The pharmacogenomics of drug-metabolizing enzymes involved in the biotransformation of tamoxifen has become a major area of interest, owing to its potential to predict a breast cancer patient’s treatment outcome before the initiation of treatment. If the tamoxifen pharmacogenomic paradigm were to be borne out in proof of principle, patients eligible for endocrine treatment would be able to exploit it by opting for their personally most powerful medication. Most breast cancers, particularly those of postmenopausal women, are hormone receptor positive; therefore, hundreds of thousands of women worldwide initiate endocrine treatment each year. On the basis of results of the Early Breast Cancer Trialist Collaborative Group, the standard recommendation has been 5 years of therapy with the selective estrogen receptor (ER) modulator tamoxifen (1). Tamoxifen is currently prescribed in >120 countries worldwide as a component of standard adjuvant therapy in early breast cancer and in the metastatic setting for patients with steroid hormone receptor–positive breast tumors. In primary breast cancer, adjuvant tamoxifen significantly decreases relapse rates and mortality in pre- and postmenopausal patients, and the therapy benefit from 5 years of adjuvant tamoxifen is maintained, even >10 years after primary diagnosis (1). In postmenopausal women with endocrine-responsive disease, tamoxifen is a valid therapy option, along with aromatase inhibitors (AIs) (2), and is considered the standard care for the prevention of invasive breast cancer in premenopausal women at high risk, including those who have had ductal carcinoma in situ (3), and for the treatment of male breast cancer (4). Tamoxifen is generally well tolerated, and menopausal symptoms, including hot flashes, are the most common side effects. Severe side effects, such as thromboembolic events or endometrial carcinoma, are rare (1). The clinical benefit of tamoxifen has been evident for more than 3 decades; however, up to 50% of patients who receive adjuvant tamoxifen relapse or die from tumor-specific resistance or host genome–associated factors.

The field of tamoxifen pharmacogenomics gained substantial impetus from the elucidation of tamoxifen metabolism and metabolite pharmacology through studies that identified major active metabolites formed by cytochrome P450 (CYP) enzymes, particularly CYP2D6, which exhibit substantial genetic and phenotypic polymorphism. Several clinical studies have reported on the relationship of genotype and/or phenotype variants with the clinical outcome of tamoxifen.
therapy, and international efforts are currently under way to clarify this relationship.

In light of the potential for a future translation of tamoxifen pharmacogenomics into clinical practice, this review seeks to impart the underlying pharmacologic, genetic, and phenotypic principles for a mechanistic explanation of tamoxifen efficacy. It highlights the biotransformation of tamoxifen into primary and secondary metabolites with an emphasis on the clinically active metabolites 4-hydroxytamoxifen and 4-hydroxy-N-desmethyltamoxifen (endoxifen). Owing to the key role of CYP2D6, this review focuses on the relationships between the CYP2D6\(^5\) (cytochrome P450, family 2, subfamily D, polypeptide 6) genotype and phenotype. This discussion also includes the phenocopying effect of CYP2D6 inhibitors, which are frequently coadministered to alleviate hot flashes in postmenopausal women treated with tamoxifen. These basic research findings provide the scientific background for a thorough discussion of the currently available literature on tamoxifen pharmacogenetic studies. Finally, there is a possibility that other drug-metabolizing enzymes and even nonmetabolic factors can influence tamoxifen efficacy. In considering these topics, this review provides an overview of the principles of the emerging practice of personalized medicine for the improvement of the outcomes of endocrine drug treatment in breast cancer.

**Tamoxifen Metabolism and Active Metabolites**

trans-Tamoxifen [(Z)-2-4-(1,2-diphenylbut-1-enyl)-phenoxy]-N,N-dimethyl-ethanamine} undergoes extensive phase I and phase II metabolism in the human liver (Fig. 1). The bioconversion of tamoxifen involves N-oxidation, N-demethylation, and hydroxylation. Formation of the major metabolite N-desmethyltamoxifen is primarily catalyzed by CYP3A4 and 3A5, with minor contributions by CYP2D6, 1A1, 1A2, 2C19, and 2B6 (5–7). The steady-state plasma concentration of N-desmethyltamoxifen after 20 mg tamoxifen is administered daily for at least 3 months is approximately twice as high as that of the parent drug (100–290 \(\mu\)g/L vs 72–160 \(\mu\)g/L) (8–14).

This fact is of utmost clinical importance because N-desmethyltamoxifen is subject to hydroxylation, predominantly at the para position, to produce the major clinically active metabolite endoxifen. Importantly, the conversion of N-desmethyltamoxifen into endoxifen is catalyzed almost exclusively by CYP2D6 (15, 16). Plasma concentrations of endoxifen have been observed to range from a mean of 8.1 \(\mu\)g/L (n = 51) for patients with 2 variant CYP2D6 alleles to 20.7 \(\mu\)g/L (n = 55) for patients with 2 wild-type alleles (17). In addition, N-desmethyltamoxifen can also be desmethylated by CYP3A4 to form N,N-didesmethyltamoxifen.

Another clinically active metabolite is 4-hydroxytamoxifen, which is formed by 4-hydroxylation, also at the para position of the phenyl ring of the parent drug. This conversion is catalyzed by a number of CYPs, including CYP2D6, 3A4, 2C9, 2B6, and 2C19 (7, 18–21). Compared with endoxifen, the steady-state concentrations of 4-hydroxytamoxifen are lower, ranging from 1.15 \(\mu\)g/L to 6.4 \(\mu\)g/L (11, 14, 22). With the exception of endoxifen and 4-hydroxytamoxifen, no other highly active metabolites have been described thus far.

Further hydroxylation also occurs at the 4’ position of the other phenyl ring system, leading to 4’-hydroxytamoxifen, which is mainly mediated by CYP2B6 and 2D6 (7), and to 4’-hydroxy-N-desmethyltamoxifen. Another hydroxylated metabolite, \(\alpha\)-hydroxytamoxifen, is produced mainly by CYP3A4 (5, 6, 23, 24).

4-Hydroxylated metabolites undergo in vitro chemical isomerization into the respective E or cis isomers (25), which are weak ER antagonists. In addition, isomerization of 4-hydroxytamoxifen is catalyzed by CYP1B1, 2B6, and 2C19 (7). Of note, an accumulation of cis-4-hydroxytamoxifen was observed in tumors of patients whose tumors showed resistance to tamoxifen treatment (26); however, because data on the plasma concentrations of cis isomers are sparse, this observation may be regarded as preliminary. Additional hydroxylation of 4-hydroxytamoxifen by CYP3A4 and 2D6 at the phenyl moiety leads to 3,4-dihydroxytamoxifen (27), a compound that is capable of binding covalently to protein and to DNA, thereby contributing to the reported toxic and carcinoenic effects associated with tamoxifen treatment (28, 29).

Another route of tamoxifen metabolism is the formation of tamoxifen-N-oxide by flavin-containing monooxygenases 1 and 3, with a chance for tamoxifen-N-oxide to be reduced back to tamoxifen by a number of different CYPs, including CYP2A6, 1A1, 3A4, and others (30, 31). From an analytical point of view, however, this metabolite cannot be ignored because of the likelihood of chemical reduction of the N-oxide during sample preparation, a reason why the quantification of
Fig. 1. Metabolic transformation of tamoxifen in humans.
Major metabolic pathways are highlighted with bold arrows. Enzymes preferentially catalyzing a distinct metabolic step are indicated in bold. Hb, hemoglobin; FMO1, flavin-containing monoxygenase 1.
Tamoxifen-N-oxide may be regarded as a problem in the analysis of tamoxifen metabolites. Thus far, few data on this issue are available, suggesting that the N-oxide in a patient’s plasma accounts for ~15% of tamoxifen (32). At the level of phase II tamoxifen metabolism, sulfation and glucuronidation are major mechanisms. O-glucuronidation of 4-hydroxytamoxifen is mainly mediated by UDP-glucuronosyltransferases (UGTs) UGT1A4, 2B15, 2B7, 1A8, and various others to produce 4-hydroxytamoxifen-O-glucuronide (33–35). Endoxifen is predominantly glucuronidated by UGT1A10 and 1A8 to the corresponding O-glucuronide. Of note is that in addition to hydroxylated metabolites that undergo phase II metabolism at the hydroxyl moiety, tamoxifen itself is conjugated by UGT1A4 to the corresponding N-glucuronide (36, 37). In contrast to endoxifen, which does not form any N-glucuronide, 4-hydroxytamoxifen is glucuronidated by UGT1A4 at the amino group to produce 4-hydroxytamoxifen-N-glucuronide (34, 37). The sulfates of 4-hydroxytamoxifen and endoxifen are catalyzed by sulfotransferases (SULTs) SULT1E1, 1A1, and 2A1 (32, 38). E isomers of both 4-hydroxytamoxifen and endoxifen are also substrates for these conjugation reactions but seem to have different affinities for different isoenzymes (33). α-Hydroxytamoxifen is sulfatized by SULT2A1 (39); the resulting α-hydroxytamoxifen sulfate is suspected to exert carcinogenic effects after covalently binding to DNA (40, 41).

Although the number of tamoxifen metabolites that have been identified in vitro is large (Fig. 1), in vivo analytical measurements of plasma samples from tamoxifen-treated patients have quantified few metabolites, including N-desmethyltamoxifen, endoxifen, 4-hydroxytamoxifen, N,N-didesmethyltamoxifen, α-hydroxytamoxifen, and tamoxifen-N-oxide (Table 1). Therefore, there may be other, yet-unidentified tamoxifen metabolites present at relevant concentrations in patients’ plasma.

**CYP2D6 Biochemistry and Genetics**

CYP2D6 is a member of CYP enzyme family 2, which in humans constitutes one third of all CYPs and is one of the largest and best studied of isoenzyme families. Human CYPs are heme-containing monoxygenases, and the human genome contains 57 CYP genes and about the same number of pseudogenes grouped into 18 families and 44 subfamilies according to sequence similarities (http://drnelson.utmem.edu/CytochromeP450.html). CYP2D6 is involved in the metabolism of many clinically important drugs, including β-blockers, anti-arrhythmics, antihypertensives, antipsychotics, antidepressants, opioids, and others. A recent analysis of the

Table 1. Tamoxifen and metabolites.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean plasma concentrations, nmol/L</th>
<th>Effect on ER/affinity for ER (estradiol = 100%)</th>
<th>Involvement of CYP2D6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>190–420</td>
<td>Weak antagonist/2%</td>
<td>Minor</td>
</tr>
<tr>
<td>N-Desmethyltamoxifen</td>
<td>280–800</td>
<td>Weak antagonist/1%</td>
<td>Minor</td>
</tr>
<tr>
<td>N,N-Didesmethyltamoxifen</td>
<td>90–120</td>
<td>Weak antagonist</td>
<td>No</td>
</tr>
<tr>
<td>Endoxifen</td>
<td>14–130</td>
<td>Strong antagonist/equal to 4-hydroxytamoxifen</td>
<td>Almost exclusively</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen</td>
<td>3–17</td>
<td>Strong antagonist/188%</td>
<td>Among others</td>
</tr>
<tr>
<td>α-Hydroxytamoxifen</td>
<td>1</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>3,4-Dihydroxytamoxifen</td>
<td>No data available</td>
<td>Weak antagonist/high affinity</td>
<td>Together with CYP3A4</td>
</tr>
<tr>
<td>Tamoxifen-N-oxide</td>
<td>15–24</td>
<td>Weak antagonist</td>
<td>No</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen-O-glucuronide</td>
<td>No data available</td>
<td>No antagonist</td>
<td>See 4-hydroxy-tamoxifen</td>
</tr>
<tr>
<td>4-Hydroxytamoxifen-N-glucuronide</td>
<td>No data available</td>
<td>No antagonist</td>
<td>See 4-hydroxy-tamoxifen</td>
</tr>
<tr>
<td>Endoxifen-O-glucuronide</td>
<td>No data available</td>
<td>No antagonist</td>
<td>See endoxifen</td>
</tr>
<tr>
<td>α-Hydroxytamoxifen sulfate</td>
<td>No data available</td>
<td>No antagonist</td>
<td>No</td>
</tr>
</tbody>
</table>

* Range of mean plasma concentrations according to different investigators [Dowsett et al. (9), Hutson et al. (10), Jin et al. (11), Lee et al. (12), Sheth et al. (14), Lim et al. (17), Stearns et al. (22), Gjerde et al. (32), Langan-Fahey et al. (108)].

b According to Wakeling and Slater (109).

c MacCallum et al. (13) reported much higher concentrations (67 nmol/L).

d Might be due to reduction to tamoxifen.

e According to Lazarus et al. (110).
routes of elimination for the “top 200 drugs” in the US (http://www.rxlist.com; most frequently prescribed 200 drugs, April 2008) showed that 15% were drugs that are CYP2D6 substrates, compared with subfamilies CYP3A (37%) and CYP2C (33%) (42).

The human CYP2D6 locus on chromosome 22 includes the CYP2D6 gene and 2 pseudogenes [CYP2D7P1 (cytochrome P450, family 2, subfamily D, polypeptide 7 pseudogene 1) and CYP2D8P1 (cytochrome P450, family 2, subfamily D, polypeptide 8 pseudogene 1)] (43). The CYP2D6 gene consists of 9 exons and 8 introns, and the sequence is highly polymorphic. By way of clinical observation (i.e., administration of the antiarrhythmic and oxocytic drug sparteine (44) and the antihypertensive agent debrisoquine (45)), the first CYP2D6 phenotypic variant (sparteine/debrisoquine polymorphism) distinct from an extensive metabolizer (EM) phenotype was identified more than 30 years ago and was termed a “poor metabolizer” (PM) phenotype. Currently, 4 CYP2D6 phenotypes are commonly observed in Caucasian populations on the basis of their drug-oxidation capacities: EM, intermediate metabolizer (IM), PM, and ultrarapid metabolizer (UM) (46–48). Among Caucasians, about 7%–10% of individuals are PMs, 10%–15% are IMs, and, at the opposite end of the activity spectrum, up to 10%–15% are UMs.

The PM status can be deduced with >99% certainty from the presence of 2 nonfunctional alleles, with >20 null alleles having been identified (43). Therefore, it is possible to exactly predict the CYP2D6 PM phenotype (i.e., lack of catalytic function of the enzyme) by genotyping the patient’s DNA without the need to phenotype (42, 46, 48, 49). The EM phenotype is due to the presence of 1 or 2 allelic variants with wild-type function, such as *1 or *2. This phenotype can be separated by genotype into homozygous or heterozygous EMs, depending on whether they carry 1 or 2 functional alleles. Because heterozygous EMs who carry one *1 or *2 allele in combination with an IM or PM allele have somewhat impaired enzyme production and function, they have been classified as IMs, assuming a gene-dosage effect such that heterozygous EMs would have only 50% of the enzyme amount and catalytic activity of homozygous EMs. This assumption is not correct, however, and there is substantial overlap between homozygous and heterozygous EMs in both enzyme content and activity. Consequently, the genotype has a rather poor predictive value. Of note is that the IM has a phenotype and genotype distinct from the heterozygous EM (47, 50–52) that involves impaired gene expression and enzyme function (these variants include *9, *10, and *41) and/or nonfunctional variants (47, 52). Within the German population, 2%–3% are carriers of a duplicated/multiplied CYP2D6 gene and therefore have very high enzyme activity (UM). These differences in enzyme activity can have profound consequences on the plasma concentrations of drug metabolites, as has been observed for the tricyclic antidepressant nortriptyline. A >30-fold difference between PMs and UMs in steady-state plasma concentrations of nortriptyline was observed when nortriptyline was prescribed as a standard daily dose of 100–150 mg (53, 54). With respect to UM phenotype, however, only 20%–30% of UM phenotypes observed in the Caucasian population are identifiable through genotyping (46, 48, 55).

Thus far, systematic genetic analyses of large numbers of individuals have led to the discovery of >100 different alleles [http://www.cypalleles.ki.se, (56)]. At least 15 of these alleles encode nonfunctional gene products caused by aberrant splicing, nonsense codons, mutations of single base pairs, small insertions/deletions, larger chromosomal deletions of the entire CYP2D6 gene, CYP2D6/CYP2D7 hybrid genes, or mutations that cause lack of heme incorporation or otherwise produce nonfunctional full-length proteins.

There are significant ethnic differences with respect to PM, IM, and UM frequencies, heralding the possibility that different ethnic groups vary with respect to the clinical outcomes of drug therapy with CYP2D6 substrates. Within this context it is important to appreciate that the frequency of gene duplication is much higher in northeastern African populations [e.g., 29% in Ethiopia (57)] and in Saudi Arabia [21% (58)] compared with populations of European descent (59, 60). In Asian populations, however, the CYP2D6*10-associated IM is prevalent (61), with the frequency in Han Chinese being 57% and the PM playing a minor role (59).

Overall, an awareness of the CYP2D6 genotype–phenotype relationship may influence treatment decisions, particularly in cases for which an effective alternative drug is available. As in the case of orally administered codeine, which in the 10% of Caucasians who are PMs is not metabolized efficiently to morphine and therefore provides little analgesic effect, there is a chance that women with a CYP2D6 PM or IM genotype/phenotype also will not benefit from the antiestrogenic effects of tamoxifen, owing to insufficient production of active metabolites. With respect to UMs, who in cases of codeine treatment develop severe opioid side effects due to rapid morphine formation (62, 63), it is important to note that such women patients may be more susceptible to hot flashes during tamoxifen therapy.
CYP2C9, 2C19, 2B6, 3A4, and 3A5 Genetics

Other important CYP isoenzymes of subfamily 2 that are involved in the bioactivation of tamoxifen are CYP2C9, 2C19, and 2B6 (15, 18); these enzymes are also polymorphic. Of the >30 variant alleles of CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9), the *2 and *3 alleles have been thoroughly investigated and found to be associated with significant but highly variable reductions in intrinsic clearance, depending on the substrate (64). The *3 allele is more strongly affected than *2, with a reduction in enzyme activity of up to 90% for some specific drugs (65). Both alleles are present in approximately 35% of Caucasians but are less prevalent in black and Asian populations (42, 66). About 2% and 24% of individuals in the Caucasian population are homozygous and heterozygous for the variants, respectively (67). Numerous clinical studies have demonstrated the clinical significance of CYP2C9 genetics with respect to an association with higher incidences of adverse drug reactions. The most prominent example is warfarin, an anticoagulant, and several retrospective and prospective studies have confirmed that CYP2C9 genetics is clinically useful for adjusting warfarin dosage to reduce serious warfarin-related bleeding events (68, 69). The anticoagulant response also depends on the genetics of vitamin K epoxide reductase (68). Moreover, gastrointestinal bleeding from nonsteroidal antiinflammatory drugs (70) and such side effects as hypoglycemia caused by sulfonylureas (71) have also been attributed to CYP2C9 polymorphisms.

For the CYP2C19 gene (cytochrome P450, family 2, subfamily C, polypeptide 19), the known null alleles (CYP2C19*2, *3, *4, *5, *6, *7, and *8) have no CYP2C19 enzyme activity (PM); the *2 allele is prevalent in Caucasians. These null alleles are due to a splice defect (*2), a premature stop codon (*3), or an alteration in CYP2C19 structure and/or stability (72) (http://www.cypalleles.ki.se/). Recently, several new CYP2C19 alleles have been identified (*9–*25) in individuals from different racial groups; however, whether these mutations produce significant alterations in enzyme activity in vivo is not clear. CYP2C19*2 and *3 are the most frequent variants. According to genotyping and phenotyping results and in analogy to CYP2D6, the distribution of PMs shows wide interethnic differences. In Caucasian Europeans, the mean frequency of PM individuals is 3%, whereas PM frequencies as high as 23% have been identified in Asian/Oceanian populations (72, 73). Carriers of heterozygous variants constitute 32% of Caucasians, however (74). A promoter variant of CYP2C19*17 has recently been identified and shown to be associated with increased CYP2C19 activity in vivo (UM) with the CYP2C19 substrate omeprazole [a proton pump inhibitor (75)] and the antidepressant escitalopram (76). Differences in CYP2C19*17 allele frequency have been reported: 18% in both a Swedish and an Ethiopian population (75), 25% in a German population (77), and 27% in a Polish population (78). A lower frequency (4%) has been reported for Chinese individuals (75). Given these genotype/phenotype relationships, there is a possibility that the CYP2C19 UM may play a role in tamoxifen metabolism and clinical outcome, as we have reported for our breast cancer tamoxifen pharmacogenetic study (79).

With respect to CYP2B6 (cytochrome P450, family 2, subfamily B, polypeptide 6), the most common variant allele, *6, occurs at frequencies of 15%–60% across different populations (80). Genotyping of CYP2B6*6 predicted increased plasma concentrations of efavirenz and nevirapine and efavirenz-related neurotoxicity in HIV-infected individuals (81, 82), and the results suggested reducing the dose by 35% in African patients who were homozygous for CYP2B6*6 (83). These findings are in agreement with the lower activities of CYP2B6*6 isozyme, which may be substrate dependent, however. At present, any contribution of CYP2B6 variants to tamoxifen outcome is unknown.

The most important CYP isoenzyme subfamilies involved in human drug metabolism are CYP3A4 and 3A5, which participate in the metabolism of 40% of the drugs that are most frequently prescribed (42). There is little evidence for a relevant contribution of CYP3A4 (cytochrome P450, family 2, subfamily A, polypeptide 4) gene expression and enzyme function, although defective CYP3A4 mutants may account for toxicity in very rare cases (84). In contrast, genetic polymorphisms define much of the variation in CYP3A5 (cytochrome P450, family 2, subfamily A, polypeptide 5) expression. The higher incidence of the inactive CYP3A5*3 variant in Caucasians (85%–95%) vs African Americans (30%–50%) causes the lower CYP3A5 activities seen in Caucasians compared with African Americans (>30% vs 50%). CYP3A5*6 and *7 lack any functional activity and occur solely in individuals of African origin. Apart from a clear effect on the immunosuppressant tacrolimus (85), the contribution of the polymorphic CYP3A5 enzyme to CYP3A-mediated metabolism remains controversial. It is difficult to delineate the relative contributions of CYP3A4 and CYP3A5 because their protein structures, functions, and substrates are so similar. In fact, one of these enzymes may functionally compensate for the lack of the other. Whether CYP3A4 and/or CYP3A5 variants contribute to tamoxifen outcome is unknown.

6 Clinical Chemistry 55:10 (2009)
Tamoxifen Pharmacogenomics

The rationale underlying the tamoxifen pharmacogenomic principle is that variant DNA sequences of drug-metabolizing enzymes that encode proteins with reduced or absent enzyme function may be associated with lower plasma concentrations of active tamoxifen metabolites, which could have an impact on the efficacy of tamoxifen treatment. About 30 years ago, Jordan et al. characterized the first potent antiestrogen metabolite, 4-hydroxytamoxifen, and reported a 100-fold greater affinity for the ER than the parent drug (86). This metabolite was later shown to be 30- to 100-fold more potent than tamoxifen in suppressing estrogen-dependent cell proliferation (86–89). Despite its potency as an antiestrogen, the contribution of this metabolite to the overall clinical effect of tamoxifen has remained unclear, because its plasma concentrations are relatively low compared with those of tamoxifen and other metabolites (86). Our knowledge of the link between tamoxifen metabolism and treatment response rapidly expanded after the characterization of endoxifen (16, 22), which, although it had been identified in the late 1980s, initially remained obscure with respect to its biological activity. Finally, a series of laboratory studies for the characterization of its pharmacology established that endoxifen has a potency equivalent to 4-hydroxytamoxifen in terms of its binding affinity for ERs (16), suppression of estrogen-dependent proliferation of breast cancer cells (16, 89, 90), and modulation of estrogen-mediated global gene expression (91). A detailed in vitro analysis showed that endoxifen is formed mainly by 4-hydroxylation of the primary metabolite N-desmethyaltamoxifen, with the CYP2D6 enzyme catalyzing this rate-limiting step (15). Owing to the dominant role of CYP2D6 in the formation of endoxifen, variation in the CYP2D6 genotype and phenotype is at the heart of tamoxifen pharmacogenetics. The currently available evidence for this notion is based on findings obtained at 2 levels of clinical investigations, which addressed (a) the association between the concentrations of active tamoxifen metabolites either with CYP2D6 genotype or by clinical outcome, and (b) the association between CYP2D6 genotype and clinical outcome. The latter approach has shown that patients with 2 functional CYP2D6 alleles benefited the most from tamoxifen treatment. Further elucidation of the relationship between plasma concentrations of endoxifen in vivo and clinical outcomes will require additional detailed investigations with large patient cohorts.

Effects of Tamoxifen Metabolite Concentrations

Prospective cohort studies of adjuvant tamoxifen treatment have shown wide interindividual variation in the formation of tamoxifen metabolites and substantial reductions in the steady-state plasma concentrations of endoxifen during tamoxifen treatment in women carrying CYP2D6 gene variants (8, 11, 22). Moreover, convincing evidence have shown that selective serotonin reuptake inhibitors (SSRIs) such as paroxetine and fluoxetine, which are known to be strong CYP2D6 inhibitors, reduce plasma endoxifen concentrations. In particular, the phenocopy of a significant reduction in endoxifen plasma concentrations induced by SSRIs was observed in breast cancer patients homozygous for the wild-type CYP2D6 genotype, whereas the concentrations of other metabolites remained unaffected by the CYP2D6 genotype/phenotype. Although the relationship between CYP2D6 variants and plasma endoxifen concentrations was first shown for patients with the PM CYP2D6*4 genotype (11), a quantitative approach that included PM, IM, and UM genotypes substantiated this relationship (8); however, endoxifen concentrations overlap across genotypes. It follows that other factors may modify plasma endoxifen concentrations.

A relationship between CYP2D6 variants and higher concentrations of N-desmethyltamoxifen (i.e., the endoxifen precursor) has been reported at the level of chemoprevention. Significantly higher plasma concentrations of N-desmethyltamoxifen were reported for mutation carriers after 1 year of tamoxifen therapy, indicating that the conversion to clinically active endoxifen may be impaired (92).

A more recent study addressed the relationship between CYP2D6 and SULT1A1 (sulfotransferase family, cytosolic, 1A, phenol-prefering, member 1) genotypes, including the effect of SULT1A1 copy number on the pharmacokinetics of tamoxifen during steady-state treatment (32). Whereas both CYP2D6 and SULT1A1 genotypes influenced the pharmacokinetics of tamoxifen metabolites, SULT1A1 copy number did not. Lower metabolic ratios with respect to the formation of endoxifen and 4-hydroxytamoxifen but higher metabolic ratios for the formation of N-desmethyltamoxifen (endoxifen precursor) were observed in carriers of CYP2D6 variant genotypes, a result consistent with a gene-dosage effect. In contrast, patients carrying CYP2D6 alleles with high predicted enzymatic activity showed higher metabolic ratios for both active metabolites. Whether such metabolic ratios are of clinical relevance remains to be determined.

Similarly, a study of a prospective cohort of Korean patients with early or metastatic breast cancer found an association between the IM CYP2D6*10 ho-
mozygous variant and lower steady-state plasma concentrations of 4-hydroxytamoxifen and endoxifen (17), and a Chinese study found that patients homozygous for CYP2D6*10 had lower serum concentrations of 4-hydroxytamoxifen (93). The high prevalence of the CYP2D6*10 allele in East Asia, together with the IM association of impaired formation of an active metabolite, confirms the CYP2D6 PM findings in Caucasians.

Clinical Outcome of Tamoxifen Therapy and Prediction

The first evidence linking CYP2D6 variants with treatment response was obtained from a prospective randomized phase III trial of postmenopausal women with ER-positive breast cancer (North Central Cancer Treatment Group adjuvant breast cancer trial) for the investigation of the effect of adding the androgen fluoxymesterone for 1 year to the standard regimen of 5 years of adjuvant tamoxifen. The pharmacogenetic investigation of patients from the tamoxifen-only arm showed that after a median follow-up of 11.4 years, the CYP2D6*4 variant allele was an independent predictor of a higher risk of relapse and a lower incidence of hot flashes (94). A follow-up study found that in addition to CYP2D6 genetics, the phenocopying due to the coprescription of CYP2D6 inhibitors (SSRIs) was an independent predictor of breast cancer outcome in postmenopausal women taking tamoxifen (95). Recently, a robust association between CYP2D6 genotype and treatment outcome was obtained from a nonrandomized retrospective cohort of ER-positive postmenopausal breast cancer patients undergoing adjuvant tamoxifen therapy (79). At a median follow-up of 71 months, carriers of PM and IM genotypes (i.e., carriers of CYP2D6*4, *5, *10, and *41 alleles) had significantly more breast cancer recurrences, shorter relapse-free times, and worse event-free survival than carriers of functional alleles (Fig. 2). This association was not observed in postmenopausal ER-positive patients not treated with tamoxifen. Interestingly, the UM CYP2C19*17 variant also had a favorable effect on tamoxifen treatment outcome. Patients with the homozygous *17 genotype had significantly fewer breast cancer recurrences, longer relapse-free times, and better event-free survival than non-*17 carriers. Overall, this study suggested that genotyping for CYP2D6*4, *5, *10, and *41 could identify patients who would derive little benefit from adjuvant tamoxifen therapy. Although the CYP2D6 EM phenotype will identify the patients likely to benefit from tamoxifen, accounting for about 50% of all patients, the benefit will be maximal for individuals with the combination of functional CYP2D6 variants and the CYP2C19 UM. The latter will apply to one third of all patients, indicating that the tamoxifen pharmacogenetics issue will be relevant for a
substantial fraction of breast cancer patients receiving endocrine treatment.

Clinical studies from Korea, China, and Japan also have linked poor clinical outcome with CYP2D6 genetics. As expected for populations with a high prevalence of the IM CYP2D6*10 allele, the *10 homozygote genotype was associated with a poor clinical outcome in a Korean cohort of metastatic breast cancer patients, whereas the *10 heterozygote and wild-type homozygote genotypes were not (17). Likewise, patients from China who were homozygous for the CYP2D6*10 allele (93) showed an association with unfavorable disease-free survival. The latter result was substantiated through comparison with a control patient group without tamoxifen treatment, in which no association between clinical outcome and the CYP2D6*10 variant was observed. Moreover, patients homozygous for CYP2D6*10 from a Japanese breast cancer cohort that underwent adjuvant tamoxifen monotherapy showed a significantly higher incidence of recurrence within 10 years of follow-up, compared with patients with wild-type CYP2D6 (96). Although some of the sample sizes were low in the Asian studies demonstrating the genotype–efficacy correlation, the findings of the clinical implications of CYP2D6 genotypes predictive for tamoxifen efficacy are in line with the findings of others.

On the other hand, a study from the US reported no association between CYP2D6 genetics and tamoxifen outcome (97), and contradictory results for this relationship were reported in a study from Sweden, which found the CYP2D6*4 isoenzyme to be associated with a better clinical outcome in tamoxifen-treated patients (98). An extended study showed favorable disease-free survival in CYP2D6*4 carriers compared with patients homozygous or heterozygous for the functional CYP2D6 allele (99).

The issue of the role of CYP2D6 in tamoxifen therapy for breast cancer has also been addressed within the context of breast cancer prevention. For example, data from the Italian Tamoxifen Trial suggest that women with a CYP2D6*4/*4 genotype may be less likely to benefit from tamoxifen as a chemopreventive agent. This finding supports the notion of CYP2D6 playing an important role in tamoxifen’s metabolic activation and efficacy (100). Moreover, the “a priori” hypothesis that hot flashes may be an independent predictor of tamoxifen efficacy has been addressed in the Women’s Healthy Eating and Living randomized trial (101). Of the 864 patients taking tamoxifen, 674 (78%) reported hot flashes, and 12.9% of these patients had experienced recurrent breast cancer after 7.3 years of follow-up, whereas only 21% of the patients who did not have hot flashes had recurrent breast cancer during this period. Because hot flashes were a stronger predictor of a breast cancer–specific outcome than age, hormone receptor status, or tumor stage at diagnosis, the authors suggested an association between side effects, tamoxifen metabolism, and efficacy. Finally, a small study of familial breast cancer patients who were carriers of either BRCA1 (breast cancer 1, early onset) or BRCA2 (breast cancer 2, early onset) mutations and treated with tamoxifen suggested a relationship between CYP2D6 PM status and a worse survival in familial breast cancer (102); however, because of the small numbers of patients as well as the inclusion of ER-positive and ER-negative patients in this investigation, clarification provided by further studies will be needed to distinguish a pharmacogenetic effect from a poor prognostic effect in carriers of these BRCA mutations.

Given the current treatment practice of long-term estrogen deprivation in ER-positive postmenopausal breast cancer patients with the use of AIs as a valid option, the question of the impact of pharmacogenetic variation on the optimal choice for adjuvant endocrine therapy has been addressed in a modeling analysis (103). A Markov model was created to examine whether the optimal treatment strategy for patients with the wild-type CYP2D6 gene differs from that for carriers of the CYP2D6*4 mutation. The study used patients from the BIG1–98 trial, information from this trial on relapse risk, and the corresponding genotype data of Goetz et al. (94). Under the assumption that AI metabolism is independent from CYP2D6, the model suggests that the 5-year benefit of adjuvant tamoxifen therapy may exceed even that of up-front AI treatment in postmenopausal CYP2D6 EM patients.

Conclusion: Clinical Relevance of CYP2D6 in Breast Cancer

Strong mechanistic, pharmacologic, and clinical evidence, as well as modeling data, now indicates that tamoxifen efficacy and clinical outcome depend on CYP2D6 metabolism controlled by CYP2D6 enzyme polymorphisms and on pharmacologic interactions. Data from international studies have consistently demonstrated that plasma concentrations of active tamoxifen metabolites are linked with genetically determined CYP2D6 metabolizer status, phenocopying by strong CYP2D6 inhibitors, and clinical outcome. The few conflicting data may be explained by variation in the studies with respect to patient-inclusion criteria, tamoxifen doses, length of treatment, additional chemotherapy regimens, or a lack of consistent ER testing. Importantly, most authors agree that CYP2D6 gene variants, as well as inhibition of CYP2D6 by prescribed comedications such as SSRIs, may decrease tamoxifen metabolism and thus negatively affect tamoxifen efficacy and treatment outcome.
There are a number of potential clinical consequences from these emerging data on CYP2D6 and the outcomes of tamoxifen treatment. First, potent SSRIs such as paroxetine or fluoxetine should not be used to relieve hot flashes in breast cancer patients receiving tamoxifen. Although SSRIs are one of the few evidence-based therapy options for menopausal vasomotor symptoms (104), convincing data now indicate that these drugs may compromise tamoxifen efficacy via a phenocopying effect due to interference with CYP2D6-dependent tamoxifen metabolism. Yet, differences in the plasma concentrations of tamoxifen metabolites have been observed, depending on the strength of the CYP2D6 inhibitor (11, 105). If treatment of hot flashes is indicated, an SSRI such as citalopram or escitalopram or a selective norepinephrine reuptake inhibitor such as venlafaxine should be used, because these compounds have shown no appreciable inhibition of CYP2D6.

Second, the relationship between CYP2D6 genotype, phenotype, and treatment outcome points to a possible benefit of up-front CYP2D6 genotyping prior to a decision on an adjuvant endocrine treatment. A comprehensive robust, standardized, and quality-controlled CYP2D6-genotyping assay will have to test for genetic variants that could affect tamoxifen metabolism. According to the data of Goetz et al. (94) and Schroth et al. (79), such assays should include testing for common PM alleles (CYP2D6*3, *4, and *5) and for IM alleles, depending on the individual’s ethnic origin. Of note, *41 is the most frequent IM allele in Europeans, *17 is the principal IM allele in Africans, and *10 dominates in Asians (*9 should also be considered) (59). Other areas of interest with respect to clinical application are the measurement of plasma endoxifen concentrations as a surrogate of CYP2D6 phenotype.

Given alternative treatment options (i.e., tamoxifen vs AI), and considering the available scientific and clinical evidence, an individualized approach for endocrine treatment of postmenopausal breast cancer patients is desirable. One may speculate that tamoxifen alone is adequate for CYP2D6 EMs and EM carriers, whereas postmenopausal patients with variant CYP2D6 alleles may fare better with up-front AI therapy. Although this approach may be regarded as straightforward for PM patients, the best treatment may be less clear for IM patients. IM is a common phenotype among many ethnic groups, including Caucasians, African Americans, and Asians, so data on linking IM genotypes with therapeutic threshold and efficacy are in demand to adequately address the clinically important question of tamoxifen dose adjustment. Similarly, any impact of UM phenotypes on metabolite concentrations, treatment efficacy, and toxicity that have potential implications for dosing requires further investigations. Formal recommendations on the integration of CYP2D6 genotypes into treatment decisions still must await validation of these genotypes in larger retrospective studies, as is being attempted by the International Tamoxifen Pharmacogenetics Consortium (http://www.pharmgkb.org/do/serve?objId=63&objCls=Project), or prospective clinical trials. Thus far, no study has addressed the question of whether genetically predisposed differences in 4-hydroxytamoxifen and endoxifen concentrations are associated with treatment response or disease progression and with side effects such as hot flashes, including phenocopying effects; therefore, therapeutic drug monitoring as a useful surrogate is currently not available in the case of tamoxifen. Whether determination of the CYP2D6 genotype will become a diagnostic tool for selecting the appropriate adjuvant endocrine therapy for ER-positive postmenopausal breast cancer patients awaits validation in prospective clinical trials that randomize tamoxifen vs AI treatment according to CYP2D6 genotypes. Such prospective clinical trials are currently being planned.

Other open questions may address the clinical relevance of other drug-metabolizing enzymes and mutations, as well as ethnic variation, in the prevalence of their treatment outcome–relevant genotypes. Finally, there is the possibility that pharmacokinetic genes will only partly explain the pharmacogenomics of tamoxifen. It will therefore be important to also explore the contribution of pharmacodynamic genes in evaluating antiestrogen resistance as a feature of the tumor cell and in addressing the role of genes associated with estrogen-mediated cell proliferation. Within this context, it will be interesting to learn whether genes encoding the ER, its coactivators, or its corepressors (106), as well as antiestrogen resistance genes (107) and their variants, will affect the response to tamoxifen. Such results may increase the overall potential of tamoxifen pharmacogenomics.

To this end, it is important to appreciate that most cancer therapies in current use have been established empirically. The recent progress in our understanding of the pharmacology and pharmacogenetics of tamoxifen, however, holds promise for the improvement of treatments through personalized medicine. Because the genome-based approach uses CYP2D6 genotyping to predict a patient’s metabolizer phenotype, ethical issues need to be sufficiently addressed. In the light of acceptable alternatives, an informed choice about adjuvant endocrine treatment and, most importantly, avoiding a therapy that may lack efficacy must be of prime interest. It will therefore be important to make patients and their caregivers aware of these issues and to initiate discussions with regulatory authorities.
Transforming the intellectual content; and (c) final approval of the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Author Contributions: Tamoxifen Pharmacogenomics

References

20. Dehali SS, Kupfer D. Evidence that the catechol 3,4-dihydroxyltamoxifen is a proximate intermediate to the reactive species binding covalently to proteins. Cancer Res 1995;55:1283–90.
26. Dehali SS, Kupfer D. Evidence that the catechol 3,4-dihydroxyltamoxifen is a proximate intermediate to the reactive species binding covalently to proteins. Cancer Res 1995;55:1283–90.
27. Dehali SS, Kupfer D. Evidence that the catechol 3,4-dihydroxyltamoxifen is a proximate intermediate to the reactive species binding covalently to proteins. Cancer Res 1995;55:1283–90.
29. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monoxygenase (FMO)
Tamoxifen Pharmacogenomics