with one normal function allele and one increased function allele	**1/*17*
CYP2C19 normal metabolizer (approximately 35–50% of individuals)	An individual with 2 normal function alleles	**1/*1*
CYP2C19 intermediate metabolizer (approximately 18–45% of individuals)	An individual with one normal function allele and one no function allele or one no function allele and one increased function allele	**1/*2* **1/*3* **2/*17*[b]
CYP2C19 poor metabolizer (approximately 2–15% of individuals)	An individual with 2 no function alleles	**2/*2* **2/*3* **3/*3*

[a] CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies. See the *CYP2C19* Frequency Tables for population-specific allele and phenotype frequencies (4).
[b] The predicted metabolizer phenotype for the**2/*17* genotype is a provisional classification. The currently available evidence indicates that the *CYP2C19*17* increased function allele is unable to completely compensate for the *CYP2C19*2* no function allele.
This CPIC table is adapted from (4).

Approximately 2% of Caucasians, 4% of African Americans, and 14% of Chinese are *CYP2C19* poor metabolizers, and up to 45% of individuals are CYP2C19 intermediate metabolizers (19).

The most common no function allele is *CYP2C19*2*, which is defined by a c.681G>A variant in exon 5 that creates an aberrant splice site that translates a truncated and non-functioning protein. The *CYP2C19*2* allele frequencies are ~15% in Caucasians and Africans, and ~29–35% in Asians (24).

Another commonly tested no function allele is *CYP2C19*3,* which is defined by a c.636G>A variant in exon 4 that creates a premature stop codon. The CYP2C19*3 allele frequencies are ~2-9% in Asian populations, but rare in other racial groups. Other no function variants occur in less than 1% of the general population, and include CYP2C19*4-*8 (24).

The *CYP2C19*17* allele is an increased function allele characterized by a promoter variant that results in increased gene expression, and *is* commonly tested for with an allele frequency of 4-21%.

Linking Gene Variation with Treatment Response

Although studies have not consistently found an association between the *CYP2C19* genotype and the toxicity or efficacy of voriconazole, *CYP2C19* genotype does contribute to the variation observed in voriconazole pharmacokinetics and potentially, could be used to guide the initial dose selection (25, 26).

The presence of *CYP2C19* variants can lead to increased or decreased voriconazole serum concentrations (27, 28). Low concentrations of voriconazole are associated with treatment failure. High concentrations are not associated with an increase in efficacy but are associated with serious adverse effects such as neurotoxicity (2, 4).

CYP2C19 Poor Metabolizers

Individuals who are CYP2C19 poor metabolizers have increased serum voriconazole concentrations, which are up to 4 times higher than normal CYP2C19 metabolizers. However, this difference is most marked in healthy

volunteers – studies with patients have found conflicting results, most likely due to factors such as drug interactions, other conditions, and organ dysfunction (2, 3).

Several studies have found that increased voriconazole serum concentrations are associated with increased risk of side effects, including hepatotoxicity, visual hallucinations and encephalopathy (4, 18, 29-32). The FDA confirms that CYP2C19 poor metabolizers have higher exposure to voriconazole, but the label does not discuss alternative dosing based on CYP2C19 metabolizer status. However, dosing guidelines based on CYP2C19 genotype have been published by CPIC and DPWG.

Therapeutic recommendations from CPIC for CYP2C19 poor metabolizers include choosing an alternative agent that is not dependent upon CYP2C19 metabolism, or if there is a strong case for using voriconazole, use a lower dose than standard with careful therapeutic drug monitoring. For all genotypes, CPIC recommend bearing in mind that further dose adjustments or selection of alternative therapy may be necessary due to other clinical factors, such as drug interactions, hepatic function, renal function, fungal species, site of infection, therapeutic drug monitoring, and comorbidities (Table 3) (4).

For CYP2C19 poor metabolizers, the DPWG recommend using 50% of the standard dose, again with careful monitoring (see Therapeutic Recommendations based on Genotype) (4, 5).

CYP2C19 Intermediate Metabolizers

Data are lacking for CYP2C19 intermediate metabolizers, therefore CPIC recommend following the standard dosing regimen, with therapeutic drug monitoring. The DPWG also recommends the standard dose with therapeutic drug monitoring (4, 5).

CYP2C19 Rapid and Ultrarapid Metabolizers

Trough concentrations of voriconazole can predict the clinical response, with low levels associated with a lower response rate and treatment failure (18, 30, 31, 33-35). Low levels of voriconazole are found in individuals who are CYPC2C19 rapid (individuals who have one copy of *CYP2C19*17*) or ultrarapid (individuals who have 2 copies of *CYP2C19*17*) metabolizers Several studies have found that the *CYP2C19*17* allele is associated with subtherapeutic voriconazole concentrations (2, 27, 36-38).

For these individuals, attempting to achieve therapeutic drug levels may be unsuccessful, or cause serious delays, allowing the invasive fungal disease to progress (3).

For CYP2C19 rapid and ultrarapid metabolizers, CPIC recommends an alternative antifungal agent that is not dependent on CYP2C19 metabolism, whereas the DPWG recommends using an initial dose of voriconazole that is 1.5 times higher than the standard dose, with therapeutic drug monitoring (Table 3, Therapeutic Recommendations based on Genotype) (4, 5).

Genetic Testing

Clinical genotyping tests are available for several *CYP2C19* alleles. The NIH's Genetic Testing Registry (GTR) provides examples of the genetic tests that are currently available for the voriconazole response and the *CYP2C19* gene. In addition, variant *CYP2C19* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (39).

Usually an individual's result is reported as a diplotype, such as *CYP2C19 *1/*1*, and may also include an interpretation of the predicted metabolizer phenotype (ultrarapid, normal, intermediate, or poor). Table 4 summarizes common CYP2C19 phenotypes.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2019 Statement from the US Food and Drug Administration (FDA)

CYP2C19, significantly involved in the metabolism of voriconazole, exhibits genetic polymorphism. Approximately 15 to 20% of Asian populations may be expected to be poor metabolizers. For Caucasians and Blacks, the prevalence of poor metabolizers is 3 to 5%. Studies conducted in Caucasian and Japanese healthy subjects have shown that poor metabolizers have, on average, 4-fold higher voriconazole exposure (AUCτ) than their homozygous normal metabolizer counterparts. Subjects who are heterozygous normal metabolizers have, on average, 2-fold higher voriconazole exposure than their homozygous normal metabolizer counterparts.

Please review the complete therapeutic recommendations that are located here: (1)

2019 Statement from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

CYP2C19 Poor Metabolizers

The gene variation can reduce the conversion of voriconazole and consequently increase the plasma concentration. This could result in improved efficacy or an increase in the risk of side effects. Initially, the risk of side effects is of particular interest.

Recommendation: Use 50% of the standard dose and monitor the plasma concentration

CYP2C19 Intermediate Metabolizers

The gene variation can reduce the conversion of voriconazole and consequently increase the plasma concentration. This could result in improved efficacy or an increase in the risk of side effects.

Recommendation: Monitor the plasma concentration

CYP2C19 Ultrarapid metabolizers

The gene variation increases the conversion of voriconazole, which increases the risk of ineffectiveness.

Recommendation: Use an initial dose that is 1.5x higher and monitor the plasma concentration

Background information

Mechanism:

Voriconazole is predominantly metabolised by CYP2C19 and otherwise by CYP2C9 and CYP3A4. The most important metabolite, voriconazole-N-oxide, is inactive.

For more information about CYP2C19 phenotypes: see the general background information about CYP2C19 on the KNMP Knowledge Bank or on www.knmp.nl (search for key word “CYP2C19”).

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

Other considerations:

Several studies indicate a higher risk of hepatotoxicity at higher plasma concentrations of voriconazole. However, the relationship between the plasma concentration and the effect or side effects (hepatotoxicity) has not been clearly identified.

The kinetics of voriconazole are non-linear at therapeutic doses.

Please review the complete therapeutic recommendations that are located here: (5).

2016 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Clinical studies have not consistently demonstrated an association between CYP2C19 genotype and adverse reactions. However, as individual patients who are poor metabolizers may have elevated levels leading to toxicity, the use of another antifungal agent is recommended. Under circumstances in which voriconazole is strongly indicated for treatment of an invasive mycosis in a patient with a poor metabolizer phenotype, administration of a lower dosage with meticulous therapeutic drug monitoring may be feasible (Table 3).

Knowledge of CYP2C19 ultrarapid and rapid metabolizer genotypes may prevent subtherapeutic concentrations of voriconazole that may lead to treatment failure. In such cases, an alternative antifungal agent also is recommended, especially as several case reports have documented voriconazole treatment failure in CYP2C19 ultrarapid metabolizers (see Supplementary Table S1 online). Attempting to obtain therapeutic levels in patients with ultrarapid metabolizer genotypes are often unsuccessful. Serious delays in achieving therapeutic concentrations in such patients with active invasive mycoses may result in disease progression.

Several alternative agents may be used instead of voriconazole for treatment of invasive mold infections. These include isavuconazole, lipid formulations of amphotericin B, and posaconazole (Table 3). The antifungal triazole isavuconazole is approved for the primary treatment of invasive aspergillosis and invasive mucormycosis and is available in intravenous and oral dosage forms. As isavuconazole is a substrate of CYP3A4, variant alleles in this gene are unlikely to affect its clearance. Only limited data for isavuconazole are currently available in the pediatric population. Liposomal amphotericin B is an alternative therapy to voriconazole for the primary treatment of invasive aspergillosis. Posaconazole is currently indicated for salvage therapy of invasive aspergillosis. The recently approved posaconazole delayed release and intravenous dosage forms achieve higher concentrations than that of the posaconazole suspension. However, intravenous posaconazole requires administration via a central line due to phlebitis with peripheral administration. Similar to voriconazole, intravenous posaconazole also contains the solubilizer sulfobutylether-beta-cyclodextrin sodium. Posaconazole is cleared largely as unchanged compound with <20% of compound being excreted as a glucuronide conjugate. Uridine 50-diphospho- glucuronosyltransferase glucuronidation of posaconazole is not significantly affected by genetic variation. Administration of posaconazole should still be guided by TDM.

Please review the complete therapeutic recommendations that are located here: (4).

Nomenclature for selected *CYP2C19* alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*2*	681G>A Pro227Pro	NM_000769.1:c.681G>A	NP_000760.1:p.Pro227=	rs4244285
*CYP2C19*3*	636G>A Trp212Ter	NM_000769.1:c.636G>A	NP_000760.1:p.Trp212Ter	rs4986893

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C19*17*	-806C>T	NM_000769.1:c.-806C>T	Not applicable - variant occurs in a non-coding region	rs12248560

dbSNP: The Single Nucleotide Polymorphism Database
Note: the normal "wild-type" allele is *CYP2C19*1*.
Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (40).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).
Nomenclature for cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.
Please note that the CYP2C19*2 defining variant (rs4244285) has recently been reported to be in high linkage disequilibrium with an intronic variant implicated in aberrant slicing (rs12769205) (41).

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Warfarin Therapy and *VKORC1* and *CYP* Genotype

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Introduction

Warfarin (brand name Coumadin) is an anticoagulant (blood thinner). Warfarin acts by inhibiting the synthesis of vitamin K-dependent clotting factors and is used in the prevention and treatment of various thrombotic disorders. Warfarin is a drug with narrow therapeutic index; thus, a small change in its plasma levels may result in concentration dependent adverse drug reactions or therapeutic failure. Therefore, the dose of warfarin must be tailored for each patient according to the patient's response, measured as INR (International Normalized Ratio), and the condition being treated.

There is a wide inter-individual variability in the dose of warfarin required to achieve target anticoagulation, and the time it takes to reach target INR. Approximately half of this variability is known to be caused by clinical or lifestyle factors (e.g., a patient's age, weight, BMI, gender, smoking status, existing conditions, and concomitant medications) and by genetic factors (known genetic factors include variants in the *VKORC1*, *CYP2C9*, *CYP4F2* genes, and the rs12777823 variant in the *CYP2C* gene cluster on chromosome 10) (1).

The *VKORC1* and *CYP2C9* genotypes are the most important known genetic determinants of warfarin dosing. Warfarin targets VKORC1, an enzyme involved in vitamin K recycling. A common variant, *VKORC1*, c.-1639G>A, is associated with an increased sensitivity to warfarin and lower dose requirements. The CYP2C9 enzyme metabolizes warfarin and the variants *CYP2C9*2* and **3*, are also associated with lower dose requirements.

The FDA-approved drug label for warfarin states that *CYP2C9* and *VKORC1* genotype information, when available, can assist in the selection of the initial dose of warfarin. The label provides 2 sets of warfarin dosing recommendations, for when the *CYP2C9* and *VKORC1* genotypes are either known (Table 1) or not known (taking into account clinical factors, the initial dose of warfarin is usually 2–5 mg once daily) (1).

In addition, the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP) has published recommendations for the initial standard dose of warfarin. A dose reduction is recommended for individuals who are CYP2C9 poor and intermediate metabolizers (with the exception of intermediate metabolizers with the CYP2C9*1/*2 genotype, no dose change is required), and a dose reduction is recommended for individuals who carry 2 copies of the variant *VKORC1* A allele *(VKORC1,* c.-1639G>A/A*)* (Table 2) (2, 3).

Recently, genetic variation in the *CYP4F2* gene, and a variant near the *CYP2C* gene cluster, rs12777823, have been associated with influencing warfarin therapy. The *CYP4F2*3* variant is associated with a modest increase in warfarin dose requirements in individuals with European or Asian ancestry, while in individuals with African ancestry, the rs12777823 A/G or A/A genotype is associated with decreased warfarin dose requirements.

The 2017 Update of the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing, provides warfarin dosing recommendations for adults with and without African ancestry, and also for pediatric patients (see Therapeutic Recommendations). CPIC recommends that these dosing guidelines are applied after a warfarin dose has been calculated using a validated pharmacogenetic algorithm, which includes genotype information for *VKORC1,* c.-1639G>A and *CYP2C9*2* and **3* (Figure 1) (4)

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Table 1. The FDA (2017) Drug Label for Warfarin. Three Ranges of Expected Maintenance Warfarin Doses based on CYP2C9 and VKORC1 Genotype.

VKORC1	*CYP2C9*					
	**1/*1*	**1/*2*	**1/*3*	**2/*2*	**2/*3*	**3/*3*
GG	5–7 mg	5–7 mg	3–4 mg	3–4 mg	3–4 mg	0.5–2 mg
AG	5–7mg	3–4 mg	3–4 mg	3–4 mg	0.5–2 mg	0.5–2 mg
AA	3–4 mg	3–4 mg	0.5–2 mg	0.5–2 mg	0.5–2 mg	0.5–2 mg

Ranges are derived from multiple published clinical studies. The *VKORC1, c.–1639G>A* (rs9923231) variant is used in this table. Other co-inherited *VKORC1* variants may also be important determinants of warfarin dose. Patients with *CYP2C9 *1/*3*, **2/*2*, **2/*3*, and **3/*3* may require more prolonged time (>2–4 weeks) to achieve a maximum international normalized ratio (INR) effect for a given dosage regimen than patients without these *CYP* variants.
Please see Therapeutic Recommendations based on Genotype for more information. This table is adapted from the FDA-approved drug label for warfarin (1).

Table 2. The DPWG (2017) Recommendations for Warfarin and *CYP2C9* and *VKORC1* Genotype.

Phenotype/diplotype	Recommendation
CYP2C9 IM	Use 65% of the standard initial dose
CYP2C9 PM	Use 20% of the standard initial dose
CYP2C9*1/*2	No action is required for this gene-drug interaction.
CYP2C9*1/*3	Use 65% of the standard initial dose
CYP2C9*2/*2	Use 65% of the standard initial dose
CYP2C9*2/*3	Use 45% of the standard initial dose
CYP2C9*3/*3	Use 20% of the standard initial dose
VKORC1 C/T	No action is required for this gene-drug interaction
VKORC1 T/T	Use 60% of the standard initial dose

Note: VKORC1 1173C>T is equivalent to c.-1639G>A. Therefore:
"VKORC1 CT" corresponds to VKORC1, c.-1639 G/A
"VKORC1 TT" corresponds to VKORC1, c.-1639 A/A
Please see Therapeutic Recommendations based on Genotype for more information from the Dutch Pharmacogenetics Working Group (DPWG). Table is adapted from (2, 3).

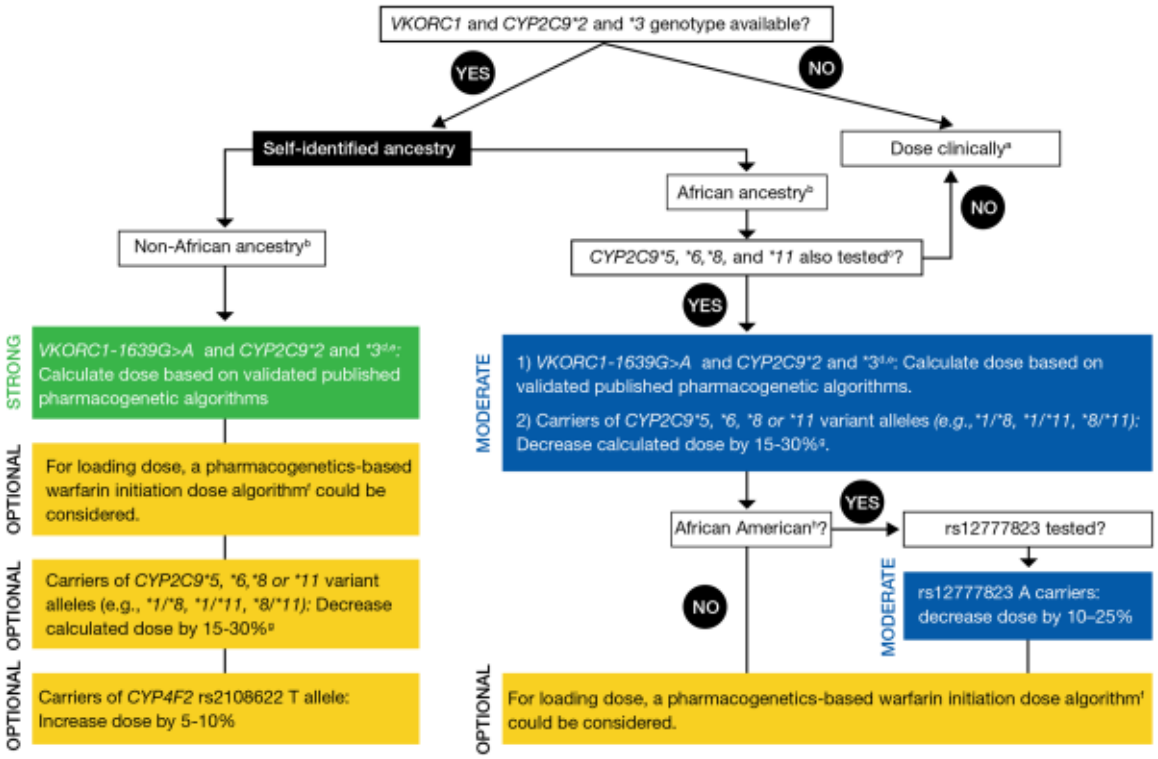

Figure 1. The CPIC (2017) Dosing Recommendations for Warfarin Dosing based on Genotype for Adult Patients. (a) "Dose clinically" means to dose without genetic information, which may include use of a clinical dosing algorithm or standard dose approach. (b) Data strongest for European and East Asian ancestry populations and consistent in other populations. (c) 45–50% of individuals with self-reported African ancestry carry CYP2C9*5, *6, *8, *11, or rs12777823. If CYP2C9*5, *6, *8, and *11 were not tested, dose warfarin clinically. Note: these data derive primarily from African-Americans, who are largely from West Africa. It is unknown if the same associations are present for those from other parts of Africa. (d) Most algorithms are developed for the target INR 2-3. (e) Consider an alternative agent in individuals with genotypes associated with CYP2C9 poor metabolism (e.g., CYP2C9*3/*3, *2/*3, *3/*3) or both increased sensitivity (VKORC1 A/G or A/A) and CYP2C9 poor metabolism. (f) See the EU-PACT trial for pharmacogenetics-based warfarin initiation (loading) dose algorithm with the caveat that the loading dose algorithm has not been specifically tested or validated in populations of African ancestry. (g) Larger dose reduction might be needed in variant homozygotes (i.e., 20–40%). (h) African-American refers to individuals mainly originating from West Africa.
This figure is adapted from (4). Please see Therapeutic Recommendations based on Genotype for more information from CPIC.

Drug: Warfarin

Warfarin is an anticoagulant used in the prevention and treatment of venous thrombosis, pulmonary embolism, and the complications associated with atrial fibrillation and/or cardiac valve replacement. Warfarin is sometimes prescribed to reduce the risk of stroke after a myocardial infarction (MI).

Warfarin has no direct effect on an established thrombus. However, once a thrombus has occurred (e.g., deep venous thrombosis), the goal of warfarin therapy is to prevent further extension of the formed clot and to prevent secondary thromboembolic complications that may be fatal (e.g., pulmonary embolism).

Warfarin is a teratogen – an agent that can cause abnormalities in a developing fetus. Therefore, warfarin use in pregnancy is contraindicated, except in women with mechanical heart valves who have a particularly high risk of thromboembolism. If warfarin is used in pregnancy, or if a patient becomes pregnant while taking warfarin, she should be informed of the potential risks to the fetus (1).

Warfarin exposure in pregnancy can cause fetal death, neonatal death, and warfarin syndrome - a pattern of developmental abnormalities that most commonly affect bone and cartilage, causing nasal hypoplasia, and a

"stippled" appearance to the ends of long bones. The risk of warfarin teratogenicity appears to be greatest between the 6th and 12th week of pregnancy, but toxicity before or after this period is still possible (5, 6).

Warfarin exerts its anticoagulant effect by inhibiting the enzyme encoded by *VKORC1*, which catalyzes the conversion of vitamin K epoxide to the active reduced form of vitamin K, vitamin K hydroquinone. Vitamin K hydroquinone is an essential cofactor in the synthesis of several clotting factors and decreased availability of vitamin K hydroquinone leads to decreased activity of the clotting factors II, VII, IX, and X, and the anticoagulant proteins C and S (7).

Warfarin is administered as a racemic mixture of the *R*- and *S*- stereoisomers. (*S*)-warfarin is 2–5 times more potent than (*R*)-warfarin and is mainly metabolized by CYP2C9. (*R*)-warfarin is mainly metabolized by other cytochrome P450 enzymes (8).

The initial and maintenance doses of warfarin must be tailored to each patient, and monitoring of the international normalized ratio (INR) should be performed in all patients treated with warfarin. The INR is a standardized measurement of prothrombin time, which is the time it takes for blood to clot. In healthy individuals, the INR is approximately one (range: 0.8–1.1). The goal of warfarin therapy is to achieve an INR in a target range for the condition being treated (most commonly 2–3).

The FDA-approved drug label for warfarin carries a boxed warning cautioning of the risk of bleeding, which can be fatal. Bleeding is more likely to occur within the first month, and risk factors include a high intensity of anticoagulation (INR greater than 4), age greater than or equal to 65, and a history of highly variable INRs. Other serious adverse events associated with warfarin therapy include necrosis of the skin and other tissues, particularly when used prematurely to manage thrombosis associated with heparin-induced thrombocytopenia (HIT).

Since warfarin is a drug with a narrow therapeutic index, an optimal starting dose may reduce the time taken to reach a stable INR and reduce the risk of having either a high INR (with a risk of bleeding) or a low INR (with a risk of thrombosis). Known factors that influence an individual's response to the initial dose of warfarin include clinical and lifestyle factors (e.g., age, race, body weight, height, gender, concomitant medications—including those that compete for binding to albumin, comorbidities, diet, nutritional status) and genetic factors (e.g., *CYP2C9* and *VKORC1* genotypes). Therefore, the initial dose should be modified to take into account these and any additional patient-specific factors that may influence warfarin dose requirement.

The FDA-approved drug label for warfarin suggests considering a lower initial and maintenance dose of warfarin for elderly and/or debilitated patients, and in Asian patients. The drug label recommends against the routine use of loading doses because this practice may increase hemorrhagic and other complications and does not offer more rapid protection against clot formation. However, loading doses are used in practice, and are addressed in CPIC recommendations (4).

The drug label also provides a dosing table of expected maintenance daily doses of warfarin based on *CYP2C9* and *VKORC1* genotypes (Table 1). The label states that if the patient's *CYP2C9* and/or *VKORC1* genotypes are known, to consider these doses when selecting the initial dose of warfarin. However, CPIC states that genetics-based algorithms, such as the International Warfarin Pharmacogenetics Consortium (IWPC), predicts warfarin dose better than the table in the drug label (9).

CPIC has provided dosing recommendations that take into account whether the patients *VKORC1* and *CYP2C9*2* and **3* genotype is available, and a patient's self-identified ancestry (African ancestry or non-African ancestry). For patients with African ancestry, the presence of *CYP2C9*5*, **6*, **8*, and **11* alleles, and rs12777823 are also taken into account (4).

Gene: *VKORC1*

Genetic variation in the *VKORC1* gene is the most important *known* genetic factor that influences warfarin dosing. Pharmacogenomic algorithms for warfarin dosing routinely include testing for *VKORC1*.

The *VKORC1* gene encodes the vitamin K epoxide reductase enzyme, which catalyzes the rate-limiting step in vitamin K recycling (converting vitamin K epoxide to vitamin K). This enzyme is also the drug target for warfarin.

A common non-coding variant, *VKORC1*, c.-1639G>A (rs9923231), is associated with an increased sensitivity to warfarin and lower dose requirements (10). The polymorphism occurs in the promoter region of *VKORC1* and is thought to alter a transcription factor binding site, leading to lower protein expression. As a result, patients starting warfarin therapy who are carrying at least one "A "allele at -1639 locus require lower initial and maintenance doses compared with patients carrying a G/G genotype at this locus.

The *VKORC1,* c.–1639G>A allele frequency varies among different ethnic groups. It is the major allele (around 90%) in Asian populations and may be one of the contributing factors for lower warfarin dosing requirements often observed in patients of Asian descent. It is also common in Caucasians (around 40%) and African-Americans (around 14%) (11-13).

Less commonly, missense mutations in *VKORC1* can lead to warfarin resistance and higher dose requirements (14, 15).

The Cytochrome P450 Superfamily

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing or detoxifying lipids, hormones, toxins, and drugs in the liver. The *CYP450* genes are very polymorphic and can result in reduced, absent, or increased enzyme activity.

CYP450 isozymes involved in the metabolism of warfarin include CYP2C9, CYP3A4, and CYP1A2. The more potent warfarin S-enantiomer is metabolized by CYP2C9 while the R-enantiomer is metabolized by CYP1A2 and CYP3A4. The FDA-approved drug label for warfarin states that drugs that inhibit or induce CYP2C9, CYP1A2, and/or CYP3A4 can influence warfarin exposure and increase or decrease the INR.

The influence of genetic variants in *CYP2C9*, *CYP4F2*, and the *CYP2C* gene cluster, is discussed below.

Gene: *CYP2C9*

Genetic variation in the *CYP2C9* gene is a well-known genetic factor that influences warfarin dosing. Pharmacogenomic algorithms for warfarin dosing routinely include testing for *CYP2C9*.

The *CYP2C9* gene is highly polymorphic, with over 60 star (*) alleles described and currently cataloged at the Pharmacogene Variation (PharmVar) Consortium. The *CYP2C9*1* allele is the wild-type allele, and is associated with normal enzyme activity and the normal metabolizer phenotype.

The frequencies of the *CYP2C9* alleles vary between different ethnic groups (16-18). In individuals of European descent, the 2 most common variant alleles associated with reduced enzyme activity are *CYP2C9*2* (c.430C>T; rs1799853) and **3* (c.1075A>C; rs1057910). The **2* allele is more common in Caucasian (10-20%) than African (0-6%) populations (19). The **3* allele is less common (<10% in most populations), but rare in African populations (20).

Compared to normal metabolizers, individuals of European ancestry who carry one or two copies of **2* or **3* are more sensitive to warfarin—they require lower doses and are at a greater risk of bleeding during warfarin initiation (21-25).

In African-Americans, *CYP2C9*5*, **6*, **8*, and **11* variant alleles contribute to the variability in patient response to warfarin (26). These alleles are found more commonly in individuals with African ancestry, and collectively, are more common than the *CYP2C9*2* and **3* alleles.

Gene: *CYP4F2*

The CYP4F2 enzyme is involved in the metabolism of vitamin K in the liver. It is a vitamin K oxidase enzyme and is an important counterpart to VKORC1, a vitamin K reductase enzyme. While VKORC1 catalyzes vitamin K recycling, CYP4F2 limits the excessive accumulation of vitamin K in the liver by catalyzing the production of hydroxylated vitamin K, which is removed from the vitamin K cycle (27).

A genetic variant *CYP4F2*3* (c.1297C>T, rs2108622), has been found to influence warfarin dosing. The frequency of the variant T allele is approximately 30% in Caucasians and Asians, and approximately 7% in African-Americans (28).

The CYP4F2 enzyme with an amino acid change due to missense *3 allele is thought to be less active, leading to a rise in hepatic vitamin K. This leads to a higher dose of warfarin being required to achieve therapeutic anticoagulation (by inhibiting vitamin K-dependent clotting factors) (27).

The first studies of *CYP4F2* and warfarin dosing reported that Caucasian individuals with the variant rs2108622 TT genotype required approximately 1 mg/day more warfarin than individuals with the rs2108622 CC genotype (28). Two more recent meta-analyses concluded that "T carriers" (individuals with CT or TT genotypes) require approximately an 8–11% increase in warfarin dose, compared to CC individuals. However, data did not support *CYP4F2* influencing warfarin requirements in African-Americans (29, 30).

The inclusion of this *CYP4F2* variant in warfarin dosing models moderately improves the accuracy of warfarin dose prediction for individuals of European or Asian ancestry, but not for individuals of African ancestry. Accordingly, CPIC recommends that the dose of warfarin should be increased by 5–10% in non-African-American individuals who carry the *CYP4F2*3* variant (optional recommendation). CPIC makes no recommendation for African-Americans, stating that data do not support an impact of this variant on warfarin dosing in those of African ancestry (moderate recommendation) (4, 29, 30).

Gene: *CYP2C* rs12777823

The genetic variant rs12777823, located in the *CYP2C* gene cluster, is a non-coding variant associated with reduced warfarin dose requirements in African-Americans. The rs12777823 variant was associated with altered warfarin clearance, and individuals with this variant require a lower maintenance dose of warfarin than individuals who do not have this variant (31).

The rs12777823 variant is common in African-Americans (allele frequency 25%) and is also common in other populations; for example, Japanese (32%), and European (15%). However, the association with warfarin dose requirement has only been found for African-Americans: individuals who are heterozygous for the rs12777823 A allele require a dose reduction of warfarin by 7 mg/week, and individuals who are homozygous for the rs12777823 A allele require a dose reduction of warfarin by 9 mg/week (31). Data are lacking for the role of rs12777823 and warfarin response in other populations.

Current pharmacogenomic dosing algorithms do not include rs12777823 status, but analysis has shown that the addition of this variant improves the dosing algorithm published by the IWPC by 21% for African-Americans (31).

CPIC has stated that for African-Americans, a dose reduction of 10–25% in individuals with the rs12777823 A/G or A/A genotype is recommended (moderate recommendation). For non-African-Americans, CPIC recommends that rs12777823 should not be considered, even if the result is available (4).

Genetic Testing

The NIH's Genetic Testing Registry (GTR) provides a list of tests for "warfarin response," and the *VKORC1*, *CYP2C9*, and *CYP4F2* genes.

The *VKORC1* and *CYP2C9* genotypes are important genetic determinants of warfarin dosing. The contribution of *VKORC1* to the variation in dose requirement is larger (approximately 30%) than the contribution of *CYP2C9* (usually less than 10%) (32). The variants that are routinely tested for are *CYP2C9*2*, *CYP2C9*3*, and *VKORC1,* c.–1639G>A. These variants are used in the FDA table to guide therapy, and also in the IWPC algorithm.

Currently, routine lab tests do not test for the presence of rs12777823. Other variants that are not routinely tested for include the *CYP2C9*5, *6, *8* and **11* alleles, the genes *CYP4F2*, *EPHX1*, and *GGCX* (which all have a role in the vitamin K cycle), and the gene *CALU* (a cofactor in the VKOR complex) (26, 33).

In African-Americans, the influence of the CYP2C9**5, *6, *8* and **11* alleles are thought to be as significant as the influence of the *CYP2C9*2* and**3* alleles on warfarin dosing in Caucasians. Requesting testing of these additional *CYP2C9* alleles, and including these genotypes in an expanded dosing algorithm improves warfarin dose prediction in African-Americans, while maintaining high performance in European-Americans (34).

Individuals who are most likely to benefit from genetic testing are those who have yet to start warfarin therapy. However, genotype-guided warfarin dosing is controversial and is generally not carried out preemptively. Some studies have reported that, in general, the current use of genotype-guided dosing algorithms did not improve anticoagulation control in the first few weeks of warfarin therapy (35-41); however, a recent study found genotype-guided warfarin dosing did improve the safety of starting warfarin, compared to clinically guided dosing (42).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2017 Statement from the US Food and Drug Administration (FDA)

Initial and Maintenance Dosing

The appropriate initial dosing of warfarin sodium tablets varies widely for different patients. Not all factors responsible for warfarin dose variability are known, and the initial dose is influenced by:

- Clinical factors including age, race, body weight, sex, concomitant medications, and comorbidities

[1] The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.

- Genetic factors (*CYP2C9* and *VKORC1* genotypes)

Select the initial dose based on the expected maintenance dose, taking into account the above factors. Modify this dose based on consideration of patient-specific clinical factors. Consider lower initial and maintenance doses for elderly and/or debilitated patients and in Asian patients. Routine use of loading doses is not recommended as this practice may increase hemorrhagic and other complications and does not offer more rapid protection against clot formation.

Individualize the duration of therapy for each patient. In general, anticoagulant therapy should be continued until the danger of thrombosis and embolism has passed.

Dosing Recommendations without Consideration of Genotype

If the patient's *CYP2C9* and *VKORC1* genotypes are not known, the initial dose of warfarin sodium tablets is usually 2 to 5 mg once daily. Determine each patient's dosing needs by close monitoring of the INR response and consideration of the indication being treated. Typical maintenance doses are 2 to 10 mg once daily.

Dosing Recommendations with Consideration of Genotype

Table 1 displays three ranges of expected maintenance warfarin sodium tablets doses observed in subgroups of patients having different combinations of CYP2C9 and VKORC1 gene variants. If the patient's *CYP2C9* and/or *VKORC1* genotype are known, consider these ranges in choosing the initial dose. Patients with *CYP2C9* *1/*3, **2/*2*, **2/*3*, and **3/*3* may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen than patients without these CYP variants.
Please review the complete therapeutic recommendations that are located here: (1)

2017 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

VKORC1 CT: warfarin

NO action is required for this gene-drug interaction.

The genetic variation results in a reduction in the required dose and an increase in the risk of excessively severe inhibition of blood clotting during the first month of the treatment. However, the effect is small and CT is also the most common genotype, meaning that the standard treatment will primarily be based on patients with this genotype.

VKORC1 TT: warfarin

The genetic variation results in increased sensitivity to warfarin. This results in an increase in the risk of excessively severe inhibition of blood clotting (INR >4) during the first month of the treatment.

Recommendation:

1 use 60% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm, as used in EU-PACT: see https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica.

From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

CYP2C9 IM: warfarin

This gene variation reduces the conversion of warfarin to inactive metabolites. This can increase the risk of bleeding.

Recommendation:

1 use 65% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm. Algorithms for Caucasian patients usually contain only the *2 and *3 allele. If the activity of the reduced-activity alleles is comparable to the activity of *2 or *3, then the algorithm can be completed as if *1/*2 or *1/*3 is present. See https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica for Excel files containing calculation modules for oral and equivalent intravenous doses. From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

Modified dose algorithms have been developed for patients of African or (East) Asian heritage.

CYP2C9 PM: warfarin

This gene variation reduces the conversion of warfarin to inactive metabolites. This can increase the risk of bleeding.

Recommendation:

1 use 20% of the standard initial dose

The genotype-specific initial dose and maintenance dose can be calculated using an algorithm. Algorithms for Caucasian patients usually contain only the *2 and *3 allele. If the activity of the reduced-activity alleles is comparable to the activity of *2 or *3, then the algorithm can be completed as if *2 or *3 is present. See https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica for Excel files containing calculation modules for oral and equivalent intravenous doses. From day 6 on the standard algorithm without genotype information can be used to calculate the dose.

Modified dose algorithms have been developed for patients of African or (East) Asian heritage.

CYP2C9*1/*2: warfarin

NO action is required for this gene-drug interaction.

Genetic variation may lead to a decrease in the required maintenance dose. However, there is insufficient evidence that this causes problems when therapy is initiated as usual.

Please review the complete therapeutic recommendations located here: (2, 3)

2017 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

Non-African ancestry recommendation

In patients who self-identify as non-African ancestry, the recommendation is to:

1. Calculate warfarin dosing using a published pharmacogenetic algorithm, including genotype information for VKORC1-1639G>A and CYP2C9*2 and *3. In individuals with genotypes associated with CYP2C9 poor metabolism (e.g., CYP2C9 *2/*3, *3/*3) or both increased sensitivity (VKORC1-1639 A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant might be considered. The bulk of the literature informing these recommendations is in European and Asian ancestry populations, but consistent data exist for other non-African populations. These recommendations are graded as STRONG.

2. If a loading dose is to be utilized, the EU-PACT loading dose algorithm that incorporates genetic information could be used. This recommendation is OPTIONAL.
3. While *CYP2C9*5*, **6*, **8*, or **11* variant alleles are commonly referred to as African-specific alleles, they can occur among individuals who do not identify as, or know of their, African ancestry. If these variant alleles are detected, decrease calculated dose by 15–30% per variant allele or consider an alternative agent. Larger dose reductions might be needed in patients homozygous for variant alleles (i.e., 20–40%, e.g., CYP2C9*2/*5). This recommendation is graded as OPTIONAL.
4. If the *CYP4F2*3* (i.e., c.1297A, p.433Met) allele is also detected, increase the dose by 5–10%. This recommendation is also considered OPTIONAL.
5. The data do not suggest an association between rs12777823 genotype and warfarin dose in non-African Americans, thus rs12777823 should not be considered in these individuals (even if available).

African ancestry recommendation

In patients of African ancestry, CYP2C9*5, *6, *8, *11 are important for warfarin dosing. If these genotypes are not available, warfarin should be dosed clinically without consideration for genotype. If CYP2C9*5, *6, *8, and *11 are known, then the recommendation is to:

1. Calculate warfarin dose using a validated pharmacogenetic algorithm, including genotype information for VKORC1 c.-1639G>A and CYP2C9*2 and *3;
2. If the individual carries a CYP2C9*5, *6, *8, or *11 variant allele(s), decrease calculated dose by 15–30%. Larger dose reductions might be needed in patients who carry two variant alleles (e.g., CYP2C9*5/*6) (i.e., 20–40% dose reduction).
3. In addition, rs12777823 is associated with warfarin dosing in African Americans (mainly originating from West Africa). Thus, in African Americans a dose reduction of 10–25% in those with rs12777823 A/G or A/A genotype is recommended. These recommendations are considered MODERATE.

In individuals with genotypes that predict CYP2C9 poor metabolism or who have increased warfarin sensitivity (VKORC1 c.-1639 A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant should be considered (see Supplemental Material for definitions of strength of recommendations). As noted above, for non-African ancestry, if a loading dose is to be used, the EU-PACT algorithm that incorporates genetic information could be used to calculate loading dose. This recommendation is OPTIONAL. The data do not support an impact on clinical phenotype for CYP4F2 on warfarin dosing in those of African ancestry and so no recommendation is made for use of CYP4F2 genotype data in blacks.

Please review the complete therapeutic recommendations, including recommendations for pediatric patients, located here: (4).

Nomenclature

Nomenclature for Selected *CYP2C9* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*2*	430C>T Arg144Cys	NM_000771.3:c.430C>T	NP_000762.2:p.Arg144Cys	rs1799853
*CYP2C9*3*	1075A>C Ile359Leu	NM_000771.3:c.1075A>C	NP_000762.2:p.Ile359Leu	rs1057910
*CYP2C9*5*	1080C>G Asp360Glu	NM_000771.3:c.1080C>G	NP_000762.2:p.Asp360Glu	rs28371686

Nomenclature for Selected continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2C9*6*	817delA Lys273Argfs	NM_000771.3:c.817delA	NP_000762.2:p.Lys273Argfs	rs9332131
*CYP2C9*8*	449G>A Arg150His	NM_000771.3:c.449G>A	NP_000762.2:p.Arg150His	rs7900194
*CYP2C9*11*	1003C>T Arg335Trp	NM_000771.3:c.1003C>T	NP_000762.2:p.Arg335Trp	rs28371685

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for Selected *VKORC1* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
-1639G>A	1173C>T	NM_024006.4:c.-1639G>A	Not applicable - variant occurs in a non-coding region	rs9923231

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for Selected *CYP4F2* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP4F2*3*	1297G>A Val433Met	NM_001082.4:c.1297G>A	NP_001073.3:p.Val433Met	rs2108622

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Nomenclature for rs12777823

HGVS reference sequence	dbSNP reference identifier for allele location
NC_000010.11:g.94645745G>A (GRCh38) NC_000010.10:g.96405502G>A (GRCh37)	rs12777823

HGVS - Human Genome Variation Society, dbSNP - Single Nucleotide Polymorphism Database

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (43).

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

Nomenclature for Cytochrome P450 enzymes is available from Pharmacogene Variation (PharmVar) Consortium.

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First edition (2012):

The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org

The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

Version History

To view the 2016 version of this summary (Created: June 8, 2016) please click here.

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Genetic variants and disease

ABO Blood Group

Laura Dean, MD[1]

Created: October 1, 2012; Updated: July 27, 2015.

Characteristics

There are four common blood groups in the ABO system: O, A, B, and AB. The blood groups are defined by the presence of specific carbohydrate sugars on the surface of red blood cells, N-acetylgalactosamine for the A antigen, and D-galactose for the B antigen. Both of these sugars are built upon the H antigen—if the H antigen is left unmodified, the resulting blood group is O because neither the A nor the B antigen can attach to the red blood cells.

Individuals will naturally develop antibodies against the ABO antigens they do not have. For example, individuals with blood group A will have anti-B antibodies, and individuals with blood group O will have both anti-A and anti-B. Before a blood transfusion takes place, routine serological testing checks the compatibility of the ABO (and Rh) blood groups. An ABO incompatible blood transfusion can be fatal, due to the highly immunogenic nature of the A and B antigens, and the corresponding strongly hemolytic antibodies (1).

Compared to other blood groups, individuals with blood group O may have a lower risk of pancreatic cancer and thromboembolic disease (2, 3). In addition, in certain African populations, individuals with the blood group O may be protected from life-threatening malaria (4). However, this blood group is not more common in some regions where malaria is endemic. This might be because individuals with blood group O are at higher risk of cholera and severe diarrhea due to *Vibrio cholerae* 01, with individuals with the AB blood group being the most protected (5, 6).

Over 80 *ABO* alleles have been reported. The common alleles include *A1*, *A2*, *B1*, *O1*, *O1v*, and *O2* (7). Whereas the *A* and *B* alleles each encode a specific glycosyl-transferring enzyme, the *O* allele appears to have no function. A single-base deletion in the *O* allele means that individuals with blood group O do not produce either the A or B antigens. Blood type frequencies vary in different racial/ethnic groups. In the US, in Caucasians, the ratio of blood group O, A, B, and AB is 45%, 40%, 11%, and 4% respectively. In Hispanics, the distribution is 57%, 31%, 10%, and 3%; and in Blacks, 50%, 26%, 20%, and 4% (8).

Diagnosis/testing

Serological testing is sufficient to determine an individual's blood type (e.g., blood group A) for the purposes of blood donation and transfusion. Molecular genetic testing can be used to determine an individual's *ABO* genotype (e.g., genotype *AO* or *AA*). This may be useful in the research setting, for example, to investigate the link between ABO blood groups and particular diseases, and also in the forensic setting (9).

Management

Determining an individual's blood group is important prior to blood transfusion and prior to the donation or receiving of a kidney transplant.

Occasionally, a person's blood type may appear to change. For example, the ABO antigens can act as tumor markers. Their presence may be decreased in particular diseases, such as acute myeloid leukemia, AML (10). In contrast, occasionally the B antigen may be acquired in certain infectious diseases. A bacterial infection with

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specific strains of *E. coli* or *Clostridium tertium* can generate a B-like antigen from an individual who has the *A1* allele (11).

Genetic counseling

The ABO blood type is inherited in an autosomal codominant fashion. The *A* and *B* alleles are codominant, and the *O* allele is recessive.

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ACHOO Syndrome

Laura Dean, MD[1]

Created: October 15, 2012; Updated: July 27, 2015.

Characteristics

Autosomal Dominant Compelling Helioopthalmic Outburst (ACHOO) Syndrome is characterized by uncontrollable sneezing in response to the sudden exposure to bright light, typically intense sunlight (1). This type of sneezing is also known as photic sneezing. About one in four individuals who already have a prickling sensation in their nose will sneeze in response to sunlight, but "pure" photic sneezing is far less common (2).

Sneezing is usually triggered by contact with infectious agents or after inhaling irritants, but the cause of photic sneezing is not fully understood. It may involve an over-excitability of the visual cortex in response to light, leading to a stronger activation of the secondary somatosensory areas (3).

Diagnosis/testing

The diagnosis of ACHOO syndrome is usually made by clinical history. Affected individuals report a "prickling sensation" or sneezing in response to a bright light. This response may be reproduced in the clinical setting by asking the individual to look at a bright light, although findings are unreliable.

The genetic basis of this syndrome is not yet known.

Management

Recommendations for management of ACHOO syndrome include using a hat or sunglasses to shield the eyes from direct sunlight whenever possible. Potential hazards include the possibility of drivers having an accident caused by sneezing brought on by, for example, exiting a road tunnel on a bright day. Similarly, airline pilots may be at risk (4).

Genetic counseling

ACHOO syndrome is inherited in an autosomal dominant manner (1). As such, if one parent is affected, their child has a 50% chance of inheriting the syndrome.

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McCune-Albright Syndrome

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Characteristics

McCune-Albright Syndrome (MAS) is a rare genetic disorder originally characterized as the triad of polyostotic fibrous dysplasia of bone, precocious puberty, and café-au-lait skin pigmentation (1-3). With time other associated endocrinopathies have been recognized, including hyperthyroidism, growth hormone excess, FGF23-mediated phosphate wasting, and hypercortisolism (4, 5).

MAS is caused by an activating mutation in the *GNAS* gene, which encodes the alpha subunit of the stimulatory G protein involved in G-protein signaling (6, 7). A missense mutation, typically Arg201Cys or Arg201His (NM_001077488.3:c.604C>T, rs11554273), impairs the intrinsic GTPase activity of the Gsα protein, resulting in the constitutive activation of the Gsα-cAMP signaling pathway in the cells that contain the mutation.

The mutation arises early in embryogenesis and is distributed in a mosaic pattern. The clinical phenotype is therefore highly variable, depending upon the location and timing of the mutation during embryologic development. Skin manifestations are common and are usually present at or shortly after birth. The café-au-lait spots typically have irregular margins giving them a "coast of Maine" appearance, and usually show an association with the midline of the body.

In MAS, fibrous dysplasia of bone typically occurs at several sites (polyostotic), and commonly presents with fracture, deformity and/or bone pain (8). Radiographs show characteristic expansile lesions with a "ground glass" appearance. Craniofacial fibrous dysplasia can be severe in individuals who have pituitary disorders leading to hypersecretion of growth hormone. Treatment can be challenging and should begin as soon as possible.

In girls, precocious puberty is a common initial manifestation, with recurrent ovarian cysts leading to episodes of vaginal bleeding and breast development. Precocious puberty is less common in boys, presenting with penile enlargement, pubic and axillary hair, acne, body odor, and sexual behavior. However, in both girls and boys, there is a high frequency of gonadal pathology (ovarian abnormalities in girls, and testicular abnormalities in boys) (9).

Diagnosis

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *GNAS* gene and the McCune-Albright Syndrome.

Currently, the diagnosis of McCune-Albright syndrome is made clinically in most cases. This is due to the mosaic nature of the disease whereby a negative genetic test result (e.g., in blood) does not exclude the presence of the mutation in other tissues. However, newer techniques such as digital PCR may improve the sensitivity of genetic testing in individuals who have clinical signs of McCune-Albright syndrome (10, 11).

Management

Treatment is individualized based on each patient's clinical presentation. Letrozole (12) and/or tamoxifen (13) may be effective for treatment of precocious puberty in girls. Medications and/or surgery may be used for

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treatment of hyperthyroidism (14, 15), growth hormone excess (16, 17), and hypercortisolism (18). Management of fibrous dysplasia of bone is palliative, with surgery as needed for fracture and deformity (19, 20). Bisphosphonates are effective for treatment of fibrous dysplasia-related pain, but have not been shown to have any long-term effect on the course of the disease (21, 22).

Genetic Counseling

McCune-Albright syndrome is caused by a new (de novo) mutation that occurs after conception, at an early stage of development. Individuals with McCune-Albright syndrome have not been observed to pass the syndrome on to their children.

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Version History

To view an earlier version (8 March 2012), please click here.

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Methylenetetrahydrofolate Reductase Deficiency

Laura Dean, MD[1]

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Characteristics

Methylenetetrahydrofolate Reductase (MTHFR) Deficiency is the most common genetic cause of elevated levels of homocysteine in the plasma (hyperhomocysteinemia).

The MTHFR enzyme plays an important role in processing amino acids, specifically, the conversion of homocysteine to methionine. Genetic variations in the *MTHFR* gene can lead to impaired function or inactivation of this enzyme, which results in mildly elevated levels of homocysteine, especially in individuals who are also deficient in folate (1). In these individuals, a daily supplement of low dose folic acid may reduce and often normalize their homocysteine levels, but this has not been demonstrated to improve health outcomes (2, 3).

A common genetic variant in the *MTHFR* gene is a 677C>T polymorphism (NM_005957.4:c.665C>T, rs1801133). This variant encodes a thermolabile enzyme that is less active at higher temperatures. Individuals who carry two copies of this variant ("TT homozygous") tend to have higher homocysteine levels and lower serum folate levels compared to controls.

More than 25% of Hispanics and around 10-15% of North America Caucasians are estimated to be homozygous for the "thermolabile" variant (TT genotype) (4). The TT genotype is least common in individuals of African descent (6%) (5, 6).

Another common *MTHFR* variant, 1298A>C (NM_005957.4:c.1286A>C, rs1801131), does not cause increased homocysteine levels in heterozygous or homozygous individuals, but combined heterozygosity of 1298A>C and 677C>T results in an outcome similar to TT homozygous individuals (7).

Until recently, it was thought that MTHFR deficiency, by causing elevated homocysteine levels, led to an increased risk of venous thrombosis, coronary heart disease, and recurrent pregnancy loss (8-11). However, more recent analysis has not found an association between elevated homocysteine levels and the risk of venous thrombosis or the risk of coronary heart disease (12).

MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia, recurrent pregnancy loss, or for at-risk family members (4).

Rarely, more severe variants in the *MTHFR* gene can be a cause of an autosomal recessive inborn error or metabolism where extremely high levels of homocysteine accumulate in the urine and plasma. This can cause developmental delay, eye disorders, thrombosis, and osteoporosis. But more commonly, homocystinuria is caused by variants in a different gene (cystathionine beta-synthase, *CBS*). To read more about homocystinuria caused by CBS deficiency, please see *GeneReviews*.

Diagnosis

A blood test that measures total homocysteine levels can diagnose hyperhomocysteinemia.

Genetic testing of the *MTHFR* gene may be used to confirm the diagnosis of an inherited hyperhomocysteinemia caused by MTHFR deficiency. However, a 2013 Practice Guideline from the American

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College of Medical Genetics and Genomics (ACMG) states that there is growing evidence that "*MTHFR* polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia" (4).

In an infant or child in whom autosomal recessive severe MTHFR deficiency is suspected, tests for plasma homocysteine and serum amino acids levels would be expected to show a pattern of extremely elevated homocysteine and low methionine. *MTHFR* full gene sequencing (as opposed to targeted polymorphism testing) can confirm the suspected clinical diagnosis.

Management

2013 Statement from the American College of Medical Genetics and Genomics (ACMG) includes the following recommendations:

- *MTHFR* polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss
- *MTHFR* polymorphism genotyping should not be ordered for at-risk family members
- A clinical geneticist who serves as a consultant for a patient in whom an *MTHFR* polymorphism(s) is found should ensure that the patient has received a thorough and appropriate evaluation for his or her symptoms
- If the patient is homozygous for the "thermolabile" variant c.665C→T, the geneticist may order a fasting total plasma homocysteine, if not previously ordered, to provide more accurate counseling
- *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines

For the complete guideline, please see *ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing*. Genetics in Medicine. 2013;15(4):153-6. (4)

The management of severe autosomal recessive MTHFR deficiency is outside the scope of this review.

Genetic Testing

The NIH Genetic Testing Registry, GTR, displays genetic tests that are currently available for the *MTHFR* gene and for homocysteinuria due to MTHFR deficiency.

Biochemical genetic tests may also be used, which assess the level of activity of the MTHFR enzyme or the level of analyte in the blood. GTR provides a list of biochemical tests that assess the level of homocysteine analytes and the activity of the MTHFR enzyme.

Genetic Counseling

The MTHFR polymorphism has been associated with many different medical complications. Individuals who are "*MTHFR* positive" carry one or two copies of variants in the *MTHFR* gene. However, in general, the following genotypes are unlikely to be of clinical significance:

- 677C>T heterozygote
- c.1286A→C homozygote
- (677C>T);(c.1286A→C) compound heterozygote

Individuals who are TT homozygous with normal homocysteine levels do not have an increased risk of venous thrombosis or recurrent pregnancy loss, according to recent evidence. However, women do have a modestly increased risk of having a child with a neural tube defect and this risk increases if the fetus is also homozygous.

If homocysteine levels are elevated, TT homozygotes may have a mildly increased risk of venous thrombosis or recurrent pregnancy loss, but not other previously associated conditions, such as cardiovascular disease.

Less is known about the c.1286A→C variant, but current evidence suggests that it is milder than the "thermolabile" c.665C→T variant (4).

For all individuals, it is important to determine whether medical disorders have been incorrectly attributed to their positive *MTHFR* status. Referral to a hematologist or maternal–fetal medicine specialist may be needed. And patients should provide their *MTHFR* genotype status to their physician before starting chemotherapy agents that require folate (e.g., methotrexate).

Finally, *MTHFR* positive individuals may decide to take vitamin B and folic acid supplements. Although safe (toxicity is rare), evidence is lacking on whether such supplements reduce the risks associated with hyperhomocysteinemia or *MTHFR* genotype status (4).

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Pitt-Hopkins Syndrome

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Introduction

Pitt-Hopkins syndrome is a rare neurodevelopmental disorder caused by loss of function of one allele of the *TCF4* gene. Most cases result from a *de novo* mutation that leads to a functional loss of one copy of the *TCF4* gene. Other cases result from a deletion of the chromosome region in which the *TCF4* gene is located (18q21.2).

Pitt-Hopkins syndrome is characterized by distinctive facial features (e.g., deep-set eyes, prominent nose, wide mouth with widely spaced teeth), global developmental delay, and moderate-severe intellectual disability. Breathing problems and epilepsy often occur.

Once Pitt-Hopkins syndrome has been suspected clinically, the diagnosis is confirmed by molecular genetic testing of the *TCF4* gene.

Characteristics

Pitt-Hopkins syndrome is rare—approximately 500 cases of Pitt-Hopkins syndrome have been reported worldwide (1). In infancy, low muscle tone can cause feeding problems. In older children, gait looks stiff because of a combination of low muscle tone (hypotonia) and balance problems (ataxia).

Children with Pitt-Hopkins tend to have a happy disposition, with hand flapping and excitability. They may develop abnormal breathing patterns, such as sudden attacks of hyperventilation followed by breath-holding until cyanosis. About half of these children have epilepsy; typically their ECG is normal (2). Subtle changes in the brain may be seen in up to 70% of patients by MRI (e.g., underdeveloped corpus callosum, dilated ventricles) (3).

Most adults with Pitt-Hopkins syndrome have severe cognitive impairment, and although they may vocalize, they are unable to use language. Other issues include gastrointestinal (e.g., constipation), ophthalmic (e.g., strabismus, severe myopia), and behavioral problems (e.g., anxiety, stereotypical movements of the head and hands).

Pitt-Hopkins syndrome may be distinguished clinically from other causes of intellectual disability and developmental delay (e.g., Angelman syndrome, Rett syndrome, Mowat–Wilson syndrome) by: 1) abnormal breathing patterns (onset from 7 months to 7 years); 2) lack of congenital abnormalities; and 3) distinctive facial features (craniofacial dysmorphism). In infants, the first sign of craniofacial dysmorphism may be the prominence of the nose and lower face. As the child grows, they may develop deep-set eyes, a high nasal root with prominent nasal bridge, wide nostrils and down-turned nasal tip; a short philtrum, and a wide mouth with widely spaced teeth.

Genetics

Pitt-Hopkins syndrome is an autosomal dominant disorder caused by haploinsufficiency of the *TCF4* gene. Haploinsufficiency occurs when one copy of the gene has been lost (e.g., by a loss-of-function mutation), and the remaining copy of the gene is not sufficient to prevent the disorder.

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TCF4 has an important role in the development of the nervous system. *TCF4* encodes a transcription factor—a protein that binds to specific DNA sequences and controls the expression of other genes. The TCF4 protein contains a basic helix-loop-helix (bHLH) domain, and is also known as an "E-protein" because it binds to a specific sequence of DNA known as an "E-box".

The TCF4 protein is expressed in the brain, heart, lungs, and muscles. TCF4 is also active during early human development, when it is thought to be involved in a series of developmental processes, including initiating the development of several regions of the nervous system (3).

Mutations in *TCF4* disrupt the ability of the protein to bind to DNA and initiate neuronal differentiation, contributing to the neurological symptoms seen in Pitt-Hopkins syndrome. In addition, other proteins that normally form heterodimers with TCF4 are unable to function normally. One of these proteins, ASCL1, is thought to be involved in development in the brain stem—after defective interaction with TCF4, impaired development of the brain stem may contribute to the breathing problems that characterize Pitt-Hopkins syndrome (3).

A spectrum of mutations can disrupt the *TCF4* gene, which is located on the long arm of chromosome 18 (18q21.2) (4, 5). The gene has 20 exons, of which exons 2 to 19 are coding. Exon 18 is thought to harbor a quarter of disease-causing mutations (6).

Approximately 30% of cases of Pitt-Hopkins syndrome are caused by whole gene deletions of TCF4, and approximately 10% caused by partial gene deletions. Missense mutations are also common, and mainly involve the bHLH domain, whereas nonsense and frameshift mutations are spread throughout the gene. Splice site mutations are less common (approximately 10%), and balanced translocations are a rare cause of Pitt-Hopkins syndrome (3, 7).

Diagnosis

The diagnosis of Pitt-Hopkins syndrome is based on the clinical presentation and confirmed by molecular genetic testing.

Currently, there is not a generally accepted diagnostic criteria, but the hallmarks of the syndrome that support a diagnosis of Pitt-Hopkins syndrome are facial dysmorphism, early onset global developmental delay, moderate to severe intellectual disability, seizures, breathing abnormalities, and a lack of major congenital abnormalities (2, 3, 8).

Management

Infants with Pitt-Hopkins syndrome should receive treatment from a multidisciplinary team specializing in the care of children with cognitive and motor impairment, including physical therapists, occupational therapists, and speech therapists. Medical specialists for pulmonary conditions, epilepsy, gastrointestinal conditions and other medical issues may also be needed.

Genetic Testing

The NIH Genetic Testing Registry, GTR, provides examples of the genetic tests that are currently available for Pitt-Hopkins syndrome and the *TCF4* gene.

Testing options include sequence analysis (to determine the nucleotide sequence of *TCF4*), chromosome microarray analysis (to detect copy number variants by determining the gain or loss of chromosome material), quantitative PCR (to determine the relative amount of DNA or RNA in a sample), and cytogenetic testing/karyotyping (to assess chromosome number and structure).

Sequencing analysis detects approximately 70% of *TCF4* variants, which may include missense, nonsense, and splice site variants, and small intragenic inserts and deletions. Typically, deletions of *TCF4* exons or the whole *TCF4* gene will not be detected by Sanger sequencing.

If a variant is not found by sequencing and a gene deletion is suspected, deletion/duplication analysis should be performed at the exon-level. Methods used include quantitative PCR and chromosome microarray analysis.

If a deletion is not found but Pitt-Hopkins syndrome is still suspected, karyotype analysis may be used to search for balanced translocations disrupting the coding region of *TCF4* (3, 9).

Genetic Counseling

Pitt-Hopkins syndrome is caused by a mutation in the *TCF4* gene, or a deletion of the chromosome region in which *TCF4* is located (18q21.2).

Most cases are caused by a *de novo* mutation (a new mutation, not present in either parent); cases of inheritance from a mosaic parent with a de novo mutation are exceedingly rare (10, 11).

Usually only one member of the family is affected. Parents are typically not affected, and although genetic testing could be offered, it would not be possible to entirely rule out a mutation because of somatic mosaicism (different cell lines may have different variants of *TCF4*).

Prenatal diagnosis and preimplantation genetic diagnosis are possible for pregnancies at increased risk of Pitt-Hopkins syndrome (e.g., if the parents have already had one affected child).

This risk of siblings being affected is low because the mutation is almost always *de novo* and not inherited. However, the risk is higher than that of the general population because of the possibility of mosaicism in parental germline cells (precursor cells of the egg or sperm).

For an individual with Pitt-Hopkins syndrome, the risk of passing on the syndrome to their offspring would be 50%. However, there are no known cases of individuals reproducing (2, 9).

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Version History

To view the 2012 version of the summary, please click here.

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Schizophrenia

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Characteristics

Schizophrenia is a severe neurodevelopmental disorder with a worldwide prevalence of around 0.3-0.7% (1). The etiology of schizophrenia is unknown, but it is thought to result from a combination of complex genetic and environmental factors. This includes physical factors e.g., complications during pregnancy and birth, infection, and autoimmune disease; as well as psychological factors that may trigger psychosis, such as stress and drug abuse (2). Several neurotransmitter systems are thought to be involved in the pathogenesis, including dopamine, glutamate, GABA, and acetylcholine.

Schizophrenia is associated with substantial morbidity and mortality. Antipsychotics are the mainstay of treatment, however, their efficacy is poor for many patients. Antipsychotics are thought to exert their therapeutic effects by the post-synaptic blockade of D2 dopamine receptors in the brain.

The symptoms of schizophrenia fall in to three main categories: positive, negative, and cognitive. Positive symptoms are generally not found in healthy individuals, but may come and go or persist in individuals with schizophrenia. Positive symptoms include reality distortion (e.g., delusions, hallucinations), and thought disorders. These symptoms often respond well to treatment.

Negative symptoms are deficits in normal emotions and behavior, and may be mistaken for depression. Symptoms divide into reduced expression of emotion (e.g., speaking without moving or with a monotonous voice) and avolition (a lack of motivation to start or continue with a task). No treatment has established efficacy for these pathologies.

Cognitive symptoms may also be difficult to recognize. They include poor executive functioning (understanding information and using it to make decisions) and trouble focusing or paying attention. And again, no treatment has established efficacy.

Genetics

Schizophrenia is highly heritable, as shown by family, twin, and adoption studies. For example, for identical twins, if one twin develops schizophrenia, the other twin has about a 50% chance of also developing the disease. The risk of the general population developing the schizophrenia is about 0.3-0.7% worldwide (3).

The search for "schizophrenia genes" has been elusive. Initial linkage studies looked at parts of the genome associated with schizophrenia, and many candidate genes were identified, including *APOE*, *COMT*, *DAO*, *DRD1*, *DRD2*, *DRD4*, *DTNBP1*, *GABRB2*, *GRIN2B*, *HP*, *IL1B*, *MTHFR*, *PLXNA2*, *SLC6A4*, *TP53*, and *TPH1* (4). However, some of these have later been questioned (5).

Microdeletions and microduplications have been found to be three times more common in individuals with schizophrenia, compared to controls. Because these deletions and duplications are in genes that are overexpressed in pathways related to brain development, it is possible that the inheritance of multiple rare variants may contribute to the development of schizophrenia (6).

Several genetic disorders feature schizophrenia as a clinical feature. The 22q11.2 Deletion Syndrome comprises many different syndromes, of which one of the most serious is DiGeorge syndrome. Children born with

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DiGeorge syndrome typically have heart defects, cleft palate, learning difficulties, and immune deficiency. Schizophrenia is a late manifestation, affecting around 30% of individuals (7). Microdeletions and duplications in chromosome 1, 2, 3, 7, 15 and 16 have also been associated with schizophrenia (8).

In 2014, a genome-wide association study looked at the genomes of over 35,000 patients and 110,00 controls. The study identified 108 SNPs that were associated with schizophrenia, 83 of which had not been previously reported. As expected, many of these loci occurred in genes that are expressed in the brain. For example, the SNPs included a gene that encodes the dopamine D2 receptor, *DRD2* (the target of antipsychotic drugs), and many genes involved in glutamine neurotransmitter pathways and synaptic plasticity (e.g., *GRM3, GRIN2A, SRR, GRIA1*). More surprisingly, however, associations were also enriched among genes expressed in tissues with important immune functions (9).

In 2016, a study based on nearly 65,000 people investigated the association between schizophrenia and variation in the Major Histocompatibility Complex (MHC) locus—a region on chromosome 6 that is important for immune function. The study focused on the *C4* gene (complement component 4) that exists as two distinct genes: *C4A* and *C4B*, which encode particularly structurally diverse alleles.

The study found that the alleles which promoted greater expression of *C4A* in the brain were associated with a greater risk of schizophrenia. By using mice models, the study showed that C4 is involved in the elimination of synapses during brain maturation. In humans, "synaptic pruning" is most active during late adolescence, which coincides with the typical onset of symptoms of schizophrenia. It is therefore possible that the inheritance of specific *C4A* alleles could lead to "run away" synaptic pruning, increasing the risk of schizophrenia. Further research may even determine C4 as a potential therapeutic target (10).

Diagnosis

Currently, the diagnosis of schizophrenia is made via a psychiatric assessment using the criteria presented in the American Psychiatric Association Manual of Psychiatric Diseases, which is now in its 5th edition, and is known as DSM-V. To make a diagnosis, specific characteristic symptoms of schizophrenia must be present for at least 6 months, together with a disruption in social or occupational function, in the absence of another diagnosis that could account for the symptoms.

The use of chromosome microarray analysis has been suggested as a diagnostic test for schizophrenia. Microarray analysis can detect copy number variants (CNVs), which are large regions of the genome that have been deleted or duplicated. The prevalence of clinically significant CNVs in schizophrenia is around 5%. For autism and intellectual disability, the prevalence is around 10-20%, and CNV testing with microarray analysis is now a routine first-line diagnostic test for these conditions.

For an individual with schizophrenia, a positive test result for CNV may have implications for medical management, because of the association of CNVs with physical diseases and genetic counseling, and because offspring have a 50% risk of inheriting the CNV (3, 11).

Management

Treatment of manifestations: Antipsychotic medications are the mainstay of treatment and help reduce symptoms and improve behaviors in patients with schizophrenia. The type, dose, and route of administration of antipsychotic medications depends upon the clinical scenario. Adverse effects are common, and may require the dose or type of drug to be altered.

Antipsychotics may be given with counseling and other types of psychosocial interventions. For refractory (treatment-resistant) symptoms, an alternative antipsychotic or an additional antipsychotic may be required.

During pregnancy, antipsychotic drugs should be given only when the benefits derived from treatment exceed the possible risks to mother and fetus. Neonates exposed during the third trimester are at risk for extrapyramidal and/or withdrawal symptoms following delivery. There have been reports of agitation, hypertonia, hypotonia, tremor, somnolence, respiratory distress, and feeding disorder. While in some cases symptoms have been self-limited, in others neonates have required intensive care unit support and prolonged hospitalization.

Surveillance: Routine monitoring for the symptoms and signs of extrapyramidal adverse effects is needed in individuals taking antipsychotics. These adverse effects include akathisia (feeling of restlessness that may be accompanied with motor restlessness), dystonias (involuntary contraction of large muscle groups), and parkinsonian syndrome. Patients should also be monitored for signs of tardive dyskinesia (involuntary facial movements) and drug-specific adverse effects. For clozapine, because of the risk of neutropenia, the patient's white blood cell count and absolute neutrophil count must be regularly monitored. For thioridazine, the risk of prolonged QT interval may lead to Torsades de pointes.

Prevention of secondary complications: Patients should be regularly monitored for weight gain and metabolic problems such as hyperglycemia and hyperlipidemia, which are common side effects of antipsychotic medications.

Genetic Testing

Genetic testing is available for several of the susceptibility loci for schizophrenia, including clinical and research tests registered in the NIH Genetic Testing Registry (GTR). Additional tests may be found in the 'Related section' of the main GTR record for schizophrenia.

GTR also has registered tests for genetic conditions with schizophrenia as a clinical feature.

Genetic Counseling

Genetic counseling is recommended for people who have a family member with schizophrenia. Recurrence risk counseling is based on empiric familial risk for families with individuals with schizophrenia (12).

The lifetime risk of schizophrenia for the general population is estimated to be 0.2 to 0.7% (13).

The recurrence risk of schizophrenia in the siblings of a patient is 10%, and in the children of patients, the risk is approximately 10%. The risk for second-degree relatives is approximately 3-4% (14, 15).

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Version History

To view an earlier version (8 March 2012), please click here.

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Authoring and Peer Review

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Medical Genetics Summaries is a growing collection of articles describing how genotypes play a role in an individual's response to drugs or predisposition to disease. This pharmacogenomics-focused resource brings clinically relevant information to point of care. Each chapter focuses on one drug and the structured format of each summary includes review and synthesis of peer-reviewed research about the variants and their impact in drug metabolism and action, strategies for genetic testing and access to available therapeutic recommendations from medical and professional societies. Medical Genetics Summaries are based on authoritative sources, driven by professional and medical guidelines, actionable, and undergo an extensive review process as described below.

Editorial Oversight

The MGS editors advise on subject matter, guide the project through developments in the field, provide final approval before publication, and assist in recruiting reviewers and resolution of key issues that may arise during the review process.

Selection of Topics

The selection of topics for new MGS chapters is influenced by drugs approved by the U.S. Food and Drug Administration (FDA) that have pharmacogenomic biomarkers in the label, the availability of clinical testing for drug response, the needs of the community, and input from the editors.

To identify new drugs not yet covered in MGS, the author consults the FDA's "Table of Pharmacogenomic Biomarkers in Drug Labeling". To prioritize the selection of new MGS chapters, the author checks the Genetic Testing Registry (GTR) for clinically available genetic tests for drug responses that lack guidelines or summary information from sources like PharmGKB or CPIC. The author takes into consideration the current needs of the community, for example adding summaries that support National Institutes of Health (NIH) initiatives like the All of Us research program's Medicine and Your DNA report, and the NIH Helping to End Addiction Long-term (HEAL) Initiative.

Upon the release of a new MGS chapter to the production site, an excerpt is displayed in the relevant GTR and MedGen drug response records. Reciprocal links between MGS, GTR, MedGen, and other National Center for Biotechnology Information (NCBI) resources are also added for better integration and accessibility.

Structured Format

Each MGS drug response chapter follows a structured format and draws from available published research evidence. Each summary has one drug section and one or more gene sections, depending on how many genetic factors have been identified to influence drug metabolism and action.

1. The introductory paragraphs detail the drug, its clinical uses, and how genetic variants influence an individual's response to the drug. Dosing recommendations from the FDA drug labels and practice guidelines from authoritative professional and medical societies are also presented.
2. The drug section begins with a description of the drug, including drug class, mechanism of action, indications for use, and common side effects. This is followed by a discussion on the factors that influence the drug response.
3. The gene section reviews important facts on the gene's role in drug metabolism or action, describes the genetic variants and the predicted impact on enzyme activity and how they influence the individual's response to the drug. It also discusses common or clinically significant variants, including their prevalence across different ethnic populations.

4. Linking genetic variation with treatment response covers in more detail the specific evidence and outcomes for variation in the gene(s) from the previous section and predicted individual responses to the drug. Key variants and alleles with impact on drug efficacy or adverse effects are discussed as well as emerging evidence that may be informative though it is not yet clinically actionable.
5. The "Genetic Testing" section describes available genetic testing options and links to currently available genetic tests for the gene(s) and drug response listed in the NIH GTR. The GTR is a resource of descriptions of clinical and research tests that includes phenotypes, test targets, methodologies and instructions on how to order the test from the laboratory.
6. The Gene-drug interactions section provides a list of interactions between the gene(s) of note and other medications, including those used in other indications separate from the current chapter. Additional resources to learn more about other medications that may be impacted by variations in the same gene are also included.
7. The "Therapeutic Recommendations based on Genotype" excerpts clinically actionable information, such as dosing recommendations from the FDA drug label, and therapeutic recommendations from pharmacogenetic societies (for example, the Clinical Pharmacogenetics Implementation Consortium [CPIC], the Canadian Pharmacogenomics Network for Drug Safety [CPNDS], The Dutch Pharmacogenetics Working Group [DPWG]) and medical societies (for example, the American Society of Clinical Oncology [ASCO], the American College of Medical Genetics and Genomics [ACMG], the National Comprehensive Cancer Network [NCCN]). The MGS does not create guidelines or recommendations.
8. The nomenclature table provides information on different terms used for genetic variants. Commonly used terms and historic terms, like the star allele nomenclature, are linked to the official Human Genome Variation Society (HGVS) terms and rs identifiers when available. The table also includes links to relevant resources like ClinVar, dbSNP, and the Pharmacogene Variation (PharmVar) Consortium.
9. Expert reviewers are a vital part of MGS and are acknowledged in every chapter. Information and access to previous versions of the summary is displayed.

Writing Process

Each summary is authored by our in-house senior medical writer, who holds a PhD with professional experience in pharmacogenomics. All phases, from authoring to production, are tracked in an internal ticket management system.

To create the first draft of a summary:

1. The author consults the most recent FDA drug label for the drug. Additionally, to gain a better understanding of the drug's context of use and the impact of genetic factors, the author uses NIH resources and other clinical sites, such as UpToDate.
2. Next, the author identifies key guidelines and primary research papers, using PubMed Clinical Queries, PubMed, CPIC, and The Pharmacogenomics Knowledgebase (PharmGKB).
3. Finally, the author searches PubMed for the most recent publications. Firstly, to find content that has not yet been cited by guidelines; and secondly, to identify external reviewers who are actively involved in relevant research.

Internal Review

Each summary undergoes internal review involving one or 2 NCBI staff members with experience in genetic counseling or molecular genetics. Once the author has finalized the first draft of a summary, it is submitted for internal review, along with the key supporting guidelines. The internal reviewers perform the first round of expert review, utilizing track changes to ask questions, provide suggestions and make corrections. This process is

documented in a ticket management system, including all versions of the document and comments from the author and reviewers.

External Review

Following the internal review, each summary goes through a scientific peer-review process involving between 2–9 experts from outside NCBI. The external review includes at least one clinical specialist experienced in prescribing the drug and with published papers on its use, and one laboratory professional experienced in pharmacogenomics. Experts are selected based on their clinical or laboratory experience and relevant publications in PubMed and any potential conflicts of interest are displayed to avoid real or perceived bias. The comments from expert reviewers are retained in our internal records. After the summary is released to production, all current and previously published versions are stored in the document management system, allowing public access.

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Once all the review comments are reconciled, the summary undergoes in-house copyediting before public release.

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Summaries are scheduled to be updated every 4–6 years or whenever there is an update to guidelines from which excerpts have been taken for the summary. Minor updates (revisions) may be needed to update citations or external links to reference materials.

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Lennard, Lynne, PhD; University of Sheffield, Sheffield, UK

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- Trastuzumab Therapy and ERBB2 (HER2) Genotype

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- Fluorouracil Therapy and *DPYD* Genotype

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- Pegloticase Therapy and *G6PD* Genotype

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- Gentamicin Therapy and *MT-RNR1* Genotype

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- Mercaptopurine Therapy and *TPMT* and *NUDT15* Genotype

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- Gentamicin Therapy and *MT-RNR1* Genotype
- Clopidogrel Therapy and *CYP2C19* Genotype

McGill, Neil William, MD; Royal Prince Alfred Hospital, Sydney, NSW, Australia

- Lesinurad Therapy and *CYP2C9* Genotype

Meijer, Berrie, MD, PhD; Noordwest Hospital Group, Alkmaar, The Netherlands

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Merl, Man Yee, PharmD, BCOP; Yale-New Haven Hospital, New Haven, CT, USA

- Irinotecan Therapy and *UGT1A1* Genotype

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- Eliglustat Therapy and *CYP2D6* Genotype

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- Risperidone Therapy and *CYP2D6* Genotype

Morton, Jenny, PhD, ScD; University of Cambridge, Cambridge, UK

- Deutetrabenazine Therapy and *CYP2D6* Genotype

Mougey, Edward B., PhD; Nemours Children's Specialty Care, Jacksonville, FL, USA

- Omeprazole Therapy and *CYP2C19* Genotype

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- Brivaracetam Therapy and *CYP2C19* Genotype
- Carisoprodol Therapy and *CYP2C19* Genotype
- Lacosamide Therapy and *CYP2C19* Genotype
- Propafenone Therapy and *CYP2D6* Genotype

Müller, Daniel, MD, PhD; University of Toronto, ON, Canada

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- ABO Blood Group

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- Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype
- Capecitabine Therapy and *DPYD* Genotype
- Carbamazepine Therapy and *HLA* Genotype
- Carisoprodol Therapy and *CYP2C19* Genotype
- Fluorouracil Therapy and *DPYD* Genotype
- Flurbiprofen Therapy and *CYP2C9* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Lesinurad Therapy and *CYP2C9* Genotype
- Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotype
- Piroxicam Therapy and *CYP2C9* Genotype
- Prasugrel Therapy and CYP Genotype
- Propafenone Therapy and *CYP2D6* Genotype
- Risperidone Therapy and *CYP2D6* Genotype
- Thioridazine Therapy and *CYP2D6* Genotype

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- Aripiprazole Therapy and *CYP2D6* Genotype
- Clozapine Therapy and *CYP* Genotype
- Codeine Therapy and *CYP2D6* Genotype
- Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotype
- Tramadol Therapy and *CYP2D6* Genotype

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Ostrov, David A., PhD; University of Florida College of Medicine, Gainesville, FL, USA

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- Irinotecan Therapy and *UGT1A1* Genotype

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- Fluorouracil Therapy and *DPYD* Genotype
- Risperidone Therapy and *CYP2D6* Genotype
- Warfarin Therapy and *VKORC1* and *CYP* Genotype

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- Dabrafenib Therapy and *BRAF* and *G6PD* Genotype
- Vemurafenib Therapy and *BRAF* and *NRAS* Genotype

Peeters, Marc, MD, PhD; University Hospital Antwerp, Antwerp, Belgium

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- Abacavir Therapy and *HLA-B*57:01* Genotype
- Rasburicase Therapy and *G6PD* and *CYB5R* Genotype

Pollock, Bruce, MD, PhD; University of Toronto, Toronto, ON, Canada

- Venlafaxine Therapy and *CYP2D6* Genotype

Pondé, Noam, MD; A.C. Camargo Cancer Center, São Paulo, Brazil

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- Irinotecan Therapy and *UGT1A1* Genotype
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- Prasugrel Therapy and *CYP* Genotype

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- Capecitabine Therapy and *DPYD* Genotype
- Oxycodone Therapy and *CYP2D6* Genotype

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- Hydroxychloroquine Therapy and *G6PD* Genotype

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- Brivaracetam Therapy and *CYP2C19* Genotype
- Carbamazepine Therapy and *HLA* Genotype
- Clobazam Therapy and *CYP2C19* Genotype
- Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotype

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- Vemurafenib Therapy and *BRAF* and *NRAS* Genotype

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- Abacavir Therapy and *HLA-B*57:01* Genotype

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- Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Thioridazine Therapy and *CYP2D6* Genotype

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- Tramadol Therapy and *CYP2D6* Genotype

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- Amitriptyline Therapy and *CYP2D6* and *CYP2C19* Genotype
- Azathioprine Therapy and *TPMT* Genotype
- Celecoxib Therapy and *CYP2C9* Genotype
- Clopidogrel Therapy and *CYP2C19* Genotype
- Deutetrabenazine Therapy and CYP2D6 Genotype
- Diazepam Therapy and *CYP2C19* Genotype
- Esomeprazole Therapy and *CYP2C19* Genotype
- Flibanserin Therapy and *CYP2C19* Genotype
- Gentamicin Therapy and *MT-RNR1* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype

- Lacosamide Therapy and *CYP2C19* Genotype
- Mercaptopurine Therapy and *TPMT* and *NUDT15* Genotype
- Omeprazole Therapy and *CYP2C19* Genotype
- Pegloticase Therapy and *G6PD* Genotype
- Phenytoin Therapy and *HLA-B*15:02* and *CYP2C9* Genotype
- Rasburicase Therapy and *G6PD* and *CYB5R* Genotype
- Simeprevir Therapy and *IFNL3* Genotype
- Tamoxifen Therapy and *CYP2D6* Genotype
- Thioguanine Therapy and *TPMT* Genotype
- Venlafaxine Therapy and *CYP2D6* Genotype

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- Risperidone Therapy and *CYP2D6* Genotype

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- Celecoxib Therapy and *CYP2C9* Genotype
- Flurbiprofen Therapy and *CYP2C9* Genotype
- Diazepam Therapy and *CYP2C19* Genotype
- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Lesinurad Therapy and *CYP2C9* Genotype
- Piroxicam Therapy and *CYP2C9* Genotype
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- Imipramine Therapy and *CYP2D6* and *CYP2C19* Genotype
- Fluorouracil Therapy and *DPYD* Genotype
- Metoprolol Therapy and *CYP2D6* Genotype
- Omeprazole Therapy and *CYP2C19* Genotype

- Propafenone Therapy and *CYP2D6* Genotype
- Risperidone Therapy and *CYP2D6* Genotype

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- Allopurinol Therapy and *HLA-B*58:01* Genotype
- Atomoxetine Therapy and *CYP2D6* Genotype
- Esomeprazole Therapy and *CYP2C19* Genotype
- Irinotecan Therapy and *UGT1A1* Genotype
- Omeprazole Therapy and *CYP2C19* Genotype

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- *CYP2D6* Overview: Allele and Phenotype Frequencies

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CYP2D6 Overview: Allele and Phenotype Frequencies

Megan Kane, PhD[1]

Created: October 15, 2021.

Introduction

Genetic variation has a significant impact on an individual's metabolism and response to a wide range of medications. The cytochrome P450 family of enzymes are key determinants in the metabolism of multiple pharmacologic compounds. One such member, CYP2D6 is involved in the metabolism of a wide range of medications including drugs for pain management, cancer, mental health disorders, some cardiovascular symptoms, chorea, and Gaucher disease (1, 2). This enzyme is encoded by the *CYP2D6* gene, which is highly polymorphic. The *CYP2D6* alleles can be classified as having no function, decreased function, normal function or increased function. The combination of alleles present in an individual (also referred to as genotype or diplotype) allows for prediction of metabolizer phenotype ranging from poor metabolizer (PMs, no enzymatic activity) through ultrarapid metabolizer (UMs, increased enzyme activity). Poor metabolizers exhibit no enzyme activity, intermediate metabolizers (IMs) have decreased enzyme activity, and UM's have increased enzyme activity relative to the normal metabolizer (NM) phenotype. Increased enzyme activity is most often due to gene copy number variation, namely, the presence of one or more functional gene copies.

As is commonly observed with genomic variation, the frequency of *CYP2D6* alleles differs from one population to another. Knowing the frequency of specific alleles and metabolizer phenotypes among sub-populations of different ethnic, or geographical groups, or both, will enable clinicians to identify individuals who can most benefit from preemptive genetic testing for actionable pharmacogenetic variation. As the *CYP2D6* locus has such a high degree of variation, there is an extensive field of literature regarding allele and phenotype frequencies for various populations. The purpose of this document is to provide a centralized resource for key information on the *CYP2D6* gene, allele function, and phenotype prediction, as well as an overview of the recent literature reporting the allele and phenotype frequencies.

Gene: *CYP2D6*

The cytochrome P450 superfamily (CYP450) is a large and diverse group of enzymes that form the major system for metabolizing lipids, hormones, toxins, and drugs in the liver. The *CYP* genes involved in drug metabolism and disposition are highly polymorphic and contribute to the wide range of activity observed among individuals (no function to increased function).

The CYP2D6 enzyme is responsible for the metabolism of many commonly prescribed drugs, including antidepressants, antipsychotics, analgesics, and beta-blockers. Many of these medications have their own chapters within the Medical Genetics Summaries.

The *CYP2D6* gene on chromosome 22q13.2 is one of the most variable among all *CYP* genes. Over 135 distinct star (*) alleles have been described and catalogued by the Pharmacogene Variation (PharmVar) Consortium, and each allele is associated with either normal, decreased, absent, or unknown enzyme function, as defined by the Clinical Pharmacogenomics Implementation Consortium (CPIC) (Table 1). (1, 3)

The combination of *CYP2D6* alleles a person harbors determines their diplotype (often also referred to as genotype). Examples are *CYP2D6*1/*2* or *CYP2D6*4/*5*. Based on function, each allele can be assigned a value

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to calculate the activity score of the diplotype, which in turn is often used to assign phenotype. Briefly, a value of 1 is assigned to each allele of a *CYP2D6*1/*2* diplotype giving rise to an activity score of 2 (NM), while both alleles of the *CYP2D6*4/*5* diplotype receive a value of 0, which results in an activity score of 0 (PM). If an individual has a gene duplication at the *CYP2D6* locus, then the additional copy is also counted toward the total activity score. An individual who has no detected variants and is determined to have triplication of the *CYP2D6* gene would thus be genotyped as *CYP2D6*1x3* and have an activity score of 3. This CPIC-recommended genotype to phenotype translation method was developed to facilitate standardization (4), it is however, not consistently utilized across clinical laboratories for reporting *CYP2D6* pharmacogenetic test results.

- Ultrarapid metabolizers (UM) have a diplotype with an activity score greater than 2.25
- Normal metabolizers (NM) have an activity score of 1.25 to 2.25
- Intermediate metabolizers (IM) have an activity score of 0.25 to 1
- Poor metabolizers (PM) have an activity score of 0

Table 1. Clinical Allele Function Assignment from CPIC of Selected *CYP2D6* Alleles

Allele type	*CYP2D6* alleles	Value for activity score calculation
Normal function	**1, *2, *35*	1
Decreased function	**9, *17, *29, *41*	0.5
"Severely" decreased function#	**10*	0.25
No function	**3, *4, *5, *6, *40*	0
Increased function	**1x2, *2x2*	2

For a comprehensive list of CYP2D6 alleles, please See PharmVar. Table updated on 13 October 2021, see the allele functionality table from CPIC for the most recent function assignments.
CPIC does not use the term "severely decreased function" to describe the CYP2D6*10 allele, this designation has been provided for clarity in this document.

The *CYP2D6*1* allele is considered the reference allele and is often inferred when no variants interrogated by a genotyping test are detected. The *CYP2D6*1* allele confers normal enzyme activity and can be found in many individuals with a NM phenotype. Some groups refer to this phenotype as an "extensive" metabolizer. Other alleles including *CYP2D6*2* and **35* (to name a few) have activities that are similar to that of **1* and are thus also classified by CPIC as having normal function.

Other *CYP2D6* alleles have one or more single nucleotide polymorphisms (SNPs, indels) that obliterate function (for example, *CYP2D6*3*, **4*, and **6*) (5, 6, 7, 8) or cause decreased function (for example, **10*, **17*, *29, and **41*) (9, 10, 11) (see Table 1) (3). Duplication of a no-function *CYP2D6* allele has the same effect on the metabolizer phenotype as a single copy, however duplicated decreased-function alleles will contribute additively to the final activity score. Clinical testing does not always specify which allele is duplicated; thus, interpretation of these testing results may be ambiguous regarding the metabolizer phenotype.

The role of CYP2D6 in drug metabolism is well established. Whether a particular medication should be administered at lower doses or be avoided entirely by individuals with altered CYP2D6 metabolism will often be discussed on the regulatory agency-approved drug prescribing Information or label (namely, the Food and Drug Administration in the United States) and in published guidelines from professional societies (for example, the Clinical Pharmacogenetics Implementation Consortium, the Canadian Pharmacogenomics Network for Drug Safety, or the Dutch Pharmacogenetic Working Group). In addition to the information presented here, additional published reviews are available that discuss *CYP2D6* gene variations and its role in personalized medicine (1, 2, 12).

Allele and Phenotype Frequencies

There are substantial differences for *CYP2D6* allele frequencies not only among ethnic groups, but also among populations within the major ethnic groups. These differences are illustrated by *CYP2D6*3*, **4*, **5*, **6,* and **41,* which are more common in people of European descent, while *CYP2D6*17* is more prevalent in Africans and their descendants, and *CYP2D6*10* is more common in Asians (13, 14, 15). While the frequency of specific alleles is useful knowledge for the implementation of pharmacogenetics and genetic testing, clinical recommendations are typically based on an individual's phenotype.

Globally, normal and IM phenotypes are the most common. Normal metabolizers comprise 43–67% of populations and IMs comprise an additional 10–44% (3). Poor and UMs are less common, but these individuals are at a higher risk for adverse reactions or treatment failure when treated with a drug that is metabolized or bioactivated by CYP2D6.

If there is any concern regarding an individual's CYP2D6 metabolism, genetic testing provides valuable information to inform drug choice or dosage for the individual. We refer to the NIH Genetic Testing Registry (GTR) and Bousman et al (16) for more information regarding whether testing should be performed and available clinical testing options. The available *CYP2D6* tests include targeted single-gene tests, as well as multi-gene panels and genome-wide sequencing tests (see comment below regarding genotyping methodology). In addition, variant *CYP2D6* alleles to be included in clinical genotyping assays have been recommended by the Association for Molecular Pathology (AMP) (17). These alleles, their defining 'core' variants and reported frequencies in the major ethnic groups are summarized in Table 2.

Table 2: The AMP Tier 1 and Tier 2 *CYP2D6* Star Alleles and Reported Population-Specific Frequencies.

	Star allele	Core SNPs	AS	African (%)	European (%)	Asian (%)	Latino (%)	Other # (%)
Tier 1	*2	rs16947, rs1135840	1	16–20	28	12–29		
	*3	rs35742686	0	<1	1.60	<1		
	*4	rs3892097	0	3–5	18.50	0.5–9.1		
	*5	Gene Deletion	0	3–5	3–5	3–5		
	*6	rs5030655	0	0.50	1	0.50		
	*9	rs5030656	0.5	<0.5	2.80	<0.5	1.60	
	*10	rs1065852, rs1135840	0.25	4–6	<2	9–44		
	*17	rs28371706, rs16947, rs1135840	0.5	17–19	<0.5	<0.5	2.30	
	*29	rs59421388, rs61736512 + rs1058164, rs16947, rs1135840	0.5	9–12	<0.5	<0.5	1.50	
	*41	rs28371725, rs16947, rs1135840	0.5	4–11.5	9	2–12		
	(any)xN	Gene duplication	ASxN	Freq. and AS varies based on the specific allele that is duplicated				
Tier 2	*7	rs5030867	0	<0.05	<0.05	0.01–0.6		
	*8	rs5030865, rs16947, rs1135840	0		0.02			American: 0.1

Table 2 continued from previous page.

	Star allele	Core SNPs	AS	African (%)	European (%)	Asian (%)	Latino (%)	Other # (%)
	*12	rs5030862, rs16947, rs1135840	0	0.08–0.3	0.02			Indigenous American: 1.7
	*14	rs5030865, rs16947, rs1135840	0.5			0.30		
	*15	rs774671100	0	0.60	<0.05	<0.05		
	*21	rs72549352, rs16947, rs1135840	0			0.40		
	*31	rs267608319, rs16947, rs1135840	0	<0.1	<1	<0.1	<1	
	*40	rs72549356, rs28371706, rs16947, rs1135840	0	0.5–1.3	<0.1			
	*42	rs72549346, rs16947, rs1135840	0	<0.5		<0.5 (Central/ South Asian)		
	*49	rs1135822, rs1065852, rs1135840	0.5			1 (East Asians)		
	*56	rs72549347, rs1135840	0	<0.2	<0.2			
	*59	rs79292917, rs16947, rs1135840	0.5		0.70			
	Hybrid Genes	Variable	0	<1	<1	1 (East Asians)		

AMP: Association for Molecular Pathology. SNP: Single Nucleotide Polymorphism; the underlined SNP (rs) identifiers are the variant(s) associated with altered function. AS: Activity score. (any)xN: any star allele duplicated N-many times. Frequency of the specific allele for the indicated population is given in percentage. # The specific population is stated before the allele frequency. Table is adapted from (17).

Literature Summaries

Many studies have sought to determine allele frequencies of interest in specific ethnic populations or study cohorts across the globe with new data being published regularly. The sections below describe the summarized findings of many of these studies, organized by geographic region of the population studied. Upon review of many of these studies, Zhang and Finklestein noted that broad race classifications are not adequate to accurately capture the ethnicity-specific allele frequencies, and many of these classifications are applied differently by different author groups (18). Thus, we have grouped the literature references based on geographic location, rather than race or ethnicity, which is often self-reported by the study participants. When provided by the authors, racial and ethnic composition of the study population is noted. The geographic groupings used herein follow the Biogeographical Groups from PharmGKB (19, 20) where possible. Further regional or country-specific divisions have been provided for clarity. Some of the larger studies that include multi-ethnic populations provide ethnicity-specific allele frequencies. These data are presented herein as reported by the authors, and readers are encouraged to verify the population definitions from the original publication.

Readers are encouraged to review any articles of interest in more detail and search out additional literature if the information regarding a specific population is not adequately described below. The following literature

summaries are not exhaustive for all *CYP2D6* allele frequency reports. Rather, the most recent literature covering any specific region or people group has been included, along with landmark studies that are commonly cited by newer publications.

There are various genotyping methods utilized in the cited literature below and used in clinical testing, each with their own caveats for accuracy of final genotype and phenotype assignment. Targeted allele testing (by TaqMan or other probe-based technologies) for the core, defining SNPs (recommended by AMP or provided by PharmVar) will provide the highest specificity in identifying known alleles. However, these targeted tests alone may not detect copy number variants (CNVs), which may change the assigned phenotype. For example, an individual whose genotype is homozygous for the *CYP2D6**2 variant SNPs may in fact have a gene duplication on one chromosome, thus they would be given a phenotype of UM rather than NM (AS of 3 for *CYP2D6*2x3*). Gene duplication testing may not include identification of which allele is duplicated in a heterozygous genotype. If the duplication is a decreased or no-function allele versus a normal-function allele, the resulting phenotype may be different. Additionally, some variants are shared among star alleles and not all author groups interrogate the frequency of the distinguishing variants. Thus, reporting the specific variant frequency overestimates the frequency of the most common allele, which is defined (partially) by that SNP. This is particularly common for the *CYP2D6*10* allele and tests of SNP rs1065852. Genotyping for gene hybrids may be beyond the scope of some of the literature herein. As such, some star allele frequencies may be overestimated due to the combined counting of the core allele without regard to the potential for hybrid alleles (for example, *CYP2D6*10+*36* hybrids may be only identified as *CYP2D6*10*). While the advances in next-generation sequencing (NGS), including whole exome (WES) and whole genome sequencing (WGS), have made this technology available to a broader range of laboratories and researchers, WES/WGS has its own limitations in accurate diplotype identification. Phasing of identified variants is difficult with short sequencing reads, thus obscuring which variants occur in *cis* or in *trans*. Other technical limitations of NGS approaches include potential interference of pseudogenes, limited information regarding structural variants, and difficulty with interpreting novel or rare haplotypes.

Global Population studies

1. Gaedigk, et al. (2017) (14) PubMedCentral: PMC5292679. This publication is frequently cited as a point of reference for specific subpopulation studies. Gaedigk and colleagues performed a literature review and summarized the predicted allele and activity score based 'phenotype' frequencies for global major ethnic groups: African American, African, American, East Asian, European, Middle Eastern/Oceanian, S. Central Asian, Jewish.

   Among these groups, PM's were present in 5.9–5.4% of the Jewish and European populations, 2.3% of African Americans and 2.8% of Africans, and ~1% or less of the other ethnicities. Intermediate metabolizer^ were most prevalent in the Jewish population at 37.7%, followed by 38–34% of African, African American, East Asian and European populations, South Central Asians were predicted to have an IM phenotype prevalence of 28.6% and Middle Eastern and American populations had the lowest frequency of IM phenotype at 24–22%. Normal metabolizer phenotype^ was most common in South Central Asian, Middle Eastern and East Asian populations (64–68%), but least common in the Jewish population at 44.8%. Ultrarapid metabolizer phenotype was predicted to be highest in Jewish and Middle Eastern populations (~11%) and least common in East Asian (~1%). (^Note: The intermediate and NM frequencies are adjusted to the CPIC activity score definitions provided above, an update from the original publication.) Figure 1 shows the predicted phenotype based on genotype; frequencies are shown in percentage (%) based on the ethnicity. A corrigendum for this table is available from PubMedCentral.

2. Koopmans, et al. (2021) (15) PubMedCentral: PMC7904867. This review summarizes the phenotype frequencies among global populations from over 300 published articles and provides the non-NM (either

poor, intermediate, or ultrarapid) phenotype frequencies for CYP2D6 and CYP2C19 to facilitate optimized dosing for psychiatric medications. The authors used allele frequency data and activity scores as described above to translate genotype to phenotype. The population with the highest prevalence of non-NMs was Algeria, and the lowest prevalence was in Gambia. The frequency of UM phenotype was nearly 40% in the Mozabite people, a Berber ethnic group in North Africa. Non-Austronesian Melanesians and Druze (from the Middle East) groups also were noted to have high frequencies of UM. The PM frequencies were highest in British (12.1%), Danish (10.6%) and Basque French (9.7%) populations. The global average for non-NM phenotypes was reported as 36.41%. It is important to note that some of the populations included in this study had small sample sizes (less than 50 individuals) and as such, the phenotype frequencies made be over- or underestimated.

3. Taliun, et al. (2021) (21) reported on the sequencing data from 53,831 diverse genomes in the National Heart Lung and Blood Institute's (NHLBI) Trans-Omics for Precision Medicine (TOPMed) program. The TOPMed program aims to combine more than 80 "omics" studies from a broad range of populations of varying race and ethnicity. The entire current set of ~155,000 selected participants consists of approximately 41% European ancestry (European, European American), 31% African ancestry (African, African American, African Caribbean), 15% Hispanic/Latino (including Mexican, Mexican American, Central American, South American, Cuban, Dominican, Puerto Rican), 9% Asian ancestry (Chinese, Taiwanese, Asian American, Pakistani) and 4% 'Other' (Samoan, Native American, multiple groups, or unknown). Among the data in the TOPMed analysis, the authors described the frequency of *CYP2D6* star alleles as identified by WGS in the supplemental data from this report. The frequencies of 99 alleles (including known star alleles, duplications, deletions, hybrid alleles and novel alleles) are reported for individuals of European, African, Hispanic/Latino, Asian, Samoan, and Amish ancestry. In all populations, the *CYP2D6*1* allele was most common, but this ranged from 29% in African ancestry up to 47% in Hispanic/Latino ancestry. The TOPMed data are available here. Detailed Tier 1 allele frequencies for AMP recommended alleles are shown in Table 3. As stated above, the identification of hybrid alleles and phasing of variants for accurate haplotype calls is difficult with NGS data. The authors cite methods using the program Stargazer to perform haplotype analysis.

Table 3: Allele Frequencies of Common *CYP2D6* Alleles from TOPMed Studies

Country/ ancestry	*2	*3	*4	*5	*6	*9	*10	*17	*29	*41	Ref
European	14.81	1.57	13.80	3.08	1.06	2.54	1.58	0.16	0.07	9.83	(21)
African	16.11	0.35	3.63	5.67	0.26	0.53	3.83	15.71	8.40	2.40	(21)
Hispanic/ Latino	16.48	0.86	9.55	2.96	0.55	1.46	1.65	1.72	1.24	5.72	(21)
Asian	9.53		0.51	6.24			12.88	0.036		4.02	(21)
Samoan	17.20	0.05	0.16	1.30		0.10	1.51	0.31		5.41	(21)
Amish	9.33		8.44	5.11			4.89			7.33	(21)

The alleles shown are commonly observed across populations and correspond to those designated by AMP as Tier 1 in their clinical allele testing recommendations (17).
Allele frequencies are given as percentages (%).
Blank values indicate that no frequency was given for that allele from the cited study.

Table 1 Phenotype predictions from genotypes

Major ethnicities	AS = 0 p^2 gPM	AS = 0.5 2pq gIM	AS = 1 q^2 gNM-S	AS = 1 2pr gNM-S	AS = 1 (sum) q^2 + 2pr gNM-S	AS = 1.5 2qr gNM-F	AS = 2 r^2 gNM-F	AS = 2 2ps gNM-F	AS = 2 (sum) r^2 + 2ps gNM-F	AS = 1 + 1.5 + 2 q^2 + 2pr + 2qr + r^2 + 2ps gNM-S+F	AS = 2.5 2qs gUM	AS = 3 2rs gM	AS = 4 s^2 gUM	AS >2 2qs + 2rs + s^2 gUM
African American	2.38	10.99	13.60	14.40	28.00	32.92	22.11	0.66	22.77	83.69	1.69	1.93	0.05	3.67
African	2.78	10.63	13.92	13.97	27.89	32.60	22.95	0.92	23.88	84.36	1.56	2.17	0.07	3.80
From the Americas	1.92	2.77	1.98	18.10	20.08	12.44	59.08	0.72	59.80	92.32	0.88	3.62	0.12	4.61
East Asian	0.41	5.48	23.92	5.14	29.06	43.16	21.46	0.11	21.57	93.79	0.69	0.67	0.01	1.37
European	5.44	5.37	1.47	28.47	29.93	14.78	43.77	0.83	44.59	89.31	0.42	2.65	0.06	3.13
Middle Eastern or Oceanian	0.91	5.19	8.99	10.68	19.67	32.91	32.93	1.38	34.31	86.89	3.46	7.32	0.43	11.20
South Central Asian	1.05	3.82	12.55	12.22	24.77	30.46	37.79	0.40	38.20	93.42	0.55	2.19	0.03	2.77
Jewish	5.95	10.64	4.75	22.35	27.10	19.97	20.98	3.90	24.88	71.95	3.49	7.33	0.64	11.46

Phenotype frequencies are averages (in %) and were calculated from average allele-frequency data. A summary table including ranges (minimum and maximum) is provided in **Supplementary Table S2** online (Summary tab).

AS, activity score; PM, IM, NM-S, NM-F, and UM, poor, intermediate, normal-slow, normal-fast, and ultrarapid metabolizer, respectively; the prefix "g" indicates genotype-predicted phenotype. p, q, r, and s, variables of the Hardy Weinberg equilibrium. (See **Supplementary Table S3** online).

Figure 1: Global ethnicity-specific predicted phenotype frequencies based on genotype. AS : activity score. gPM: genetic poor metabolizer, gIM: genetic intermediate metabolizer, gNM: genetic normal metabolizer, gUM: genetic ultrarapid metabolizer. This article predates the adjustment of the AS to phenotype translations described above. This figure is reproduced under Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. © A Gaedigk et al. (2016)

American Allele Studies

Table 4: Allele Frequencies of Common *CYP2D6* Alleles from North American Studies

Country/ ancestry	*2	*3	*4	*5	*6	*9	*10	*17	*29	*41	(any)xN	Ref
American	17.30	1.40	16.75	3.40	1	2.40	1.80	2.72	1.40	8.20	2.70	(22)
American/AFR		0.20	6.10	6.10	0.10	0.10	4.10	21.80	6.50	8.70	4.50	(23)
American/EUR		1.90	18.60	2.80	2.00	2.60	2.80	2.00	0.10	8.70	3.40	(23)
American	14.30	1.10	13.50	3.80	1	3.50	2.40	0.60	0.20	11.10	1.20	(24)
African American	21.30	0.00	6.90	5.60	0.00	0.00	4.40	12.50	10.60	2.50	9.40	(25)
Asian	14.20	0.60	5.10	4.60	0.00	0.60	13.60	0.00	0.60	7.40	1.10	(25)
Caucasian	16.90	1.80	16.90	1.80	0.60	1.20	3.00	0.00	0.00	12.70	1.80	(25)
Hispanic	22.60	0.60	9.10	3.00	0.00	2.40	0.00	0.60	0.60	7.30	2.40	(25)
Ashkenazi	9.90	0.50	20.30	0.50	0.50	0.50	7.80	1.00	0.00	14.10	10.40	(25)
non-EUR	15.00	0.52	9.20	3.40	0.49	1.10	7.70	3.10	1.60	5.80	3.18	(26)
AFR	13.00	0.16	3.90	5.90	0.22	0.32	3.20	17.00	7.50	1.80	6.21	(26)
AMR	17.00	0.53	11.00	2.20	0.53	1.40	1.20	0.81	0.64	4.40	2.73	(26)
EAS	15.00	0.02	0.26	3.70	0.02	0.10	34.00	0.00	0.00	3.00	2.84	(26)
EUR		1.50	18.00	3.00	1.00	2.50	1.30	0.19	0.04	9.30	1.75	(26)
SAS	21.00	0.41	9.90	2.80	0.08	0.08	5.30	0.00	0.00	15.00	2.10	(26)
Other	16.00	0.71	12.00	3.30	0.67	1.40	4.20	2.60	1.40	7.00	3.02	(26)
American	23	1.40	20	3.90	1	2.50	1.70	<1		9	3.80	(27)
Caucasian Canadian		3	19				21			12		(28)

Table 4 continued from previous page.

Country/ ancestry	*2	*3	*4	*5	*6	*9	*10	*17	*29	*41	(any)xN	Ref
Inuit Canadian		0	6.7–8.3				2.20					(29)
Native Indian Canadian		0	3				3					(30)
Mexican Native and Mestizo	77.8–79.6 §						10.2–12.4	0.5–0.7		0–2.8		(31)
Mexican Amerindian	12–28	0	0.6–5.3	0.50	0		0			1	1.50	(32)
Mexican Mestizo	10.7–19.3	0.9–1.4	11.2–13.1	1.3–2.7	0		2.3–12.4			2.20	4.1–12.8	(32)

The alleles shown are commonly observed across populations and correspond to those designated by AMP as Tier 1 in their clinical allele testing recommendations (17).
AFR: Africa; EUR: Europe; AMR: Americas; EAS: East Asia; SAS: South Asia.
Allele frequencies are given as percentages (%).
Blank values indicate that no frequency was given for that allele from the cited study.
§ This frequency is for the rs16947 SNP, which is found in multiple *CYP2D6* haplotypes.

US-Based Allele Studies

4. Del Tredici, et al. (2018) (22) reported on a study of a multi-ethnic population selected from across the United States, though ethnicity was self-reported and optional. The study covered 104,509 individuals. Based on those who chose to self-report their ethnicity (44.6%), Caucasians were most prevalent across all regions of the US and both Asian and Hispanic-descent individuals were underrepresented (based on comparison to US census data). A total of 37 different alleles were detected in the assay panel data (counting CNV haplotypes as distinct from single-copy haplotypes of the same star allele). Normal-function alleles were most prevalent in the study, comprising roughly 60% of the alleles, with 2% having duplications of normal-function alleles. No-function alleles were present at a frequency of 23% and decreased-function alleles were observed at a frequency of nearly 17%. Frequencies determined by this study for the AMP recommended Tier 1 alleles are shown in Table 4. Overall, 2.2% of the study participants were predicted to be UM, 81.4% NMs, 10.7% IMs, and 5.7% PMs.
5. Chanfreau-Coffinier, et al. (2019) (23) examined the prevalence of actionable *CYP2D6* genotypes among veterans who utilized the U.S. Veterans Health Affairs (VHA) pharmacy services between 2011–2017. Out of the 7,769,359 individuals studied, roughly 90% of the subjects enrolled were men. A comparison between individuals of European (EUR) or African (AFR) descent was made to determine if any heritage-specific allele frequencies could be identified. The alleles studied were *CYP2D6*3, *4, *5, *6, *7, *9, *10, *17, *29, *41* and *CYP2D6* duplications; the absence of these specific alleles was assumed to be the *CYP2D6*1* reference allele. Overall, 7.6% of the individuals studied had an "actionable" *CYP2D6* genotype, suggesting they were at risk for an undesirable outcome for a CYP2D6 substrate medication. Within the AFR sub population, 10.5% of individuals had actionable genotypes and 7.1% of the EUR subpopulation had actionable genotypes. The results projected that UMs are more frequent at 4.5% among the AFR group compared with 3.3% of the EUR group, with an overall projected frequency within the VHA of 3.4%. The AFR subpopulation was predicted to have a frequency of 12.6% IMs, with EUR predictions of IMs were at 7.2% and overall frequency of 8% in the VHA. The authors report PMs were more prevalent in the EUR group at 6.1% while only 1.9% of the AFR group were predicted to have this phenotype. Detailed allele frequencies for AMP recommended Tier 1 alleles are shown in Table 4.

6. Dalton, et al. (2020) (24) studied human liver tissue samples from 2 biobanks to examine the relationship between *CYP2D6* genotype and actual metabolic activity. Over 300 samples were examined (predominantly of European descent) and allele frequencies determined. Next-generation sequencing-based testing detected 23 alleles; if no variants or structural variation were found, the *CYP2D6*1* reference allele was assigned. Overall, the *CYP2D6*1* allele was observed with a frequency of 32%, followed by *CYP2D6*2* at 14.3%, both of these alleles are categorized as normal function. The no-function *CYP2D6*4* allele was observed at a frequency of 13.5% and the *CYP2D6*41* decreased-function allele was observed at 11.1%. The authors found novel *CYP2D6* alleles and suggested that structural variation should be considered alongside SNP genotyping for improved phenotype prediction from genotype. The authors reported the prevalence of UMs was 1.3%, NM frequency was 51.6%, IM frequency was 39.5%, and PM frequency was 7.6% (scored based on the revised CPIC definitions). Detailed allele frequencies for AMP recommended Tier 1 alleles are shown in Table 4.
7. Qiao, et al. (2018) (25) studied 854 blood donors with self-reported ethnic and racial backgrounds. Blood donors were from the New York Blood Center and unrelated healthy Ashkenazi Jewish donors were from the greater New York City, NY area. Ethnicities represented in the study were African American (AA), Asian (A), Caucasian (C), Hispanic (H) and Ashkenazi Jewish (AJ). Sixteen variant alleles and gene duplications were analyzed using a Luminex *CYP2D6* genotyping kit, and the absence of detected variation led to *CYP2D6*1* allele assignment; CNVs were also detected by a multiplex ligation-dependent probe amplification (MLPA) kit. They report that UMs were most common among the AJ (10–20%), followed by AA (~5%) with H, A, and C least likely to have UM phenotype. The authors provide frequencies for the different phenotypes under 2 different scoring scenarios: in the first an AS of one results in an NM phenotype, and in the second an AS of one results in an IM phenotype. Under the latter scoring rubric (more in alignment with the latest CPIC scoring described above), the NMs were most common (~70%) in H, with C, A and AA groups demonstrating 50–60% frequency; NMs were found to be the least frequent (40–50%) in AJ. A phenotype of IM was most common in AA (~40%), next most common in C and AJ (<40%) and least in H (~25%). The PM frequency was 3–4% in C, AJ, and AA, 1% in H and none were detected in the Asian group. Detailed Tier 1 allele frequencies for AMP recommended alleles are shown in Table 4. The authors noted that among individuals of Asian descent, the *CYP2D6*36+*10* tandem had a frequency of 15% (many studies do not discriminate between *CYP2D6*10* and *CYP2D6*36+*10* in which case the reported frequency captures both [and depending on the platform used, other alleles as well]).
8. Luo, et al. (2021) (33) published an additional resource of multi-ethnic US *CYP* allele frequencies and phenotypes, termed the Helix DNA project. This study reported all 86,490 study participants were residing in the USA at the time of sample collection but did not rely on self-reported ethnicity. Relevant variants of interest were extracted from exome sequencing data and used to impute genetic heritage from one of these 5 reference populations: AFR (Africa), AMR (America), EAS (East Asia), EUR (Europe), SAS (South Asia). Individuals who were admixed but had >80% markers for a specific population were called as member of that group. Any sample with <80% markers for any of the genetic groups was classified as "other." The authors noted their study population was less diverse than the broader US population. Authors observed that for a subset of very rare alleles in the EUR cohort, these alleles were present at a higher frequency in other ethnic groups. A total of 1,288 unique genotypes were observed at the *CYP2D6* locus in the study cohort. Notably, the decreased-function allele *CYP2D6*29* was present in 7.5% of individuals with AFR genetic markers; this allele was present in only <0.05% in EUR ancestry individuals. This study also reports the non-functional *CYP2D6*36* gene being present at a frequency of 22% in the EAS genetic group, much higher than the frequency provided by the PharmGKB reported 1.2% for the same group. The Helix DNA project also reports a prevalence of 5.2% for the *CYP2D6*68* loss-of-function allele in their EUR individuals. It is likely that due to the limitations of NGS genotyping, these allele frequencies reflect the combined frequencies of the single and hybrid alleles, thus are over estimations. Access to the full resource is available via GitHub where users can

download tables of either specific allele frequencies or genotype-phenotype frequencies (26). Detailed Tier 1 allele frequencies for AMP recommended alleles are shown in Table 4.

9. Zhu, et al. (2021) (34) performed genotype to phenotype predictions for 2,877 individuals who were enrolled in the Right Drug, Right Rose, Right Time-Using Genomic Data to Individuals Treatment (RIGHT) study who were also prescribed a CYP2D6-substrate opioid medication. The frequency of UMs in this study was 2%, NMs comprised 50% of the cohort, 41% were IMs and 7% were PMs. Among this cohort, 94% of the individuals identified as "white," 11 individuals (<1%) were African American, 22 (1%) individuals identified as Asian/Native and the remaining 5% of the study cohort identified as "Other", which included unknown and mixed heritage. Only 1% of the cohort separately identified as "Hispanic" for their ethnicity. Preliminary sequencing data for the RIGHT protocol (1,013 individuals) was reported in 2014 by Bielinski and colleagues (27). Detailed Tier 1 allele frequencies for AMP recommended alleles are shown in Table 4.
10. Salyakina, et al. (2019) (35) studied a diverse population of 413 individuals. These individuals self-reported their ethnicity; 75% of the study population identified as Hispanic and 62% of the population identifying as "White-Hispanic." Overall, the most common phenotype was NM (90%), followed by IM (4%), with UM and PM both seen in 3% of the study participants. White non-Hispanic participants had a low frequency of decreased-function alleles (2.8%) but mixed non-Hispanics had a higher rate (50%). The low frequency of decreased-function alleles in White, non-Hispanic study participants may be an underestimate due to the low number of these individuals in the study cohort (18 out of 413). The study authors concluded there are differences in *CYP2D6* allele frequencies among the various distinct racial and ethnic populations of south Florida but acknowledged this study had limited power to detect differences between smaller ethnic groups. The specific allele studied were *CYP2D6*2*, **2A, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *14A, *15, *17, *19, *20, *29, *35, *36*, and **41*; however only 14 were detected in the study population by the targeted testing panel. The specific allele frequencies with 95% confidence intervals are displayed in the publication.
11. Oshikoya, et al. (2019) and Rossow, et al. (2021) (36, 37) studied a pediatric population from the Vanderbilt University Medical Center. The 257 individuals were predominantly white (84%), with the next most prevalent ethnicity being African American (11%) and most participants identifying as "non-Hispanic" (96%). Next-generation sequencing data was used to detect variants; deletions or duplications were detected by long-range polymerase chain reaction (PCR). At total of 23 distinct *CYP2D6* star alleles were interrogated in this study. The authors reported ~13% of the study cohort genotyped as PM or IM and the remaining 87% were NM, UM or "rapid" metabolizers.
12. Davis, et al. (2021) (38) reported on the prevalence of actional pharmacogenetic genotypes in a population of 4,230 individuals enrolled in the Alabama Genomic Health Initiative (AGHI). The genotyping data available to the authors from the AGHI study only interrogated 5 *CYP2D6* alleles *(*6, *7, *9, *17,* and **41*), yielding only 7.6% of the study population as carriers of actionable *CYP2D6* alleles. The frequency of actionable *CYP2D6* alleles was 5.9% in individuals of African ancestry and 8.3% in individuals of European ancestry. The authors note that this is likely an underestimate based on the limited alleles covered by the genotyping array.

Canadian Allele Studies

Canadians of European descent likely have a similar frequency of the *CYP2D6*10* allele compared with indigenous populations (2% versus 2–3%). The *CYP2D6*4* allele is also more common in European Canadians (19%) as compared with native populations (3–8%). Most of the native Canadians were predicted to be NMs, though there are limited studies for these populations in the recent literature.

1. Gulilat, et al. (2019) (28) reported on the utility of a targeted exome NGS sequencing panel for pharmacogenetic testing, using a study cohort of 246 'Caucasian' individuals. Targeted genotyping was performed to validate variants found in the *CYP2D6* alleles. The validated variants were found in the no-

function alleles *CYP2D6*3A* and **4*, as well as the decreased-function *CYP2D6*10* and *CYP2D6*41* alleles. The concordance rate was high for individual allele calls (98–100%), but the *CYP2D6*4* and **10* alleles share the SNP rs106585.2 The *CYP2D6*10* allele was most common, observed in 21% of the alleles sequenced, however this does not account for the overlap with *CYP2D6*4*. The second variant in *CYP2D6*4* was present in 19% of the samples. (Thus, the unique frequency of the **10* allele is likely only 2%.) The *CYP2D6*3A* allele was least common at 2% (the rationale for reporting the *CYP2D6*3A* sub-allele over the **3* allele is unclear). These variant frequencies all agreed with the published EUR group frequencies from 1000 Genomes Project and Exome Aggregation Consortium (ExAC). The *CYP2D6*41* allele was observed at a rate of 12%, higher than the published rate of 9% from ExAC. Most individuals with these decreased or no-function alleles were heterozygous, indicating a probable IM phenotype. Detailed allele frequencies for AMP recommended alleles are shown in Table 4.

2. Jurima-Romet, et al. (1997) (29) studied the CYP2D6 phenotype and genotyped for 152 individuals from a Canadian Inuit population. Three individuals phenotyped as PM based on dextromethorphan metabolism, the other 149 individuals were classified as NM. Genotyping did not detect any *CYP2D6*3* alleles. However, the frequency of the **4* allele was estimated to be between 6.7% and 8.3% in this population. The frequency of the **10* allele was estimated to be 2.2%. Detailed allele frequencies for AMP recommended alleles are shown in Table 4.
3. Nowak, et al. (1997) (30) reported on the CYP2D6 phenotypes in 115 Canadian Native Indian (also called First Nation) individuals. One individual in the study demonstrated a PM phenotype based on dextromethorphan metabolism (1.1% frequency). Genotyping did not detect any individuals with the *CYP2D6*3* allele, while the **4* and **10* alleles were both seen with a frequency of 3%. Detailed allele frequencies for AMP recommended alleles are shown in Table 4.

Mexico and Amerindian Allele Studies

1. Leitao, et al. (2020) (39) reviewed 13 distinct studies that reported on Amerindian populations from multiple countries. Seven of the reviewed studies focused on Mexican populations, but others studied populations from Argentina, Chile, Costa Rica, Paraguay, Peru, and the United States. Phenotype frequencies varied among the Amerindian populations in these countries. The highest frequency of PMs was observed in Costa Rica (30%), with Argentina and Paraguay next at 13%, and Venezuelan and US cohorts at 6%. The frequency of IMs ranged from 1% in Argentina/Paraguay up to 22% in Mexico. The NMs were most commonly seen in the US (90%), with rates between 80–87% in most other studies. Notably Mexico and Costa Rica reported much lower NM frequencies: 69% in Mexico and 45% in Costa Rica. Diplotypes resulting in UM phenotype were seen at higher rates in Mexico (9%) and Costa Rica (7%) but less often in the US (1%).
2. Henderson, et al. (2018) (40) performed a literature review of 27 studies focused on Amerindian (also called Indigenous North American) populations residing in Canada, the United States of America, and Mexico, 13 of those studies included some analysis of *CYP2D6* variation. Indigenous populations in the USA had a reported frequency of 14.6–20.9% for *CYP2D6*4*, 1.3–2.8% for *CYP2D6*5*, 1.3–2.0% for **10*, 1.1% or less for *CYP2D6*25*, and 6.9–11.2% for *CYP2D6*41*. A large number of studies of Mexican Amerindian groups were also reviewed, with a notably low frequency of *CYP2D6*3, *6,* and **10* alleles. The authors note that the low frequencies of no-function *CYP2D6* alleles in these populations appear to correlate well with the phenotype studies of dextromethorphan metabolism in a subset of the Mexican indigenous studies. The American Indian populations in the northwest USA were notably different from populations in Mexico with regard to higher frequencies of *CYP2D6*4* and **41* alleles. Overall, allele frequencies in these indigenous populations vary as compared with European descent populations. The authors note that not all studies reviewed were comprehensive in their genotyping approaches and alleles of altered function may be present in these populations that were not detected.

3. Gonzales-Covarrubias, et al. (2019) (31) studied pharmacogenetic markers among natives (94 individuals) and Mestizos (1284 individuals) in Mexico. By performing WES, they studied genetic variation in 17 different pharmacogenes. They observed 76 variants in the *CYP2D6* gene among their study participants. A handful of variants were notably different in population frequency among the native and Mestizo study cohort compared with global minor-allele frequencies. The variant associated with the *CYP2D6*10* allele was more common in the Native population than Mestizo. However, this variant is shared among other haplotypes, so it likely represents an over-estimate of this allele frequency. In contrast, the variant associated with the **41* allele was more common the Mestizo population. Detailed allele frequencies for AMP recommended alleles are shown in Table 3.
4. Sosa-Macias, et al. (2013) (32) reviewed published allele and phenotype frequencies among the indigenous populations of Mexico, focusing primarily on 4 studies from Mexico while comparing to data from 8 other studies from different populations from the Americas. Among the studies of Mexican indigenous populations, 993 individuals were genotyped for the *CYP2D6*4* allele, though some studies reported on the frequency of as many as 8 star alleles and the frequency of gene duplications. Most notably, there was a near total absence of PM-predicting genotypes for Mexican Amerindian populations. This corresponded with the absence of PMs based on dextromethorphan metabolism. Overall, the genetic and phenotypic diversity at the *CYP2D6* locus among the indigenous groups reported is notably lower than the Mestizo population. Detailed allele frequencies for AMP recommended alleles are shown in Table 3.

Latin American Allele Studies

Similar to reports of Mestizo and Native populations in Mexico, these populations in Latin America as a whole show differences from one another in allele frequencies for *CYP2D6*. The frequencies of NM and UM were high in native populations from the south (NM) and northern regions (UM). Owing to their ancestry, most reported "mestizo" populations had allele frequencies similar to reported European frequencies. The mestizo population of Columbia stands out as noteworthy for a higher incidence of UMs (18%) compared with other Hispanic mestizo populations. The *CYP2D6*10* allele frequencies reported in Brazilian populations may be overestimated (10.2–94%), though the possibility remains that this allele is more common in Brazil as compared with global "Caucasian" frequencies. However, the *CYP2D6*41* allele had a lower population frequency in Brazil. Some native people groups were found to have a higher frequency of the *CYP2D6*4/*4* diplotype (25% in the Bari group from Venezuela), leading to a higher proportion of PMs.

1 Naranjo, et al. (2018) (41) reported a large cohort (6,060 individuals) study spanning multiple countries in Latin America. The groups studied were Native Americans (1395 individuals either from Mexico [North], Costa Rica [Central] or Peru [South]), Afro-Latin Americans (96 individuals, self-reported as being of African descent and living in Costa Rica or determined to have 4 black grandparents and living in Cuba), White Latin Americans (287 individuals from Cuba with 4 white or Caucasian grandparents), Admixed Latin Americans (2571 individuals who may be described as "Mestizo" in other studies), Iberians (1537 individuals from Spain or Portugal), and Argentinian Ashkenazi Jews (174 individuals). The study interrogated the presence of distinct star alleles *(*2, *3, *4, *6, *10, *17, *29, *35,* and **41*) via real-time PCR with TaqMan allelic discrimination assays, the *5 allele and gene duplications were detected by long-range PCR, and overall gene copy number was assessed by TaqMan copy-number assay. A *CYP2D6*1* allele was assigned in the absence of any variants. The PM phenotype was observed at rates between 0–10.2%, being most prevalent in Central native Americans. The prevalence of IMs (an AS of either 0.5 or 1) was between 0–8.67% (AS=0.5) or 12.62–31.12% (AS=1). The combined frequencies of these 2 IM AS were highest in Central native Americans (~33%) and Argentinean Ashkenazi Jews (~34%). Normal metabolizers were observed at rates ranging from 29–86%, based on an AS of either 1.5 or 2. Overall, 64% of all individuals studied were NM, but the highest frequency was observed highest in South Native American (~87%), ~64–65% in Afro-Latin Americans and Admixed Latin Americans, ~51% in Central

Native Americans. The frequency of UM ranged from 0.47–11.56%, with Argentinian Ashkenazi Jews having the highest frequency, followed closely by North Native Americans (~9.5%) and an overall average frequency of 6.15%. The authors noted that the *CYP2D6*10* and **41* alleles did not fit Hardy-Weinberg equilibrium in the admixed population, but all other specific alleles were within the expected equilibrium range. Notably, the admixed populations and Native American populations showed the widest difference in population frequencies across the alleles studied, supporting the main hypothesis that Native Americans are notably different from other populations in Ibero-America.

BRAZIL

1. Ameida Melo, et al. (2016) (42) studied the *CYP2D6*4, *10,* and **17* alleles in Brazilian individuals undergoing tamoxifen treatment. Though the cohort was small (80 females), they noted a higher frequency of the decreased-function *CYP2D6*10* allele in their population relative to Caucasians, an allele often associated with the IM phenotype. However, they used only a single variant to define each of the 3 star alleles, which likely means the authors overestimated the *CYP2D6*10* frequency.
2. Suarez-Kurtz (2004) (43) reported the development of a database specifically for Brazilian pharmacogenetic variation. The Brazilian populations is highly heterogeneous and the author states that racial classifications used in a county like the United States may not be similarly applied in Brazil, thus determination of regional allele frequencies within Brazil is more useful than comparing to global classifications. The author introduces an online database from the Brazilian National Pharmacogenetics/pharmacogenomics Network (REFARAGEN) that provides allele frequencies for multiple pharmacogenes (including *CYP2D6*) broken out by 4 regional populations as well as overall county-wide frequencies. http://www.refargen.org.br/
3. Suarez-Kurtz (2018) (44) discussed the role of pharmacogenetics in oncology treatment in Brazil. Several drug-gene interactions are discussed but notably the author summarizes studies relating to *CYP2D6* variation and tamoxifen response and notes that the NM phenotype was most common (83.5%), with PM (2.5%) and UM (3.7%) phenotypes being less prevalent in one study, and similar phenotype frequencies were projected from the data of other studies. At the time of this publication, the Refargen database held data from 2 studies: one with 1,034 healthy individuals from 4 different regions of Brazil and a second with 270 healthy individuals from the Southeast region of Brazil.
4. Salles, et al. (2021) (45) studied the frequency of 4 star alleles in specific regions of the Amazon: Novo Repartimento, Goianésia do Pará, Macapá, Porto Velho, and Plácido de Castro in a total of 180 individuals. The authors interrogated a single variant for each of the 4 alleles via targeted Sanger sequencing methods, which may have resulted in an over estimation of the allele frequencies. They reported that the frequency of the *CYP2D6*2* (normal-function) allele within all 5 Amazonian regions was less than other published studies (5.6–16.7%). The reported *CYP2D6*10* (decreased-function) allele frequency varied within the 5 regions studied, similar to other Brazilian reports (ranging between 10.2–94.4%). The *CYP2D6*41* (decreased-function) allele was seen only in a single study participant, in the heterozygous state. They did, however, note that the no-function *CYP2D6*4* allele was more frequent in their study than in other reports (31–35% in the current study). However, the authors discussed that these alleles were primarily observed in the heterozygous state and were unlikely to affect primaquine therapy. It is likely, given the genotyping methods utilized in this study, that the reported *CYP2D6**4 allele frequency is overestimated and includes hybrid alleles, given the lack of homozygotes for this genotype.
5. Silvino, et al. (2020) (46) reported CYP2D6 phenotype and genotype results from a 10-year retrospective study of 261 individuals who were treated for malaria in Rio Pardo in the Amazon region. The authors performed real-time PCR genotyping with TaqMan assays for 8 variants and one small deletion, then inferred haplotypes using PHASE software and assigned alleles using the PharmVar allele definitions. However, their phenotype assignment did not follow CPIC scoring outlined above. The frequency of PMs (AS=0) was 1.2%, IMs (AS=0.5) were observed in 2.4%, and "normal-slow" metabolizers (AS=1, which is

IM per CPIC scoring) were observed at a frequency of 21.8%. The frequency of UMs (AS>2) was reported to be 4.4%. The remaining 70.2% were classified as "normal-fast" metabolizer (AS=1.5–2) phenotype.

VENEZUELA

6 Cirillo, et al. (2014) (47) reviewed several reports of pharmacogene variation in Venezuela. The authors reported on a previous study where most altered-function alleles were present in the admixed Venezuelan population at similar frequencies to Western Eurasian and other Central or South American populations. The predicted PM phenotype was present at a rate of 2% in the analyzed Venezuelan population. Among the Amerindian populations in Venezuela, the Bari population had a notably high frequency of individuals with the *CYP2D6*4/*4* diplotype (25%) predicting PM status.

CHILE

1. Munoz, et al. (1998) (48) reported on polymorphisms in multiple *CYP* genes in the South-Amerindian population of Chile. The specific population studied was the Mapuche Indian population living in the national reservation of the Haupi Island, Lake Ranco. A total of 84 individuals enrolled in the study. The authors reported the *CYP2D6*1* and *CYP2D6*2* normal-function alleles were present in roughly 84% of the population, with the *CYP2D6*10* decreased-function allele present in less than 2% and no-function alleles present in roughly 8% of the population. The authors noted technical difficulties in full genotyping of some individuals and suggested unknown variation may have interfered with their analyses. However, the genotyping methods and limitations of known alleles at the time of publication likely contributed to difficulties in genotyping and an overestimation of the *CYP2D6*1* allele.
2. Varela, et al. (2015) (49) genotyped 4 selected alleles as well as gene duplications, and analyzed in vivo metabolism of debrisoquine, a known CYP2D6 substrate, to confirm genotype predictions in a smaller sample of the cohort. The 321 genotyped study participants were described as Chilean mestizo. The frequencies of the tested alleles (40.6% for *CYP2D6*2* [normal-function], 1.09% for *CYP2D6*3* [no-function], and 11.8% for *CYP2D6*4* [no-function]) were similar to reported frequencies in the Spanish population. The *CYP2D6*17* allele was not observed. Some discordance between predicted and actual metabolizer phenotype (5 out of the 23 subjects tested) was observed, but most of the genetic predictions were validated with the in vivo metabolic study. The authors noted that a non-coding genetic variation at the promoter that is associated with UM phenotype was not assessed in the genetic analysis, potentially contributing to genotype-phenotype mismatch. It is also possible that incorrect haplotype assignments were made based on limited variant genotyping data, further contributing to the discordance of phenotype.

COLUMBIA

1 Sarmiento, et al. (2020) (50) reported on the genotype and phenotype frequency among 212 healthy mestizo individuals from Columbia. The authors report that this study improves upon previously published worked by Isaza, et al. (51) by more closely characterizing gene duplications before automatically assigning a UM phenotype. The authors performed genotyping for 8 known star alleles and tested for gene duplications and deletions (the *CYP2D6*5* allele). Based upon verification that duplicated alleles were indeed functional, Sarmiento and colleagues reported 18.4% of their study cohort were classified as UMs. However, only 4.7% were classified as PMs. The prevalence of the IM phenotype was reported to be 22.6%. The authors noted that the prevalence of UMs in this study is significantly higher than in other Hispanic mestizo populations, while the PM prevalence is similar across this and other cited studies.

European Allele Studies

Gene duplication events are more common in south-eastern Europe, while loss-of-function alleles are more common in north-western Europe. The frequency of UM phenotype in Denmark and the Netherlands are between 0–5% and PM frequency is between 4.2–11%. Lithuanian populations may have a lower frequency of the **5* allele as compared with the European average. Studies from the Czech Republic and Bosnia reported a higher frequency of the **10* allele among these populations and the Romani people living in the Czech Republic as compared with other Caucasian European groups.

1 Petrovic, et al. (2019) (52) published a systematic review of *CYP2C19* and *CYP2D6* allele frequencies from multiple European countries and compared the relative frequencies of alleles. The article provides detailed summaries of the literature but further notes a trend of frequencies of *CYP2D6* gene duplications formed a clear South-East to North-West gradient ranging from <1% in Sweden and Denmark to 6% in Greece and Turkey. The inverse trend was observed for the no-function *CYP2D6*4* and *CYP2D6*5* alleles (higher in the Nordic, north-west countries, lower in the south-east).

DENMARK

1 Lunenberg, et al. (2021) (53) studied 77,684 individuals from Denmark to determine pharmacogenetic genotype and phenotype frequencies in this population. A genotyping array was used to detect variants in pharmacogenes, this included 8 variants in *CYP2D6* as well as deletions (*CYP2D6*5* allele) and duplications at this locus. The frequency of NM was 62.4%, IM was 33.4%, and PM was 4.2%. The authors noted there was an absence of *CYP2D6* gene deletions or duplications in their cohort, thus no UM predicted phenotypes were detected.

NETHERLANDS

1 Poulussen, et al. (2019) (54) reported on the allele and phenotype frequencies of 7 different *CYP2D6* variants and gene duplications among 105 hospitalized individuals from the Netherlands who were being treated with metoprolol. The authors reported the frequency of PM was 11%, IM frequency was 45%, NM frequency was 39% and UM frequency was 5%. These frequencies were based on the Royal Dutch Pharmacist Association genotype-phenotype translation guidelines as of January 2019, before the publication of the above AS and phenotype harmonization report.

LITHUANIA

1 Dlugauskas, et al. (2019) (55) studied 179 individuals from Lithuania for *CYP2D6* polymorphisms. Variants were identified by Sanger sequencing of *CYP2D6* exons 1, 2, 3–4, 5–6, 6–7, and 9; CNVs were detected by MLPA. The authors noted similar allele frequencies to other European populations, with the exception that the *CYP2D6*5* allele was less common in Lithuania. A total of 9 star alleles were detected by sequencing as well as one individual with a duplication of the *CYP2D6*2* allele. The predicted phenotype frequencies among the Lithuanian population are 1.15% for PM, 0.56% for UM, 4.47% for IM and the remainder were predicted NM. (Note: the phenotypes were predicted based on these activity score values: PM 0, IM 0.5, NM 1–2 and UM >2.)

SPAIN

1. Lopez de Frutos, et al. (2020) (56) reported on the frequency of genetic and phenotypic variation at the *CYP2D6* locus in individuals from Spain who were diagnosed with type I Gaucher disease. Out of the 109 individuals enrolled in the study, 87 were predicted to be NM (80%), 14 were predicted to be IM (13%), 6 were predicted to be PM (5%), and only 2 individuals were predicted to be UM (1.8%). These frequencies were compared with previous reports from other Iberian populations, noting a higher incidence of IM phenotype in their report versus previous publications. The study utilized an xTAG assay on the Luminex

platform that detects 19 different variants, most of which were previously associated with known star alleles, as well as duplications and deletions to characterize the metabolizer phenotypes.

2. Barreda-Sanchez, et al. (2019) (57) studied a cohort of 50 individuals in southern Spain with acute intermittent porphyria. They performed targeted genotyping via TaqMan SNP or CNV assays at multiple CYP loci; at the *CYP2D6* locus, they tested for the presence of the *CYP2D6*4* and *CYP2D6*5* alleles. The frequency of the *CYP2D6*4* allele in this small cohort was 12% and the *CYP2D6*5* allele was observed with a frequency of 1% of the cohort. Phenotype predictions were not given for metabolizer status.

ITALY

1 Dagostino, et al. (2018) (58) reported on the utility of *CYP2D6* genotyping for opioid safety in the treatment of chronic back pain among a cohort of 196 Italians in the PainOMICS study. They observed 79.6% of their study participants were predicted to be NM, 16.8% were predicted as either IM or PM, and 3.6% were predicted UM. They examined 10 different star alleles and also tested for duplication of the *CYP2D6*1* and *CYP2D6*2* alleles on the Luminex platform.

SERBIA

1 Skadric and Stojkovic (2020) (59) published a description of multiple cytochrome P450 gene variants among more than 7,000 DNA samples from Serbia. The authors divided the samples into 5 distinct groups based on geography, historic precedent, and ethnically distinguishable populations. These regions are north Serbia, west Serbia, east and south Serbia, central Serbia, and Belgrade. Based on the sample size and number of genes interrogated in this study, only 4 variants were tested at the *CYP2D6* locus. In general, the data from the Serbian population closely followed other European allele frequencies, including 3 out of the 4 variants associated with altered CYP2D6 function. The authors noted that rs28371706 in *CYP2D6* was very rare and thus may not be an informative variant in the Serbian population. This variant is associated with several no-function alleles. Because limited variants were genotyped at the *CYP2D6* locus, accurate star allele calls are not feasible from this dataset.

BOSNIA

1 Nefic (2018) (60) reported on the frequency of gene duplication and the frequency of the c.100C>T variant (commonly associated with the *CYP2D6**10 decreased-function allele, among other known haplotypes) in 151 unrelated, healthy individuals from Bosnia. Gene duplications were observed at a frequency of 2.73%, similar to other Caucasian populations. The variant associated with the decreased-function allele was observed at a higher frequency of 15.56%, more commonly than was reported in other Caucasian groups, but less common than in Asian populations.

CZECH REPUBLIC

1. Dlouhá, et al. (2020) (61) compared the frequencies of variants in multiple CYP enzymes between the Roma (302 individuals) and non-Roma (298 individuals) populations residing in the Czech Republic. Study participants were genotyped for 2 variants in *CYP2D6* and one variant each in *CYP1A2*, *CYP2A6*, and *CYP2B6*. The authors reported a higher frequency of the variant that defines the no-function *CYP2D6*4* allele in the Roma populations as compared with the Czech population (39.2% versus 38.2%). The variant commonly associated with the *CYP2D6*10* allele was observed at a similar frequency in the 2 populations (24%). However, the frequency of the predicted *CYP2D6*10/*10* IM genotype, was higher in the Czech population (9.1%) than the Roma population (6.5%). This study does not account for potential hybrid gene structures or other alleles incorporating the c.100C>T variant that may contribute to an overestimate of homozygosity for this variant.

RUSSIA

The prevalence of the no-function *CYP2D6*4* allele ranges from 17.4–27.1% in the Russian population but is notably lower in the Nanai people group (1.4%), Tatar group (11.5%), and Mari group (8.98%). The predominant predicted phenotype is NM, with IM's present at a frequency of 21.5–33%.

2. Sychev, et al. (2017) (62) compared allele frequencies at several pharmacogenes between Russian and Nanai populations. The study enrolled 70 individuals of the Nanai people group and 642 individuals from the broader Russian population. At the *CYP2D6* locus, they interrogated a variant associated with the no-function *CYP2D6*4* allele. The *CYP2D6*4* allele was more common the Russian population (17.4%) than the Nanai (1.4%), and consequently the authors predicted a higher frequency of NM phenotypes in the Nanai population. (Note: The Nanai population in Russia live in the eastern region of the country, an area that is technically part of the "East Asian" region defined by PharmVar. Because this study compares this ethnic group to the broader Russian population, it is grouped here with other Russian population studies.)

3. Muradian, et al. (2021) (63) studied the effects of *CYP2D6* and *CYP2C9* variants on pain management with tramadol and ketorolac. A total of 107 individuals were genotyped for the *CYP2D6*4* allele. The authors reported 21.5% of the individuals in the study were heterozygous or homozygous for the no-function *CYP2D6*4* allele, resulting in either an IM or PM phenotype, depending upon the functional status of the other allele in heterozygotes.

4. Zastrozhin, et al. (2021) (64) analyzed the effect of the no-function *CYP2D6*4* allele on the efficacy and safety of fluvoxamine in 96 males treated for major depressive disorder. They performed targeted variant genotyping for a defining SNP variant (rs3892097, G>A) and determined that 72.9% of the cohort were homozygous WT and 27.1% were heterozygous (*CYP2D6*4* present for one allele, leading to a possible IM phenotype). No individuals in the study were determined to be homozygotes for the *CYP2D6*4* allele.

5. Ivashchenko, et al. (2021) (65) studied the pharmacogenetics of *CYP2D6*, *CYP3A4/5* and *ABCB1* variants and the efficacy and safety of antipsychotics in adolescents with acute psychotic episodes. A total of 101 individuals were enrolled, aged 10–18, and targeted variant genotyping was performed to detect the *CYP2D6*4, *9,* and **10* alleles. Individuals with at least one decreased or no-function allele were assumed to be IMs, and those with 2 no-function alleles were phenotyped as PMs. Individuals with no variation detected were assumed to be NMs. The authors reported 68/101 individuals were NMs, 33/101 were IMs, and there were no PMs detected in the study cohort.

6. Abdullaev, et al. (2020) (66) examined clinically relevant pharmacogenetics markers in Tatars and Balkars, 2 ethnic groups living in in the Volga and Caucasus regions of Russia. A total of 341 individuals were enrolled in the study. Targeted genotyping at 10 variants was performed, but only the *CYP2D6*4* allele was tested at the *CYP2D6* locus. The authors observed that 77% of the 141 Tatar subjects did not have the *CYP2D6*4* allele and the remaining 23% of individuals were heterozygous or homozygous for the *CYP2D6*4* variant. The *CYP2D6*4* allele frequency in Europeans is similar to the Balkar group, but the Tatar group had a slightly lower allele frequency. The PM phenotype in Europeans is often associated with the *CYP2D6*4* allele.

7. Mirazev, et al. (2020) (67) studied polymorphisms in several pharmacogenes in 845 healthy individuals from the Volga and northern Caucasus regions of Russia. These individuals were from multiple ethnic groups: 238 from the Chuvash ethnic group, 206 Mari, 157 Kabardins and 244 Ossetians. The only allele studied at the *CYP2D6* locus was *CYP2D6*4*. The frequency of heterozygotes for the defining *CYP2D6*4* variant was 22.69% in the Chuvash group, 17.96% in the Mari group, 30.74 in the Ossetians and 32.48% in the Kabardins. The Ossetians and Kabardins' *CYP2D6*4* allele frequencies were most similar to the overall Russian population (15–16% versus 18%), the frequency in the Chuvash and Mari groups was statistically significantly lower. These frequencies suggest the rates of PM and IM due to the presence of

the *CYP2D6*4* allele will be lower in the Chuvash and Mari as compared with the Ossetians, Kabardins, and Russian population at large.

Near Eastern Allele Studies

The prevalence of the decreased-function *CYP2D6*41* allele may be higher in the United Arab Emirates, Saudi Arabia, and Turkey than other populations. The frequency of NM phenotype ranges between 54–82%.

1 Khalaj, et al. (2019) (68) reported on the distribution of various *CYP2D6* alleles across the Middle East. A total of 32 studies were reviewed, with the total number of individuals in each study ranging from 43–552. Overall, the most common allele was the *CYP2D6*1*, normal-function allele at 68% average frequency, though this may be overestimated due to default assignment when no variants are detected. The *CYP2D6*3*, **4*, and **5* no-function alleles combined for an average frequency of 37.5%. As one might expect, the NM phenotype was most common in every country reported, ranging from a peak of ~82% in Saudi Arabia to 54% in Egypt. The UM phenotype was most commonly seen in Saudi Arabia (20%), Syria (15%), Jordan (14%), and Emirates (13%). Individuals with PM phenotype were most frequently observed in Egypt (19%) and Iran (9%) with other countries having PM frequencies fewer than 5%.

SAUDIA ARABIA

1 Almeman (2020) (69) reviewed CYP450 gene polymorphism in Saudi individuals from 10 different studies in this population. The number of individuals in each study ranged between 90 and 200, though not all reviewed studies examined *CYP2D6* function or genotype. Overall, the author summarizes the findings of these studies and notes there are rare PM individuals and associated no-function allele frequencies were also low (with some alleles notably absent). Gene duplication at the *CYP2D6* locus was noted to be frequent in the Saudi population. The author reported the frequency of the *CYP2D6*41* decreased-function allele within the Saudi population was higher than in other populations, such as Chinese, Mexican, Caucasian and Ghanaians. Overall, the Saudi population demonstrates similar allele frequencies for the reported loci as other Middle Eastern populations.

TURKEY

1 Arici & Ozhan (2016) (70) reported on multiple CYP gene profiles and susceptibility to drug response in a Turkish population of 160 individuals. This study examined the frequency of variation leading to the decreased-function *CYP2D6*9* and **41* alleles within the *CYP2D6* locus. They observed a minor-allele frequency of 4% for the *CYP2D6*9* allele (a 3-nucleotide deletion) and 15% for the *CYP2D6*41* allele. Thus, the **9* decreased-function allele was more prevalent in Turkish individuals than in European or Asian populations. Similarly, the prevalence of the **41* allele was higher in the Turkish study cohort as compared with Caucasian or Chinese populations.

EGYPT

2 Mutawi, et al. (2021) (71) reported on a genotyping study of 145 healthy Egyptian individuals with the goal of elucidating the frequency or major allelic variants at the *CYP2D6* locus. From the variant genotyping data of 5 *CYP2D6* alleles and CNVs (detected with CNV TaqMan assay), the authors concluded the NM phenotype was the most common among their cohort, with a frequency of 67.6%. They did not identify any study participants with 2 no-function alleles suggesting that PM phenotype in Egypt is rare. Gene duplications, however, were observed and the authors predict a frequency of UMs of 4.8% in this Egyptian cohort.

Sub-Saharan Africa Allele Studies

The IM phenotype has been reported as the most common phenotype in Kenya and Madagascar. The *CYP2D6*17* and *CYP2D6*29* decreased-function alleles both have frequency greater than 10) in multiple regions of Africa (Zimbabwe, Kenya, Ethiopia, and Madagascar for *CYP2D6*17*; Tanzania, Kenya for *CYP2D6*29*). Multiple studies report a very low frequency (<2%) of PMs.

1 Rajman, et al. (2017) (72) performed a literature-based review (80 articles total) of cytochrome P450 variants across the continent of Africa. While results from multiple genes are presented, the *CYP2D6* alleles discussed specifically are **3, *4, *9, *10, *17,* and **29*. The no-function *CYP2D6*3* and *CYP2D6*4* alleles were most common the San population from Zimbabwe (9% allele frequency). The decreased-function *CYP2D6*17* and *CYP2D6*29* alleles were notably higher in certain populations; *CYP2D6*17* was seen at a frequency of 34% in Zimbabwe (Shona), and *CYP2D6*29* was observed in 20% of the alleles in Tanzania. The *CYP2D6*9* decreased-function allele was not present in any population reviewed, while the *CYP2D6*10* decreased-function allele was most common in the South African Venda population (19%), but not present in multiple other populations. The other decreased-function allele, *CYP2D6*29*, was present in nearly 30% of the Igbo (Nigeria) population, but only 2% of the San population.

ETHIOPIA

1 Ahmed, et al. (2019) (73) studied the genotype and predicted phenotype of female individuals being treated for breast cancer with tamoxifen in Ethiopia. The authors reported the frequency of 5 specific star alleles detected via variant genotyping and the duplication of the *CYP2D6*1* and *CYP2D6*2* alleles, detected via TaqMan CNV assays. Among the 181 participants, 22.2% were predicted to be UM, roughly 60% were NM, approximately 16% had activity scores resulting in a current predicted phenotype of IM, and 1.2% were PM. The authors reported differences in tamoxifen metabolites among individuals with the same metabolizer phenotype but distinct diplotypes (for example, *CYP2D6*1/*1* individuals had different endoxifen levels as compared with *CYP2D6*2/*2* genotyped individuals).

KENYA

1 Rico, et al. (2020) (74) reported on the *CYP2D6* genotype frequencies and functional characterization of novel variants found in the Ni-Vanuatu (Melanesia/Polynesia) and localized Kenyan populations. The 278 Ni-Vanuatu study participants were residents of 6 different islands from Melanesia or Polynesia. Within Kenya, the authors enrolled 195 individuals residing on islands or the shore of Lake Victoria in western Kenya. All study participants were healthy and unrelated. Eight variant star alleles were identified by PCR-based sequencing, allele fusions and duplications were also detected along with 6 novel variants. Detailed allele frequencies are presented in tables 1 and 2 within the publication. Among the Kenyan population, 34.4% of the individuals were predicted to be IM, 1% UM, and 0.5% PM.

MADAGASCAR

1 Mehlotra, et al. (2021) (75) conducted a study of 211 individuals from 2 health regions in Madagascar to determine the frequency of CYP2D6 metabolizer phenotypes associated with primaquine therapy failure for *Plasmodium vivax* (*P. vivax)*-caused malaria. A total of 29 variants were tested, allowing for identification of 27 distinct star alleles. Duplications, deletions, and complex gene arrangements (hybrids or tandem genes) were detected by previously published multiplex methods. The authors predicted 51.2% of individuals in the study population were IM. The frequency of UM phenotype in the study population was 4.88% and NM phenotype comprised 43.9% of the study population. No PM genotypes were identified in the study population, but the predicted population-wide PM phenotype rate was 0.32%.

East Asian Allele Studies

The most common altered-function allele in this region of the world is the *CYP2D6*10* allele, with frequencies near 40–60% in most countries. Notably, Japan has a slightly lower frequency of the *CYP2D6*10* allele (36%). The IM phenotype is significantly enriched in populations from to this region. The no-function *CYP2D6*5* allele (gene deletion) is rare, observed at a rate of 9% or less.

2 Dorji, et al. (2019) (76) performed a systematic literature review of 86 studies of CYP gene allele frequencies in South-East and East Asian (SEEA) populations. Multiple tables in the original publication delineate allele frequencies for the specific CYP loci by specific population. In total, 8 variant *CYP2D6* star alleles were commonly reported in the reviewed articles. Overall, the authors report that no-function alleles (namely *CYP2D6*3*, **4*, and **6*) are exceedingly rare (or absent) in Asian populations, whereas the *CYP2D6*5* no-function allele is present at rates similar to other populations. The decreased-function *CYP2D6*10* allele is present at a rate of up 50% in many SEEA populations, making that allele the major contributor to the IM phenotype in Asians. Specifically, the *CYP2D6*5/*10* diplotype may be of clinical significance to individuals of Asian descent, given the prevalence of these alleles. The normal-function alleles that were most commonly seen were *CYP2D6*1* and *CYP2D6*2*. Duplication of the *CYP2D6*1* and *CYP2D6*2* (or even **10* alleles) are rarely seen among SEEA populations, leading to a very low frequency of the UM phenotype.

SINGAPORE

1. Goh, et al. (2017) (77) reported on an analysis of CYP450 genes and allele frequencies among residents of Singapore. At the *CYP2D6* locus, 12 different variants were examined for a total of 10 star alleles. A total of 506 individuals were enrolled: 126 Malays, 179 Indians, and 201 Chinese. Overall, the authors report that NM phenotype was most common, followed by IM. The frequency of PMs ranged from 0.7–3.4% but was not observed in any of the Chinese subjects. In fact, the highest frequency of UM phenotype was observed in Chinese study participants (11%), followed by Indian (5%), and Malay (4.8%).
2. Bakar (2021) (38) reviewed pharmacogenetic variation of common alleles, making a comparison between Singaporean/Malaysian populations and European populations. Special emphasis was given to the decreased-function alleles *CYP2D6*4* and **10*, as described in 3 of the reviewed studies. As in Chinese populations, the *CYP2D6*10* allele is more common in Singapore and Malaysia than in European populations. The author reports that individuals from Asia who are homozygous for the *CYP2D6*10* allele are more likely to have altered tamoxifen pharmacogenetics and have higher odds of developing metastatic cancer.

KOREA

1. Byeon, et al. (2018) (78) studied the frequency of 4 *CYP2D6* alleles and gene duplications in 3,417 individuals from Korea and compared their results to published frequencies from east Asian, Caucasian, and African populations. The authors report the *CYP2D6*10* decreased-function allele was most prevalent at 46.2%, more common than the wild-type *CYP2D6*1* allele (34.6% allele frequency). Among studies of east Asian populations, the *CYP2D6*10* allele was observed with a frequency between 38–53%, notably higher than the 1.4% reported for Caucasians. The no-function *CYP2D6*4* allele was also noted to be far less frequent in east Asians than Caucasians or Africans. Overall, the Korean population showed an NM phenotype in roughly 22% and only 7 out of the 3,417 (0.2%) individuals had a predicted PM phenotype.
2. Ryu, et al. (2017) (79) reported on the effects of *CYP2C19* and *CYP2D6* on individual responses to amitriptyline in healthy Koreans. A total of 53 volunteers enrolled in the study and were genotyped for the *CYP2D6*10* and **5* alleles. The authors reported 12 individuals (22.6%) had 2 normal-function alleles, indicative of NM phenotype. There were 17 individuals with genotype combinations including one

function allele, leading to IM phenotype (32%). The authors reported 24 additional individuals with decreased function diplotype associated with IM phenotype (45%). Thus, most study participants would be predicted to be IM.

3. Han, et al. (2021) (80) reported on variation of multiple pharmacogenes among the Korean Genome and Epidemiology Study (KoGES) cohort, a total of 69,027 individuals were genotyped via SNP array and copy number variation (CNV) data was available from 947 individuals (614 individuals were both genotyped and tested for CNVs). Three variants at the *CYP2D6* locus were reported. Variation at the *CYP2D6* locus was notable for the SNP rs1065852, also called c.100C>T, which is associated with the decreased-function allele *CYP2D6*10* (among other alleles). The frequency of the variant was 48.23% of all alleles genotyped. Variation at rs16947 (G>A) was observed in 25% of the alleles; this variant is associated with multiple *CYP2D6* star alleles. Variation at rs1135840 (G>C) was observed in 46.3% of the tested alleles; this variation is associated with decreased and no-function star alleles. Based on the nature of the study and how the genomic data was obtained, there was no data regarding CNVs for the *CYP2D6* locus, nor could specific star allele haplotypes be assigned to this cohort.

HONG KONG

1 Chan, et al. (2018) (81) reported on the allele and phenotype frequencies from 800 individuals residing in Hong Kong who self-reported their ethnicity. The authors tested for 12 different star alleles via targeted variant genotyping. The vast majority identified as Asian descent, with the other members of the cohort either identifying as Caucasian or "mixed race." Among the individuals of Asian descent, the UM phenotype was seen at a rate of 3.3%, NM at 49.9%, IM at 46.4%, and PM at only 0.4%. The frequencies of the specific alleles within the Asian study participants showed a frequency of 32% for the *CYP2D6**36-**10* fusion allele (characterized by a tandem arrangement of the no-function *CYP2D6*36* allele and the decreased-function *CYP2D6*10* allele, see PharmVar's page on structural variants for additional information).

CHINA

1. Liu, et al. (2020) (82) reported on the genetic variation in a cohort of 105 individuals from the Zhuang people group of southern China. Overall, among several "very important pharmacogenes" (VIPs), the genetic variation of the Zhuang population was notably similar to Han Chinese in Beijing, southern Han Chinese, and the Japanese population in Tokyo, Japan. Two variants in *CYP2D6* were interrogated. The SNP rs1065852 in the *CYP2D6* locus was a notable deviation between the Zhuang and other global people groups, including some Asian populations. The data suggests this variant may be present at a higher frequency in this subpopulation. This variant has been associated with the decreased-function *CYP2D6*10* allele and the authors mention that variation at this position has been previously associated with altered responses to multiple drugs.
2. Qi, et al. (2019) (83) reported on the frequency of specific *CYP2D6* alleles in the Chinese Millinome WGS database. The database comprises data from 141,431 individuals from 31 different provinces in China. Note: the genotyping data is obtained from WGS, and as such there may be inaccuracies in calling of the specific haplotype frequencies. The frequency of the *CYP2D6**2, normal-function allele was reported as 27% and the *CYP2D6*10* decreased-function allele was present at 68%. Because the genotype calls were determined by WGS, the study examined known variants associated with star alleles as well as identified novel genomic variants with potential functional impact. The identified variants in *CYP2D6* were not listed in full, but no novel variants were reported.
3. Huang, et al. (2021) (84) studied *CYP2D6* genotype in 120 individuals living in the Yunnan province, China to identify correlation between genotype and malarial *P. vivax* infection relapse following chloroquine and primaquine therapy. The authors interrogated 12 variants at the *CYP2D6* locus and 5 known star alleles. They observed the most common allele was the decreased-function *CYP2D6*10* allele,

present in 45.4% of all alleles tested. Additionally, 60 individuals carried at least one copy of the *CYP2D6*10* allele, suggesting the IM phenotype was present in 50% of the study population.

4. Lu, et al. (2021) (85) reported on *CYP2D6* genotype in 76 individuals prescribed risperidone who were seen at a hospital in the Jiangsu province, China. Five common variants were tested, translating into 3 variant star alleles. The authors observed the *CYP2D6*10*, decreased-function allele was present in 81.6% of the individuals in the study. The authors reported that the *CYP2D6*65* allele was present in 17.1% of the individuals in the study and the *CYP2D6*2* allele was present in just 9.2% of the individuals. The functional classification of the *CYP2D6*65* allele is uncertain (as reported by the authors and per CPIC (3)).

JAPAN

1 Kisoi, et al. (2020) (86) reported on a method for genotyping variants and CNVs (CNVs) in a cohort of 216 healthy females from Japan. The specific star alleles studied were *CYP2D6*2, *5, *10, *14*, and *41*. Similar to other studies from Asia, the most commonly observed variant allele in this cohort was the *CYP2D6*10* decreased-function allele (36.3%). Diplotype combinations including the *CYP2D6*10* allele, likely leading to an IM phenotype, were observed in over 60% of the study cohort. The NM-associated diplotype of 2 WT (*CYP2D6*1*) alleles was observed in 16.2% of the cohort.

VIETNAM

1 Nguyen, et al. (2019) (87) studied the frequency of single nucleotide variants and structural variations in a cohort of 136 Kinh Vietnamese unrelated individuals by Sanger sequencing the coding region and targeted copy number analysis of *CYP2D6*. The authors reported 7 novel variants in the sequencing data as well as 23 known variants. The normal-function alleles (*CYP2D6*1* and *2) comprised less than 30% of the alleles identified in this study. The decreased-function *CYP2D6*10* allele had a frequency of approximately 44%, the most common allele in the study. Consequently, the 3 most common diplotypes all involved the *CYP2D6*10* allele, resulting in at least 50% of the cohort having allele combinations that would predict an IM phenotype. No individuals with increased copy number were detected in this cohort, suggesting an extreme rarity for the UM phenotype in this population.

THAILAND

1. Puaprasert, et al. (2018) (88) reported on targeted allele genotyping of 6 star alleles the *CYP2D6* locus among members of the Karen population living in Tak, a western province of Thailand. The authors reported a high frequency of the decreased-function *CYP2D6*10* allele (40%), with the next most common variant allele being the normal-function *CYP2D6*2* allele (33%). However, only one variant (c.100C>T) was used to define the *CYP2D6*10* allele, as this frequency may be indicative of multiple haplotypes. Duplication (1%) and deletion (*CYP2D6*5* allele, 3%) alleles were relatively rare in this study cohort. Based on the high prevalence of the decreased-function allele, the authors advise the efficacy of the malaria medication primaquine may be affected in this population.
2. Mauleekoonphairoj, et al. (2020) (89) performed WGS on 291 individuals of self-reported Thai ethnic origin. While several pharmacogenes were studied, the *CYP2D6* sequencing revealed 20 distinct star alleles in the study cohort. There were 5 duplications (1.9% of the identified alleles), 1 deletion (*CYP2D6*5* allele, 4.5% of the alleles), and 6 rearrangements (34.7% of the alleles). The most common decreased-function alleles in the cohort were the *CYP2D6*36+*10* fusion and the *CYP2D6*10* allele. Overall, 25% of the Thai cohort was found to have a "high risk" diplotype for at least one of the studied pharmacogenes. This study utilized WGS and Stargazer analysis to impute haplotypes and diplotypes.

CAMBODIA

1 Spring, et al. (2020) (90) reported the prevalence of 18 different *CYP2D6* star alleles as well as gene duplications and reported the predicted phenotypes among 96 Cambodians at high risk for Malaria. Genotyping was performed using the Luminex xTAG kit, which detects specific variants and identifies copy number variants. The overall phenotype frequencies were 46% for NM, 52% for IM, and 1% for PM. The authors also provide a table comparing the specific allele frequencies in their study and other publications focused on the greater Mekong subregion.

Central/South Asian Allele Studies

The phenotype frequencies in India range from 1–3% for PMs, IMs were observed in 7.3%, and NMs were most common at 91.7%.

INDIA

1. Manoharan, et al. (2019) (91) published a study on the allele frequency of *CYP2D6* variants in South India. The study cohort was comprised of 105 individuals with mild to moderate depression but no other noted medical conditions. Targeted sequencing of the *CYP2D6* locus led to the identification of 18 distinct star alleles, and the *CYP2D6*1* allele was assigned in the absence of variants of interest. Based on the predicted activity scores, the NM phenotype was most common at 91.7%, with IM frequency of 7.3%, and the PM phenotype was present in 1% of their cohort. The authors noted that the *CYP2D6 *41* allele was the most common decreased-function allele in their cohort and reported on 4 novel missense variants that have been integrated into the star allele nomenclature. The *CYP2D6*1* and **2* normal-function alleles were most common, with a combined frequency of approximately 70%.
2. Dhuya, et al. (2020) (92) tested 97 individuals to assess their metabolism of dextromethorphan as a measure of CYP2D6 metabolizer status. In contrast to other recent publications, genotyping was not performed. Their data suggests 3/97 (~3%) individuals to be PM's and the rest (94/97, ~97%) were NMs. The authors conclude the prevalence of *CYP2D6* polymorphisms is similar in the region of east India to other regions in the country, with overall a low frequency of PMs.

PAKISTAN

1. Ahmed, et al. (2018) (93) reported on the frequency of variation in multiple pharmacogenes among 244 healthy individuals in an indigenous Pakistani population. Fifteen variants were genotyped via targeted sequencing and the frequencies were compared with published frequencies of these variants in other populations. The SNP rs16947, which defines the *CYP2D6*41* allele but has been found in haplotypes for normal, decreased and no-function *CYP2D6* alleles was observed in nearly 40% of the individuals in the study, but was slightly higher in the Azad Kashmir region. This specific variant genotype distribution varies notably from east Asians, admixed Americans, most Africans and Europeans, but was similar to other south Asians. Another SNP, rs1135840, was present at a lower rate (35.2%) in the Pakistani population compared with all other populations. This variant has also been associated with haplotypes of varying functional activity, though the authors conclude in this study that both this and the rs16947 SNP are responsible for a UM phenotype. The combination of these 2 variants defines the *CYP2D6*2* normal-function allele. The authors did not interrogate CNVs, thus the presumption of a UM phenotype is dubious.
2. Ullah, et al. (2020) (94) reported the frequency of the no-function *CYP2D6*4* allele in 16 different ethnic groups from Pakistan. Over 900 individuals were enrolled in the study. The frequency of the *CYP2D6*4* allele in these groups was highest at 13.64% in the Meo population, and lowest in the Kalash population at 3.73%. The frequency of homozygotes for the *CYP2D6*4* allele, with a predicted PM phenotype, was highest in the Sindhi group (4.49%) and lowest in the Pathan group (0.83%).

BHUTAN

1 Dorji, et al. (2019) (95) reported a study of CYP allele frequencies in a Bhutanese population cohort. Specifically, the authors studied the *CYP2D6*10* and presumed *CYP2D6*1* allele frequencies via targeted analysis in 443 individuals. The *CYP2D6*10* allele was observed at frequency of 21%, lower than other Asian populations (see Dorji et al., 2019 (64)) but more commonly than Caucasians and Africans. The genotyping method (PCR with restriction fragment polymorphism) may not accurately differentiate between the *CYP2D6*10* allele and alleles with similar variants. The frequency of the *CYP2D6*1* allele is overestimated here, due to limited testing for *CYP2D6* variants.

Oceanian Allele Studies

Australian studies reported a frequency for UMs in this population to be around 2%, NMs were predicted in slightly more than 50% of the population, IM frequencies were more discordant and ranged between 11–30%. The frequency of PMs ranged between 5.7–19.7%. The no-function **4* allele was seen, on average, at a frequency of 20%.

AUSTRALIA

1. Chen, et al. (2019) (96) studied *CYP2D6* allele frequency among members of the Australian Defense Forces personnel who were deployed to regions with endemic malaria and were subsequently treated for *P. vivax* infection with primaquine. While the primary purpose was to examine whether *CYP2D6* genotype correlated with malarial relapse, the authors reported the prevalence of 14 star alleles and duplication of the *CYP2D6*1* allele within the 157 individuals enrolled in the study. The most common allele was the normal-function *CYP2D6*1* allele (~32%), but the *CYP2D6*4* no-function allele was also relatively common (24.5%). The authors found roughly 1.3% of the study participants were predicted to have a UM phenotype, 50.9% had an NM-associated diplotype, approximately 29% had an IM-associated diplotype, 19.7% had a PM-predicting diplotype and one individual had 2 novel, unknown function alleles.
2. Mostafa, et al. (2019) (97) analyzed genotype and phenotype frequencies in 5,408 Australian individuals at several pharmacogene loci. Fifteen variant star alleles were studied via Sanger sequencing and CNV analysis was based on published methods (14). The *CYP2D6*1* allele was assigned in the absence of detected variants. This large cohort was ethnically diverse and subsequent analysis of study participants with medication histories enabled further analysis of the frequency of phenoconversion in a subset of the cohort. The most common altered-function allele was *CYP2D6*4*, at 17.8%. Genotyping results led to the predicted prevalence of UMs at 2.8%, NMs at 53.2%, IM at 11.4% (with an additional 26.2% of "low normal metabolizer" diplotypes that would be reclassified as IM based on the current CPIC definition), PMs were observed at 5.7% and one individual could not be genotyped. When considering medications that may alter the predicted metabolizer phenotype, the frequency of CYP2D6 PMs increased from 5.4% in the sub-cohort to 24.7%, all the result of other metabolizer phenotype groups being decreased in predicted enzyme activity levels.

MELANESIA/POLYNESIA

3 Rico, et al. (2020) (74) reported on the *CYP2D6* genotype frequencies and functional characterization of novel variants found in the Ni-Vanuatu (Melanesia/Polynesia) and localized Kenyan populations. The 278 Ni-Vanuatu study participants were residents of 6 different islands from Melanesia or Polynesia. Within Kenya, the authors enrolled 195 individuals residing on islands or the shore of Lake Victoria in western Kenya. All study participants were healthy and unrelated. Eight variant star alleles were identified by PCR-based sequencing, allele fusions and duplications were also detected along with 6 novel variants. Detailed allele frequencies are presented in tables 1 and 2 within the publication. Overall, the phenotype frequencies in the Ni-Vanuatu population were predominantly NM, with only 5.8% as predicted IM, 5% UM, and 0% PM.

Genetic Variation Frequency Resources

At the National Library of Medicine at the National Institutes of Health, the National Center for Biotechnology Information (NCBI) has several resources for learning more about genetic variation. These resources do not focus on established star allele haplotypes but are typically limited to the data available for single SNPs or other short variants. The dbSNP database is a repository of known "short" genetic variants, ranging from single base changes to small deletions or insertions and microsatellite repeats. In the nomenclature table below, the dbSNP-assigned identifiers are provided as a link to the entry for that specific variant. Within dbSNP, users can access allele frequency data from the Allele Frequency Aggregator (ALFA) project, explore the clinical significance data stored in ClinVar and access literature citations from PubMed for the variant of interest. Specific dbSNP identifiers, star alleles, and gene names can also be searched in the LitVar database. When available, dbSNP pages also display frequency data from other studies including the 1000Genomes project, the Exome Aggregation Consortium (ExAC), the genome Aggregation Database (gnomAD), and country-specific studies that have been submitted to the BioProject database at NCBI. Additionally, the data from TOPMed are available at the NHLBI website. These individual variant frequencies do not inherently translate to star allele frequencies.

Genetic variation data from the *CYP2D6* locus is also available in databases such as Ensembl and gnomAD, though these data do not specifically address pharmacogenetics.

Other resources specific to pharmacogenomics data include PharmVar, PharmGKB, and CPIC. The Allele Frequency Table maintained by CPIC and available from PharmGKB, is an excellent resource for star allele frequency data. These tables are periodically updated by these pharmacogenomic expert communities based on peer-reviewed scientific publications. Furthermore, the PharmVar Consortium provides data on the known star alleles for multiple pharmacogenes. Data on *CYP2D6* includes the key variants that define each star allele, the assigned CPIC clinical function category, citations for the initial reports of the specific alleles, and a detailed description of the more complex structural variants at the *CYP2D6* locus. The PharmGKB database provides a wide range of data and resources, including specific variant annotations, clinical annotations, and drug label annotations for the *CYP2D6* gene, as well as multiple pharmacogenes. It should be noted that PharmGKB is a resource for drug-specific data, as well. The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an authoritative source for clinical recommendations based on drug-gene interactions. The CPIC page for *CYP2D6* guidelines links to their specific guidelines and each guideline further provides information on allele definitions, functionality, frequency data and diplotype-phenotype translation tables. It should be noted that PharmVar, PharmGKB, and CPIC coordinate much of their data and cooperatively update their shared resources.

Resources from additional publications described in detail above include:

REFARGEN (Brazil)

Helix DNA project (USA)

TOPMed (USA; NHLBI, NIH)

Nomenclature for Selected *CYP2D6* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*2*	2851C>T (Arg296Cys)	NM_000106.6:c.457G>C	NP_000097.3:p.Arg296Cys	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*3*	2550delA (Arg259fs)	NM_000106.6:c.775del	NP_000097.3:p.Arg259fs	rs35742686

Table continued from previous page.

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*CYP2D6*4*	1846G>A	NM_000106.6:c.506-1G>A	Variant occurs in a non-coding region (splice variant causes a frameshift)	rs3892097
*CYP2D6*5*	Gene deletion			
*CYP2D6*6*	1707 del T	NM_000106.6:c.454delT	NP_000097.3:p.Trp152Glyfs	rs5030655
*CYP2D6*10*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*17*	1023C>T[1] (Thr107Ile)	NM_000106.6:c.320C>T	NP_000097.3:p.Thr107Ile	rs28371706
	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*27*	3854G>A (Glu410Lys)	NM_000106.6:c.1228G>A	NP_000097.3:p.Glu410Lys	rs769157652
*CYP2D6*31*	2851C>T (Arg296Cys)	NM_000106.6:c.886C>T	NP_000097.3:p.Arg296Cys	rs16947
	4043G>A (Arg440His)	NM_000106.6:c.1319G>A	NP_000097.3:p.Arg440His	rs267608319
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*36* [3]	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	4129C>G (Pro469Ala)	NM_000106.6:c.1405C>G	NP_000097.3:p.Pro469Ala	rs1135833
	4132A>G (Thr470Ala)	NM_000106.6:c.1408A>G	NP_000097.3:p.Thr470Ala	rs1135835
	4156C>T+4157A>C (His478Ser)	NM_000106.6:c.1432C>T + NM_000106.6:c.1433A>C	NP_000097.3:p.His47Ser	rs28371735 + rs766507177
	4159G>C (Gly479Arg)	NM_000106.6:c.1435G>C	NP_00097.3:p.Gly479Arg	
	4165T>G (Phe481Val)	NM_000106.6:c.1441T>G	NP_00097.3:p.Phe481Val	
	4168G>A+4169C>G (Ala482Ser)	NM_000106.6:c.1444G>A + NM_000106.6:c.1445C>G	NP_000097.3:p.Ala482Ser	rs74478221 + rs75467367
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*41*	2851C>T[2] (Cys296Arg)	NM_000106.6:c.886T>C	NP_000097.3:p.Cys296Arg	rs16947
	2988G>A	NM_000106.6:c.985+39G>A	Variant occurs in a non-coding region (impacts slicing).	rs28371725
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840
*CYP2D6*49*	100C>T (Pro34Ser)	NM_000106.6:c.100C>T	NP_000097.3:p.Pro34Ser	rs1065852
	1612T>A (Phe120Ile)	NM_00106.6:c.358T>A	NP_000097.3:p.Phe120Ile	rs1135822
	4181G>C (Ser486Thr)	NM_000106.6:c.1457G>C	NP_000097.3:p.Ser486Thr	rs1135840

Allele definitions are maintained by the Pharmacogene Variation (PharmVar) Consortium. If there is a discrepancy between this table and information from PharmVar, the authors defer to PharmVar's authority.

[1] In the literature, 1023C>T is also referred to as 1111C>T

[2] In the literature, 2851C>T is also referred to as 2938C>T

[3] *CYP2D6*36* is a gene conversion with *CYP2D7;* variants provided here are from PharmVar.

Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting (98).

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS).

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