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REVIEW

CYP2D6 as a treatment decision aid for ER-positive non-metastatic breast cancer patients: a systematic review with accompanying clinical practice guidelines

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Abstract

Purpose Tamoxifen is one of the principal treatments for estrogen receptor (ER)-positive breast cancer. Unfortunately, between 30 and 50% of patients receiving this hormonal therapy relapse. Since *CYP2D6* genetic variants have been reported to play an important role in survival outcomes after treatment with tamoxifen, this study sought to summarize and critically appraise the available scientific evidence on this topic.

Methods A systematic literature review was conducted to identify studies investigating associations between *CYP2D6* genetic variation and survival outcomes after tamoxifen treatment. Critical appraisal of the retrieved scientific evidence was performed, and recommendations were developed for *CYP2D6* genetic testing in the context of tamoxifen therapy.

Results Although conflicting literature exists, the majority of the current evidence points toward *CYP2D6* genetic variation affecting survival outcomes after tamoxifen treatment. Of note, review of the *CYP2D6* genotyping assays used in each of the studies revealed the importance of comprehensive genotyping strategies to accurately predict CYP2D6 metabolizer phenotypes.

Conclusions and recommendations Critical appraisal of the literature provided evidence for the value of comprehensive *CYP2D6* genotyping panels in guiding treatment decisions for non-metastatic ER-positive breast cancer patients. Based on this information, it is recommended that alternatives to standard tamoxifen treatments may be considered in *CYP2D6* poor or intermediate metabolizers.

Keywords Clinical practice guidelines · CYP2D6 · Pharmacogenomics · Systematic review · Tamoxifen

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Introduction

Tamoxifen and the treatment of estrogen receptor-positive breast cancer

Breast cancer is the most common cancer in women, with 1.8 million individuals affected worldwide [1]. More than half of all breast cancer patients are estrogen receptor (ER)-positive, and the endocrine therapy, tamoxifen, is one of the principal treatments for these cases [2, 3]. Tamoxifen is a selective ER modulator, which acts as an estrogen agonist or antagonist in a tissue-dependent manner. Tamoxifen exerts its pharmacological effect by binding to the ERs, causing a conformational change in these receptors, and subsequently altering the transcription of estrogen-dependent genes [4, 5]. The drug is used in all stages of breast cancer and is employed for both the treatment and prevention of the disease [3]. In ER-positive breast cancer, the introduction of tamoxifen in the 1970s has helped decrease the recurrence rate by almost 40% and risk of mortality by approximately 30% [6, 7]. Unfortunately, although tamoxifen treatment is highly effective, not all patients respond optimally to this therapy, with between 30 and 50% of patients relapsing [8, 9].

Metabolism of tamoxifen by CYP2D6

The majority of tamoxifen's anti-tumor effects are exerted by two of its major metabolites, endoxifen and 4-hydroxy-tamoxifen (4-HT) [10, 11]. Compared to tamoxifen, these metabolites have a 100-fold higher affinity for the ER and 30- to 100-fold greater suppression of estrogen-dependent cell proliferation [12–14]. Metabolism of tamoxifen to *N*-desmethyl-tamoxifen and subsequent conversion to endoxifen is predominantly mediated by the polymorphic drug metabolizing enzyme, cytochrome P450 2D6 (CYP2D6) (Fig. 1). Metabolism to endoxifen contributes to approximately 92% of overall metabolism, while metabolism to 4-HT contributes approximately 7% to overall tamoxifen metabolism [15]. Concordantly, the concentration of endoxifen is 5–10 times higher than 4-HT [9, 16, 17]. Therefore, endoxifen may play a more significant role in tamoxifen outcomes and may result in greater potency upon binding to ER sites [14, 18, 19].

CYP2D6 inhibitors and their effect on endoxifen levels

Selective serotonin reuptake inhibitors (SSRIs) as well as serotonin and norepinephrine reuptake inhibitors (SNRIs) are used to treat the depressive symptoms associated with cancer therapy [20, 21] as well as hot flashes, which are a common adverse effect of tamoxifen [22, 23]. Unfortunately, many SSRIs and SNRIs are moderate or strong CYP2D6 inhibitors [24], which reduce or eliminate the metabolism of tamoxifen [25, 26]. Studies have shown that potent CYP2D6 inhibitors result in 50–72% lower endoxifen levels [26–29]. This reduction in endoxifen levels may have important implications for the efficacy of tamoxifen [27]. As a result, the Health Canada drug label recommends that the concurrent

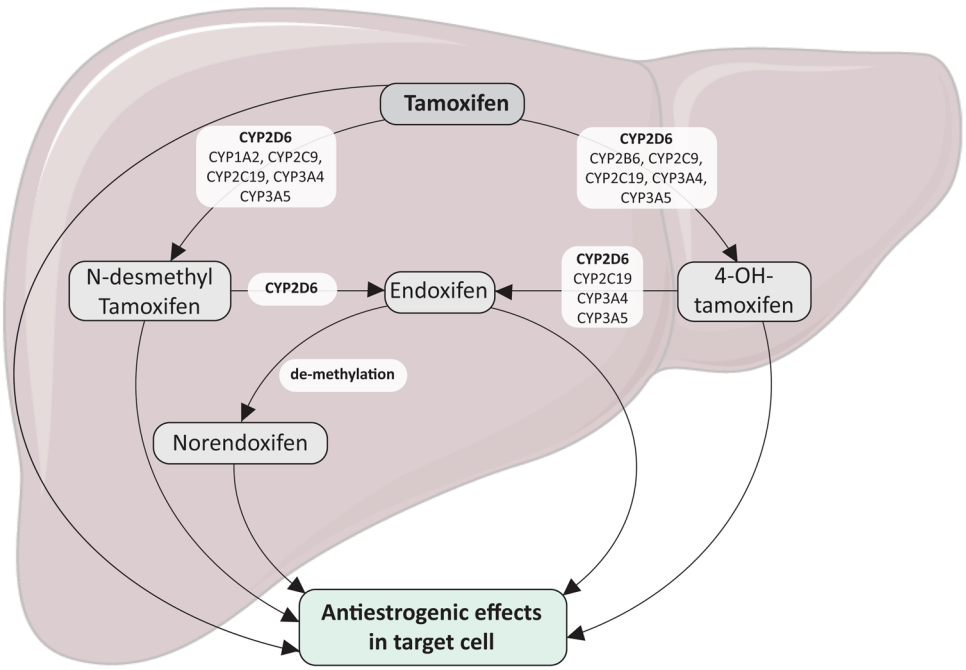

Fig. 1 Role of CYP2D6 and other CYP enzymes in the metabolism of tamoxifen to its antiestrogenic metabolites as depicted by PharmGKB [83]. For a more detailed overview of this pharmacokinetic pathway refer to: https://www.pharmgkb.org/pathway/PA145011119

use of CYP2D6 inhibitors should be avoided in patients taking tamoxifen [30].

CYP2D6 genetic variants and their effect on endoxifen levels

CYP2D6 genetic variation can influence enzyme activity, the extent of which can be defined in vivo using CYP2D6 probe drugs [31]. The predicted effect of this variation on CYP2D6 function can be categorized according to the activity score (AS) system (Supplementary Table 1). Patients who carry two non-functional genes are classified as having an AS of 0.0 and are also traditionally referred to as CYP2D6 poor metabolisers (PMs). In the case of certain CYP2D6-metabolized drugs, individuals with an AS of 1.0 are considered normal metabolizers (NMs). However, prospective tamoxifen pharmacology studies have shown that individuals with an AS of 1.0 have decreased baseline endoxifen levels [32–35]. Therefore, for tamoxifen, patients who have one non-functional gene and one normal/decreased function gene, or two decreased function genes (AS = 0.5/1.0) are referred to as CYP2D6 intermediate metabolisers (IMs). Both PMs and IMs have decreased levels of endoxifen due to a lower CYP2D6 metabolism rate and are theoretically more likely to demonstrate treatment failure [36]. Further to the influence of genetic variation on the activity of CYP2D6, non-genetic factors such as strong or moderate CYP2D6 inhibitors can also impact the AS of individuals. This phenomenon is known as phenoconversion. For example, if an individual who is a genetic EM receives a strong CYP2D6 inhibitor, this individual will be considered a PM, while if that same individual receives a moderate inhibitor, the phenoconverted status of that individual will be an IM [37].

Evidence for the role that genetic variants play on influencing endoxifen levels has been provided by four *CYP2D6*-based tamoxifen dose-adjustment studies. These studies, which incorporated either an individualized dose escalation approach or a doubling in tamoxifen dose (from 20 to 40 mg/day) in CYP2D6 PM and IM individuals, consistently showed that: (i) baseline endoxifen levels were significantly lower in PM and IM individuals when compared to NM individuals; (ii) tamoxifen dose escalation in IM and PM individuals significantly increased endoxifen levels, with endoxifen levels normalizing in IM individuals in the majority of cases; and (iii) the increase in tamoxifen dose did not increase short-term adverse events [32–35].

As detailed above and summarized in Box 1, there is strong evidence that genetic variants in *CYP2D6* affect endoxifen levels. Therefore, investigators have hypothesized that the inclusion of *CYP2D6* genotyping to guide treatment decisions could improve survival outcomes. However, the value of *CYP2D6* genotyping in this context has been under debate and further examination of this topic is required. The purpose of this systematic review with clinical practice recommendations is to therefore evaluate the evidence regarding the impact of *CYP2D6*-related genetic variation on survival outcomes after treatment with tamoxifen and provide evidence-based recommendations concerning:

1. Which patients should undergo *CYP2D6* genotyping
2. Which variants should be included in *CYP2D6* genetic tests
3. Which biological samples should be used for genetic testing

Box 1 Summary of the available evidence prior to systematic review

There is strong evidence that CYP2D6 is the major drug metabolizing enzyme of tamoxifen

There is strong evidence that CYP2D6 metabolizer status correlates with the levels of endoxifen produced from tamoxifen

There is strong evidence that the presence of strong CYP2D6 inhibitors correlates with the levels of endoxifen produced from tamoxifen

Endoxifen is identified as tamoxifen's active metabolite. There is evidence that its levels correlate with inhibition of tumor growth

These recommendations are intended for all clinicians but are especially useful to clinical oncologists who treat women with diagnoses of ER-positive breast cancer. Evidence was only evaluated regarding the treatment of ER-positive breast cancer with tamoxifen. Therefore, these recommendations do not apply to ER-negative or triple-negative breast cancer.

Methods

Systematic literature search

A systematic literature search was conducted using Embase (studies between 1980 and 7 November 2017) and Ovid Medline (studies between 1946 and 7 November 2017) on all published, peer-reviewed English-language articles. This search was performed to identify articles describing associations with genetic variants (pharmacogen* or genetic* or genom* or gene varia* or genotype* or polymorphism*) related to *CYP2D6* (cyp2d6 or "cytochrome p450 2d6") and tamoxifen (tamoxifen or nolvadex) treatment survival outcomes (survival or disease-free or mortality or recurrence or cancer-free). All original research publications describing genetic association analyses between *CYP2D6* and tamoxifen outcomes were considered eligible for inclusion. The details of the search strategy are summarized in Fig. 2 and Supplementary Table 2. Each article was reviewed by two independent investigators, and discrepancies were discussed in conjunction with a third reviewer. A grading scheme

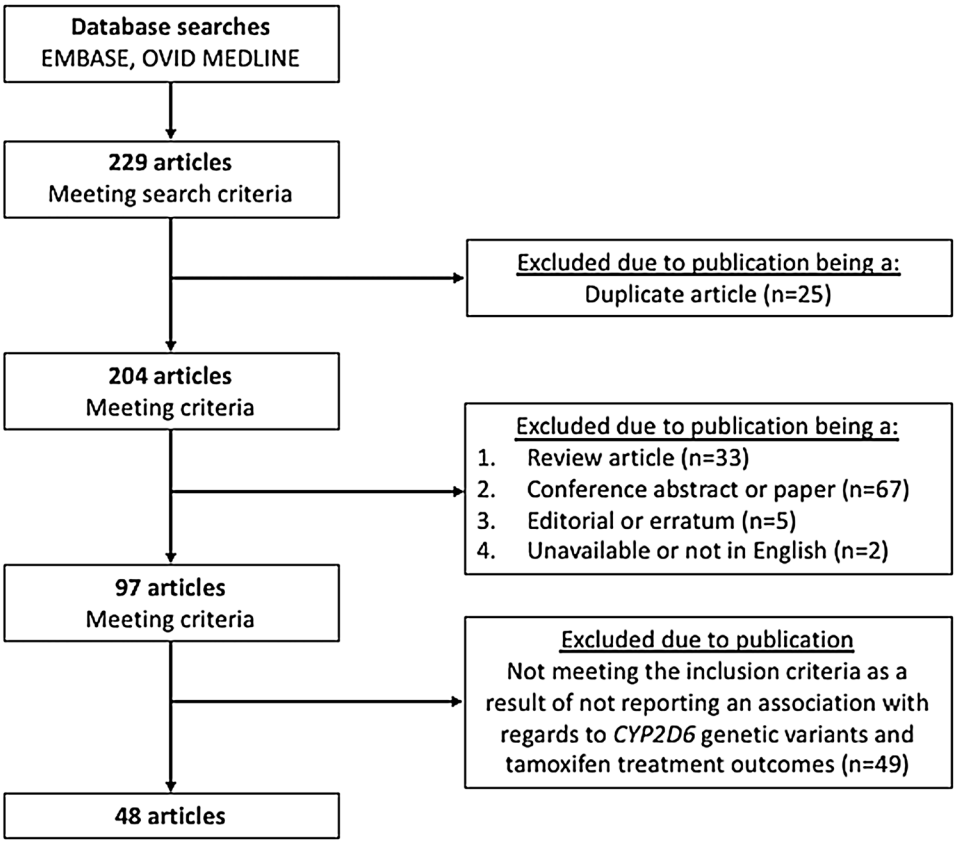

Fig. 2 PRISMA flow diagram of the articles identified through systematic literature search, indicating those included in the final critical appraisal

based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group [38, 39] was used by the authors for the critical appraisal of the literature (Supplementary Table 3) and grading of clinical practice recommendations (Supplementary Table 4). The main findings from these articles are summarized in Supplementary Table 5.

Critical appraisal of the literature

For each article, the following information was recorded and carefully evaluated where available: sample size (continuous variable), source of DNA (i.e., DNA derived solely from tumor samples or formalin-fixed, paraffin-embedded (FFPE) samples versus DNA derived from other sources), ethnicity of the cohort (i.e., European versus South East Asian versus East Asian versus Admixed versus Other) and the comprehensiveness of the *CYP2D6* genotyping assay (i.e., comprehensive versus non-comprehensive) (Supplementary Table 5). In order for an assay to be considered comprehensive, it was *a priori* determined that this would require inclusion of commonly tested non-functional/decreased function *CYP2D6* alleles reported by Crews et al. [40], with minor allele frequencies > 1% in European or South Asian or East Asian populations [41] (since > 80% of studies retrieved from the literature search focused on these populations). Genotyping was therefore considered comprehensive if at least *CYP2D6*3*, **4*, **5*, **10* and **41* were genotyped. In cohorts consisting of only East Asian patients, genotyping of *CYP2D6*3* was not required, as this allele is not present in this population.

The relationship between these variables and reported *CYP2D6*-tamoxifen survival outcome associations was assessed using a Fisher's exact test or a Wilcoxon–Mann–Whitney rank-sum test where applicable. All analyses were implemented in R and were performed irrespective of the specific survival outcome.

Results

Critical appraisal of the literature

The systematic literature search and subsequent filtering strategy identified a total of 48 articles that were included for critical appraisal purposes. To prevent duplication of information, the following articles were removed: (i) five articles that utilized samples that were already captured in other studies included in our literature search and (ii) five meta-analyses that included cohorts already captured by our literature search (Supplementary Table 5). Although the results reported by Province et al. [42] were also obtained

from a meta-analysis, these analyses included samples from cohorts that had not yet been reported in the published literature. Therefore, this article was included.

Critical appraisal of the remaining 38 articles revealed that 20 articles (52.6%) reported at least one statistically significant association with *CYP2D6* and tamoxifen survival outcomes ($P < 0.05$), while 18 articles (47.4%) reported no statistically significant associations ($P > 0.05$).

Examination of confounding factors

An assessment of confounding factors revealed no significant relationships between *CYP2D6*-survival outcome associations and sample size ($P = 0.83$), ethnicity ($P = 0.33$) or source of DNA (i.e., FFPE- or tumor-derived DNA versus other sources) ($P = 0.14$). A significant association was, however, observed when examining the type of *CYP2D6* genotyping assay used ($P = 0.006$), with comprehensive genotyping panels being more likely to report a significant association with *CYP2D6*-survival outcome.

CYP2D6 genotyping comprehensiveness and predicted metabolizer status

CYP2D6 is a highly polymorphic gene, with over 100 functional alleles identified to date, including whole gene duplications and deletions [31, 43] (Supplementary Table 6). Inadequate assessment of the variation present in the gene may therefore result in inaccurate prediction of CYP2D6 metabolizer phenotypes. This may therefore have an impact on the accuracy of association results due to unrecognized PM or IM genotypes being classified as NMs. Supporting this observation, of the studies that used comprehensive genotyping ($n = 13$), only two studies did not report significant associations between *CYP2D6* and tamoxifen survival outcomes (Fig. 3). The two studies reporting nonsignificant associations had relatively small sample sizes ($n = 92$ and $n = 106$), which may have limited their power to detect statistically significant results (Supplementary Tables 5, 7).

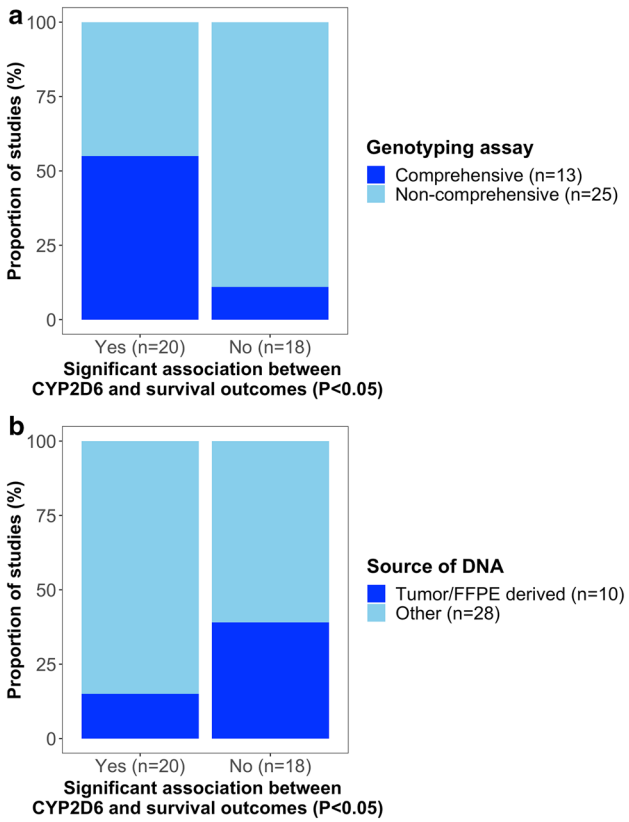

Fig. 3 Effect of source of DNA and comprehensiveness of *CYP2D6* genotyping on the reporting of significant associations between *CYP2D6* and tamoxifen survival outcomes. **a** Studies using non-comprehensive genotyping panels were less likely to identify statistically significant associations between *CYP2D6* and tamoxifen treatment outcomes ($P = 0.006$). **b** Studies using DNA derived from FFPE or tumor samples were less likely to identify statistically significant associations between *CYP2D6* and tamoxifen treatment outcomes, although this difference is not statistically significant ($P = 0.14$). *FFPE* formalin-fixed, paraffin-embedded

DNA source and the accuracy of *CYP2D6* genotyping

Due to the complexity of the *CYP2D6* locus, which includes two homologous pseudogenes, the accuracy of *CYP2D6* genotyping may be affected by the source of DNA used. Although studies have shown that there is good concordance between the genotype results obtained from DNA derived from FFPE and whole blood for certain *CYP2D6* variants [44], DNA derived from FFPE tissue is more likely to be degraded resulting in higher genotyping failures and making this tissue unsuitable for the examination of copy number variations present in *CYP2D6* [44–46]. Furthermore, a substantial proportion of breast cancers exhibit somatic loss of heterozygosity at the *CYP2D6* locus on chromosome 22q13, with *CYP2D6* loss of heterozygosity rates in The Cancer Genome Atlas data reported to be approximately 40% for luminal A and B tumors [46, 47]. Therefore, *CYP2D6* genotyping based on tumor-derived DNA may suffer from genotyping error due to the loss of alleles present in non-tumor cells. Both of these factors may reduce the sensitivity to detect associations between *CYP2D6* and tamoxifen survival outcomes and the accuracy of estimated effect sizes.

Indeed, although this difference was not statistically significant, 70% of the studies included in our literature search that used DNA derived solely from tumor or FFPE samples did not detect an association of *CYP2D6* genotype with tamoxifen survival outcomes, compared to 39% of studies using other DNA sources. Genotyping accuracy can, to some extent, be measured by testing for deviations from observed and expected genotype frequencies using

the Hardy–Weinberg equilibrium (HWE) test. In line with this, substantial deviations from HWE were observed for one of the largest tamoxifen genomic studies to date (HWE *P* values ranging from 2.1×10^{-6} to 2.1×10^{-174}) that used tumor- and FFPE-derived DNA and did not detect any significant associations between *CYP2D6* and tamoxifen survival outcomes [48, 49], raising serious concerns relating to genotyping accuracy in this study [48, 50].

Additional confounding factors

Further to genotyping considerations, other limitations that were described in the reviewed literature which may have contributed to discrepant study findings included: differences in survival outcome definitions, differences in metabolizer group classifications (e.g., differences in whether individuals with AS = 1.0 are considered NMs or IMs), low consent rates and not controlling for compliance or the use of CYP2D6 inhibitors [51]. The inability to detect associations between *CYP2D6* and tamoxifen treatment outcomes has also specifically been shown to be attributed to short follow-up times [52], not controlling for the administration of additional chemotherapy treatments [53], the use of retrospective studies performed in cohorts of convenience [54, 55] and inadequately powered studies [56].

In addition to these important considerations, the most recent and comprehensive meta-analysis of *CYP2D6* and tamoxifen treatment outcomes highlighted other important confounding factors [42]. This meta-analysis included 4,973 breast cancer patients, who were treated with tamoxifen. Although only genotyping of *CYP2D6*4* was required and the genotyping coverage varied from between sites, this study included *CYP2D6*2-*6*, **10*, **17* and **41* in the analyses to examine associations with metabolizer class (AS = 0.0 vs. AS = 0.5–1.5 vs. AS > 1.5) and tamoxifen survival outcomes. Initial meta-analyses performed in the entire cohort did not identify any significant associations. However, when the cohort was restricted to postmenopausal women with surgically resected non-metastatic invasive ER-positive breast cancer who received adjuvant tamoxifen at 20 mg/day for 5 years and were followed at least annually, statistically significant associations with both invasive disease-free survival (HR 1.25, 95% CI 1.06–1.47, $P = 0.009$) and breast cancer-free interval (HR 1.27, 95% CI 1.01–1.62, $P = 0.041$) were observed. These restricted meta-analyses did not include patients with metastatic cancer, and studies examining these individuals are limited [57, 58]. Therefore, future prospective studies should be performed in such cohorts to provide further evidence relating to whether individuals with metastatic cancer will also benefit from *CYP2D6* genotyping.

Discussion and recommendations

Critical appraisal of the literature confirmed that conflicting information exists with regard to the role that *CYP2D6* genetic variation plays in survival outcomes after treatment with tamoxifen. However, when confounding factors, such as DNA source and genotyping panels are accounted for, the majority of evidence points toward the influence that *CYP2D6* genetic variation exerts on survival outcomes after treatment with tamoxifen (+++ evidence; based on Supplementary Table 3). Based on these data, recommendations for the inclusion of *CYP2D6* genotyping in the treatment of non-metastatic ER-positive breast cancer patients are provided using the criteria listed in Supplementary Table 4. Therapeutic recommendations were developed by incorporating *CYP2D6*-based treatment decisions into the current standard of care guidelines [59, 60]. All members of this Clinical Recommendation Group agreed to the final recommendations presented in these guidelines.

Which patients should be tested for *CYP2D6?*

Postmenopausal women with non-metastatic ER-positive breast cancer in whom tamoxifen is being considered as a therapeutic alternative to aromatase inhibitors

Genetic testing for CYP2D6 is RECOMMENDED prior to initiation of adjuvant tamoxifen treatment (Grade B—Moderate)

For postmenopausal women, aromatase inhibitors (AIs) have been shown to result in superior survival outcomes when compared to tamoxifen alone [61, 62]. Of note, a recent study has shown that there are no differences in disease-free survival between patients who receive 5 years of AI treatment alone compared to those that receive 2 years of treatment with tamoxifen followed by 3 years of AIs [63]. In cases where initial tamoxifen treatment is being considered, *CYP2D6* genotyping should be performed and individuals who are CYP2D6 PMs/IMs should opt for 5 years of AI treatment. Further to this, *CYP2D6* genotyping may guide treatment decisions related to the prevention of adverse drug reactions. In postmenopausal women, estrogen synthesis is predominantly situated in adipose and breast tissue, and AIs suppress the final step of estrogen synthesis, inhibiting the conversion of androgens to estrogens [64]. However, reduction in estrogen levels during menopause increases the risk of osteoporosis and bone fractures [22] and a postmenopausal diagnosis of breast cancer accentuates this risk [65].

AIs, which reduce estrogen levels, further increase this risk [66]. In contrast, tamoxifen has protective functions against osteoporosis in both healthy women and in women with breast cancer [67–70]. Therefore, in patients in whom concerns exist regarding the adverse effects of AIs, additional information to aid in determining the benefit/risk ratio of tamoxifen versus AI treatment would be of great clinical utility [71]. In such cases, *CYP2D6* genotyping can be used to guide treatment decisions in postmenopausal women at increased risk of adverse effects from AIs, as described in Table 1.

Premenopausal women with non-metastatic ER-positive breast cancer

Genetic testing for CYP2D6 is RECOMMENDED prior to initiation of adjuvant treatment in higher-risk breast cancer patients (Grade B—moderate)

In premenopausal woman with higher-risk breast cancer, consideration of a therapy consisting of ovarian suppression combined with endocrine therapy (i.e., tamoxifen or AI) is recommended [60]. In this context, information regarding *CYP2D6* genotype and predicted metabolizer phenotype may aid in assessing the relative risks and benefits of different therapeutic options for an individual patient. Without ovarian suppression, AIs are not a viable therapeutic alternative in premenopausal women. In such patients, if tamoxifen is the only therapeutic option, *CYP2D6* genotyping prior to initiation of treatment may inform dosing decisions. Studies have shown that endoxifen levels in PMs/IMs can be significantly increased in comparison with baseline levels through increasing the dose of tamoxifen. These studies have also reported that the increase in tamoxifen dose did not result in an increase in short-term adverse events [32–35]. Based on this information, *CYP2D6* genotyping can be used to guide treatment decisions, as described in Table 1.

Which genetic variants should be tested for *CYP2D6*?

Testing of all common genetic variants is RECOMMENDED (Grade A—strong)

The evidence synthesis has provided empirical support for the importance of the comprehensiveness of *CYP2D6* genotyping for the prediction of treatment outcomes. Due to the large number of genetic variants observed in *CYP2D6*, exhaustive genotyping panels including both common and rare variants are preferred. However, where this is not feasible, patients should preferably be *genotyped for variants affecting the function of CYP2D6 occurring at a frequency of > 1%* in major ethnic groups as reported by Gaedigk et al. [31] (Supplementary Table 8). Although limited evidence is available for UMs and tamoxifen, genotyping of *CYP2D6* duplications would allow for more accurate prediction of metabolizer status and therefore should also be considered important. Subsequent to genotyping, we recommend the use of the AS system [31, 72] (Supplementary Table 1) for classification of individuals into metabolizer classes.

Which biological samples are recommended for genotyping?

The use of DNA that is derived from a source other than tumor or FFPE samples is RECOMMENDED (Grade A—strong)

Due to the susceptibility of tumor samples to accumulate additional variants and loss of heterozygosity at the *CYP2D6* locus, genomic DNA extracted from tumor samples should

Table 1 Treatment guidelines for non-metastatic ER-positive breast cancer patients based on *CYP2D6* activity scores

CYP2D6 metabolizer status	Therapeutic recommendation		Strength of recommendation
	AIs are viable treatment option	AIs are contraindicated	
PM (AS = 0.0)	AI (with ovarian suppressor in premenopausal women)	Tamoxifen (40 mg/day)[a,b]	Grade B—moderate
IM (AS = 0.5–1.0)	AI (with ovarian suppressor in premenopausal women)	Tamoxifen (40 mg/day)[b]	Grade B—moderate
NM/UM (AS ≥ 1.5)[c]	AI (with ovarian suppressor in premenopausal women) or tamoxifen (20 mg/day)[b]	Tamoxifen (20 mg/day)[b]	Grade B—moderate

AI aromatase inhibitor, *AS* activity score, *NM* normal metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer, *UM* ultra-rapid metabolizer

[a]Studies have shown that in individuals with AS = 0.0, although a dosage change increases endoxifen levels, these levels do not completely normalize. Therefore, AIs may be a preferred treatment in these individuals

[b]In individuals receiving tamoxifen, moderate or strong CYP2D6 inhibitors (refer to the Flockhart P450 Drug Interaction Table for classification of CYP2D6 inhibitors) should be avoided

[c]Evidence is currently limited regarding whether individuals with an AS = 1.0 should be considered IMs. Therefore, dosing decisions for these individuals may be made in accordance with NM/IM dosing guidelines

be avoided and *we recommend that DNA for CYP2D6 genotyping should be extracted from whole blood or saliva*. Further, due to the sequence complexity associated with *CYP2D6*, the genomic DNA used to genotype patients *should be of high quality* and should not be degraded to avoid genotyping failures and potential genotyping errors associated with pseudogenes (i.e., *CYP2D7* and *CYP2D8*).

Conclusions and future directions

The recommendations provided in this guideline are based on a large body of literature and are further supported by studies showing a relationship between endoxifen and tamoxifen efficacy [73–75], as well as a prospective study reporting an association between *CYP2D6* variants and tamoxifen response [76]. The summation of information encapsulated by the current manuscript supports the recommendations provided by Goetz and colleagues [77] and builds on these data by (i) using GRADE criteria to systematically review the available evidence, (ii) providing a comprehensive analysis of the various confounding factors that have impacted the results reported in the literature, (iii) providing specific recommendations regarding which patients should be prioritized for *CYP2D6* genotyping, what DNA should be used and which variants should be tested and (iv) targeting breast cancer audiences.

In terms of future directions, therapeutic drug monitoring (TDM) has been proposed as a promising approach to personalizing tamoxifen treatment [78]. Although TDM provides the ability to account for all factors influencing endoxifen levels, *CYP2D6* genotyping offers the unique benefit of providing predictive information that can guide treatment decisions prior to dosing, and since *CYP2D6* genotype remains constant throughout a patient's life, the test only needs to be performed once. Nonetheless, where feasible, by combining genotype-guided treatments with TDM, ER-positive non-metastatic breast cancer treatments can be further personalized. These strategies could be used to complement each other by carrying out an approach similar to that described by de Vries Schultink et al. [79] as follows:

i. All patients undergo *CYP2D6* genotyping prior to treatment initiation. Treatment decisions are made in accordance with the recommendations provided in the "Discussion and Recommendations" section.
ii. Patients in whom tamoxifen was recommended as a first-line treatment undergo TDM after 3 months of treatment. Individuals with endoxifen levels less than 6 ng/ml receive tamoxifen dose increments.
iii. Patients who exhibited endoxifen levels less than 6 ng/ml after 3 months of tamoxifen treatment are re-evaluated after another 3 months using TDM. Individuals whose endoxifen levels remain less than 6 ng/ml are switched to AI treatments.

To better inform the utility of these approaches, additional carefully designed prospective studies, further unraveling the relationship between *CYP2D6*, tamoxifen, endoxifen and treatment outcomes would be of value. Further, in vitro studies have reported that the role of endoxifen differs based on the levels of estrogen observed in postmenopausal and premenopausal women [80, 81]. Therefore, future studies should examine the relevance of these findings in real world settings. Finally, additional research should be performed to examine the effects of genetic variation outside of *CYP2D6* on tamoxifen treatment outcomes, the consequences of genetic variation on the adverse effects of AIs and the role of interpatient tumor differences on treatment outcomes. Although concerns regarding limitations in short read sequencing technologies for the analysis of complex regions of the genome such as *CYP2D6* have been brought to light [43, 82], as these technologies and accompanying bioinformatic tools continue to improve, the generation of whole genome and tumor sequences may be harnessed to provide more detailed information to guide cancer treatments.

In closing, we believe that as the body of literature surrounding the utility of *CYP2D6* genotyping for the optimization of tamoxifen continues to grow, the recommendations provided in this article will serve as a valuable tool to aid in optimizing treatment decisions prior to treatment initiation in non-metastatic ER-positive breast cancer patients. Ultimately, these recommendations will be translated into improved treatment outcomes for these patients.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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