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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 30-50% 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 towards *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

1. INTRODUCTION

1.1. 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 30-50% of patients relapsing [8, 9].

1.2. Metabolism of tamoxifen by CYP2D6

The majority of tamoxifen's anti-tumor effects are exerted by two of its major metabolites, endoxifen and 4-hydroxytamoxifen (4-HT) [10, 11]. Compared to tamoxifen, these metabolites have a 100-fold higher affinity for the ER and 30-100-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) (Figure 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].

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

1.4. 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 mg/day 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

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.

2. METHODS

2.1. 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 Figure 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 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.

2.2. 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.

3. RESULTS

3.1. 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 and colleagues [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 ($P>0.05$).

3.2. 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.

3.2.1. *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 (Figure 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 Table 5 and Supplementary Table 7).

3.2.2. 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 tumour- 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].

3.2.3. 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 ($AS=0.0$ versus $AS=0.5-1.5$ versus $AS>1.5$) 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.

4. DISCUSSION AND RECOMMENDATIONS

Critical appraisal of the literature confirmed that conflicting information exists with regards 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 towards 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.

4.1. Which patients should be tested for *CYP2D6*?

4.1.1. *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 five years of AI treatment alone compared to those that receive two years of treatment with tamoxifen followed by three 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 five 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 of 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.

4.1.2 *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

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4 this context, information regarding *CYP2D6* genotype and predicted metabolizer phenotype may aid in
5 assessing the relative risks and benefits of different therapeutic options for an individual patient.
6 Without ovarian suppression, AIs are not a viable therapeutic alternative in premenopausal women. In
7 such patients, if tamoxifen is the only therapeutic option, *CYP2D6* genotyping prior to initiation of
8 treatment may inform dosing decisions. Studies have shown that endoxifen levels in PMs/IMs can be
9 significantly increased in comparison to baseline levels through increasing the dose of tamoxifen. These
10 studies have also reported that the increase in tamoxifen dose did not result in an increase in short-term
11 adverse events [32-35]. Based on this information, *CYP2D6* genotyping can be used to guide treatment
12 decisions, as described in Table 1.
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15 16 **4.2. Which genetic variants should be tested for *CYP2D6*?**

17 *Testing of all common genetic variants is RECOMMENDED (Grade A – strong)*
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19 The evidence synthesis has provided empirical support for the importance of the comprehensiveness of
20 *CYP2D6* genotyping for the prediction of treatment outcomes. Due to the large number of genetic
21 variants observed in *CYP2D6*, exhaustive genotyping panels, including both common and rare variants
22 are preferred. However, where this is not feasible, patients should preferably be **genotyped for variants**
23 **affecting the function of *CYP2D6* occurring at a frequency of >1%** in major ethnic groups as reported by
24 Gaedigk *et al* 2016 [31] (Supplementary Table 8). Although limited evidence is available for UMs and
25 tamoxifen, genotyping of *CYP2D6* duplications would allow for more accurate prediction of metabolizer
26 status and therefore should also be considered important. Subsequent to genotyping, we recommend
27 the use of the AS system [31, 72] (Supplementary Table 1) for classification of individuals into
28 metabolizer classes.
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32 33 **4.3. Which biological samples are recommended for genotyping?**

34 *The use of DNA that is derived from a source other than tumor or FFPE samples is RECOMMENDED*
35 *(Grade A – strong)*
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37 Due to the susceptibility of tumour samples to accumulate additional variants and loss of heterozygosity
38 at the *CYP2D6* locus, genomic DNA extracted from tumour samples should be avoided and **we**
39 **recommend that DNA for *CYP2D6* genotyping should be extracted from whole blood or saliva.** Further,
40 due to the sequence complexity associated with *CYP2D6*, the genomic DNA used to genotype patients
41 **should be of high quality** and should not be degraded to avoid genotyping failures and potential
42 genotyping errors associated with pseudogenes (i.e. *CYP2D7* and *CYP2D8*).
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45 46 **4.4. Conclusions and Future Directions**

47 The recommendations provided in this guideline are based on a large body of literature, and are further
48 supported by studies showing a relationship between endoxifen and tamoxifen efficacy [73-75], as well
49 as a prospective study reporting an association between *CYP2D6* variants and tamoxifen response [76].
50 The summation of information encapsulated by the current manuscript supports the recommendations
51 provided by Goetz and colleagues [77] and builds on these data by (i) using GRADE criteria to
52 systematically review the available evidence, (ii) providing a comprehensive analysis of the various
53 confounding factors that have impacted the results reported in the literature, (iii) providing specific
54 recommendations regarding which patients should be prioritized for *CYP2D6* genotyping, what DNA
55 should be used and which variants should be tested and (iv) targeting breast cancer audiences.
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58 In terms of future directions, therapeutic drug monitoring (TDM) has been proposed as a promising
59 approach to personalizing tamoxifen treatment [78]. Although TDM provides the ability to account for
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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 using an approach similar to that described by de Vries Schultink and colleagues [79] as follows:

- (i) All patients undergo *CYP2D6* genotyping prior to treatment initiation. Treatment decisions are made in accordance with the recommendations provided in Section 4.
- (ii) Patients in whom tamoxifen was recommended as a first-line treatment undergo TDM after three months of treatment. Individuals with endoxifen levels less than 6 ng/ml receive tamoxifen dose increments.
- (iii) Patients who exhibited endoxifen levels less than 6ng/ml after three months of tamoxifen treatment are re-evaluated after another three 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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4 **COMPLIANCE WITH ETHICAL STANDARDS:**
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12
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14

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REFERENCES

1. Global Burden of Disease Pediatrics Collaboration, Fitzmaurice C, Dicker D, et al. (2015) The Global Burden of Cancer 2013. *JAMA Oncol* 1:505-527. doi: 10.1001/jamaoncol.2015.0735.
2. Dunnwald LK, Rossing MA, Li CI (2007) Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Res* 9:R6. doi: 10.1186/bcr1639.
3. Jordan VC (2003) Tamoxifen: a most unlikely pioneering medicine. *Nat Rev Drug Discov* 2:205-213. doi: 10.1038/nrd1031.
4. Maximov PY, Lee TM, Jordan VC (2013) The discovery and development of selective estrogen receptor modulators (SERMs) for clinical practice. *Curr Clin Pharmacol* 8(2):135-155.
5. Jordan VC (2007) Chemoprevention of breast cancer with selective oestrogen-receptor modulators. *Nat Rev Cancer* 7(1):46-53.
6. Cole MP, Jones CT, Todd ID (1971) A new anti-oestrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. *Br J Cancer* 25:270-275.
7. Early Breast Cancer Trialists' Collaborative Group, Davies C, Godwin J, Gray R, Clarke M, Cutter D,
8. Early Breast Cancer Trialists' Collaborative Group (1998) Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet* 351:1451-1467.
9. Kiyotani K, Mushiroda T, Nakamura Y, Zembutsu H (2012) Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters. *Drug Metab Pharmacokinet* 27:122-131.
10. Coezy E, Borgna JL, Rochefort H (1982) Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. *Cancer Res* 42:317-323.
11. Katzenellenbogen BS, Norman MJ, Eckert RL, Peltz SW, Mangel WF (1984) Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxy-tamoxifen isomers in MCF-7 human breast cancer cells. *Cancer Res* 44:112-119.
12. Barginear MF, Jaremko M, Peter I, Yu C, Kasai Y, Kemeny M, Raptis G, Desnick RJ (2011) Increasing tamoxifen dose in breast cancer patients based on CYP2D6 genotypes and endoxifen levels: effect on active metabolite isomers and the antiestrogenic activity score. *Clin Pharmacol Ther* 90:605-611. doi: 10.1038/clpt.2011.153.
13. Jordan VC (1982) Metabolites of tamoxifen in animals and man: identification, pharmacology, and significance. *Breast Cancer Res Treat* 2:123-138.
14. Lim YC, Desta Z, Flockhart DA, Skaar TC (2005) Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen. *Cancer Chemother Pharmacol* 55:471-478. doi: 10.1007/s00280-004-0926-7.
15. Klein DJ, Thorn CF, Desta Z, Flockhart DA, Altman RB, Klein TE (2013) PharmGKB summary: tamoxifen pathway, pharmacokinetics. *Pharmacogenet Genomics* 23:643-647. doi: 10.1097/FPC.0b013e3283656bc1.
16. Singh MS, Francis PA, Michael M (2011) Tamoxifen, cytochrome P450 genes and breast cancer clinical outcomes. *Breast* 20:111-118. doi: 10.1016/j.breast.2010.11.003.
17. Ahmad A, Shahabuddin S, Sheikh S, Kale P, Krishnappa M, Rane RC, Ahmad I (2010) Endoxifen, a new cornerstone of breast cancer therapy: demonstration of safety, tolerability, and systemic bioavailability in healthy human subjects. *Clin Pharmacol Ther* 88:814-817. doi: 10.1038/clpt.2010.196.
18. Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV, Desta Z, Flockhart DA, Skaar TC (2004) Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat* 85:151-159. doi: 10.1023/B:BREA.0000025406.31193.e8.

19. Wu X, Hawse JR, Subramaniam M, Goetz MP, Ingle JN, Spelsberg TC (2009) The tamoxifen metabolite, endoxifen, is a potent antiestrogen that targets estrogen receptor alpha for degradation in breast cancer cells. *Cancer Res* 69:1722-1727. doi: 10.1158/0008-5472.CAN-08-3933.
20. Thompson DS, Spanier CA, Vogel VG (1999) The Relationship Between Tamoxifen, Estrogen, and Depressive Symptoms. *Breast J* 5:375-382.
21. Lash TL, Fox MP, Westrup JL, Fink AK, Silliman RA (2006) Adherence to tamoxifen over the five-year course. *Breast Cancer Res Treat* 99:215-220. doi: 10.1007/s10549-006-9193-0.
22. Perez EA (2007) Safety profiles of tamoxifen and the aromatase inhibitors in adjuvant therapy of hormone-responsive early breast cancer. *Ann Oncol* 18 Suppl 8:viii26-35. doi: 10.1093/annonc/mdm263.
23. Shen W, Stearns V (2009) Treatment strategies for hot flushes. *Expert Opin Pharmacother* 10:1133-1144. doi: 10.1517/14656560902868217.
24. Flockhart Table [™] [<http://medicine.iupui.edu/clinpharm/ddis/main-table>]
25. Dehal SS, Kupfer D (1997) CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. *Cancer Res* 57:3402-3406.
26. Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z, Flockhart DA (2003) Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst* 95:1758-1764.
27. Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, Blanchard R, Nguyen A, Ullmer L, Hayden J, Lemler S, Weinshilboum RM, Rae JM, Hayes DF, Flockhart DA (2005) CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J Natl Cancer Inst* 97:30-39. doi: 10.1093/jnci/dji005.
28. Teft WA, Gong IY, Dingle B, Potvin K, Younus J, Vandenberg TA, Brackstone M, Perera FE, Choi YH, Zou G, Legan RM, Tirona RG, Kim RB (2013) CYP3A4 and seasonal variation in vitamin D status in addition to CYP2D6 contribute to therapeutic endoxifen level during tamoxifen therapy. *Breast Cancer Res Treat* 139:95-105. doi: 10.1007/s10549-013-2511-4.
29. Borges S, Desta Z, Li L, Skaar TC, Ward BA, Nguyen A, Jin Y, Storniolo AM, Nikoloff DM, Wu L, Hillman G, Hayes DF, Stearns V, Flockhart DA (2006) Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther* 80:61-74. doi: 10.1016/j.clpt.2006.03.013.
30. Tamoxifen Product Monograph. Mylan Pharmaceuticals ULC.2014.
31. Gaedigk A, Sangkuhl K, Whirl-Carrillo M, Klein T, Leeder JS (2016) Prediction of CYP2D6 phenotype from genotype across world populations. *Genet Med* 19:69-76 doi: 10.1038/gim.2016.80.
32. Welzen ME, Dezentje VO, van Schaik RH, Colbers AP, Guchelaar HJ, van Erp NP, den Hartigh J, Burger DM, van Laarhoven HW (2015) The Effect of Tamoxifen Dose Increment in Patients With Impaired CYP2D6 Activity. *Therapeutic drug monitoring* 37:501-507. doi: 10.1097/FTD.0000000000000195.
33. Dezentje VO, Opdam FL, Gelderblom H, Hartigh den J, Van der Straaten T, Vree R, Maartense E, Smorenburg CH, Putter H, Dieudonne AS, Neven P, Van de Velde CJ, Nortier JW, Guchelaar HJ (2015) CYP2D6 genotype- and endoxifen-guided tamoxifen dose escalation increases endoxifen serum concentrations without increasing side effects. *Breast Cancer Res Treat* 153:583-590. doi: 10.1007/s10549-015-3562-5.
34. Hertz DL, Deal A, Ibrahim JG, Walko CM, Weck KE, Anderson S, Magrinat G, Olajide O, Moore S, Raab R, Carrizosa DR, Corso S, Schwartz G, Graham M, Peppercorn JM, Jones DR, Desta Z, Flockhart DA, Evans JP, McLeod HL, Carey LA, Irvin WJ, Jr. (2016) Tamoxifen Dose Escalation in Patients With Diminished CYP2D6 Activity Normalizes Endoxifen Concentrations Without Increasing Toxicity. *Oncologist* 21:795-803. doi: 10.1634/theoncologist.2015-0480.

35. Kiyotani K, Mushiroda T, Imamura CK, Tanigawara Y, Hosono N, Kubo M, Sasa M, Nakamura Y, Zembutsu H (2012) Dose-adjustment study of tamoxifen based on CYP2D6 genotypes in Japanese breast cancer patients. *Breast Cancer Res Treat* 131:137-145. doi: 10.1007/s10549-011-1777-7.
36. Zhou SF (2009) Polymorphism of human cytochrome P450 2D6 and its clinical significance: part II. *Clin Pharmacokinet* 48:761-804. doi: 10.2165/11318070-000000000-00000.
37. Shah RR, Smith RL (2015) Addressing phenoconversion: the Achilles' heel of personalized medicine. *Br J Clin Pharmacol*. 79(2):222-240.
38. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ, Group GW (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 336:924-926. doi: 10.1136/bmj.39489.470347.AD.
39. Guyatt GH, Oxman AD, Kunz R, Falck-Ytter Y, Vist GE, Liberati A, Schunemann HJ, Group GW (2008) Going from evidence to recommendations. *BMJ* 336:1049-1051. doi: 10.1136/bmj.39493.646875.AE.
40. Crews KR, Gaedigk A, Dunnenberger HM, Klein TE, Shen DD, Callaghan JT, Kharasch ED, Skaar TC, Clinical Pharmacogenetics Implementation C (2012) Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. *Clin Pharmacol Ther* 91:321-326. doi: 10.1038/clpt.2011.287.
41. CYP2D6 Frequency Table [<https://www.pharmgkb.org/page/cyp2d6RefMaterials>]
42. Province MA, Goetz MP, Brauch H, *et al.* (2014) CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. *Clin Pharmacol Ther* 95:216-227. doi: 10.1038/clpt.2013.186.
43. Wright GE, Carleton B, Hayden MR, Ross CJ (2018) The global spectrum of protein-coding pharmacogenomic diversity. *Pharmacogenomics J*. 18:187-195 doi: 10.1038/tpj.2016.77.
44. Rae JM, Regan MM, Thibert JN, Gersch C, Thomas D, Leyland-Jones B, Viale G, Pustai L, Hayes DF, Skaar T, Van Poznak C (2013) Concordance between CYP2D6 genotypes obtained from tumor-derived and germline DNA. *J Natl Cancer Inst* 105:1332-1334. doi: 10.1093/jnci/djt204.
45. Gaedigk A, Ndjountche L, Divakaran K, Dianne Bradford L, Zineh I, Oberlander TF, Brousseau DC, McCarver DG, Johnson JA, Alander SW, Wayne Riggs K, Steven Leeder J (2007) Cytochrome P4502D6 (CYP2D6) gene locus heterogeneity: characterization of gene duplication events. *Clin Pharmacol Ther* 81:242-251. doi: 10.1038/sj.clpt.6100033.
46. Goetz MP, Sun JX, Suman VJ, Silva GO, Perou CM, Nakamura Y, Cox NJ, Stephens PJ, Miller VA, Ross JS, Chen D, Safgren SL, Kuffel MJ, Ames MM, Kalari KR, Gomez HL, Gonzalez-Angulo AM, Burgues O, Brauch HB, Ingle JN, Ratain MJ, Yelensky R (2014) Loss of heterozygosity at the CYP2D6 locus in breast cancer: implications for germline pharmacogenetic studies. *J Natl Cancer Inst* 107. doi: 10.1093/jnci/dju401.
47. Castells A, Gusella JF, Ramesh V, Rustgi AK (2000) A region of deletion on chromosome 22q13 is common to human breast and colorectal cancers. *Cancer Res* 60:2836-2839.
48. Regan MM, Leyland-Jones B, Bouzyk M, Pagani O, Tang W, Kammler R, Dell'orto P, Biasi MO, Thurlimann B, Lyng MB, Ditzel HJ, Neven P, Debled M, Maibach R, Price KN, Gelber RD, Coates AS, Goldhirsch A, Rae JM, Viale G, Breast International Group 1-98 Collaborative G (2012) CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. *J Natl Cancer Inst* 104:441-451. doi: 10.1093/jnci/djs125.
49. Stanton V, Jr. (2012) Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. *J Natl Cancer Inst* 104:1265-1266; author reply 1266-1268. doi: 10.1093/jnci/djs305.
50. Nakamura Y, Ratain MJ, Cox NJ, McLeod HL, Kroetz DL, Flockhart DA (2012) Re: CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the

- Breast International Group 1-98 trial. *J Natl Cancer Inst* 104:1264; author reply 1266-1268. doi: 10.1093/jnci/djs304.
51. Goetz MP, Ingle JN (2014) CYP2D6 genotype and tamoxifen: considerations for proper nonprospective studies. *Clin Pharmacol Ther* 96:141-144. doi: 10.1038/clpt.2014.99.
 52. Zeng Z, Liu Y, Liu Z, You J, Chen Z, Wang J, Peng Q, Xie L, Li R, Li S, Qin X (2013) CYP2D6 polymorphisms influence tamoxifen treatment outcomes in breast cancer patients: a meta-analysis. *Cancer Chemother Pharmacol* 72:287-303. doi: 10.1007/s00280-013-2195-9.
 53. Kiyotani K, Mushiroda T, Hosono N, Tsunoda T, Kubo M, Aki F, Okazaki Y, Hirata K, Takatsuka Y, Okazaki M, Ohsumi S, Yamakawa T, Sasa M, Nakamura Y, Zembutsu H (2010) Lessons for pharmacogenomics studies: association study between CYP2D6 genotype and tamoxifen response. *Pharmacogenet Genomics* 20:565-568. doi: 10.1097/FPC.0b013e32833af231.
 54. Henry NL, Hayes DF, Rae JM (2009) CYP2D6 testing for breast cancer patients: is there more to the story? *Oncology (Williston Park)* 23:1236, 1243, 1249.
 55. Goetz MP, Suman VJ, Hoskin TL, Gnant M, Filipits M, Safgren SL, Kuffel M, Jakesz R, Rudas M, Greil R, Dietze O, Lang A, Offner F, Reynolds CA, Weinshilboum RM, Ames MM, Ingle JN (2013) CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. *Clin Cancer Res* 19:500-507. doi: 10.1158/1078-0432.CCR-12-2153.
 56. Schroth W, Goetz MP, Hamann U, Fasching PA, Schmidt M, Winter S, Fritz P, Simon W, Suman VJ, Ames MM *et al* (2009) Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA* 302(13):1429-1436
 57. Lammers LA, Mathijssen RH, van Gelder T, Bijl MJ, de Graan AJ, Seynaeve C, van Fessem MA, Berns EM, Vulto AG, van Schaik RH (2010) The impact of CYP2D6-predicted phenotype on tamoxifen treatment outcome in patients with metastatic breast cancer. *Br J Cancer* 103:765-771. doi: 10.1038/sj.bjc.6605800.
 58. Lim HS, Ju Lee H, Seok Lee K, Sook Lee E, Jang IJ, Ro J (2007) Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J Clin Oncol* 25:3837-3845. doi: 10.1200/JCO.2007.11.4850.
 59. Visvanathan K, Hurley P, Bantug E, Brown P, Col NF, Cuzick J, Davidson NE, Decensi A, Fabian C, Ford L, Garber J, Katapodi M, Kramer B, Morrow M, Parker B, Runowicz C, Vogel VG, 3rd, Wade JL, Lippman SM (2013) Use of pharmacologic interventions for breast cancer risk reduction: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol* 31:2942-2962. doi: 10.1200/JCO.2013.49.3122.
 60. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Solky AJ, Stearns V, Winer EP, Griggs JJ (2016) Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update on Ovarian Suppression. *J Clin Oncol* 34:1689-1701. doi: 10.1200/JCO.2015.65.9573.
 61. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hocht-Boes G, Houghton J, Locker GY, Tobias JS, Group AT (2005) Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 365:60-62. doi: 10.1016/S0140-6736(04)17666-6.
 62. Early Breast Cancer Trialists' Collaborative Group (2015) Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* 386:1341-1352. doi: 10.1016/S0140-6736(15)61074-1.
 63. De Placido S, Gallo C, De Laurentiis M, Bisagni G, Arpino G, Sarobba MG, Riccardi F, Russo A, Del Mastro L, Cogoni AA, Cognetti F, Gori S, Foglietta J, Frassoldati A, Amoroso D, Laudadio L, Moscetti L, Montemurro F, Verusio C, Bernardo A, Lorusso V, Gravina A, Moretti G, Lauria R, Lai A, Mocerino C, Rizzo S, Nuzzo F, Carlini P, Perrone F, Investigators GIM (2018) Adjuvant anastrozole versus

- exemestane versus letrozole, upfront or after 2 years of tamoxifen, in endocrine-sensitive breast cancer (FATA-GIM3): a randomised, phase 3 trial. *Lancet Oncol* 19:474-485 doi: 10.1016/S1470-2045(18)30116-5.
64. Miller WR (2003) Aromatase inhibitors: mechanism of action and role in the treatment of breast cancer. *Semin Oncol* 30:3-11.
65. Kanis JA, McCloskey EV, Powles T, Paterson AH, Ashley S, Spector T (1999) A high incidence of vertebral fracture in women with breast cancer. *Br J Cancer* 79:1179-1181. doi: 10.1038/sj.bjc.6690188.
66. Lester J, Dodwell D, McCloskey E, Coleman R (2005) The causes and treatment of bone loss associated with carcinoma of the breast. *Cancer Treat Rev* 31:115-142. doi: 10.1016/j.ctrv.2005.01.008.
67. Ramaswamy B, Shapiro CL (2003) Osteopenia and osteoporosis in women with breast cancer. *Semin Oncol* 30:763-775.
68. Powles TJ, Hickish T, Kanis JA, Tidy A, Ashley S (1996) Effect of tamoxifen on bone mineral density measured by dual-energy x-ray absorptiometry in healthy premenopausal and postmenopausal women. *J Clin Oncol* 14:78-84.
69. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL (1992) Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *N Engl J Med* 326:852-856. doi: 10.1056/NEJM199203263261302.
70. Kristensen B, Ejlersen B, Dalgaard P, Larsen L, Holmegaard SN, Transbol I, Mouridsen HT (1994) Tamoxifen and bone metabolism in postmenopausal low-risk breast cancer patients: a randomized study. *J Clin Oncol* 12:992-997.
71. Ganz PA, Cecchini RS, Julian TB, Margolese RG, Costantino JP, Vallow LA, Albain KS, Whitworth PW, Cianfrocca ME, Brufsky AM, Gross HM, Soori GS, Hopkins JO, Fehrenbacher L, Sturtz K, Wozniak TF, Seay TE, Mamounas EP, Wolmark N (2016) Patient-reported outcomes with anastrozole versus tamoxifen for postmenopausal patients with ductal carcinoma in situ treated with lumpectomy plus radiotherapy (NSABP B-35): a randomised, double-blind, phase 3 clinical trial. *Lancet* 387:857-865. doi: 10.1016/S0140-6736(15)01169-1.
72. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS (2008) The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. *Clin Pharmacol Ther* 83:234-242. doi: 10.1038/sj.clpt.6100406.
73. Gong IY, Teft WA, Ly J, Chen YH, Alicke B, Kim RB, Choo EF (2013) Determination of clinically therapeutic endoxifen concentrations based on efficacy from human MCF7 breast cancer xenografts. *Breast Cancer Res Treat* 139:61-69. doi: 10.1007/s10549-013-2530-1.
74. Saladores P, Murdter T, Eccles D, Chowbay B, Zgheib NK, Winter S, Ganchev B, Eccles B, Gerty S, Tfayli A, Lim JS, Yap YS, Ng RC, Wong NS, Dent R, Habbal MZ, Schaeffeler E, Eichelbaum M, Schroth W, Schwab M, Brauch H (2015) Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. *Pharmacogenomics J* 15:84-94. doi: 10.1038/tpj.2014.34.
75. Madlensky L, Natarajan L, Tchu S, Pu M, Mortimer J, Flatt SW, Nikoloff DM, Hillman G, Fontecha MR, Lawrence HJ, Parker BA, Wu AH, Pierce JP (2011) Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. *Clin Pharmacol Ther* 89:718-725. doi: 10.1038/clpt.2011.32.
76. Zembutsu H, Nakamura S, Akashi ST, Kuwayama T, Watanabe C, Takamaru T, Takei H, Ishikawa T, Miyahara K, Matsumoto H, Hasegawa Y, Kutomi G, Shima H, Satomi F, Okazaki M, Zaha H, Onomura M, Matsukata A, Sagara Y, Baba S, Yamada A, Shimada K, Shimizu D, Tsugawa K, Shimo A, Tan EY, Hartman M, Chan CW, Lee SC, Nakamura Y (2016) Significant Effect of Polymorphisms in CYP2D6 on Response to Tamoxifen Therapy for Breast Cancer; a Prospective Multicenter Study. *Clin Cancer Res* 23:2019-2026 doi: 10.1158/1078-0432.CCR-16-1779.

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46
47
48
49
50
51
52
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54
55
56
57
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61
62
63
64
65
77. Goetz MP, Sangkuhl K, Guchelaar HJ, Schwab M, Province M, Whirl-Carrillo M, Symmans WF, McLeod HL, Ratain MJ, Zembutsu H, Gaedigk A, van Schaik RH, Ingle JN, Caudle KE, Klein TE (2018) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. *Clin Pharmacol Ther* 103:770-777. doi: 10.1002/cpt.1007.
 78. Binkhorst L, Mathijssen RH, Jager A, van Gelder T (2015) Individualization of tamoxifen therapy: much more than just CYP2D6 genotyping. *Cancer Treat Rev* 41:289-299. doi: 10.1016/j.ctrv.2015.01.002.
 79. de Vries Schultink AHM, Huitema ADR, Beijnen JH (2018) Therapeutic Drug Monitoring of endoxifen as an alternative for CYP2D6 genotyping in individualizing tamoxifen therapy. *Breast* 42:38-40.
 80. Maximov PY, McDaniel RE, Fernandes DJ, Korostyshevskiy VR, Bhatta P, Murdter TE, Flockhart DA, Jordan VC (2014) Simulation with cells in vitro of tamoxifen treatment in premenopausal breast cancer patients with different CYP2D6 genotypes. *Br J Pharmacol* 171:5624-5635. doi: 10.1111/bph.12864.
 81. Maximov PY, McDaniel RE, Fernandes DJ, Bhatta P, Korostyshevskiy VR, Curpan RF, Jordan VC (2014) Pharmacological relevance of endoxifen in a laboratory simulation of breast cancer in postmenopausal patients. *J Natl Cancer Inst* 106. doi: 10.1093/jnci/dju283.
 82. Drogemoller BI, Wright GE, Niehaus DJ, Emsley R, Warnich L (2013) Next-generation sequencing of pharmacogenes: a critical analysis focusing on schizophrenia treatment *Pharmacogenetics and genomics* 23(12):666-674.
 83. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, Altman RB, Klein TE (2012) Pharmacogenomics Knowledge for Personalized Medicine *Clin Pharmacol Ther* 92(4): 414-417.

FIGURE LEGENDS

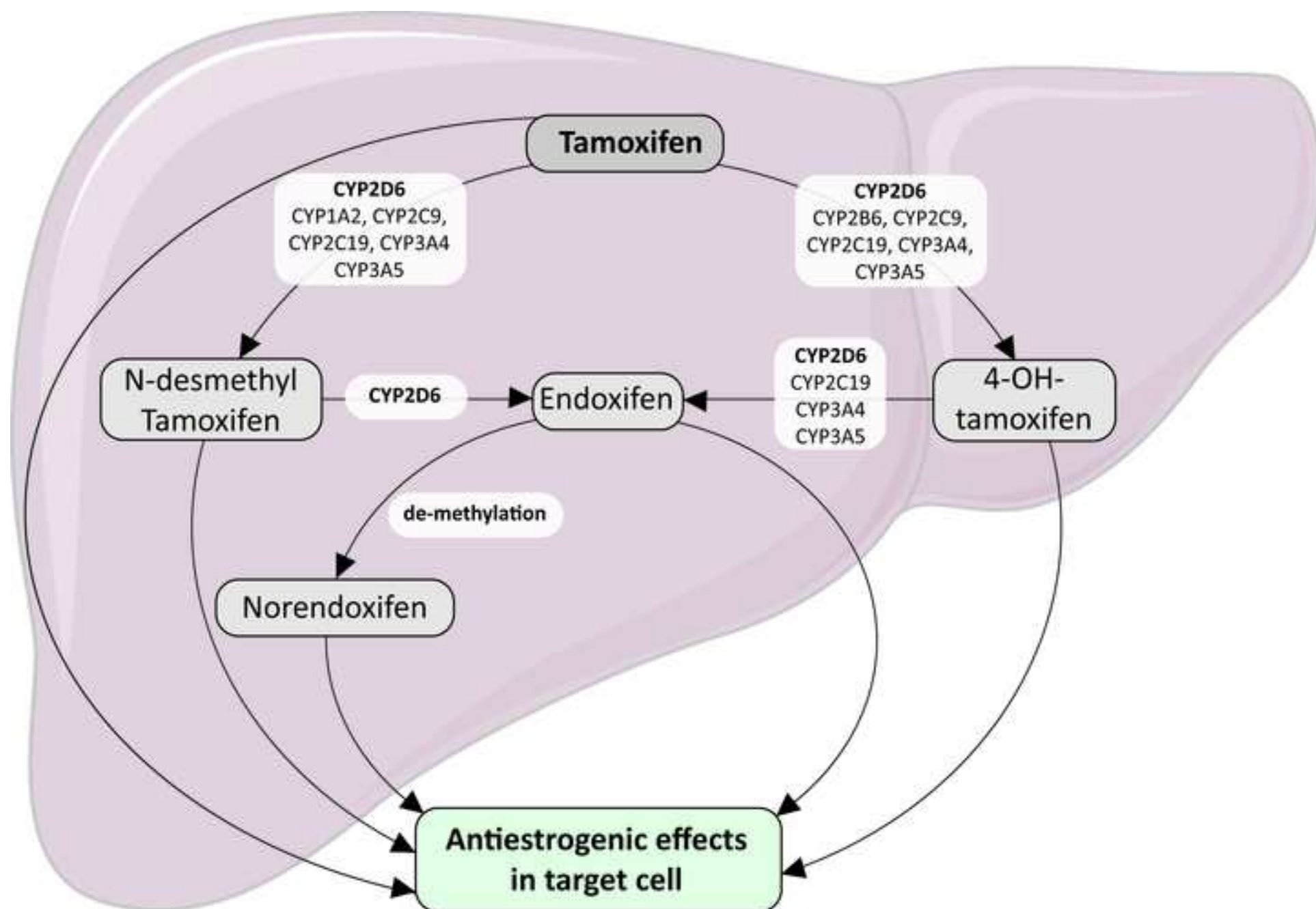
Fig. 1 The role of CYP2D6 and other CYP enzymes in the metabolisms of tamoxifen to its antiestrogenic metabolites as depicted by PharmGKB [83]. For a more detailed overview of this pharmacokinetic pathway refer to: <https://www.pharmgkb.org/pathway/PA145011119>

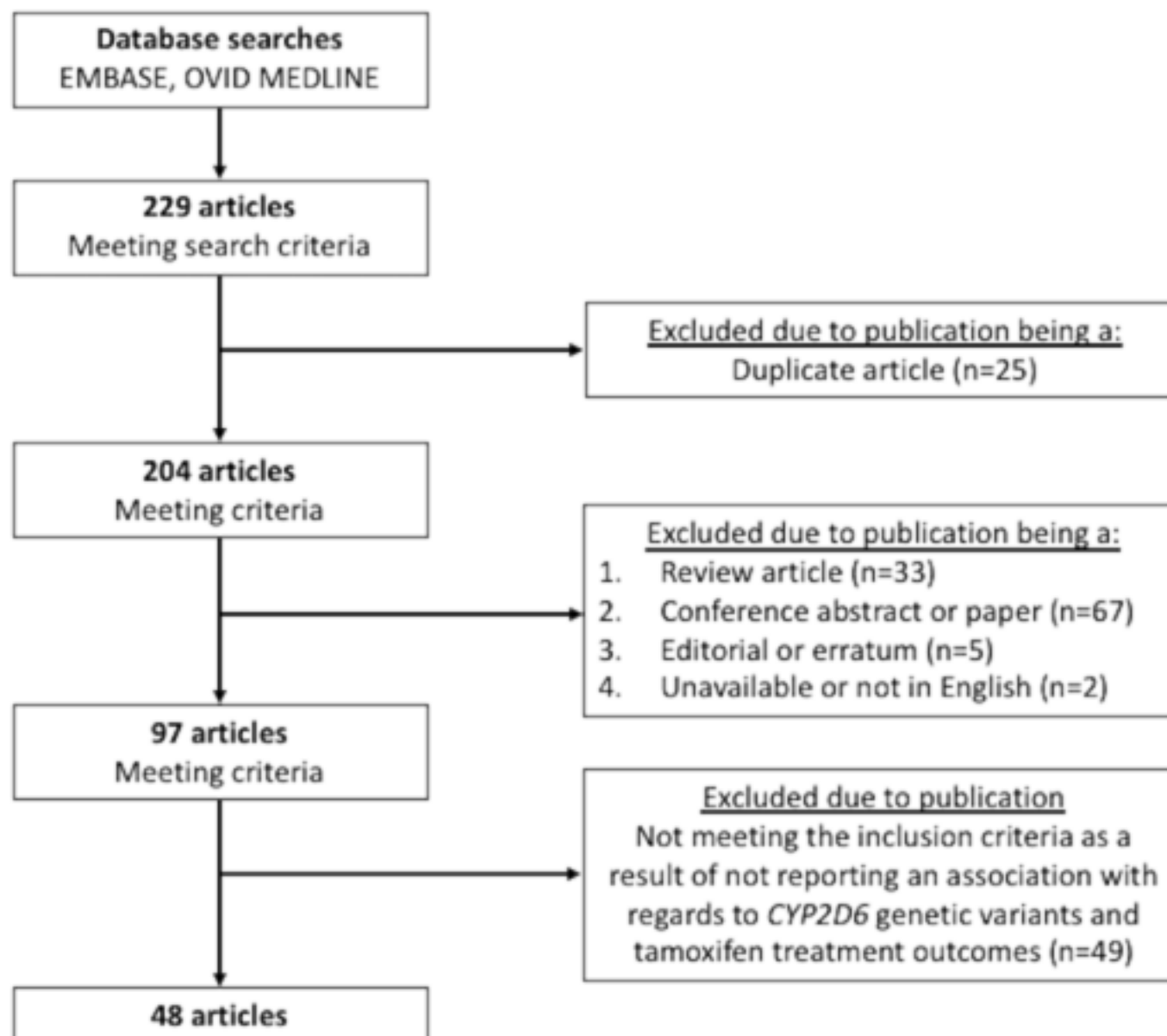
Fig. 2 PRISMA flow diagram of the articles identified through systematic literature search, indicating those included in the final critical appraisal

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

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 tumour growth





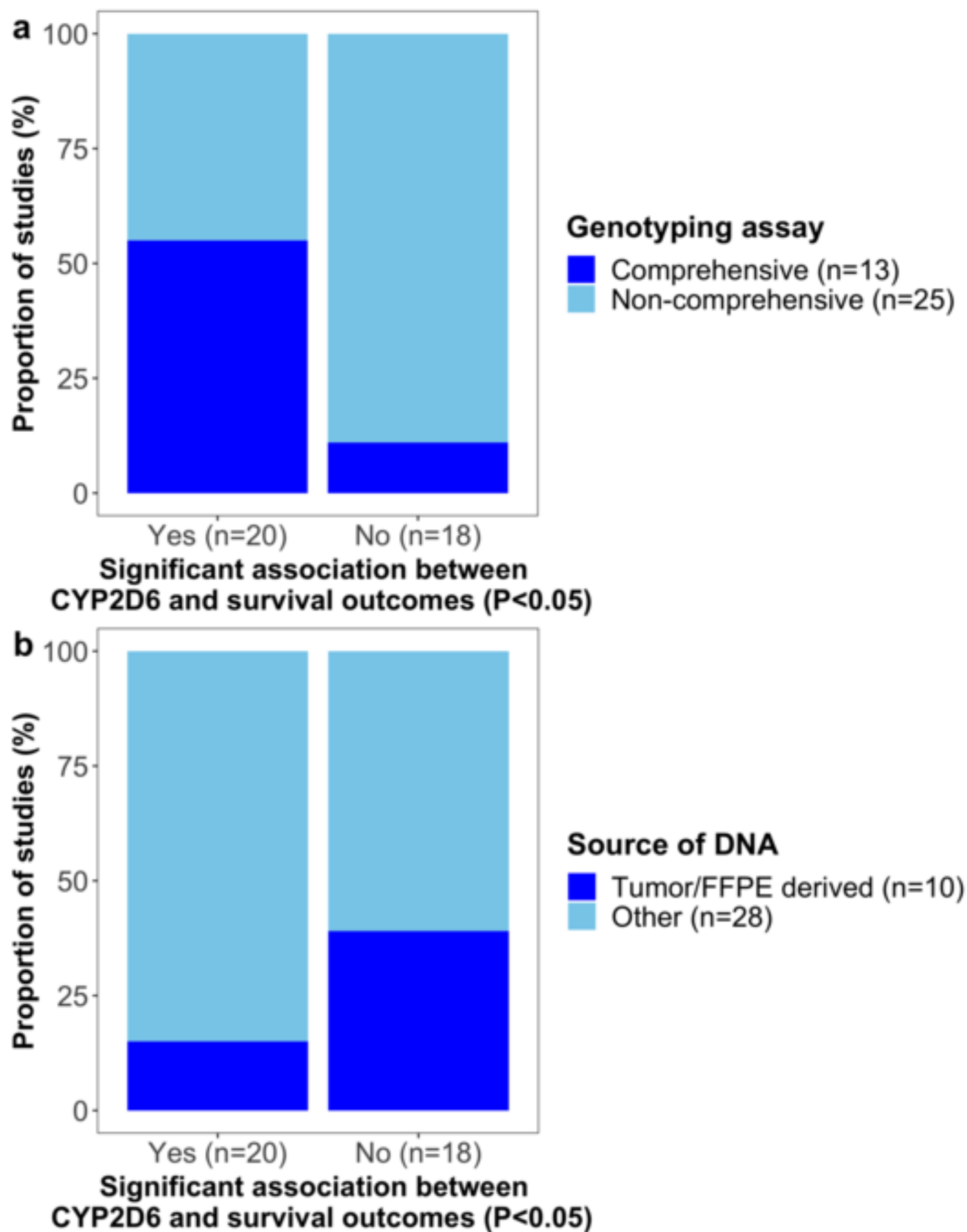


Table 1. Treatment guidelines for non-metastatic ER-positive breast cancer patients based on *CYP2D6* activity scores

<i>CYP2D6</i> metabolizer status	Therapeutic recommendation		Strength of recommendation
	Als are viable treatment option	Als are contraindicated	
PM (AS=0.0)	AI (with ovarian suppressor in premenopausal women)	Tamoxifen (40mg/day) ^{a,b}	Grade B – Moderate
IM (AS=0.5-1.0)	AI (with ovarian suppressor in premenopausal women)	Tamoxifen (40mg/day) ^b	Grade B – Moderate
NM/UM (AS≥1.5) ^c	AI (with ovarian suppressor in premenopausal women) or Tamoxifen (20mg/day) ^b	Tamoxifen (20mg/day) ^b	Grade B - Moderate

^aStudies 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; ^bIn individuals receiving tamoxifen, moderate or strong CYP2D6 inhibitors (refer to the Flockhart P450 Drug Interaction Table for classification of CYP2D6 inhibitors) should be avoided; ^cEvidence 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.

AI: Aromatase inhibitor, AS: Activity score, NM: Normal metabolizer, IM: Intermediate metabolizer, PM: Poor metabolizer, UM: Ultra-rapid metabolizer



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Category of disclosure	Description of Interest/Arrangement

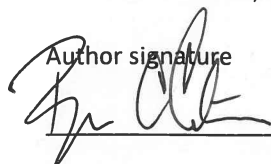
Article title CYP2D6 as a treatment decision aid for ER-positive non-metastatic breast cancer patients: a systematic review with accompanying clinical practice guidelines

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Herewith I confirm, on behalf of all authors, that the information provided is accurate.

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Date

16 August 2018