

Clinical Significance of CYP2D6 Polymorphisms and Tamoxifen in Women with Breast Cancer

Cindy Gaston, RPh, and Jill Kolesar, PharmD

Cindy Gaston is a Senior Clinical Pharmacist at the University of Wisconsin Hospitals and Clinics, and Dr. Kolesar is Associate Professor of Pharmacy at the University of Wisconsin in Madison, Wisconsin

Address correspondence to:
Jill Kolesar, PharmD
Associate Professor of Pharmacy (CHS)
600 Highland Ave
K4/544 Clinical Sciences Center
Madison, WI 53792
Phone: 608-262-5549; Fax: 608-265-8133
E-mail: jmkolesar@pharmacy.wisc.edu

Abstract: Tamoxifen has been used as adjuvant hormonal therapy for estrogen receptor positive breast cancer for over 30 years and is also widely used for the treatment of metastatic breast cancer. Tamoxifen is metabolized to its more active form by cytochrome P450 2D6 (CYP2D6); decreases in CYP2D6 activity, either by inactivating polymorphisms or drug interactions, can reduce concentrations of tamoxifen's active metabolites. Clinical studies demonstrate that breast cancer patients treated with adjuvant tamoxifen who have decreased CYP2D6 due to genetic polymorphisms or drug interactions may have an increased risk of recurrence and reductions in disease-free survival. Pharmacogenetic testing is currently available to predict CYP2D6 phenotypes and individualize tamoxifen therapy.

The American Cancer Society estimates there will be 184,450 new cases of breast cancer diagnosed and 40,930 deaths caused by breast cancer in the U.S. in 2008, making breast cancer the second leading cause of cancer-related death in women.¹ Between 1995 and 2004 the mortality rate for breast cancer in the United States decreased by 2.3% in females.² This decline is largely attributed to a combination of improvement in early detection, a decline in postmenopausal estrogen replacement therapy, and improved breast cancer treatment.³ Tamoxifen, a mixed estrogen receptor (ER) antagonist and agonist, has been used as adjuvant treatment of breast cancer for over 30 years. Until recently, it has been the standard treatment for women with early-stage breast cancer with node-positive disease. In addition to adjuvant therapy, tamoxifen is used for primary prevention of breast cancer and treatment of metastatic breast cancer.

Tamoxifen for the Prevention and Treatment of Breast Cancer

Over an expected lifetime of 80 years, 1 in 8 women will develop breast cancer.⁴ Women with an increased risk of breast cancer, defined as a 1.67% risk of developing breast cancer in the next

Keywords

CYP2D6, polymorphisms, tamoxifen, breast cancer

5 years, may benefit from chemoprevention.⁵ Until the recent approval of raloxifene (Evista, Eli Lilly), the only drug approved by the Food and Drug Administration (FDA) for the prevention of breast cancer was tamoxifen. A statistically significant 49% reduction ($P < .001$) in the incidence of invasive breast carcinoma after a median follow-up of 54 months was demonstrated by the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 trial involving 13,388 women over 35 years old at high risk for breast cancer.⁶ Three subsequent randomized prevention trials and a meta-analysis supported the use of tamoxifen for 5 years for chemoprevention of ER-positive breast cancer.⁷⁻¹⁰ However, the incidence of venous thromboembolic events increased by almost 2-fold and the incidence of endometrial cancer by almost 2.5-fold in the tamoxifen arm.¹⁰ A trial of 7,154 women with an increased risk of breast cancer followed for a median of 96 months demonstrated persistent benefit for 10 years after randomization, despite only 5 years of active treatment.¹¹ This trial also demonstrated an increased risk of thromboembolic disease and endometrial cancer during active treatment, but the incidence declined after active treatment was discontinued. In select patients at high risk for breast cancer and low risk for thromboembolic disease or endometrial cancer, tamoxifen is effective chemoprevention of breast cancer for 8 years or possibly longer.

Tamoxifen is also indicated for adjuvant therapy in ER-positive and/or progesterone receptor (PR)-positive early stage breast cancer after systemic chemotherapy. Five years of adjuvant tamoxifen decreased breast cancer mortality by 31% in ER-positive pre- and postmenopausal women, without regard to age, prior chemotherapy, nodal status, or progesterone status.¹² In addition, tamoxifen demonstrated a reduction in the annual recurrence rate by almost half. For premenopausal women, tamoxifen continues to be the standard of care, but for postmenopausal women, aromatase inhibitors (AIs) alone or subsequent to 2–3 years of tamoxifen may be more beneficial.¹³ An extended analysis (after a median of 100 months) of a trial comparing 5 years of anastrozole (Arimidex, AstraZeneca) therapy to tamoxifen therapy in 6,241 postmenopausal women with hormone-receptor-positive invasive breast cancer revealed a lower recurrence rate for women treated with anastrozole, with a hazard ratio (HR) of 0.76 (0.76–0.87; $P = .0001$). However, overall survival (OS; $P = .7$) and death rate after recurrence ($P = .2$) were not significant. In the treatment of ER-positive disease, tamoxifen continues to be the treatment of choice for premenopausal women; but for postmenopausal women, AIs and tamoxifen are both important options. Tamoxifen may have a role in postmenopausal patients in sequential therapy with an AI. The challenge, however, is in determining which patients

will benefit from sequential therapy with tamoxifen.

In premenopausal women with ER-positive metastatic breast cancer, first-line therapies include both tamoxifen and ovarian ablation via surgery, radiation, or medical ablation with luteinizing hormone-releasing hormone (LHRH) analogs.¹⁵ Endocrine therapy with tamoxifen or an AI is standard first-line therapy for postmenopausal patients with metastatic breast cancer. A recent Cochrane review demonstrated that while response rates were improved with chemotherapy compared to endocrine therapy, with a relative risk of 1.25 (1.01–1.54; $P = .04$) patients receiving chemotherapy do not have improvements in survival (HR, 0.94; 95% confidence interval [CI], 0.79–1.12; $P = .5$) and experience more toxicity.¹⁶ Third generation AIs such as anastrozole, exemestane, and letrozole demonstrate improved survival in this patient population and are now preferred agents.¹⁷

Tamoxifen remains a fundamental agent for primary prevention and hormonal treatment of breast cancer in premenopausal women; given that an estimated 1.1 million new cases of breast cancer are diagnosed globally each year, it continues to be a critical drug in the treatment of breast cancer.¹⁸

Cytochrome P450 2D6

Since alternative therapies such as AIs, LHRH analogs, and flutestrant are available for endocrine treatment of ER-positive breast cancer, it is vital to optimize treatment on an individual basis and maximize each patient's outcome.¹⁹ Variability in response to a drug can be due to age, sex, weight, renal and liver function, drug interactions, nutrition, and smoking status.²⁰ In addition, genetic differences in drug transporters and metabolizing enzymes can impact absorption and metabolism of certain drugs, including tamoxifen. Variability in metabolic activity is significant for tamoxifen since the metabolites are substantially more active than the parent compound.^{21,22}

Cytochrome P (CYP) 450 enzymes are responsible for the metabolism of over 90% of commonly used drugs and are located predominantly in the liver and small intestine.²³ One of the most extensively studied CYP450 enzymes is the CYP450 2D6 (CYP2D6) enzyme. Approximately 20–25% of clinically used drugs, including tamoxifen, are metabolized by this enzyme.²⁴ Other commonly used medications metabolized by CYP2D6 are tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), opioid analgesics, antipsychotic agents, and antiarrhythmic agents.²⁰

Genetic factors play a role in drug metabolism and can account for differences in response in both the efficacy and the toxicity of some drugs.²⁰ A specific gene encodes

Table 1. Common CYP2D6 Variants, Phenotypes, and Frequency^{27,42}

Phenotype	Common CYP2D6 Variant Alleles	Frequency of Phenotype		
		Caucasians	African Americans	Asians
Ultra rapid metabolizer	*1, *2, gene duplication *35 enhanced activity	2–10%	<1%	<1%
Extensive metabolizer	*1	71%	37%	51.5%
Intermediate metabolizer	*9, *10, *17, *29, *41	11%	30%	30%
Poor metabolizer	*3, *4, *5, *6	7–10%	7%	1–2%

CYP2D6, but inherited genetic alterations can result in differences in enzymatic activity. Variations in genetic sequence from the wild-type or common gene are often caused by alterations in single nucleotides and are thus designated single nucleotide polymorphisms. Over 100 allele variants of CYP2D6 have been identified; some cause inactivation of CYP2D6, some reduce or increase CYP2D6, while others do not alter normal activity.^{20,25} Most individuals have 2 alleles, so the combined activity level of the alleles determines the metabolic rate of CYP2D6. A small percentage of individuals have more than 2 alleles by means of gene duplication, which can result in higher than usual enzymatic CYP2D6 activity. Studies have documented individuals having from 2 to 13 copies of CYP2D6, and metabolic rate has been correlated with the number of active alleles.^{20,26} Although numerous allele variants exist, patients can generally be grouped into classifications of poor, intermediate, extensive, or ultrarapid metabolizers²³ (See Table 1). Poor metabolizers have 2 nonfunctional alleles. In Caucasians, 97% of poor metabolizers can be identified by 4 alleles with null activity (*3, *4, *5, *6).²⁸ Intermediate metabolizers have 2 alleles with decreased function or 1 allele with decreased function and 1 nonfunctional allele, resulting in metabolism slower than extensive metabolizers. The CYP2D6*10 allele causes decreased function and occurs frequently in Asians, causing 30% of the population to be intermediate metabolizers.²⁷ The standard or usual CYP2D6 metabolism is termed extensive, with 71% of Caucasians and 52% of Asians being classified under this term.²⁷ Ultrarapid metabolizers have active gene duplication of a functional allele. The influence of CYP2D6 polymorphism depends on the characteristic of the metabolized drug. If a drug requires activation or is converted to an active metabolite by CYP2D6, poor metabolizers may have less activation and less pharmacologic effect than extensive metabolizers. If a drug is primarily metabolized by CYP2D6 to an inactive metabolite, poor metabolizers may have less metabolism and more therapeutic effect compared to extensive metabolizers.

Several CYP450 microsomal enzymes are responsible for primary and secondary metabolism of tamoxifen to active and inactive metabolites.²² In the primary metabolic pathway, tamoxifen undergoes oxidative metabolism via the CYP450 3A4/5 (CYP3A) enzyme to an intermediary metabolite, N-desmethyltamoxifen (NDM), which is further metabolized by CYP2D6 to 4-hydroxy-N-desmethyl-tamoxifen (endoxifen).²⁹ In a separate minor metabolic pathway, tamoxifen is converted by CYP2D6, CYP3A, CYP2C9, and CYP2C19 to 4-hydroxytamoxifen (4-OH-tamoxifen). Both endoxifen and 4-OH-tamoxifen have a higher affinity for the ER and are 30 to 100 times more active than tamoxifen.^{21,22} Although both metabolites are equipotent in suppressing breast cancer, endoxifen concentrations are 6 times higher than 4-OH-tamoxifen concentrations and it is subsequently considered the primary active metabolite responsible for tamoxifen activity.^{30,31} Since the metabolism of tamoxifen to more active metabolites is primarily dependent upon CYP2D6 and CYP3A enzymes, alterations of these enzymes can affect the concentrations of active metabolites.²⁹⁻³¹

Clinical Studies

Endoxifen concentrations vary widely in clinical studies which may be attributable to interindividual differences in genetic metabolism and drug interactions.³⁰⁻³² Women with dysfunctional or nonfunctional alleles of CYP2D6 who are categorized as intermediate or poor metabolizer phenotypes and are not taking interacting drugs have lower endoxifen concentrations ($P < .05$).³² The decreased concentrations could impact many women since approximately 20% of Caucasian, 37% of African Americans, and 32% of Asians are categorized as CYP2D6 intermediate or poor metabolizer phenotypes.^{27,32} Analogous to dysfunctional or null alleles, drugs that inhibit CYP2D6 activity reduce endoxifen concentrations.³² It is not uncommon for women experiencing hot flashes related to adjunctive tamoxifen treatment to also take SSRIs,

some of which are potent CYP2D6 inhibitors.³³ Since endoxifen is the major active metabolite of tamoxifen, lower concentrations due to genetics or drug interactions could result in poorer outcomes in the prevention and treatment of breast cancer.

Several studies indicate endoxifen concentrations are lower in subjects with variant CYP2D6 alleles³⁰⁻³² (See Table 2). A small open-label trial of 12 women with ER-positive breast cancer treated with adjunct tamoxifen therapy compared tamoxifen and metabolite concentrations in women with wild-type CYP2D6*1 and variant *4, *6 and *8 alleles.³⁰ Baseline endoxifen concentrations were significantly lower in participants with a variant allele than in those with wild-type alleles ($P=.002$). A larger prospective observational trial evaluated plasma concentrations of tamoxifen and 3 metabolites in 80 pre- and postmenopausal women with newly diagnosed breast cancer, who were also genotyped for functional and select variant CYP2D6, CYP3A4, and CYP2C9 alleles.³¹ Endoxifen concentrations in participants who were either heterozygous or homozygous for nonfunctional alleles were statistically lower than endoxifen concentrations in women homozygous for the functional allele ($P<.001$) in the primarily Caucasian population. Heterozygous individuals had concentrations that were 55% (95% CI, 16.9–147.4) of concentrations observed in women homozygous for the wild-type allele. Those homozygous with the CYP2D6*4/*4 genotype had concentrations that were 26% (95% CI, 1–638.6) of participants homozygous for the wild-type allele. Although significance was established, a wide range of endoxifen concentrations were observed with each genotype which the authors attributed to untested null alleles and drug interactions. The CYP2D6 genotype was not significantly associated with concentrations of tamoxifen ($P=.92$), 4-hydroxytamoxifen ($P=.86$), or NDM ($P=.62$). Similarly, no significant difference was seen between high performance liquid chromatography plasma concentrations of tamoxifen and its metabolites and CYP2C9 and CYP3A4 genotypes. This trial only demonstrated decreased endoxifen concentrations in women with dysfunctional or null CYP2D6 alleles and no change was observed in the concentrations of the other tamoxifen metabolites.

The same trial evaluated tamoxifen and metabolite concentrations in participants concomitantly taking CYP3A4 or CYP2D6 inhibitors.³¹ Five of 78 (6%) participants taking CYP3A inhibitors along with tamoxifen had statistically higher tamoxifen concentrations ($P=.044$), but no significant change was observed in the concentrations of metabolites NDM, 4-OH-tamoxifen, and endoxifen. Twenty-four (30%) subjects were concomitantly taking medications that inhibit CYP2D6 and in those homozygous for the functional allele, the plasma

endoxifen concentration was 58% lower than those not taking CYP2D6 inhibitors ($P=.0025$). Mean endoxifen plasma concentrations of subjects taking paroxetine, a potent CYP2D6 inhibitor, were similar to those of subjects homozygous for nonfunctional CYP2D6 alleles not taking inhibitors.³³ Endoxifen concentrations in subjects taking venlafaxine, a weak CYP2D6 inhibitor, were minimally changed. Both CYP2D6 genotypes with decreased function and CYP2D6 inhibitors reduce plasma concentrations of tamoxifen's primary active metabolite and the effects can be additive.

A further report of the ongoing multicenter trial confirmed the significant reduction in endoxifen concentrations in patients also taking CYP2D6 inhibitors.³² Extensive metabolizers taking CYP2D6 inhibitors had lower plasma endoxifen concentrations ($P<.01$) than those not taking inhibitors. Weak CYP2D6 inhibitors, citalopram and sertraline, decreased mean plasma endoxifen concentrations less than the potent CYP2D6 inhibitors, paroxetine and fluoxetine ($P<.01$).³³ Endoxifen concentrations in women on potent CYP2D6 inhibitors ($n=19$) were similar to patients with CYP2D6 poor metabolizer phenotype (19.4 ± 6.1 nmol/L; $P=.43$). Similar results were observed in other CYP2D6 phenotypes ($P=.003$). The only phenotype not converted to a poor metabolizer by potent CYP2D6 inhibitors was the ultrarapid metabolizer. Another study evaluated endoxifen concentrations before and after the administration of paroxetine.³⁰ Baseline tamoxifen and metabolite concentrations were measured in women on chronic tamoxifen therapy and again 4 weeks after taking paroxetine concomitantly. Mean plasma concentrations of endoxifen decreased by 56% after adding paroxetine ($P=.02$), but concentrations of NDM and 4-OH-tamoxifen were not significantly altered. These studies consistently demonstrate a correlation between CYP2D6 inhibitors and decreased endoxifen concentrations.

Since endoxifen is the primary active metabolite of tamoxifen, decreased concentrations could impair pharmacologic activity and clinical outcomes in breast cancer treatment. Relapse-free time, disease-free survival (DFS), and OS, along with the incidence of hot flashes, were studied in a prospective trial of 223 women taking tamoxifen for early stage ER-positive breast cancer after tumor resection.³⁴ Patient tumor and/or buccal samples were genotyped for CYP2D6 *4 and *6 and CYP3A5*3 in the predominantly Caucasian population. The wild-type allele was defined by the absence of variant CYP2D6 *4 and *6 alleles. The mean follow-up time of patients alive at the end of the study was 11.4 years (range, 4.7–14.1 years). Relapse-free time, defined as the time from randomization to the documentation of local, regional, or distant breast cancer, was shorter for women homozygous

Table 2. Studies Evaluating the Effect of CYP2D6 in Breast Cancer Patients Treated with Tamoxifen

Article	Design	Patient population	Patient number	Outcome
Borges et al ³²	Multi-center prospective open-label trial of plasma concentrations of tamoxifen and its metabolites at 4, 8, and 12 months was correlated to genetic polymorphisms.	Pre- and post-menopausal women with newly diagnosed breast cancer starting on tamoxifen 20 mg/day after completion of primary surgery, radiation and adjuvant chemotherapy.	158	<ul style="list-style-type: none"> No significant difference in mean plasma concentrations of tamoxifen, NDM and 4-hydroxytamoxifen ($P=.85$, $P=.42$, $P=.29$, respectively) between patients on CYP2D6 inhibitors and those not on CYP2D6 inhibitors. Mean endoxifen concentrations were lower in patients taking CYP2D6 inhibitors than in those not taking CYP2D6 inhibitors ($P<.01$).
Stearns et al ³⁰	Prospective, open-label observational trial in women taking tamoxifen, measuring tamoxifen and its metabolites before and 4 weeks after co-administration of paroxetine 10 mg/day.	Women taking tamoxifen 20 mg/day for adjuvant treatment of breast cancer for at least 4 weeks.	12	<ul style="list-style-type: none"> Baseline plasma endoxifen concentration were lower in women with a variant CYP2D6 allele than those with the wild-type genotype ($P=.002$). Plasma concentrations of endoxifen decreased from 12.4 ng/mL to 5.5 ng/mL after paroxetine administration ($P=.004$). In women with a wild-type genotype, endoxifen concentrations decreased by 64% but only by 24% in women with a variant genotype ($P=.03$).
Jin et al ³¹	Multi-center, prospective, open label observational trial of plasma concentrations of tamoxifen and its metabolites after 1 and 4 months of therapy. Concentrations were correlated and evaluated by genotype and concurrent administration of CYP2D6 inhibitors.	Pre- and postmenopausal women with newly diagnosed breast cancer starting on tamoxifen 20 mg/day after completion of primary surgery, radiation, and adjuvant chemotherapy.	80	<ul style="list-style-type: none"> Endoxifen concentrations were significantly lower in women with CYP2D6 homozygous variant genotype ($P=.003$) or heterogenous genotype ($P=.003$) than those with the homozygous wild-type genotype. Tamoxifen, 4-hydroxytamoxifen, and NDM concentrations were not significantly different among various genotypes ($P=.92$, $P=.86$, $P=.62$, respectively). Women with homogenous wild-type genotype also taking CYP2D6 inhibitors had endoxifen concentrations 58% lower than those not on CYP2D6 inhibitors ($P=.0025$). Variant alleles of CYP3A5 and CYP2C9 did not alter endoxifen concentrations.
Goetz et al ³⁴	Multi-center prospective trial comparing RFT, DFS, and OS to genotype.	Postmenopausal women with resected ER-positive breast cancer, taking tamoxifen 20 mg daily for 5 years. No patients received chemotherapy.	223	<ul style="list-style-type: none"> Patients with CYP2D6*4/*4 genotype had worse RFT ($P=.023$) and DFS ($P=.012$), but not OS ($P=.169$). Incidence of hot flashes was lower in women with the CYP2D6*4/*4 genotype ($P=.06$).
Goetz et al ³⁵	Multi-center retrospective review of medical records; patients with decreased metabolism due to variant alleles and concomitant inhibitors were classified as PM, IM, or EM and evaluated for clinical outcome.	Postmenopausal, resected ER-positive breast cancer, treated with tamoxifen but no chemotherapy.	180	<ul style="list-style-type: none"> Compared to women with extensive CYP2D6 metabolism, patients with decreased metabolism due to phenotype or co-administration of a CYP2D6 inhibitor had a shorter time to breast cancer recurrence ($P=.015$), RFS ($P=.007$), and DFS ($P=.009$), and tended to have worse OS ($P=.083$).

(Table continues on following page)

Table 2. (Continued) Studies Evaluating the Effect of CYP2D6 in Breast Cancer Patients Treated with Tamoxifen

Article	Design	Patient population	Patient number	Outcome
Kiyotani et al ³⁷	Retrospective trial genotyped for CYP2D6 alleles and compared genotype with recurrence free survival.	Japanese women with ER- and/or PR-positive invasive breast cancer with surgical treatment followed by 5 years of tamoxifen 20 mg/day. No chemotherapy was administered.	67	<ul style="list-style-type: none"> • Patients with CYP2D6*10/*10 genotype had a significantly higher recurrence within 10 years of surgery than the CYP2D6*1/*1 genotype ($P=.0057$; OR, 16.63; 95% CI, 1.75–158.12). • Multivariate analysis indicated CYP2D6*10/*10 genotype was associated with shorter RFS ($P=.036$; adjusted HR, 10.04; 95% CI, 1.17–86.27).
Lim et al ³⁸	Prospective 2-part study using whole blood to genotype CYP2D6. Genotypes were compared to concentrations of tamoxifen and metabolites in the first portion of the study. The second portion evaluated clinical outcomes of women with metastatic breast cancer taking tamoxifen.	Korean women with ER- or PR-positive metastatic breast cancer treated with tamoxifen for a mean of 9 months; chemotherapy was administered as required and no patients were taking known CYP2D6 inhibitors.	202 for genotype-kinetic study 21 for genotype-clinical outcome study	<ul style="list-style-type: none"> • Steady state concentrations of endoxifen and 4-hydroxytamoxifen were significantly lower in the CYP2D6*10/*10 genotype than wild/*10 or wild/wild genotypes ($P<.0001$ for both metabolites). • Among nonresponders with metastatic breast cancer, CYP2D6*10/*10 was significantly more frequent ($P=.0186$).
Schroth et al ³⁹	Retrospective review of single-center database with at least 8 months of follow-up from 1986–2000; compared genotype with recurrence.	Women with primary invasive, ER-positive breast cancer receiving adjunctive tamoxifen but not chemotherapy.	206	<ul style="list-style-type: none"> • When adjusted for tumor size and nodal status, a shorter relapse time was reported for women with at least 1 variant allele (HR, 2.24; 95% CI, 1.16–4.33). • A trend in decreased OS was associated with at least 1 variant allele ($P=.11$; HR, 1.73; 95% CI, 0.88–3.41).
Nowell et al ⁴⁰	A retrospective review of a tumor registry comparing OS and progression-free survival in women with 1 or 2 variant alleles to the wild-type alleles. Concomitant administration of CYP2D6 inhibitors was not taken into account.	Pre- and postmenopausal women treated with tamoxifen and chemotherapy and/or radiation, or tamoxifen alone treated for ER- and PR-positive and negative breast cancer.	162	<ul style="list-style-type: none"> • No correlation between recurrence of disease and CYP2D6*4 genotype in tamoxifen-treated women (HR, 0.67; 95% CI, 0.33–1.35). • No association between CYP2D6 genotype and OS (HR, 0.77; 95% CI, 0.32–1.81)
Wegman et al ⁴¹	Retrospective review of RFS was compared to CYP2D6*4 genotype. Inhibitors of CYP2D6 were not taken into account.	Postmenopausal women with stage II and III ER-positive breast cancer treated with adjunctive tamoxifen 20 or 40 mg/day.	677	<ul style="list-style-type: none"> • Women with 2 CYP2D6*4 alleles had significantly better DFS than women with 1 or 2 CYP2D6*1 alleles ($P=.05$ and $P=.04$, respectively). • In a multivariate analysis with tumor stage, tumor size, and lymph node status, the result was not as strong ($P=.055$).

CI=confidence interval; CYP2D6=Cytochrome P450 (CYP450) 2D6; CYP2C9=CYP450 2C9; CYP3A5=CYP450 3A4/5; DFS=disease-free survival; EM=extensive metabolizer; ER=estrogen receptor; HR=hazard ratio; IM=intermediate metabolizer; NDM=N-desmethyltamoxifen; OR=overall response; OS=overall survival; PM=poor metabolizer; PR=progesterone receptor; RFS=relapse-free survival; RFT=relapse-free time.

for variant CYP2D6 alleles ($P=.023$). DFS time, determined as the time from randomization to documentation of any recurrence of breast cancer, contralateral breast cancer, or death from any cause, was also significantly lower ($P=.012$) in women homozygous for variant alleles. OS, the time from registration to death from any cause, was not significantly improved ($P=.169$). After controlling for node status and tumor size, women in the CYP2D6*4/*4 genotype still tended to have worse relapse-free time (HR, 1.85; $P=.176$) and DFS (HR, 1.86; $P=.089$) compared to the women with one or fewer variant alleles, although this did not reach statistical significance. No difference was found in terms of CYP3A5*3 and relapse-free time, DFS, or OS. Poorer outcomes tended to occur in women with the CYP2D6*4/*4 genotype, but other null alleles were not tested and concomitant administration of CYP2D6 inhibitors was not evaluated. Both considerations could minimize the number of women with poor metabolism and alter study results. The analysis was further updated by a retrospective chart review of CYP2D6 inhibitor administration and evaluated for clinical outcome.³⁵ Patients were placed into extensive, intermediate, and poor metabolizer groups based on the number of CYP2D6*4 alleles and co-administration of moderate or potent CYP2D6 inhibitors. After accounting for tumor size and node status, intermediate and poor metabolizers demonstrated a shorter time to recurrence ($P=.034$; HR, 1.91) and shorter time of risk free survival ($P=.017$; HR, 1.74) compared to extensive metabolizers. OS between these metabolizer groups did not differ significantly ($P=.223$; adjusted HR, 1.34). Decreased tamoxifen metabolism due to either CYP2D6 null alleles and/or CYP2D6 inhibitors can impact time to recurrence and DFS in patients treated with adjunctive tamoxifen.

In Asians, the CYP2D6*4 null allele occurs in less than 1% of the population, but the CYP2D6*10 allele, which has markedly reduced activity, occurs in 40–50% of the population.^{27,36} The effect of the CYP2D6*10 allele on clinical outcome was studied in a prospective trial of 67 Japanese women with ER- and/or PR-positive invasive breast cancer.³⁷ They were treated with adjuvant tamoxifen for 5 years and genotyped for variant alleles. No women received chemotherapy or concurrent SSRIs. Recurrence-free survival (RFS) was defined as the time from surgery to time of recurrence of local, distant or contralateral breast cancer and followed for a mean of 8 years (range, 1.6–21.6 years). Women with the CYP2D6*10/*10 genotype had a significantly higher rate of recurrence within 10 years than those with homozygous CYP2D6*1 alleles ($P=.0057$; odds ratio, 16.63; 95% CI, 1.75–158). CYP2D6 genotype and tumor size were independent variables affecting RFS. After adjustment for other prognostic factors CYP2D6*10/*10 genotype was associated

with a significantly shorter RFS ($P=.036$; adjusted HR, 10.01; 95% CI, 1.17–86.27). Another trial evaluated 21 Korean women with ER- or PR-positive metastatic breast cancer treated with tamoxifen for a median follow-up of 19.6 months (range, 6.6–53.8 months).³⁸ Chemotherapy was given as indicated, but women concurrently taking CYP2D6 inhibitors or inducers were excluded. The mean time to progression in the metastatic breast cancer group, defined as the first day of tamoxifen to progression of cancer, was shorter for women with the CYP2D6*10/*10 genotype compared to women with other genotypes (5.0 vs 21.8 months; $P=.0032$). Although the CYP2D6*10 allele does confer some activity, Asian women homozygous for this allele have poorer outcomes for both metastatic and nonmetastatic breast cancer.

A larger study evaluating multiple variant CYP2D6 alleles comparing clinical outcome also reported poorer outcomes in patients with dysfunctional or null alleles.³⁹ The retrospective review of women with primary invasive ER-positive breast cancer treated with adjunctive tamoxifen compared genotypes CYP2D6*4, *5, *10, and *41 to clinical outcome for a median of 71 months (range, 4–227 months). Those followed for less than 8 months were not included in the analysis and concomitant administration of CYP2D6 inhibitors was not evaluated due to incomplete information. Relapse-free time was defined as the time from surgery to the time of recurrence of any breast cancer, including in the contralateral breast. Women with at least 1 CYP2D6 allele with decreased or no function had shorter relapse-free time than women with the extensive metabolizer phenotype ($P=.12$). This difference was not observed in a control group of women with ER-positive breast cancer not treated with tamoxifen at the same center ($P=.32$). Analysis adjusted for tumor size and nodal status demonstrated shorter relapse time (HR, 2.24; 95% CI, 1.16–4.33) for women with at least 1 variant allele. A trend in OS, defined as the time from surgery to death from any cause, was associated with at least 1 variant allele ($P=.11$; HR, 1.73; 95% CI, 0.88–3.41). Irrespective of concomitant CYP2D6 inhibitor administration, women with genotype CYP2D6 *4, *5, *10, and *41 treated with adjunct tamoxifen therapy, but not chemotherapy, had a shorter time to recurrence of breast cancer in this study.

Two separate studies did not demonstrate poorer outcomes in women with variant CYP2D6 alleles.^{40,41} One retrospective study compared OS and progression-free survival (PFS) of CYP2D6*4 heterozygous and homozygous women to CYP2D6*1 homozygous women.⁴⁰ No association was determined for either OS (HR, 0.77; 95% CI, 0.32–1.81) or PFS (HR, 0.67; 95% CI, 0.33–1.35) for women with one or more CYP2D6*4 alleles. Concomitant administration of CYP2D6 inhibitors was not taken into account and the results could

further be impacted by the diversity of chemotherapy and radiation therapy some women received. In addition, 19% of the participants were African American and 15–26% of African Americans have a CYP2D6*17 allele that was unaccounted for in this study.²⁷ The other study evaluated the prognostic value of CYP2D6*4 in a large number of women with ER-positive stage II or III breast cancer treated with 20 or 40 mg per day of tamoxifen for 2 or 5 years.⁴¹ Frozen tumor specimens of women treated in Sweden were genotyped for CYP2D6*4 and followed for a mean length of 7.3 years (range, 0.04–17.9 years). The study results indicated that women with 2 CYP2D6*4 alleles had significantly better DFS than women with 1 or 2 CYP2D6*1 alleles ($P=.05$ and $P=.04$, respectively); however, in a multivariate analysis with tumor stage, tumor size, and lymph-node status, the result was not as strong ($P=.055$). Inhibitors of CYP2D6 were not accounted for in this study, but the investigators considered this minor since these drugs were rarely used in the study population. Comparison of these 2 studies with the others is difficult since there are multiple differences in study design, including dose of tamoxifen, duration of tamoxifen therapy, use of chemotherapeutic treatments, inclusion of CYP2D6 inhibitors, and the number of variant alleles genotyped.

Practical Implications

Multiple studies have shown a relationship between CYP2D6 genotype status, tamoxifen metabolite concentrations, and breast cancer outcomes.^{34,35,37-41} There are no large prospective trials currently available to demonstrate improved outcomes for CYP2D6 directed treatment with tamoxifen, but recent studies indicate lower endoxifen concentrations are associated with less pharmacologic activity and poorer outcomes in the poor metabolizer phenotype and concomitant administration of CYP2D6 inhibitors. The FDA considers CYP2D6 a valid biomarker for tamoxifen treatment.⁴² In 2006 an FDA advisory panel met and made recommendations to update the tamoxifen label to indicate that postmenopausal women with ER-positive breast cancer who are treated with tamoxifen are at an increased risk of recurrence if they are poor metabolizers of CYP2D6.⁴³ The panel did not reach a consensus on recommendations for genetic testing for tamoxifen because some members considered it optional rather than required.

Currently, women being started on tamoxifen therapy should consider CYP2D6 genotyping if they are candidates for alternative treatment options. While the available clinical trials were conducted in patients receiving tamoxifen as adjuvant treatment of breast cancer, the effect of CYP2D6 on tamoxifen metabolism is pharma-

cologically mediated and independent of disease setting and applies to all women receiving tamoxifen. If a woman is genotyped and determined to be a poor metabolizer, she should be treated with other therapeutic modalities. Higher doses of tamoxifen in poor metabolizers have not been studied and there is no current therapeutic concentration identified for endoxifen.

To obtain the most accurate information from CYP2D6 genotyping, the common variant alleles of the patient's ethnic or national origin need to be tested. An effective multiethnic program should test for at least CYP2D6*3–6, *9, *10, *17, *41 alleles; however, testing for multiple alleles via polymerase chain reaction can be time prohibitive. Until recently, CYP2D6 genotyping was not readily available for commercial use. In January 2005, the FDA approved a CYP450 array (AmpliChip, Roche), which quickly analyzes variants of CYP2D6 and CYP2C19. It can precisely and accurately predict 20 CYP2D6 alleles from a whole blood sample.⁴⁴ Commercial laboratories commonly utilize this technology and are able to provide results within a few days. The cost of CYP2D6 genotyping is \$500 to \$600 per patient and generally is not covered by insurance, but should be viewed as an investment in individualized patient therapy for the prevention of life-threatening recurrences. Of the expected 180,000 newly diagnosed cases of breast cancer in 2008,² roughly 6,000 of these women will have ER-positive disease and will be poor metabolizers of CYP2D6. Pharmacogenetic testing could potentially alter their outcomes.

Conclusion

Studies evaluating genotype and tamoxifen concentrations or outcomes have included premenopausal women as well as postmenopausal women, and stages of breast cancer ranging from newly diagnosed invasive cancer to metastatic breast cancer.^{30-32,34-41} Poor metabolizer phenotype and concomitant administration of potent CYP2D6 inhibitors decrease the concentrations of endoxifen at all stages of breast cancer and may also impact women taking tamoxifen for primary prevention. Further study is required to evaluate the significance of poor metabolizers and CYP2D6 inhibitors in primary prevention, evaluate tamoxifen dosage adjustment in poor metabolizers, and determine the optimal sequence of tamoxifen and AIs in ultrarapid metabolizers. The metabolism of AIs and raloxifene is unaffected by CYP2D6 metabolism and these agents may be considered as therapeutic alternatives in poor metabolizers of CYP2D6. With further study, genotyping could provide a means for optimizing outcomes in the prevention and treatment of ER-positive breast cancer with hormonal therapy.

References

- Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin*. 2008;58:71-96.
- Ries LAG, Melbert D, Krapcho M, et al. SEER cancer statistics review 1975-2004. National Cancer Institute. Available at http://seer.cancer.gov/cgi-bin/csr/1975_2004/search.pl. Accessed January 25, 2008.
- Berry DA, Cronin KA, Plevritis SK, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med*. 2005;353:1784-1792.
- Feuer EJ, Wun LM, Borning CC, et al. The lifetime risk of developing breast cancer. *J Natl Cancer Inst*. 1993;85:892-897.
- SEER Cancer Statistics. Available at http://seer.cancer.gov/cgi-bin/csr/1975_2004/search. Accessed April 12, 2008.
- Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst*. 1998;90:1371-1388.
- Cuzick J, Forbes J, Edwards R, et al; International Breast Cancer Intervention Study I Investigators. First results from the International Breast Cancer Intervention Study (IBIS-1): a randomized prevention trial. *Lancet*. 2002;260:817-824.
- Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomized chemoprevention trial. *Lancet*. 1998;352:98-101.
- Veronesi U, Maisonneuve P, Costa A, et al; Italian Tamoxifen Prevention Study Group. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomized trial among hysterectomized women. *Lancet*. 1998;352:93-97.
- Cuzick J, Powles T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. *Lancet*. 2003;361:296-300.
- Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer – 96-month follow-up of the randomized IBIS-1 trial. *J Natl Cancer Inst*. 2007;99:272-282.
- Early Breast Cancer Trialists' Collaborative Group (EBCTG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomized trials. *Lancet*. 2005;365:1687-1717.
- Winer EP, Hudis C, Burstein HJ, et al. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer: status report 2004. *J Clin Oncol*. 2005;23:619-629.
- The Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100 month analysis of the ATAC trial. *Lancet*. 2008;9:45-53.
- Crump M, Sawka CA, DeBoer G, et al. An individual patient-based meta-analysis of tamoxifen versus ovarian ablation as first line endocrine therapy for premenopausal women with metastatic breast cancer. *Breast Cancer Res Treat*. 1997;44:201-210.
- Wilcken N, Hornbuckle J, Ghersi D. Chemotherapy alone versus endocrine therapy alone for metastatic breast cancer. *Cochrane Database Syst Rev*. 2003;(2):CD002747.
- Gibson LJ, Dawson CK, Lawrence DH, Bliss JM. Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. *Cochrane Database Syst Rev*. 2007;(1):CD003370.
- Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. *CA Cancer J Clin*. 2005; 55:74-108.
- Pruthi S, Boughey JC, Brandt KR, et al. A multidisciplinary approach to the management of breast cancer, part 2: therapeutic considerations. *Mayo Clin Proc*. 2007;82:1131-1140.
- Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med*. 2005;352:2211-2221.
- Johnson MD, Zuo H, Lee KH, et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat*. 2004;85:151-159.
- Lim YC, Desta Z, Flockhart DA, Skaar TC. Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen. *Cancer Chemother Pharmacol*. 2005;55:471-478.
- Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med*. 2003;348:538-556.
- Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*. 2005;5:6-13.
- Home of the Human Cytochrome P450 Allele Nomenclature Committee. Available at <http://www.cypalleles.ki.se/cyp2d6.htm>. Accessed 2/21/08.
- Dalen P, Dahl ML, Ruiz ML, et al. 10-hydroxylation of nortriptyline in white persons with 0, 1, 2, 3 and 13 functional CYP2D6 genes. *Clin Pharmacol Ther*. 1998;63:429-439.
- Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002;3:229-243.
- Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequence. *Am J Hum Genet*. 1997;60:284-295.
- Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles of CYP3A and CYP2D6. *J Pharmacol Exp Ther*. 2004;310:1062-1075.
- Stearns V, Johnson MD, Rae JM, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst*. 2003;95:1758-1764.
- Jin Y, Desta Z, Stearns V, et al. CYP2D6 genotype, antidepressant use and tamoxifen metabolism during adjuvant breast cancer treatment. *J Natl Cancer Inst*. 2005;95:30-39.
- Borges S, Desta Z, Li L, et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther*. 2006;80:61-74.
- Hemeryck A, Belpaire FM. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. *Curr Drug Metab*. 2002;3:13-37.
- Goetz MP, Rae JM, Suman VJ, et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes and efficacy and hot flashes. *J Clin Oncol*. 2005;23:9312-9318.
- Goetz MP, Knox SK, Suman VJ, et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res Treat*. 2007;101:113-121.
- Yokota H, Tamura S, Furuya H, et al. Evidence for a new variant CYP2D6 allele CYP2D6*10 in Japanese populations associated with lower in vitro rates of sparteine metabolism. *Pharmacogenetics*. 1993;3:256-263.
- Kiyotani K, Mushiroya T, Sasa M, et al. Impact of CYP2D6*10 on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy. *Cancer Sci*. 2008;99:995-999. Epub 2008 Feb 24.
- Lim HS, Lee HJ, Lee KS, et al. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J Clin Oncol*. 2007;24:3837-3845.
- Schroth W, Antoniadou L, Fritz P, et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol*. 2007;25:5187-5193.
- Nowell SA, Ahn J, Rae JM, et al. Association of genetic variation in tamoxifen-metabolizing enzymes and overall survival and recurrence of disease in breast cancer patients. *Breast Cancer Res Treat*. 2005;91:249-258.
- Wegman P, Elingarami S, Casrtenen J, et al. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. *Breast Cancer Res*. 2005;7:R284-R290.
- Food and Drug Administration Website. Available at http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm. Accessed April 24, 2008.
- Food and Drug Administration. Available at <http://www.fda.gov/ohrms/dockets/ac/cder06.html#PharmScience>. Accessed April 24, 2008.
- Heller T, Kirchheiner J, Armstrong W, et al. AmpliChip CYP450 Gene Chip®: A new gene chip that allows rapid and accurate CYP2D6 testing. *Ther Drug Monit*. 2006;28:673-677.