Supplement to:

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *CYP2D6* and Tamoxifen Therapy

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GUIDELINE UPDATES

The Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *CYP2D6* Genotype and Tamoxifen Therapy is published in full on the CPIC website (1). Relevant information will be reviewed periodically and updated guidelines published online.

LITERATURE REVIEW

We searched the PubMed® database (1966 to February 2017) for the following keyword searches: 1) tamoxifen or endoxifen or n-desmethyl tamoxifen or 4-hydroxy tamoxifen AND CYP2D6 and 2) CYP2D6 OR endoxifen AND breast. Using these search terms, 631 publications were identified. In addition, studies annotated in PharmGKB (http://www.pharmgkb.org) were identified. Study inclusion criteria included publications that incorporated analyses for the association between *CYP2D6* genotypes and metabolism of tamoxifen or tamoxifen-related clinical outcomes (i.e. breast cancer-specific survival, event-free or recurrence-free survival, distant disease-free survival, overall survival, and recurrence). Non-English manuscripts were excluded. Tamoxifen dose escalation studies were not included. For studies with overlapping cohorts, the appropriate studies were identified through discussion with the study authors. Following application of these inclusion criteria, 40 publications were reviewed and included in the evidence table (**Supplemental Table S2**). Studies that evaluated only one *CYP2D6* allele (e.g. **4*) were excluded based on Schroth *et al* (2) demonstrating that *CYP2D6*4* genotyping alone is inconclusive for predicting CYP2D6 phenotype. Based on these findings, the several studies were excluded (2-12). In addition, if a study performed comparisons to a single allele only (e.g., *CYP2D6*4* vs all genotypes) these were also excluded regardless of how many alleles were genotyped (13-16).

The *CYP2D6* allele frequency table (**CYP2D6 frequency table (1, 17, 18))** is an update of the tables previously published in CPIC guidelines (19-21). Updates to the *CYP2D6* allele frequency tables were made by searching the PubMed® database (1995 to August 2017). The following criteria were used for *CYP2D6*: (CYP2D6 or 2D6 or cytochrome P4502D6) AND (genotype OR allele OR frequency OR minor allele OR variant OR ethnic OR race OR racial OR ethnicity) with filter limits set to retrieve "full-text" and "English" literature. In addition, reports were also identified from citations by others or review articles. Studies were considered for inclusion in the *CYP2D6* frequency table if: (1) the ethnicity of the population was clearly indicated, (2) either

allele frequencies or genotype frequencies were reported, (3) the method by which the genes were genotyped was indicated, (4) the sample population consisted of at least 50 individuals with a few exceptions (e.g., smaller cohorts that were part of larger studies) and (5) the study represented an original publication (no reviews or meta-analyses).

GENE: *CYP2D6*

Genetic Test Interpretation

CYP2D6 genetic variants are typically reported as haplotypes, which are defined by a specific combination of single nucleotide polymorphisms (SNPs) and/or other sequence variants including insertions and deletions that are interrogated during genotyping analysis. *CYP2D6* haplotypes are assigned a star-allele (*) nomenclature to allow for the standardization of genetic polymorphism annotation (22). A complete list of *CYP2D6* star-allele nomenclature along with the genetic variants that define each star-allele is available at https://www.pharmvar.org/. Information regarding *CYP2D6* haplotypes (star-alleles) is also available at PharmGKB (***CYP2D6* Allele Definition Table (1, 18))**. Knowing which SNPs or other genetic variants a particular test interrogates is important as the inclusion or exclusion of certain genetic variants in a pharmacogenetic test could affect the reported star-allele result.

Reference laboratories usually report a diplotype, which is the summary of inherited maternal and paternal star-alleles (e.g. *CYP2D6*1/*10*, where an individual inherited a **1* allele and a **10* allele). Commonly reported *CYP2D6* star-alleles are categorized into functional groups (e.g., normal function, decreased function, or no function) based on the predicted activity of the encoded enzyme (***CYP2D6* Allele Definition Table (1, 18)**). The predicted phenotype (**Table 1, main manuscript**) is influenced by the expected function of each reported allele in the diplotype. A CYP2D6 gentotype to phenotype translation table have been developed by CPIC and are updated on an ongoing basis on the CPIC website (1).

Calculating CYP2D6 Activity Score. Gaedigk *et al*. developed a scoring system to provide a uniform approach to assigning a predicted CYP2D6 phenotype (23). *CYP2D6* alleles are assigned an activity value as detailed in **Supplemental Table S1.** The activity value of each allele reported in the diplotype is added together to calculate the CYP2D6 activity score. For example, to calculate the activity score of a *CYP2D6*1/*17* diplotype, the activity value of **1*

(activity value = 1) and the activity value of **17* (activity value = 0.5) are totaled to provide the CYP2D6 activity score of 1.5. Note that a value of 0.5 indicates decreased activity and not that the activity conveyed by an allele is half of that encoded by a normal function allele. For this guideline, the CYP2D6 activity score is used to assign a predicted phenotype as follows: activity score of 0 = poor metabolizer, activity score of 0.5 = intermediate metabolizer, activity scores ranging from 1.0-2.0 = normal metabolizer, and activity score greater than 2.0 = ultrarapid metabolizer. Therefore, a pharmacogenetic test result of *CYP2D6*1/*17* would result in a CYP2D6 activity score of 1.5 and a predicted phenotype of normal metabolizer.

There is a lack of consensus in regards to whether patients with a CYP2D6 activity score of 1.0 should be assigned a normal or intermediate phenotype (20). Pharmacokinetic data suggest that patients with an activity score of 1.0 have a higher CYP2D6 metabolic capacity compared to patients with an activity score of 0.5, but less CYP2D6 enzyme activity compared to patients with an activity score of 2.0 (23). However, the activity score of 1.0 has less activity towards tamoxifen compared to those with an AS of 1.5 or 2.0 and patients with an activity score of 1.0 may be classified as IMs by some reference laboratories. Thus, for this guideline, an activity score of 1.0 is classified as a CYP2D6 normal metabolizer or intermediate metabolizer, (**Table 1**). This is in contrast to the classification used in previous guidelines (19, 21). A group of *CYP2D6* experts are currently working to standardize the *CYP2D6* genotype to phenotype translation system. Note that genotypes with an activity score of 1 are classified as NMs in the ***CYP2D6* Genotype to Phenotype Table** (1, 18) and CPIC will update the CPIC website and this table accordingly when the CYP2D6 genotype to phenotype standardization is complete (1).

CYP2D6 Structural and Gene Copy Number Variants. Because *CYP2D6* is subject to copy number variation (gene duplications, multiplications, or deletions), clinical laboratories may report gene copy number if directly tested. Most patients will have a normal copy number of 2, with one gene copy inherited maternally and one gene copy inherited paternally. When two *CYP2D6* gene copies are present, the diplotype may be reported as follows: *CYP2D6*1/*1* or *CYP2D6 (*1/*1)2N*, where "2" represents the gene copy number. A copy number of "1" indicates the presence of a *CYP2D6* gene deletion (the patient possesses only one gene copy), and a copy number of "0" indicates both *CYP2D6* genes are deleted. *CYP2D6* gene deletions are indicated by the *CYP2D6*5* allele. A gene deletion that is present on one chromosome may be

reported as follows: *CYP2D6*2/*5* or *CYP2D6 (*2/*2)1N*, where "1" represents gene copy number and the *CYP2D6*5* allele is inferred. Typically, clinical laboratories will report a homozygous gene deletion as *CYP2D6*5/*5* or *CYP2D6 (*5/*5)0N*.

A copy number greater than two indicates the presence of a *CYP2D6* gene duplication or multiplication. When a *CYP2D6* gene duplication is present, the diplotype may be reported as *CYP2D6 (*1/*2)3N*, where "3" represents gene copy number. A clinical laboratory may not report an exact copy number, but rather indicate that additional copies of the *CYP2D6* gene are present (e.g., *CYP2D6*1/*2* duplication or *CYP2D6 (*1/*2)xN*). In instances where a duplication/multiplication is present and the exact copy number is not reported, most patients will likely have a *CYP2D6* gene copy number of 3. However, individuals carrying as many as 13 *CYP2D6* gene copies have been reported (24). Clinical laboratories typically do not determine which allele is duplicated, therefore when calculating CYP2D6 activity score the duplication must be considered for each allele reported in the diplotype (25). For example, a genotype result of *CYP2D6 (*1/*4)3N* indicates a patient has three copies of the *CYP2D6* gene, with either two copies of the *CYP2D6*1* allele and one copy of the *CYP2D6*4* allele, or one copy of the *CYP2D6*1* allele and two copies of the *CYP2D6*4* allele. If the *CYP2D6*1* allele carries the duplication, the CYP2D6 activity score of this diplotype will be 2, whereas if the *CYP2D6*4* allele carries the duplication, the activity score will be 1. Likewise, if the number of gene copies is not determined and it remains unknown which allele carries the duplication/multiplication, a *CYP2D6 (*4/*9)xN* genotype, for example, can be consistent with an IM (intermediate metabolizer) phenotype (*CYP2D6*4xN/*9*; activity score of 0.5) or an NM (normal metabolizer) phenotype (*CYP2D6*4/*9xN* assuming that *xN* does not exceed four copies in which case the activity score is 1 for *xN*=2, 1.5 for *xN*=3 and 2 for *xN*=4). As these examples illustrate, phenotype prediction will be considerably more accurate if testing determines which allele carries the duplication/multiplication and determines the number of gene copies present. Studies have been published describing the translation of *CYP2D6* genotypes into predicted phenotypes when gene duplications or multiplications are present (19, 23, 25-27).

Note that a duplication may not be detected by copy number assays when paired with the *CYP2D6*5* allele (gene deletion). A *CYP2D6*2x2/*5* diplotype, for example, has a gene

duplication on one allele and a gene deletion on the other for a total number of two gene copies. This diplotype may be reported as *CYP2D6*2/*2*.

Other structural variants include gene copies that consist of *CYP2D6* and *CYP2D7*-derived sequences (28, 29). The no function *CYP2D7-2D6* hybrid genes, collectively assigned as *CYP2D6*13* (30), may not be detected by a particular genotype test or gene copy number testing. In such cases the test may detect only the allele present on the second chromosome and report the diplotype as homozygous for that allele. For example, a test that does not detect *CYP2D6*13* will report a *CYP2D6*1/*13* diplotype as *CYP2D6*1/*1*. Hybrid genes can also occur in duplication configurations and cause positive gene duplication test results that may lead to an overestimation of activity and false-positive prediction of ultrarapid metabolism (17, 29). For example, a *CYP2D6*1/*13+*2* diplotype (activity score = 2 predicting normal metabolism) may be assigned as *CYP2D6*1/*2xN* (activity score =3 predicting ultrarapid metabolism).

Limitations of the Star (*) Nomenclature and Allele Assignments. The star (*) nomenclature has defined multiple subvariants for an allele (e.g., *CYP2D6*2* and **4*), but generally, these are not distinguished by current testing. This is of no consequence for *CYP2D6*4*, because all **4* subvariants share 1846G>A causing aberrant splicing and absence of functional protein. For *CYP2D6*2*, however, it is uncertain whether any of the sequence variations defining the suballeles convey a functional consequence. Also, there is no, or little, information regarding their frequencies because test laboratories do not discriminate the suballeles. In addition, there are numerous known variants and subvariants of uncertain function that have not been designated by the nomenclature committee.

It also needs to be realized that the accuracy of a genotype test depends on the number of sequence variations/allelic variants tested. If no variation is found, a *CYP2D6*1* will be the 'default' assignment. Depending on which sequence variations are found, the default assignment will be *CYP2D6*2* (or other). For example, if 2850C>T is present, but 1023C>T is not, the default assignment is *CYP2D6*2*. Also see 'CYP2D6 Other Considerations' below.

Recent findings indicate that a SNP in a distal enhancer region impacts allele activity on the transcriptional level (31, 32). It is not fully understood on which allelic variants this enhancer

SNP is located. Emerging knowledge, however, suggests that a portion of *CYP2D6*2* alleles carrying the enhancer SNP convey normal function while others lacking the enhancer SNP have decreased function; the effect of the enhancer SNP in other haplotypes remains unknown. Presence or absence of the enhancer SNP likely also impacts the activity encoded by *CYP2D6*2xN* (duplications and multiplications). This SNP is, however, not included in current test panels. The activity score will be updated, if warranted, as new information becomes available.

Available Genetic Test Options

Commercially available genetic testing options change over time. Additional information about pharmacogenetic testing can be found at the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/). The American College of Medical Genetics and Genomics (ACMG) established guidelines for laboratory testing of *CYP2D6* in relation to tamoxifen therapy (33).

Clinical laboratories may analyze for different SNPs or other genetic variants, which are dependent on the genotyping platforms used and may affect the reported diplotype leading to discrepant results between methodologies. Additionally, laboratories may differ in how *CYP2D6* copy number variants are reported, which can potentially affect phenotype prediction. Therefore, it is important to not only know the alleles interrogated by each laboratory, but also which sequence variants (e.g., SNPs, insertions, or deletions) are tested and how copy number variants are reported. Clinical laboratories commonly give an interpretation of the genotype result and provide a predicted phenotype. Phenotype assignment for this guideline is defined in the main manuscript and supplementary data, but may differ from some clinical laboratory interpretations. Any *CYP2D6* genotyping results used to guide patient pharmacotherapy and/or deposited into patient medical records should be derived from validated genotyping platforms in clinical laboratories that implement the appropriate regulatory standards and best practices (e.g., CAP, CLIA).

CYP2D6 Other Considerations

There are several factors that cause potential uncertainty in *CYP2D6* genotyping results and phenotype predictions as follows: **1)** Because it is currently impractical to test for every variation

in the *CYP2D6* gene, genotyping tests may not detect rare variants resulting in patients being assigned a default genotype. It also needs to be stressed that genotyping tests are not designed to detect unknown/de novo sequence variations. Depending on the sequence variations (or alleles present) in a given patient, the default genotype may be *CYP2D6*1/*1* (or wild-type) or another diplotype. If the rare or *de novo* variant adversely affects CYP2D6 enzyme function, then the patient's actual phenotype may differ from the predicted phenotype. **2)** Sub-alleles of *CYP2D6*4* have been identified that harbor additional SNPs with limited or no added functional consequence (e.g., *CYP2D6*4A*, **4B*, **4C*, and **4D*). Therefore, only analyzing for the defining *CYP2D6*4* SNPs (100C>T and 1846G>A) is usually sufficient to determine a CYP2D6 phenotype. **3)** There are multiple gene units involved in duplication and other major rearrangements. Additionally, the pseudogenes *CYP2D7* and *CYP2D8* may be misinterpreted as functional duplications (34). If the specific gene units involved in the duplication or other rearrangements are not specifically tested for, the phenotype prediction may be inaccurate and CYP2D6 activity over-estimated. **4)** Some SNPs exist on multiple alleles. For example, *CYP2D6*69* carries the defining SNPs for *CYP2D6*41* (2850C>T, 2988G>A, and 4180G>C) and the defining SNPs for *CYP2D6*10* (100C>T and 4180G>C) in addition to multiple other SNPs. If a patient carries these genetic variants (in the absence of 1846G>A), a *CYP2D6*10/*41* diplotype is typically assigned, because this is the most likely result based on allele frequencies. However, a *CYP2D6*1/*69* genotype cannot be excluded with certainty. Testing for additional SNPs (e.g., 1062A>G, 3384A>C, and 3584G>A) could exclude *CYP2D6*1/*69* with certainty. Therefore, to unequivocally determine the presence of certain alleles, testing for multiple SNPs may be required. **5)** Allele frequencies may vary considerably among individuals of different ethnic backgrounds. For instance, *CYP2D6*10* is common in Asian populations while *CYP2D6*17* is common in people of Sub-Saharan African ancestry. These alleles, however, have a considerably lower prevalence in other ethnic groups such as Caucasians of European ancestry. As another example, *CYP2D6*14* is present in Asian populations and therefore its defining SNP (1758G>A) has been incorporated into Asian genotyping panels (35). Thus, the alleles that should be tested for a given population may vary considerably. **6)** Certain alleles carry genes in tandem arrangements. One such example is *CYP2D6*36+*10* (one copy of the non-functional *CYP2D6*36* and one copy of the decreased function *CYP2D6*10*). This tandem can be found in Asians and is typically reported as a default assignment of *CYP2D6*10*.

LEVELS OF EVIDENCE LINKING GENOTYPE TO PHENOTYPE

The evidence summarized in **Supplemental Table S2** is graded (36) on a scale of high, moderate, and weak, based upon the level of evidence:

> **High:** Evidence includes consistent results from well-designed, well-conducted studies.
>
> **Moderate:** Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies, generalizability to routine practice, or indirect nature of the evidence.
>
> **Weak:** Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

Every effort was made to present evidence from high-quality studies, which provided the framework for the strength of therapeutic recommendations (**Table 2**, main manuscript).

STRENGTH OF RECOMMENDATIONS

CPIC's therapeutic recommendations are based on weighing the evidence from a combination of preclinical functional and clinical data, as well as on some existing disease-specific consensus guidelines. Some of the factors that are taken into account in evaluating the evidence supporting therapeutic recommendations include: in vivo pharmacokinetic and pharmacodynamic data, in vitro enzyme activity of tissues expressing wild-type or variant-containing CYP2D6, in vitro CYP2D6 enzyme activity from tissues isolated from individuals of known *CYP2D6* genotypes, and in vivo pre-clinical and clinical pharmacokinetic and pharmacodynamic studies.

Overall, the therapeutic recommendations are simplified to allow rapid interpretation by clinicians. CPIC uses a slight modification of a transparent and simple system for just three categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of antiretroviral agents (37):

Strong recommendation for the statement**:** "The evidence is high quality and the desirable effects clearly outweigh the undesirable effects."

Moderate recommendation for the statement: "There is a close or uncertain balance" as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.
Optional recommendation for the statement: The desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
No recommendation: There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time

RESOURCES TO INCORPORATE PHARMACOGENETICS INTO AN ELECTRONIC HEALTH RECORD WITH CLINICAL DECISION SUPPORT

Clinical decision support (CDS) tools integrated within electronic health records (EHRs) can help guide clinical pharmacogenetics at the point of care (38-42). Resources to support the adoption of CPIC guidelines within an EHR are available on the CPIC website (1, 43). Based on the capabilities of various EHRs and local preferences, we recognize that approaches may vary across organizations. Our intent is to synthesize foundational knowledge that provides a common starting point for incorporating *CYP2D6* genotype results in an EHR to guide tamoxifen use.

Effectively incorporating pharmacogenetic information into an EHR to optimize drug therapy should have some key attributes. Pharmacogenetic results, an interpreted phenotype, and a concise interpretation or summary of the result must be documented in the EHR (27). To incorporate a phenotype in the EHR in a standardized manner, genotype test results provided by the laboratory must be consistently translated into an interpreted phenotype (**Table 1, main manuscript; *CYP2D6* Diplotype to Phenotype Table (1, 18)**). Because clinicians must be able to easily find the information, the interpreted phenotype may be documented as a problem list entry or in a patient summary section; these phenotypes are best stored in the EHR at the "person level" rather than at the date-centric "encounter level". Additionally, results should be entered as standardized and discrete terms to facilitate using them to provide point-of-care CDS (see **Tamoxifen Pre- and Post-Test Alerts and Flow Chart** for example CDS alerts; (1, 18)) (44, 45).

Because pharmacogenetic results have lifetime implications and clinical significance, results should be placed into a section of the EHR that is accessible independent of the test result date to allow clinicians to quickly find the result at any time after it is initially placed in the EHR. To facilitate this process, CPIC is providing gene-specific information figures and tables that include full diplotype to phenotype tables, diagram(s) that illustrate how *CYP2D6* pharmacogenetic test results could be entered into an EHR, example EHR consultation/genetic test interpretation language and widely used nomenclature systems (see (1, 42)**.** Point-of-care CDS should be designed to effectively notify clinicians of prescribing implications at any time after the test result is entered into the EHR. CPIC is also providing gene-drug specific tables that provide guidance to achieve these objectives with diagrams that illustrate how point-of-care CDS should be entered into the EHR, example pre- and post-test alert language, and widely used nomenclature systems for relevant drugs (1).

SUPPLEMENTAL TABLE S1. ASSOCIATION BETWEEN ALLELIC VARIANTS[A] AND CYP2D6 ENZYME ACTIVITY

Functional Status (19, 23)	Activity Value[c,d]	Alleles
Increased function	>1	**1xN, *2xN, *35xN, *45*[g]*xN*
Normal or Increased function	1 or >1[h]	**9xN, *10xN>2, *17xN, *29xN, *41xN*
Normal function[b]	1	**1*[e]*, *2, *27, *33, *34*[f]*, *35, *39*[f]*, *45*[g]*, *46*[g]*, *48, *53*
Decreased function	0.5	**9, *10, *10x2*[i]*, *14B, *17, *29, *41, *49, *50, *54, *55, *59, *72, *84*
No function	0	**3, *3xN, *4, *4xN, *5, *6, *6xN, *7, *8, *11, *12, *13, *14A, *15, *18, *19, *20, *21, *31, *36, *36xN, *38, *40, *42, *44, *47, *51, *56, *57, *60, *62, *68, *69, *92, *96, *99, *100, *101*
Uncertain	N/A	**22, *23, *24, *25, *26, *28, *30, *32, *37, *43, *43xN, *52, *61, *63, *64, *65, *70, *71, *75, *81, *87, *88, *89, *90, *91, *93, *94, *95, *97, *98, *106*
Unknown	N/A	**58, *73, *74, *82, *83, *85, *86, *102, *103, *104, *105, *107, *108, *109, *110, *111, *112, *113*

[a]See https://www.pharmvar.org/ or ***CYP2D6*** **Allele Definition Table (1, 18)** for updates on *CYP2D6* allelic variants and nomenclature.

[b]An important caveat for all genotyping tests is that the decision to assign an allele a wild-type status is based upon a genotyping test that interrogates only the most common and already-proven sites of functional variation. It is always possible that a new, previously undiscovered (and therefore un-interrogated) site of variation is defaulted to a functional allele assignment (wild-type). There is a rare possibility that such variation confers decreased or no function in an individual and that the person's CYP2D6 function is not accurately predicted.
[c]For some allelic variants there is no or sparse information regarding their activity; therefore, no value can be assigned and no CYP2D6 activity score can be calculated. In such cases, the activity score may be estimated based on the second/known allele. A recent in vitro investigation using tamoxifen as substrate provides preliminary information for alleles listed here as uncertain (46).
[d]For certain *CYP2D6* alleles *in vivo* data are lacking or are uncertain to unambiguously assign an activity value. Activity of an allele may also be substrate dependent, and therefore the actual activity of a decreased function allele could be closer to 1 (normal function) or 0 (no function). For instance, there is evidence that the *CYP2D6*10* decreased function allele has less activity towards tamoxifen *in vivo* compared to other substrates and that the activity is closer to 0 than 1. It should be noted that the CYP2D6 activity score is an ordinal scale to bin alleles of similar activity. An allele with an activity score of 0.5 does not necessarily have half the metabolic activity of an allele with an activity score of 1. Rather the score of 0.5 indicates the allele has decreased metabolic activity when compared to the *CYP2D6*1* reference allele.
[e]*CYP2D6*1* serves as reference and is defined as wild-type.
[f] Function of *CYP2D6*34* and **39* is extrapolated from **2*. Both star alleles have SNP(s) that are part of the **2* haplotype.
[g]Limited data are available to determine the predicted activity value of *CYP2D6*45* and **46*. Although an activity value of 1 (normal function) is assigned to *CYP2D6*45* and **46* in this guideline, others may assign an activity value of 0.5 (decreased function).
[h]Activity value is dependent on the number of duplications/multiplications present.
[i]The *CYP2D6*10* allele has considerable decrease in activity. The function of *CYP2D6*10x2* was conservatively placed into the decreased function category.

SUPPLEMENTAL TABLE S2. EVIDENCE LINKING CYP2D6 TO TAMOXIFEN PHENOTYPE

Type of experimental model	Major findings	References (PMID)	Allele combinations[a]	Activity scores	Level of evidence
Clinical (PK/PG)	CYP2D6 Poor Metabolizers (AS=0) have lower plasma endoxifen concentrations among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Madlensky**, *et al.* (2011) (47) **Ruddy**, *et al*. (2013) (48) **Rangel**, *et al.* (2014) (49) **Henning**, *et al*. (2015) (50)	**Madlensky**: *3/*4, *4/*4, *3/*6, *15/*4, *5/*4, *6/*4, *4/*4xN, *5/*6 vs UM/PM + UM/IM + EM/EM + EM/IM + EM/PM; **Ruddy**: PM vs EM, alleles not discussed; **Rangel**: *3/*4, *4/*4, *4/*6 vs *1/*1, *1/*2, *2/*2; **Henning**: PM/PM vs EM/EM	**Madlensky**: 0 vs 1 + 1.5 + 2; **Ruddy**: 0 vs EM not specified; **Rangel**: 0 vs 2; **Henning**: 0 vs 2	**High**
Clinical (PK/PG)	Reduced CYP2D6 activity (AS=0 to 1) is associated with lower plasma endoxifen concentrations among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Fernandez-Santander**, *et al*. (2013) (51) **Borges**, *et al*. (2006) (52) **Henning**, *et al.* (2015) (50)	**Fernandez-Santander**: PM/PM + IM/PM + IM/IM vs EM/PM + EM/IM + EM/EM ($p<0.002$) AND PM/PM + IM/PM + IM/IM vs EM/EM ($p<0.001$); **Borges**: PM/PM + IM/PM vs EM/PM + EM/IM AND EM/PM + EM/IM vs EM/EM + UM/EM AND PM/PM + IM/PM vs IM/PM vs EM/PM vs	**Fernandez-Santander**: 0 + 0.5 + 1 (IM/IM) vs 1 (EM/PM) + 1.5 +2 AND 0 + 0.5 + 1 vs 2; **Borges**: 0 + 0.5 + 1 vs 1.5 vs 2 + >2; **Henning**: 0 or 0.5 or 1 vs 2	**High**

			EM/EM + UM/EM (cohort w/o 2D6 inhibitors); **Henning**: PM/PM or PM/IM or IM/IM or EM/PM vs EM/EM		
Clinical (PK/PG)	Reduced CYP2D6 activity (AS=0 to 1, predominantly *10) is associated with lower plasma endoxifen concentrations among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Love**, *et al*. (2013) (53) **Lim**, *et al.* (2011) (54) **Lim**, *et al*. (2007) (55) **Kiyotani**, *et al.* (2010) (56) **Park**, *et al.* (2012) (57)	**Love**: *10/*10, *10/*41, *5/*10, *1/*5, *2/*5 vs *1/*10, *2/*10, *1/*41 vs *1/*1, *1/*2, *2/*2; **Lim2011**: *5/*10 vs *1/*1 OR *1/*5 OR *1/*10 AND *10/*10 vs *1/*1 OR *1/*5 OR *1/*10; **Lim2007**: *1/*1 OR *1/*10 vs *10/*10; **Kiyotani**: v/v vs v/*1 vs *1/*1 with v: specific alleles not reported but at least 75% were *1/*10 or *10/*10; **Park:** (*5, *10, *41 = v) IM/IM (mainly) + IM/PM + PM/PM (n=2) vs PM/EM + IM/EM + EM/EM	**Love**: 0.5 + 1 vs 1.5 vs 2; **Lim2011**: 0.5 vs 1 (EM/PM) or 1.5 or 2 AND 1 (IM/IM) vs 1 (EM/PM) or 1.5 or 2; **Lim 2007**: 1 vs 1.5 or 2; **Kiyotani**: (0.5 +)? 1 vs 1.5 vs 2; **Park**: 0.5 + 1 (IM/IM) vs 1 (PM/EM) + 1.5 + 2	**High**

Clinical (PK/PG)	CYP2D6 genotype (activity score) is positively correlated with endoxifen plasma concentrations.	**Safgren**, *et al.* (2015) (58) **Saladores**, *et al*. (2015) (59) **Mürdter**, *et al.* (2011) (60) **Antunes**, *et al.* (2015) (61)		**Safgren**: 0 to 3; **Saladores**: 0 to 3; **Mürdter**: 0 to 3; **Antunes**: 0 to 3	**High**
Clinical (PG/PD) (*pre-operative tamoxifen window trial with Ki-67 as endpoint)	*CYP2D6*10/*10* and *CYP2D6*5/*10* are associated with a lower Ki-67 response compared to *CYP2D6*1/*1*.	**Zembutsu**, *et al*. (2017) (62)*	**Zembutsu**: v/v vs v/*1 + *1/*1 (v: mainly *5 and *10 but a few *4, *14, *18, *21, *41)	**Zembutsu**: 0.5 + 1 (IM/IM) vs 1 (PM/NM) + 1.5 + 2	**High**
Clinical (side effects)	There is a positive correlation between CYP2D6 activity and tamoxifen-related side effects (e.g. hot flashes, weight gain).	**Supports statement:** **Rolla**, *et al*. (2012) (63) **No significant difference:** **Baxter**, *et al.* (2014) (64) **Dezentjé**, *et al*. (2014) (65) [**Regan**, *et al*. (2012) (16)] **Zembutsu**, *et al*. (2017) (62)* * pre-operative tamoxifen window trial with Ki-67 as endpoint	**Rolla**: EM + IM + PM vs UM; **Baxter**: UM/EM + EM/EM vs EM/IM + EM/PM + IM/IM + IM/PM vs PM/PM; **Dezentjé**: PM/PM or IM (IM/PM + IM/IM + PM/EM) vs EM (EM/EM + IM/EM); **Regan**: PM/PM vs EM/EM and IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM	**Rolla**: 0 + 0.5 + 1 + 1.5 + 2 vs >2; **Baxter**: 0 vs 0.5 + 1 + 1.5 vs 2 + >2; **Dezentjé:** 0 or 0.5 + 1 vs 1.5 + 2; **Regan**: 0 vs 2 AND 0.5 + 1 +1.5 vs 2	**Weak**
<u>Recurrence</u>: Comparing Poor Metabolizers with Normal Metabolizers					

Clinical	CYP2D6 Poor Metabolizers (AS=0) have a higher risk of breast cancer recurrence among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Supports statement:** **Schroth**, *et al*. (2009) (66) **No significant difference:** [**Rae**, *et al*. (2012) (67)] [**Regan**, *et al*. (2012) (16)] **Newman**, *et al.* (2008) (68)	**Schroth**: PM/PM vs EM/EM; **Rae**: AS score: 0 vs 0.5 or 1 or 1.5 or 2; **Regan**: PM/PM vs EM/EM; **Newman**: PM/PM vs EM/EM + EM/PM + EM/IM	**Schroth**: 0 vs 2; **Rae**: 0 vs 2; **Regan**: 0 vs 2; **Newman**: 0 vs 1 + 1.5 + 2	**Moderate**
<u>Recurrence</u>: Comparing Poor Metabolizers with Intermediate Metabolizers					
Clinical	CYP2D6 Poor Metabolizers (AS=0) do **NOT** have a higher risk of breast cancer recurrence among patients taking adjuvant tamoxifen compared to CYP2D6 Intermediate Metabolizers.	[**Rae**, *et al.* (2012) (67)]	**Rae**: AS score: 0 vs 0.5 or 1 or 1.5 or 2	**Rae**: AS score: 0 vs 0.5 or 1 or 1.5 or 2	**Weak**
<u>Recurrence</u>: Comparing Poor Metabolizers and Intermediate Metabolizers with Normal Metabolizers					
Clinical	Poor (AS = 0) and Intermediate (AS = 0.5) metabolizers combined have a higher risk of	**Damodaran**, *et al*. (2012) (69)	**Damodaran**: (*2, *4, *5, and *10) AS 0 (n=3) + 0.5 (n=8) vs 1 (n=22) + 1.5 (n=10) + 2 (n=89)	**Damodaran**: 0+0.5 vs 1+1.5+2	**Weak**

	breast cancer recurrence among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.				
<u>Recurrence</u>: Comparing CYP2D6 activity scores of 0.5 – 1.5 with normal CYP2D6 activity					
Clinical	Activity scores of 0.5 – 1.5 have a higher risk of breast cancer recurrence among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Supports statement:** **Schroth**, *et al.* (2009) (66) **No significant difference:** [**Regan**, *et al*. (2012) (16)]	**Schroth**: IM/IM + IM/PM + EM/PM + EM/IM vs EM/EM (including xN) **Regan**: IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM AND PM/PM + IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM	**Schroth**: 0.5 + 1 +1.5 vs 2 + >2; **Regan**: 0.5 + 1 + 1.5 vs 2	**Weak**
<u>Recurrence</u>: Comparing CYP2D6 activity scores of 0.5 – 1.5 with normal CYP2D6 activity					
Clinical	Activity scores of 0.5 – 1.5 (predominantly *10) have a higher risk of breast cancer recurrence among patients taking adjuvant tamoxifen compared to	**Supports statement:** **Teh**, *et al*. (2012) (70) **No significant difference:** **Chamnanphon**, *et al*. (2013) (71)	**Teh**: IM/IM + IM/PM + EM/PM (mainly *****10/*10) or *1/*10 vs *****1/*1 (including xN) (*1/*10 vs *****1/*1 not significant); **Chamnanphon**: *10/*10 vs *1/*10 vs *1/*1 and *1/*1 vs EM/IM: *1/*10, *2/*10, *10/*35,	**Teh**: 0.5 + 1 or 1.5 vs 2; **Chamnanphon**: 1 vs 1.5 vs 2	**Weak**

	normal CYP2D6 activity.		*1/*36, *1/*41 vs IM/IM: *10/*10, *41/*10 and *1/*1 vs *1/v vs v/v		
<u>Recurrence:</u> Comparing CYP2D6 activity scores of 0 – 1.5 with normal CYP2D6 activity					
Clinical	Reduced CYP2D6 activity (AS 0 to 1.5) have a higher risk of breast cancer recurrence among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Supports statement:** **Schroth**, *et al.* (2009) (66) **Margolin**, *et al.* (2013) (72)* **No significant difference:** **[Regan**, *et al*. (2012) (16)] **Morrow**, *et al.* (2012)(73) **Mwinyi**, *et al*. (2014) (74) *study separately analyzed pre- and post-menopausal individuals	**Schroth**: PM/PM and/or IM/IM, IM/PM + EM/IM + EM/PM vs EM/EM (including xN; **Margolin**: > 50% activity vs. ≤ 50% activity with *1, *3, *4, *5, *10, *17, *41; **Regan**: PM/PM and/or IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM; **Morrow**: PM/PM + IM/IM + IM/PM vs EM/EM + EM/UM; **Mwinyi**: PM/PM + IM/IM + EM/IM + EM/PM + IM/PM vs EM/EM + EM/UM	**Schroth**: 0 and/or 0.5+1+1.5 vs 2+>2; **Margolin**: 0 + 1 vs 2 + >2; **Regan**: 0 and/or 0.5+1+1.5 vs 2+>2; **Morrow**: 0+0.5+1 vs 2+>2; **Mwinyi**: 0+0.5+1+1.5 vs 2+>2	**Weak**
<u>Event-free survival</u>: Comparing Poor Metabolizers with Normal Metabolizers					
Clinical	CYP2D6 Poor Metabolizers (AS=0) have worse event-free and recurrence-	**Supports statement:** **Goetz**, *et al.* (2013) (75) **No significant difference:** **Markkula**, *et al.* (2014) (76)	**Goetz**: PM/PM vs EM/EM; **Markkula**: PM/PM vs EM/EM; **Dezentje**: PM/PM vs EM/EM; **Thompson**:	**Goetz**: 0 vs 2; Markkula: 0 vs 2; **Dezentje**: 0 vs 2; **Thompson**: 0 vs 2 + >2;	**Moderate**

	free survival among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Dezentje**, *et al.* (2013) (77) **Thompson**, *et al.* (2011) (78) **Argalacsova**, *et al.* (2015) (79)* **Newman**, *et al*. (2008) (68) * in premenopausal women only	PM/PM vs EM/EM + EM/UM; **Argalacsova:** not specified; **Newman**: PM/PM vs EM/EM + EM/PM + EM/IM	**Argalacsova:** not specified; **Newman**: 0 vs 1+1.5+2	
<u>Event-free survival</u>: Comparing Poor and Intermediate Metabolizers with Normal Metabolizers					
Clinical	CYP2D6 Poor (AS=0) and Intermediate Metabolizers (AS=0.5) combined have worse recurrence-free survival among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Damodaran**, *et al*. (2012) (69)	**Damodaran**: (*2, *4, *5, and *10) AS 0 (n=3) + 0.5 (n=8) vs 1 (n=22) + 1.5 (n=10) + 2 (n=89)	**Damodaran**: 0 + 0.5 vs 1+1.5 + 2	**Weak**
<u>Event-free survival</u>: Comparing Poor Metabolizer with Intermediate and Normal Metabolizers					
Clinical	CYP2D6 Poor Metabolizer (AS=0) is **NOT** associated with worse recurrence-free survival	**[Hertz**, *et al*. (2017) (80)]	**Hertz:** PM/PM vs non-PM	**Hertz:** 0 vs >0	**Weak**

	among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.				
<u>Event-free survival</u>: Comparing CYP2D6 activity scores of 0.5 – 1.5 with normal CYP2D6 activity					
Clinical	Activity score of 0.5-1.5 do NOT have worse event-free and recurrence-free survival among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Markkula**, *et al.* (2014) (76) **Goetz**, *et al.* (2013) (75)	**Markkula**: IM/PM + IM/IM + IM/EM + PM/EM vs EM/EM; **Goetz**: IM/EM + PM/EM or EM/IM + IM/IM vs EM/EM	**Markkula**: 0.5+1+1.5 vs 2; **Goetz**: 0.5+1 (PM/EM) vs 2 OR 1 (IM/IM) +1.5 vs 2	**Weak**
<u>Event-free survival</u>: Comparing CYP2D6 activity scores of 0.5 – 1.5 with normal CYP2D6 activity					
Clinical	Activity score of 0.5-1.5 (predominantly *10) have worse event-free and recurrence-free survival among patients taking adjuvant tamoxifen compared to	**Supports Statement:** **Sukasem**, *et al.* (2012) (81)* **Kiyotani**, *et al.* (2010) (56) **No significant difference:** **Chamnanphon**, *et al.* (2013) (71) **Park**, *et al.* (2011) (82) **Park**, *et al.* (2012) (57)	**Sukasem**: EM (*1/*2, *1/*1, *1/*5, *1/*10, *1/*36, *1/*41, *10/*35, *2/*2, *2/*4, *2/*10) vs IM (*5/*10, *10/*10, *41/*10) AND *10/*10 vs other genotypes excluding het/*10 with no association for het/*10	**Sukasem**: 0.5 + 1 (IM/IM) vs 1 (EM/PM; 3 out 34) + 1.5 + 2 AND 1 (*10/*10) vs 1 + 1.5 + 2 (all other excluding het/*10); **Kiyotani**: 0 + 0.5 + 1 (IM/IM) vs 2 and 1 (EM/PM) +	**Weak**

	normal CYP2D6 activity.	* Association only in post-menopausal cohort but not overall cohort	vs all other excluding *10/*10); **Kiyotani**: v/v or v/*1 vs *1/*1 with v: *4, *5, *10, *10-*10, *14, *21, *36-*36, *41; **Chamnanphon**: *10/*10 vs *1/*10 + *1/*1; **Park 2011**: IM/PM (mainly)+ PM/PM vs IM/IM + EM/PM + EM/IM vs EM/EM; **Park 2012:** (*5, *10, *41 = v) IM/IM (mainly) + IM/PM + PM/PM (n=2 at the most, only listed for total patient cohort not specific for treatment group) vs PM/EM + IM/EM + EM/EM	1.5 vs 2; **Chamnanphon**: 1 vs 1.5 + 2; **Park 2011**: 0.5 vs 1 + 1.5 AND 0.5 vs 2; **Park 2012**: 0.5 + 1 (IM/IM) vs 1 (PM/EM) + 1.5 + 2	
<u>Event-Free Survival:</u> CYP2D6 activity scores of 0 – 1.5 with activity score to normal CYP2D6 activity					
Clinical	Reduced CYP2D6 activity (AS 0 to 1.5) is associated with worse event-free and recurrence-free survival among patients taking adjuvant tamoxifen	**Supports statement:** **Schroth**, *et al.* (2009) (66) **Thompson**, *et al.* (2011) (78) **No significant difference:** **Martins**, *et al.* (2014) (83) **Margolin**, *et al.* (2013) (72) **Ramon y Cajal**, *et al.* (2010) (84)	**Schroth**: PM/PM + IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM (including xN) **Thompson**: PM/PM + IM/PM + IM/IM + EM/IM + EM/PM vs EM/EM + EM/UM; **Martins**:	**Schroth**: 0 + 0.5 + 1 + 1.5 vs 2 + >2; **Thompson**: 0 + 0.5 + 1 + 1.5 vs 2 + >2; **Martins**: 0.5 + 1 (IM/IM) vs 1 (EM/PM) + 1.5 + 2; **Margolin**: 0 +1 vs 2 + >2; **Ramon**	**Moderate**

	compared to normal CYP2D6 activity.		*4/*4, *4/*10, *10/*10 vs *1/*4, *1/*10, *1/*1; **Margolin**: > 50% activity vs. ≤ 50% activity; **Ramon y Cajal**: *4/*4, *4/*41, *1/*5, *2/*5 vs all other genotypes (*3/*4, *4/*9, *9/*10, *9/*41, *41/*41, *1/*4, *1/*6, *2/*4, *2/*20, *1/*10, *1/*41, *1/*9, *10/*35, *9/*35, *2/*2, *1/*2, *1/*1, *1/*35, *2/*35, *1xN/*2, *2xN/*41	**y Cajal**: 0 + 0.5 + 1 vs 0 + 0.5 + 1 + 1.5 + 2 + >2	
<u>Distant Relapse Free Survival</u>: Comparing Poor Metabolizers with Normal Metabolizers					
Clinical	CYP2D6 Poor Metabolizers (AS=0) do not have worse distant relapse free survival among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Saladores**, *et al.* (2015) (59)** * in pre-menopausal women	**Saladores**: PM/PM vs EM/EM + UM/EM;	**Saladores**: 0 vs 2 + >2;	**Weak**

<u>Breast Cancer Specific Survival</u>: **Comparing activity scores of 0 – 1.5 with normal CYP2D6 activity**					
Clinical	Reduced CYP2D6 activity (AS 0 to 1.5) is associated with worse breast cancer-specific survival among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Supports statement:** **Margolin***, *et al.* (2013) (72) **No significant difference:** **Abraham**, *et al.* (2010) (14) *study separately compared pre- and post-menopausal individuals	**Margolin**: > 50% activity vs. ≤ 50% activity; **Abraham**: v/v vs *1/v + *1/*1 AND v/v + *1/v vs *1/*1	**Margolin**: 0 +1 vs 2 + >2; **Abraham**: 0 + 1 (IM/IM) vs 1 + 1.5 + 2 AND 0 + 0.5 +1 + 1.5 vs 2	**Weak**
<u>Overall Survival:</u> **Comparing Poor Metabolizers with Normal metabolizers**					
Clinical	CYP2D6 Poor Metabolizers (AS=0) do **NOT** have worse overall survival among patients taking adjuvant tamoxifen compared to CYP2D6 Normal Metabolizers.	**Newman**, *et al.* (2008) (68)	**Newman**: PM/PM vs EM/EM + EM/PM + EM/IM	**Newman**: 0 vs 1+1.5+2	**Weak**
<u>Overall Survival:</u> **Comparing activity scores of 0 – 1.5 with normal CYP2D6 activity**					

Clinical	Reduced CYP2D6 activity (AS 0 to 1.5) is **NOT** associated with worse overall survival among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Schroth**, *et al*. (2009) (66) **Abraham**, *et al.* (2010) (14)	**Schroth**: PM/PM + IM/IM + IM/PM + EM/IM + EM/PM vs EM/EM; **Abraham**: v/v vs *1/v + *1/*1 AND v/v + *1/v vs *1/*1	**Schroth**: 0 + 0.5 + 1 +1.5 vs 2 + >2; **Abraham**: 0 + 1 (IM/IM) vs 1 + 1.5 + 2 AND 0 + 0.5 +1 + 1.5 vs 2	**Weak**
<u>Overall Survival</u>: Comparing activity scores of 0 – 1.5 with normal CYP2D6 activity					
Clinical	Reduced CYP2D6 activity (AS 0 to 1.5, predominantly *10) is **NOT** associated with worse overall survival among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity.	**Park**, *et al.* (2011) (82)	**Park**: IM/PM (mainly, n=47) + PM/PM (n=2) vs IM/IM + EM/PM + EM/IM vs EM/EM	**Park**: 0.5 vs 1 + 1.5 AND 0.5 vs 2	**Weak**

PM = no function allele, IM = decreased function allele, EM = normal function allele, UM = increased function allele. [ ] brackets indicate that DNA was isolated from fresh-frozen tumor or FPPE tumor tissue with evidence for substantial deviation from Hardy Weinberg Equilibrium and therefore considered weak support for the statement based on genotyping errors. See discussion in guideline (*Other considerations* section) for more information.

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