Published in final edited form as:

Pharmacol Ther. 2013 July ; 139(1): 1–11. doi:10.1016/j.pharmthera.2013.03.001.

Pharmacogenomics of Breast Cancer Therapy: An Update

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Abstract

Clinical and histopathologic characteristics of breast cancer have long played an important role in treatment decision-making. Well-recognized prognostic factors include tumor size, node status, presence or absence of metastases, tumor grade, and hormone receptor expression. High tumor grade, presence of hormone receptors, and HER2-positivity are a few predictive markers of response to chemotherapy, endocrine manipulations, and anti-HER2 agents, respectively. However, there is much heterogeneity of outcomes in patients with similar clinical and pathologic features despite equivalent treatment regimens. Some of the difference in response to specific therapies can be attributed to somatic tumor characteristics, such as degree of estrogen receptor expression and HER2 status. In recent years, there has been great interest in evaluating the role that pharmacogenetics/pharmacogenomics, or variations in germline DNA, play in alteration of drug metabolism and activity, thus leading to disparate outcomes among patients with similar tumor characteristics. The utility of these variations in treatment decision-making remains debated. Here we review the data available to date on genomic variants that may influence response to drugs commonly used to treat breast cancer. While none of the variants reported to date have demonstrated clinical utility, ongoing prospective studies and increasing understanding of pharmacogenetics will allow us to better predict risk of toxicity or likelihood of response to specific treatments and to provide a more personalized therapy.

Introduction

Breast cancer continues to be the leading malignancy diagnosed in women in Western societies. It is estimated that 226,870 women will be diagnosed with and 39,510 women will die of breast cancer in the United States in 2012 (National Cancer Institute, 2012). Treatment for breast cancer is constantly evolving as new technologies, agents, and

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Conflict of interest:

VS received investigator-initiated research funding from Abraxis (Celgene), Merck, Novartis and Pfizer.

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strategies are discovered. Advances in the early detection and adjuvant treatment of breast cancer have already led to a significant reduction in disease-related relapse and death (Berry, et al., 2005; Early Breast Cancer Trialists' Collaborative, 2012). However, there is significant variation in drug response and survival outcomes in individuals treated with equivalent regimens, including hormonal agents, cytotoxic agents, and novel targeted therapies. Traditionally, clinical and histopathologic factors alone have been used to guide choice of therapy. These factors include tumor stage, tumor size, nodal status, and intra-tumoral characteristics such as grade, expression of estrogen and progesterone receptors, and HER2 status. These factors may be prognostic, indicating the aggressiveness of a tumor and likelihood of relapse without systemic therapy, predictive of response to specific treatments, or both. In recent years, advances in technology such as the sequencing of the human genome, development of high-throughput DNA analysis, and popularization of the idea of "personalized medicine" have led to a significant interest in how differences in genetic makeup may be used to predict treatment safety and efficacy. In the last decade there has been an increase in the number of studies investigating the role of pharmacogenetics in the treatment of breast and other cancers.

The term pharmacogenetics (here used synonymously with pharmacogenomics) refers to the study of the influence of a patient's genetic makeup on their response to drug therapy, including toxicity and efficacy. Technologic advances have allowed the rapid assessment of gene expression and function. This includes assessment of both tumor (somatic) and host (germline) genetic variation. Tissue microarrays, for example, permit the evaluation of expression patterns of thousands of tumor genes, which have proven critical in providing prognostic and predictive information regarding specific biologic subsets of cancer. Genetic variations may be in the form of DNA alterations including nucleotide repeats, insertions, deletions, or substitutions. The alteration of one nucleotide, a single nucleotide polymorphism (SNP), can lead to absence or altered enzyme activity and thus to a significant impact on the disposition of and/or response to a drug. These alterations may affect drug toxicity and efficacy in a variety of ways. Changes in the coding region of DNA may result in amino acid substitutions in the translated protein, and changes in the noncoding regions of DNA can alter different aspects of protein function compared to a wild type protein. The goal of pharmacogenomic studies is to identify genetic alterations such as SNPs that considerably affect the function or expression of proteins involved in the pharmacokinetics or pharmacodynamics of therapeutic drugs.

The ultimate goal of selecting a particular drug for a patient based on their genetic makeup is to improve efficacy and safety. To date, numerous studies have been conducted not only focusing on drug targets but also on cell cycle control and apoptosis, DNA damage and repair, and drug metabolism and transport. These studies have attempted to correlate SNPs with breast cancer outcomes and to translate the results to clinical applications. Several pharmacogenetic tests are commercially available and can be used to determine SNPs in individual patients, but whether these tests should be used in the clinic, and how they should be interpreted, remain challenging questions. In this article we seek to review the evidence to date regarding the role of patient genetics in predicting both toxicity and response to therapies that are commonly used in breast cancer. We will review hormonal agents, trastuzumab and other targeted therapies, as well as common cytotoxic agents (Table 1).

Hormonal Therapy

Nearly two thirds of breast cancers are classified as estrogen receptor (ER)-positive, which is prognostic for improved survival outcomes and predicts responsiveness to endocrine manipulations. By binding to either ER-alpha or -beta, estrogen regulates a wide variety of cellular effects and physiologic conditions including breast cancer cell proliferation. Though

not always considered in this manner, endocrine therapies are indeed breast cancer targeted therapies, as they treat cancer by blocking specific receptors, which prevents or inhibits tumor growth. Several hormonal agents are approved for the prevention or treatment of breast cancer, including the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene, as well as the third generation aromatase inhibitors (AIs) anastrozole, letrozole and exemestane. While the final common pathway of both SERMs and AIs is to disrupt estrogen signaling, both classes of drugs undergo vastly different metabolic routes of elimination and activation, and each of these metabolic steps is under genetic control. Thus, genetic variations that alter the proteins involved in the metabolism, uptake, or distribution of each drug could provide useful predictive information for therapeutic recommendations.

Tamoxifen

Tamoxifen is the most widely used SERM and is the standard adjuvant hormonal agent initially prescribed to women with hormone-responsive early breast cancer for over 30 years. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) reported that five years of tamoxifen therapy is associated with a reduction in relative risk of breast cancer recurrence of approximately 39%, and that breast cancer mortality is reduced by about one-third yearly throughout the first fifteen years following randomization (Early Breast Cancer Trialists' Collaborative, 2011). Even more recently, analysis of the worldwide Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial has suggested that ten years of effective endocrine therapy can approximately halve breast cancer mortality during years 10–14 after diagnosis (Davies, et al., 2012). In addition to widespread use in the adjuvant setting, tamoxifen is an effective palliative therapy in women with hormone receptor-positive metastatic breast cancer (Rose & Mouridsen, 1984). It is also approved for women with ductal carcinoma in situ and for the prevention of breast cancer in high-risk women (Cuzick, et al., 2002). Tamoxifen remains the drug of choice for premenopausal women with breast cancer. In postmenopausal women, tamoxifen is often used in sequence with AIs or as a single agent in women with a contraindication to AI therapy.

Despite decades of data supporting its efficacy, not all women taking tamoxifen benefit from the agent, and the adverse effect profile varies substantially among individuals. Although approximately 70% of ER-positive patients with metastatic breast cancer respond initially to tamoxifen treatment, 30–40% of them experience relapse and eventually die from the disease (Early Breast Cancer Trialists' Collaborative, 2012). Of the possible explanations for this inter-individual variation, one hypothesis is that pharmacogenetic variation or pharmacologic enzyme inhibition can lead to changes in the plasma concentrations of active tamoxifen metabolites and may affect outcomes (Stearns, et al., 2003). The complex metabolism of tamoxifen, which is itself a weak anti-estrogen, involves primary and secondary hepatic biotransformation via the cytochrome p450 (CYP450) enzyme system. During this process, tamoxifen and its primary metabolites, N-desmethyl-tamoxifen and 4-hydroxy-tamoxifen, undergo extensive oxidation, principally by the CYP3A and CYP2D6 enzymes, to metabolites that exhibit a range of pharmacological effects (Desta, Ward, Soukhova, & Flockhart, 2004). Of these, 4-hydroxy-tamoxifen is an active metabolite associated with 30- to 100-fold greater potency in suppressing estrogen-dependent cell proliferation compared with tamoxifen, and nearly a 100-fold higher affinity for ER (Fabian, Tilzer, & Sternson, 1981). Another active metabolite, endoxifen is associated with plasma concentrations that are five to 10 times higher than 4-hydroxy-tamoxifen's in women on chronic adjuvant tamoxifen (Stearns, et al., 2003). Endoxifen may also induce ER protein degradation, block ER transcriptional activity, and inhibit estrogen-dependent breast cancer cell proliferation (Wu, et al., 2009).

CYP2D6 activity as a predictor of tamoxifen-associated outcomes—While variants in several genes encoding for tamoxifen metabolizing enzymes have been correlated with tamoxifen-associated outcomes (Table 1). Variable activity of the pertinent cytochrome P450 enzymes (CYP), brought about by genetic polymorphisms and drug interactions, may alter the balance of tamoxifen metabolites, thereby altering its effects. While there are several genes of interest in tamoxifen's metabolism, the one that has generated the most press has been *CYP2D6*, which encodes the enzyme responsible for metabolism of N-desmethyl-tamoxifen to endoxifen. *CYP2D6* is a polymorphic gene with more than 100 reported allelic variants, often due to SNPs (Sim, 2012). Common allelic variants in this gene are associated with the extent of N-desmethyl-tamoxifen metabolism and subsequently with in vivo concentrations of endoxifen. This observation has led to the hypothesis that allelic variation may predict responsiveness to therapy with tamoxifen. This theory has generated great interest, studies and even practice changes over the last decade. In general, patients have been classified as extensive, intermediate or poor metabolizers based upon their *CYP2D6* allelic variants.

Multiple laboratory, cohort, and case control studies were conducted and included patients with metastatic disease, or those receiving tamoxifen in adjuvant and prevention trials, to test the hypothesis of a relationship between *CYP2D6* genetic variation and breast cancer outcomes and have been previously reviewed (Higgins, Rae, Flockhart, Hayes, & Stearns, 2009; Higgins & Stearns, 2011). The majority of studies were conducted in the adjuvant setting, mostly using archival tissue banks of specimens and outcomes collected either through retrospective analysis or through prospective trials designed to ask other questions. Newer studies continue to provide mixed support for the hypothesis that *CYP2D6* metabolic status due to genetic variants or concomitant use of inhibitors of the enzyme is an important predictor of response. In a 2010 cohort study of metastatic breast cancer patients on tamoxifen, overall survival was significantly shorter in patients with a poor *CYP2D6* metabolizer phenotype, compared with extensive metabolizers (Hazard Ratio [HR]=2.09; 95% confidence interval [CI] 1.06–4.12; P=0.034) and concurrent use of *CYP2D6* inhibitors was independently associated with worse survival and time-to-progression survival (Lammers, et al., 2010).

Despite significant findings in this and other studies, more recent retrospective analysis of several large adjuvant trials has been disappointing. In ten years of follow-up after the adjuvant trial of Arimidex, Tamoxifen Alone or in Combination (ATAC) study, authors have recently published a retrospective analysis that failed to demonstrate a significant association between *CYP2D6* genotype and breast cancer recurrence (HR for distant recurrence in poor vs. extensive metabolizers = 1.25, 95% CI 0.55–3.15; P=0.64; HR for any recurrence = 0.99, 95% CI 0.48–2.08; P=0.99) (Rae, et al., 2012). Similarly, analysis of the BIG 1-98 trial was without a significant association between *CYP2D6* genotype and cancer-free interval (Regan, et al., 2012). Of note, both studies used tumor DNA, and the genotyping quality in the BIG 1-98 analysis has been called into question due to inconsistencies with the expected genotype frequencies based on the Hardy Weinberg equilibrium (Nakamura, et al., 2012; Pharoah, Abraham, & Caldas, 2012; Stanton, 2012).

Analysis of large prevention trials has yielded similarly disappointing results in regards to *CYP2D6* genotype and breast cancer outcomes. In a retrospective review of women enrolled in the Italian Tamoxifen Prevention Trial, women classified as poor metabolizers who were enrolled in the tamoxifen arm showed higher risk of developing breast cancer (P=0.035) (Serrano, et al., 2011). Additionally, retrospective analysis of the National Surgical Adjuvant Breast and Bowel Project (NSABP) prevention trials (P1 and P2) revealed no specific association between any *CYP2D6* parameter and breast cancer events in tamoxifen and raloxifene-treated patients (Goetz, et al., 2011).

Several studies have focused on the effect of CYP2D6 inhibitors administered with tamoxifen. Certain selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine, and selective noradrenaline reuptake inhibitors (SNRIs), such as venlafaxine, which are commonly prescribed for alleviation of tamoxifen-induced hot flashes or for mood disorders, inhibit the CYP2D6 enzyme (Henry, Stearns, Flockhart, Hayes, & Riba, 2008). The impact of co-administration of drugs that utilize CYP2D6 in patients on tamoxifen has been examined in multiple studies. In one series, use of the CYP2D6 inhibitors paroxetine, fluoxetine, sertraline and citalopram with tamoxifen was associated with a worse overall survival (HR=3.55; 95% CI 1.59–7.96; P=0.002) and time-to-progression (HR=2.97; 95% CI 1.33–6.67; P=0.008) compared with patients not concurrently on CYP2D6 inhibitors (Kelly, et al., 2010; Lammers, et al., 2010). In another large population-based cohort study in Ontario, risk of death from breast cancer after completion of tamoxifen treatment was increased in women who were co-prescribed the strong inhibitor paroxetine, but not with other inhibitors (Kelly, et al., 2010). Other investigators were not able to demonstrate an association between the use of the weak inhibitor citalopram and outcomes in women prescribed tamoxifen (Henry, et al., 2008). Likewise, assessment of a pharmacy database in the Netherlands did not reveal an association between concomitant use of the strong inhibitors bupropion, paroxetine, fluoxetine, and quinidine, the moderate inhibitors duloxetine and terbinafine, and the weak inhibitors amiodarone, cimetidine, and sertraline and breast cancer recurrence in patients treated with adjuvant tamoxifen (Dezentje, et al., 2010; Dusetzina, Alexander, Freedman, Huskamp, & Keating, 2013). Nonetheless, a recent analysis of antidepressant prescribing demonstrated a substantial decline in strong CYP2D6-inhibitor use among tamoxifen users following the time period in which the CYP2D6 information was disseminated (Dusetzina, et al., 2013). In a prospective assessment of drug interactions, investigators have presented preliminary results suggesting that concurrent use of tamoxifen and venlafaxine is associated with reduction in endoxifen to a concentration associated with a higher risk of recurrence in patients treated with adjuvant tamoxifen (Goetz, et al., 2012). The investigators are continuing assessment of changes in plasma concentrations of tamoxifen metabolites in women concurrently taking citalopram hydrobromide, escitalopram oxalate, gabapentin, or sertraline hydrochloride (Goetz, 2012).

Unfortunately, evidence about CYP2D6 metabolic activity due to concurrent use of inhibitors has been as mixed as data regarding *CYP2D6* genetic variation and outcomes on tamoxifen. Initial evidence was so compelling that in 2006, a United States Food and Drug Administration (FDA) advisory committee recommended tamoxifen's label be changed to note that postmenopausal women with estrogen receptor-positive breast cancer who are considered poor CYP2D6 metabolizers either by genotype or by drug interactions may be at increased risk of cancer recurrence (Center for Drug Evaluation and Research, 2006). Though the label for tamoxifen was never actually changed due to conflicting results, as discussed above, we recommend use of a weak inhibitor whenever possible in women taking chronic tamoxifen.

The inconsistent results from retrospective cohorts have led to the general conclusion that there are insufficient data to support assessment of CYP2D6 in treatment decision-making (Fleeman, et al., 2011; Kelly & Pritchard, 2012). The American Society of Clinical Oncology (ASCO) 2010 Clinical Practice Guidelines update included a formal recommendation against using *CYP2D6* testing to select adjuvant endocrine therapy, while simultaneously encouraging caution with concurrent use of CYP2D6 inhibitors and tamoxifen (Burststein, et al., 2010). Current National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines for Breast Cancer specify that symptom management of hot flashes and depression in women on tamoxifen should be undertaken with

medications that have only minimal effect on tamoxifen metabolism, as inhibitors of CYP2D6 may decrease plasma concentration of endoxifen (Carlson, et al., 2009).

Although retrospective analysis has been disappointing, we await more information from prospective trials designed to collect data on *CYP2D6* polymorphisms and response to tamoxifen (Table 2). The Eastern Cooperative Oncology Group (ECOG) E3108 trial, represent an ongoing attempt to correlate CYP2D6 activity with progression-free survival in metastatic breast cancer patients through collection of germline DNA and high quality prospective data (Stearns, 2012). Another example is a French neoadjuvant phase II study evaluating *CYP2D6* polymorphisms in non-metastatic patients and response to tamoxifen prior to surgery, that was expected to complete accrual in September 2012 (Gauducheau, 2012). Finally, the Korean ASTRRA study aims to assess the impact of *CYP2D6* genotype on the clinical effects of tamoxifen using samples from a prospective randomized multicenter study, and should complete accrual in 2016 (Lee, 2012).

Other genetic variants associated with tamoxifen outcomes—In addition to CYP2D6 mediated-activation, another key component of the metabolism of tamoxifen is its breakdown and clearance (Table 1). Active tamoxifen metabolites are converted to inactive and soluble metabolites by UDP-glucuronosyl-transferase (UGT) and sulfotransferase 1A1 (SULT1A1). Only a few analyses evaluated the pharmacogenomic importance of the genes encoding for these enzymes. Researchers reported increased risk of recurrence and death in tamoxifen-treated patients with variation in *SULT1A1* and *UGT2B15* alleles (Nowell, et al., 2005). In the Italian Tamoxifen Prevention Trial, variants of *SULT1A1* were not associated with tamoxifen efficacy (Serrano, et al., 2011). Another small study reported no association between *SULT1A1* copy number and disease free survival (Moyer, et al., 2011).

Other pharmacogenomic targets of interest in the metabolism of tamoxifen have been examined, but these studies have yielded similarly conflicting results. In the analysis of the ATAC trial, a near null association was observed between *UGT2B7* genotypes and recurrence in 603 tamoxifen-treated patients (Rae, et al., 2012). An analysis of *ABCB1* genetic variants, a gene which is associated with the multi-drug resistance (MDR) phenotype, demonstrated that certain *ABCB1* variants among metastatic breast cancer patients treated with tamoxifen were associated with a shorter time-to-recurrence (Teh, et al., 2012). *ESR1* and 2, genes encoding the ER-alpha and beta respectively, have been examined for pharmacogenomic significance. Of identified SNPs in *ESR1*, RS3798577 was correlated with disease-free survival (P=0.05) in a small cohort (Anghel, et al., 2010). Other *ESR1* variants such as high frequency of the exon-5-deletion variant (d5) may confer increased risk for relapse (Gallacchi, et al., 1998).

Pharmacogenetic predictors of tamoxifen-related adverse effects and secondary benefits—In addition to evaluation of breast cancer outcomes based on *CYP2D6* genotype, others assessed differential side effects among patients with variable *CYP2D6* alleles treated with tamoxifen (Table 1). In a retrospective analysis based on medical records' documentation, Mayo Clinic investigators reported that poor metabolizers were not likely to report hot flashes (Goetz, et al., 2005). In a prospective analysis of germline variation and patient-reported outcomes, investigators from the Consortium on Breast Cancer Pharmacogenomics (COBRA) reported a trend toward fewer severe hot flashes in poor metabolizers compared to intermediate plus extensive metabolizers (P=0.062) (Henry, et al., 2009). In this prospective cohort study, significantly increased hot flashes were observed in patients classified as intermediate metabolizers compared to poor metabolizers (P=0.038) and extensive metabolizers (P=0.011). In the BIG 1-98 trial, patients classified as poor or intermediate metabolizers had increased risk of hot flashes compared with extensive metabolizers, however, concomitant medication use was not available

(Regan, et al., 2012). The significance of this increased prevalence of hot flashes in extensive metabolizers remains a topic of interest, but how the emergence of these symptoms may correlate with outcomes is unclear. A prospective assessment found a strong correlation between higher *CYP2D6* activity and likelihood of tamoxifen discontinuation rates ($P=0.018$), suggesting that patients most likely to benefit from tamoxifen may be paradoxically more likely to discontinue it prematurely (Rae, et al., 2009). In another prospective cohort study, women who had variant *ESR2* genotype were significantly less likely to experience tamoxifen-induced hot flashes (Jin, et al., 2008). SNPs in *ESR1* and *ESR2* may predict hot flashes and should be examined further, however, given inconclusive results to date, the presence or absence of hot flashes should not be used to determine likelihood of drug efficacy

Another well-known adverse effect of tamoxifen is increased risk of thromboembolic events. An assessment of the Factor V Leiden gene (*FVL*) and thromboembolism in women with early-stage breast cancer on adjuvant tamoxifen found that those who had a blood clot were nearly five times more likely to carry an *FVL* mutation than those that did not have a clot (Garber, et al., 2010). This led study authors to the question whether *FVL* mutation testing should be undertaken before prescription of adjuvant tamoxifen if positive testing would alter prescribing practices.

Tamoxifen is associated with improved bone density in postmenopausal women. Preliminary results offer no association between SNPs in *CYP2D6*, *ESR1* or *ESR2* and bone density (Henry, et al., 2010). However, another study suggested an association between a SNP in the gene encoding for steroid receptor coactivator-1 (*SRC-1*), which increases transcriptional activity of the ER in bone, and altered tamoxifen response in bone, possibly due to disruption of GSK3 β phosphorylation site (Hartmaier, et al., 2012). Finally, although *ESR* polymorphisms have been associated with circulating lipid concentrations, there is no conclusive evidence that SNPs in *CYP2D6*, *ESR1* or *ESR2* influence tamoxifen-associated lipid profile modulation (Hayes, et al., 2010).

Pharmacogenetics and tamoxifen associated outcomes: Conclusions—

Although research into pharmacogenetic variations in tamoxifen metabolism on breast cancer outcomes has been ongoing for over a decade now, the evidence available does not yet allow us to recommend routine testing of *CYP2D6* genotype for those initiating adjuvant hormonal therapy. And, while there are other genetic targets of interest, no one genetic variant has yet sufficiently piqued the interest of researchers. Nonetheless, interest in the pharmacogenomics of important genes in tamoxifen metabolism still abounds, and prospective DNA collection in future tamoxifen trials will be critical to determine if a pharmacogenetic test can predict who will gain benefit or experience harm from tamoxifen treatment.

Prospective studies may provide more definitive conclusions regarding a correlation between *CYP2D6*, other genes, and tamoxifen associated-outcomes. As we have discussed, ongoing trials in the metastatic, neoadjuvant, and adjuvant setting are designed to assess *CYP2D6* activity in a prospective fashion. Studies have demonstrated the feasibility of tamoxifen dose escalation driven by *CYP2D6* genotype, and suggested that increasing tamoxifen dose in intermediate metabolizers and poor metabolizers can increase serum endoxifen concentrations (Irvin, et al., 2011). Whether an increased tamoxifen dose will correlate with improved outcomes in intermediate or poor metabolizers is not known. Other ongoing studies also assess whether escalating the dose of tamoxifen in patients with genetic polymorphisms of *CYP2D6* will increase endoxifen concentration to the same range of most patients who have wild type *CYP2D6* (Gurney, 2012).

Aromatase Inhibitors

In contrast to tamoxifen, the third generation AIs anastrozole, letrozole and exemestane are considered active in the parent form, and metabolism serves as a means of inactivation. These drugs function by inhibiting the enzyme aromatase, which is encoded by the *CYP19A1* gene and is responsible for the conversion of androgens to estrogens. As this is the primary source of estrogen in postmenopausal women, the third generation AIs inhibit 96–99% of *in vivo* aromatase enzyme activity, and thereby decrease endogenous estrogen concentrations far below those caused by natural menopause. Several large randomized trials comparing AIs with tamoxifen as adjuvant hormonal therapy have demonstrated significant improvement in disease-free survival and reduction in breast cancer events (Dowsett, et al., 2010). AIs are, however, associated with bone loss and musculoskeletal adverse effects, and it has been postulated that these AI-related effects may correlate with pharmacogenetic variation and may predict improved treatment efficacy (Cuzick, 2008).

The principal pharmacogenomic target of interest in the metabolism of AIs has been the *CYP19A1* gene. Sequencing of *CYP19A1* has revealed 88 polymorphisms in a heterogeneous population comprising equal numbers of white Americans, African Americans, Han Chinese Americans, and Mexican Americans, with substantial variation in polymorphisms between ethnic groups, and genomic studies revealed that some SNPs were associated with decreased aromatase activity compared with wild-type aromatase (Ma, et al., 2005). Studies which have evaluated the impact of genetic polymorphisms of *CYP19A1* on response to AIs have yielded conflicting results. In the metastatic setting, a small study of patients treated with letrozole demonstrated that time to progression was significantly improved in patients with a variant in an untranslated region of *CYP19*, compared with the wild-type gene (17.2 versus 6.4 months, $P=0.02$) (Colomer, et al., 2008). Another recent analysis noted that, while certain polymorphisms were significantly associated with improved time-to-treatment-failure among metastatic breast cancer patients on AIs, none of these results maintained independent prognostic significance in multivariate analysis of other predictive factors (Ferraldeschi, et al., 2012).

SNPs also predict improved efficacy of letrozole in the neoadjuvant and adjuvant settings. In a small series of postmenopausal women treated with adjuvant AI, two tightly linked SNPs in *CYP19* were significantly associated with a greater change in aromatase activity following AI therapy ($P=0.038$ for both SNPs) and higher plasma estradiol levels pre and post-AI (Wang, et al., 2010). Based on these findings, authors concluded that these SNPs have an important functional role in variation in response to AI therapy, and may even play a role in breast cancer risk. Other studies have identified SNPs which predict poor response to letrozole, such as a polymorphism in the 3'-UTR region of *CYP19* (Garcia-Casado, et al., 2010).

In addition to pharmacogenomic analyses relating AIs to breast cancer outcomes, studies have investigated the pharmacogenetic relationships of AI-associated adverse effects. In a nested case-control assessment of patients enrolled on NCIC MA.27, a phase III trial comparing anastrozole with exemestane, cases with musculoskeletal toxicities or treatment discontinuation due to toxicity were genotyped and genome-wide association studies (GWAS) identified four SNPs within the *TCL1A* gene associated with musculoskeletal adverse events in women treated with AIs (Ingle, et al., 2010). A prospective COBRA evaluation of reasons for treatment discontinuation in women with hormone receptor-positive breast cancer initiating adjuvant AI, examined SNPs in 24 candidate genes and found that two inherited genetic variants in *ESR1* and one in *CYP19A1* were associated with increased risk of discontinuation of AI therapy because of toxicity, and one variant in *NCOR1* (ER co-repressor) was associated with decreased risk of discontinuation of letrozole (Henry, 2012).

Further investigation is clearly warranted to determine the impact of pharmacogenetic variation in aromatase and other candidate genes on adverse effects and breast cancer outcomes in women receiving AIs. It may be that patients with lower aromatase activity may derive reduced benefit from AIs and that inter-ethnic variability in response to AIs may impact treatment decisions. Ongoing clinical trials aim to elucidate these answers in a prospective manner.

Targeted Biologic Therapy

Trastuzumab

Trastuzumab is a humanized monoclonal antibody that binds specifically to the HER2 receptor and suppresses cell proliferation that is driven by overexpression of the HER2 protein. The combination of trastuzumab with chemotherapy has led to significant reduction in breast cancer recurrence and mortality in HER2 overexpressing or amplified tumors ("HER2-positive") when used in the adjuvant setting (Piccart-Gebhart, et al., 2005; Romond, et al., 2005; Slamon, et al., 2011). Trastuzumab efficacy is believed to be, in large part, due to engagement of Fc gamma receptors on immune effector cells, which leads to destruction of tumor cells, a process known as antibody dependent cell-mediated cytotoxicity (ADCC) (Clynes, Towers, Presta, & Ravetch, 2000). Despite extreme improvement in outcomes with the use of trastuzumab, not all women whose tumors are HER2-positive will benefit equally from adjuvant trastuzumab, and metastatic patients treated with trastuzumab will eventually progress during therapy.

Given variable response, significant risks associated with treatment, including cardiotoxicity in 2–7% of patients, and substantial cost involved, identification of genetic markers predictive of trastuzumab response is quite attractive. Studies on the pharmacogenomics of trastuzumab have been mostly focused on polymorphisms of the Fc gamma receptors. In an analysis of 54 metastatic breast cancer patients treated with trastuzumab, investigators reported that the Fc gamma RIIIA-158 V/V genotype was significantly correlated with objective response rate and progression-free survival, and possibly associated with higher trastuzumab-mediated cytotoxicity (Musolino, et al., 2008). Similarly, in a single-arm study of trastuzumab in patients with metastatic breast cancer Fc gamma RIIIA-158V/V genotype was significantly correlated with response and progression-free survival (Tamura, et al., 2011). However, other studies do not support these findings. In the largest trial to date on the Fc gamma receptor IIa and IIIa polymorphisms, investigators tested samples from 1,189 patients enrolled in the BCIRG006 trial and analyzed several Fc gamma receptor polymorphisms, without any significant relationship between the multiple SNPs and disease-free survival in trastuzumab-treated patients (Hurvitz, et al., 2012). Unfortunately, these conflicting results preclude use of tests for these SNPs to predict trastuzumab response and further study into the factors underlying variability in trastuzumab response is warranted.

Bevacizumab

Bevacizumab is a humanized monoclonal antibody targeted against Vascular Endothelial Growth Factor A (VEGF-A), which stimulates angiogenesis and thus can promote tumor growth. The first molecularly targeted anti-angiogenesis drug, the story of bevacizumab in breast cancer has been dynamic. Bevacizumab was initially approved for metastatic breast cancer in 2008 following the E2100 Intergroup phase III trial comparing weekly paclitaxel alone with paclitaxel plus bevacizumab as first-line therapy for metastatic breast cancer (Miller, et al., 2007). This approval was contingent upon further data collection. When data failed to demonstrate a significant benefit in 2010, the FDA advisory panel recommended against breast cancer as an indication and this was officially withdrawn from the list of approved uses in 2011 (Lyman, Burstein, Buzdar, D'Agostino, & Ellis, 2012). Additional

studies to further determine the role of this agent in the adjuvant treatment of breast cancer are ongoing.

In a retrospective analysis of the E2100 data, investigators assessed available archived tumor samples for SNPs in genes encoding for VEGF and VEGF receptor, and found polymorphic genotypes associated with differential overall survival in the bevacizumab arm but not in the control arm. There were no significant differences in response rate or progression-free survival correlating with the variant alleles, regardless of the treatment arm, but some genotypes correlated with a lower likelihood of grade 3 or 4 hypertension when compared with the alternate genotypes (Schneider, et al., 2008). More recently, a prospective analysis assessed the role of *VEGF-A* polymorphisms in women with locally recurrent or metastatic breast cancer receiving first-line bevacizumab-containing therapy. Although none of the polymorphisms were significantly linked to clinical response, some allelic variants exhibited a marked tendency for a shorter time-to-progression than others (Etienne-Grimaldi, et al., 2011). Certainly these observations are limited as bevacizumab is not FDA approved for the treatment of breast cancer. However, further delineation of which patients may benefit from bevacizumab treatment and which may have less toxicity could make this drug much more appealing for treatment of breast cancer in the future.

Cytotoxic Chemotherapy

It is well known that most patients have a narrow therapeutic index to cytotoxic drugs and may react differently to administration of equal doses. As with use of hormonal agents and other targeted therapies, not only clinical and histopathologic features but also pharmacogenetics may partly explain individual differences in safety and efficacy of cytotoxic agents.

Antimetabolites

Capecitabine is an orally administered prodrug of the pyrimidine analog 5-fluorouracil (5-FU), which is used frequently in the treatment of metastatic breast cancer. Capecitabine is enzymatically converted to 5-FU, and inhibits DNA synthesis within tumors, thereby slowing growth. Dihydropyrimidine dehydrogenase (DPD), an enzyme encoded by *DPYD*, is the rate-limiting step in pyrimidine catabolism and deactivates more than 80% of standard doses of 5-FU and the oral 5-FU prodrug capecitabine (Lee, Ezzeldin, Fourie, & Diasio, 2004). Up to 5% of the population lacks the DPD enzyme, and this has been associated with excess drug accumulation and toxicity (Lee, et al., 2004). Additionally, an estimated 3% to 5% of the population has sequence variations in *DPYD*, and these pharmacogenetic variants may be linked with 5-FU toxicity. Multiple *DPYD* variants have been identified, including well-known non-synonymous and splice site variations within the coding regions of the gene, and more novel variations within noncoding regions (Amstutz, Froehlich, & Largiader, 2011), and their association with 5-FU toxicity has been replicated in multiple studies. Future metabolic profiling of patients and comprehensive genetic screening of *DPYD* has been proposed to further refine the understanding of relative contribution of individual *DPYD* variants to the risk of severe 5-FU-related toxicity (Amstutz, et al., 2011).

In addition to *DPYD*, variations in the genes encoding for thymidylate synthase (TS) and methylenetetrahydrofolate reductase (MTHFR) may affect outcomes in treatment with capecitabine as well as toxicity. In a prospective pilot study of advanced breast cancer patients, increased toxicity and significantly shortened duration of response was observed in patients homozygous for a *TS* variant allele (Largillier, et al., 2006).

Similar to capecitabine, gemcitabine is a nucleoside analog that inhibits DNA replication and thus induces apoptosis of tumor cells. Gemcitabine is approved in treatment of

metastatic breast cancer either alone or in combination with other agents. In patients with advanced breast cancer treated with gemcitabine, the degree of hematologic toxicity, tumor response, and survival may be affected by the presence of variant enzymes. In one study which analyzed SNPs in genes encoding for deoxycytidine kinase (dCK), deoxycytidine monophosphate deaminase (DCTD), and ribonucleotide reductase M1 polypeptide (RRM1), investigators demonstrated that the a ribonucleotide reductase haplotype was associated with a lower frequency of chemotherapy-induced toxicity, such as neutropenia and growth factor requirement with gemcitabine monotherapy in breast cancer patients (Rha, et al., 2007). Variant ribonucleotide reductase *RRM1* has been associated with response to gemcitabine in multiple diseases and studies are ongoing to better define its prognostic and predictive utility (Jordheim, Seve, Tredan, & Dumontet, 2011).

Antimicrotubules

The taxanes paclitaxel and docetaxel are some of the most effective chemotherapeutic agents against breast cancer and are indicated in both metastatic and adjuvant settings. Taxanes disrupt microtubule depolymerization and spindle formation during cell replication, thereby causing cell death. Both paclitaxel and docetaxel are hydroxylated in the liver by CYP3A4; paclitaxel undergoes further metabolism by CYP2C8, and docetaxel undergoes further metabolism by CYP3A5 (Cresteil, et al., 2002). Transport of taxanes across the cell membrane occurs via influx transporter *SLCO1B3* and efflux transporters *ABCB1*, *ABCC1*, *ABCC2* and *ABCG2* (Cresteil, et al., 2002). Added to a standard anthracycline-based regimen in the adjuvant setting, taxanes result in significant reduction in the risk of cancer recurrence, breast cancer mortality and overall mortality (Early Breast Cancer Trialists' Collaborative, 2012).

One of the major genes involved in paclitaxel metabolism, *CYP2C8*, is a polymorphic gene with several variant genotypes that have been shown to impact rates of paclitaxel clearance (Bergmann, et al., 2011). Investigators have prospectively collected data for breast cancer patients treated with paclitaxel-containing regimens in the neoadjuvant setting and correlated treatment response by Response Evaluation Criteria In Solid Tumors (RECIST) criteria with multiple genotypes and haplotypes involved in paclitaxel metabolism. Patients with the variant allele *CYP2C8**3 were more likely to achieve clinical complete response from neoadjuvant paclitaxel treatment, but also had increased rates of severe peripheral neurotoxicity (Hertz, et al., 2012). As with many pharmacogenomic studies, results have been conflicting. Another analysis of breast cancer patients receiving paclitaxel found no association between *ABCB1*, *ABCG2*, *CYP1B1*, *CYP3A4*, *CYP3A5* and *CYP2C8* genotypes and response, but patients homozygous for a different allele, *CYP1B1**3, had a significantly longer progression-free survival, though this finding was independent of paclitaxel clearance (Marsh, et al., 2007). Several other studies have attempted to examine the impact of drug transporter genes on the pharmacogenetics of taxanes, but show discordant results. In patients being treated with paclitaxel for metastatic breast cancer, two *ABCB1* SNPs were associated with efficacy and resistance to paclitaxel (Chang, et al., 2009). *SLCO1B3* has been identified as the most efficient influx transporter for docetaxel as well as a key regulator for hepatic uptake of paclitaxel. In a small study, investigators demonstrated that a variant form of *SLCO1B3* was significantly associated with risk of docetaxel-induced leukopenia and neutropenia (Kiyotani, et al., 2008). But others have not found any relationship between membrane transporter genotypes and docetaxel clearance (Baker, et al., 2009).

Another common side effect of taxane therapy is chemotherapy-induced peripheral neuropathy (CIPN), which can often be quite debilitating and even dose-limiting. There has been much interest in delineating genetic factors that may be associated with taxane neurotoxicity, as it has been difficult to predict in which patients this will occur. In an

analysis of patients enrolled on SWOG 0221, a trial of cyclophosphamide, doxorubicin and paclitaxel, patients with haplotypes in *FANCD2*, a Fanconi anemia/*BCRA* family gene, had almost double the risk of CIPN, suggesting a genetic link (Sucheston, et al., 2011).

Anthracyclines

The anthracyclines doxorubicin and epirubicin have been widely used in breast cancer treatment for several decades. This class of drugs inhibits topoisomerase II and thereby induces apoptosis of cells. Pharmacogenetic variations have been observed in genes encoding for anthracycline-metabolizing enzymes, drug transporters, and enzymes influencing oxidative stress and apoptosis (Ambrosone, et al., 2005). Approximately 50% of infused doxorubicin is eliminated in its intact form. The metabolism of remainder of doxorubicin is complex and involves a variety of enzymes, principally aldo-ketoreductase (*AKR1A1*), carbonyl reductases (*CBR1* and *CBR3*), NADH dehydrogenase (*NQO1*), and nitric oxide synthases (*NOS1*, *NOS2* and *NOS3*).

Of the genes encoding for enzymes involved in doxorubicin metabolism, variants of the carbonyl reductases (*CBR1* and *CBR3*) have been shown to be correlated with doxorubicin pharmacokinetics and clinical outcomes in pediatric cancer survivors, but when *CBR3* polymorphisms were evaluated in breast cancer patients receiving combination CMF (cyclophosphamide, methotrexate, and 5-FU) or CAF (cyclophosphamide, doxorubicin, and 5-FU) with or without tamoxifen in SWOG 8897, there were no associations with disease-free survival or toxicity (Choi, et al., 2009). In a study of southeast Asian breast cancer patients treated with doxorubicin, the *CBR3* 11A variant was associated with greater tumor reduction and more significant neutropenia (Fan, et al., 2008).

Though the data regarding genetic variants involved in doxorubicin metabolism are conflicting, there are two major genes encoding for cellular membrane transporters, *SLC22116* and *ABCB1*, which may have pharmacogenetic implications. *ABCB1* (*MDR1*, P-glycoprotein) is a drug transporter known to cause efflux of drugs from malignant cells, leading to resistance to multiple chemotherapeutic agents. In a study investigating 68 women with locally advanced breast cancer, there was a significant correlation between likelihood of clinical complete response to neoadjuvant chemotherapy and the *ABCB1* C3534T polymorphism (Kafka, et al., 2003). In breast cancer patients treated with doxorubicin and cyclophosphamide, *SLC22A16* polymorphisms were correlated with dose delay but not with survival, and variant alleles of *ABCB1* were correlated with shorter time-to-progression and overall survival (Bray, et al., 2010).

In contrast to doxorubicin, metabolism of epirubicin involves conjugation with glucuronic acid by glucuronosyltransferase *UGT2B7*. In an analysis of patients who received adjuvant epirubicin for breast cancer, the *UGT2B7*-His268T polymorphism was associated with increased risk of breast cancer recurrence (Parmar, et al., 2011). The cytotoxicity of both epirubicin and doxorubicin is believed to be influenced by oxidative stress, which has led to evaluation of antioxidant and pro-oxidant genes and their relationship with clinical outcomes. Polymorphisms of the myeloperoxidase gene (*MPO*) linked to increased oxidative activity have been associated with better survival in early stage breast cancer patients treated with anthracyclines (Ambrosone, et al., 2009). In addition to *MPO*, polymorphisms of manganese superoxide dismutase (*SOD2*) have been correlated with lower rates of anthracycline-induced neutropenia but also with inferior disease-free survival (Yao, et al., 2010), and Nitric Oxide Synthase (*NOS3*) variants have been correlated with increased risk of breast cancer progression (Choi, et al., 2009).

Given the toxicity profile of anthracyclines, identification of markers that can predict improved response to therapy remains paramount, and prospective research into the pharmacogenetics of anthracyclines is ongoing.

Cyclophosphamide

Cyclophosphamide, a backbone of breast cancer treatment, is incorporated into most adjuvant breast cancer chemotherapy regimens. Like tamoxifen, cyclophosphamide is a prodrug that undergoes two phases of hepatic metabolism, with initial metabolism to 4-hydroxy-cyclophosphamide primarily mediated by CYP3A4, CYP2B6 and CYP2C9. The main active metabolite is aldophosphamide, which is then detoxified by aldehyde dehydrogenase 1A1 (ALDH1A1). Many other cyclophosphamide metabolites can be deactivated by irreversibly binding with glutathione, a process which is catalyzed by Glutathione S-transferases (GST) A1 and P1. There is significant variability in plasma concentrations of cyclophosphamide metabolites between patients, which suggests that there may be a role for genetic variability in determining cyclophosphamide metabolism.

Investigation into genetic variants in both phases of cyclophosphamide metabolic pathways has yielded variable results (Pinto, Ludeman, & Dolan, 2009). Some studies have demonstrated an association between certain *CYP2B6* genotypes and shorter time-to-progression or overall survival, as increased risk for dose delays and expected toxicities (Bray, et al., 2010). However, these results have not been observed in other studies, such as SWOG 8897, which found no associations between *CYP2B6* genotypes and disease-free survival or rates of neutropenia (Yao, et al., 2010).

Inactivation of much of cyclophosphamide occurs via GSTP1, which is the most abundant GST in human breast tissue. Expression of GSTP1 in tumor cells has been linked to resistance to chemotherapy. In one study of ER-negative breast cancer patients treated with cyclophosphamide, GSTP1 expression was predictive of inferior pathological complete response (Miyake, et al., 2012). Other studies, however, have not revealed this association. For example, in SWOG 8897 an association between *GSTP1* genotype and disease-free survival was not identified, though one *GSTP1* variant was associated with reduced risk of hematologic toxicity (Yao, et al., 2010).

Cyclophosphamide clearance may also be under variable genetic control that could have pharmacogenetic implications for breast cancer patients. The ALDH1A1 enzyme is highly involved in the detoxification of cytotoxic cyclophosphamide metabolites, and has also been linked with the basal-like subtype of breast cancer, which confers a poorer prognosis (Ginestier, et al., 2007). In addition, expression of ALDH1A1 has been associated with poorer outcomes in breast cancer patients receiving neoadjuvant cyclophosphamide-based chemotherapy (Khoury, et al., 2012).

Given the frequency with which cyclophosphamide is used in adjuvant and neoadjuvant breast cancer regimens, identification of which patients will most benefit, and who is at risk for most side effects, is clearly a goal of future investigation.

Ongoing investigation and Confounding Variables

Despite multiple conflicting studies and a lack of definitive conclusions within the realm of pharmacogenomics of breast cancer therapy, interest in augmenting the knowledge base of genetic alterations that impact drug metabolism remains great. As we gain more data regarding long-term patient outcomes, it is natural to question the variability that occurs between patients treated with similar doses of the same drugs. Along the same lines, the variable toxicities can be substantial and have far-reaching consequences on patient quality

of life and function after treatment. The promise of identification of which patients are most likely to benefit from which therapies, and those who will experience significant adverse effects, remains an exciting possibility and allows us to envision a time when cancer therapy is tailored not only based on tumor characteristics, but also on individual patient's host factors.

As in the story of tamoxifen and *CYP2D6*, much focus has been placed on identification of a single test that can predict patients less likely to benefit from therapy or, as with 5-fluorouracil and *DYPD*, more likely to experience toxicities. Hypotheses to explain conflicting results in the many studies mentioned above include variable stage of disease, tumor biology, statistical power, method of genotype assessment, and different doses and combinations of drugs. These sources of heterogeneity among breast cancer pharmacogenomics studies often prohibit multivariate analysis and supply confounding variables that can make comparison of results challenging

The role of combination therapy as a source of heterogeneity among breast cancer pharmacogenomic studies remains an interesting question. Many studies assessed genetic variability in patients undergoing multimodality therapy. As many key enzymes are involved in metabolism of multiple drugs, the degree to which multimodality therapy may alter the significance of pharmacogenetic studies remains unclear. In assessment of the tamoxifen and *CYP2D6* story, some have argued that when tamoxifen is given as monotherapy, association between germline *CYP2D6* genotype and disease outcome trends toward significance (especially in studies not using tumor DNA), but that for studies in which tamoxifen was given as a part of combination chemotherapy, the *CYP2D6* association does not persist (Wheeler, Maitland, Dolan, Cox, & Ratain, 2013). In a follow-up assessment of their 2008 analysis of 282 Japanese breast cancer patients receiving tamoxifen monotherapy, investigators evaluated 167 women receiving tamoxifen-combined therapy to assess the effects of combination therapy on genotype association, and reported no significant association between *CYP2D6* genotype and recurrence-free survival ($P=0.44$). In subgroup analyses, they observed a positive association between *CYP2D6* genotype and clinical outcome only when tamoxifen was administered as monotherapy, leading them to conclude that combination therapy may explain some of the discrepancies in overall *CYP2D6* associations (Kiyotani, et al.).

In assessing the pharmacogenomics of cytotoxic chemotherapy for this paper, we have predominantly reviewed studies that evaluate single-agent therapy for breast cancer since only a few studies have assessed candidate genes among patients receiving combination therapy. It has been demonstrated that pharmacogenetic targets of interest maintain significance in some combination regimens. For example, variant alleles in the *ABCB1*, *SLC22A16* and *CYP2B6* genes were associated with response to the combination of doxorubicin and cyclophosphamide in the treatment of breast cancer (Bray, et al.). In a study that correlated twenty-six gemcitabine and platinum-based DNA repair polymorphisms in breast cancer patients receiving gemcitabine/carboplatin, all patients who possessed either genotype developed grade 3/4 neutropenia, compared to 38% with neither genotype ($P=0.001$) (Wong, et al.). One group has recently reported that among 79 SNPs in *CYP450*, only a single SNP within *CYP1A1* had significant correlation with progression-free survival ($P=0.0003$) when analyzed in metastatic breast cancer patients receiving combination docetaxel and capecitabine (Dong, et al.).

As drug metabolism and clearance may well be affected by concomitant administration of chemotherapeutic agents, it is easy to understand how this may affect assessment of pharmacogenomics outcomes in combination regimens. Equally important, it is difficult to know how to translate pharmacogenomics data obtained from single-agent studies into the

realm of combination chemotherapy, which is often how drugs are administered in practice. In an attempt to get around this confounding variable, a study of patients with acute lymphoblastic leukemia evaluated the endophenotype of drug clearance, which is likely to be less affected by concomitant drugs than some other phenotypes. They were thus able to analyze pharmacogenomics within three different dosing regimens that included different drug combinations (Trevino, et al., 2009). Ongoing investigation such as this may help minimize the significance of confounding variables such as combination therapies in pharmacogenomics studies and allow for comparison across heterogeneous study populations.

Another important variable among pharmacogenomics studies is that there is a general lack of high quality prospective data collected within the realm of pharmacogenomics to date. Though a handful of the studies involved prospective data collection, most included retrospective analysis of previously banked tumor samples and were not designed to assess pharmacogenetics. Tumor genetic analysis via microarray data is known to be difficult to analyze and is not reproducible. As discussed, many adequately-powered ongoing studies seek to rectify this with prospective collection of data intended to evaluate primary pharmacogenetic outcomes.

Among the future endeavors to delineate pharmacogenomic relationships within breast cancer therapies, the evolving technology of DNA sequencing holds much promise. High-throughput sequencing technologies enabling the generation of large amounts of sequence data at greatly reduced cost should simplify genotyping in future studies and increase the cost-effectiveness of DNA sequencing, making even a comprehensive pretreatment diagnostic screening potentially feasible in the near future.

As the field of pharmacogenomics has taken off, there has been an explosion of data, as suggested by the many studies reviewed above, and the realm of bioinformatics has developed to keep pace with increasing amounts of data. In 2000 the NIH initiated the Pharmacogenetics Knowledge Base (PharmGKB) initiative with a goal of creating a repository of primary data to track associations between genes and drugs, and to catalog the location and frequency of genetic variations known to impact drug response (Thorn, Klein, & Altman, 2010). As new technologies shifted research from candidate gene pharmacogenetics to phenotype-based pharmacogenomics over the past decade, the PharmGKB has refocused on curating knowledge rather than housing primary genotype and phenotype data, and now captures more complex relationships between genes, variants, drugs, diseases and pathways. Going forward, the challenges are to provide the tools and knowledge to plan and interpret genome-wide pharmacogenomics studies, predict gene-drug relationships, and support data-sharing among associations investigating clinical applications of pharmacogenomics (Thorn, et al., 2010).

Sequencing of the whole genome is something that has created quite a bit of interest in determining pharmacogenetic links of interest. Several GWAS based on prospective cooperative group clinical trials are underway to search for genetic markers predictive of response and toxicity in breast cancer patients. The benefit of a GWAS over the candidate gene approach is that it does not require previous knowledge of the genomic regions to be examined for alleles of interest. In addition to GWAS, specific algorithms may be designed to predict drug sensitivity. Indeed, researchers reported on the use of Random Forests Algorithm, a machine-learning algorithm which uses an ensemble approach based on classification and regression trees to predict multidrug sensitivity in colon cancer (Midorikawa, Tsuji, Takayama, & Aburatani, 2012). Also in colon cancer, a study from Korea suggested a novel 3-step approach of genome-wide screening, clinical association, and biological validation to identify SNP markers of chemosensitivity to cetuximab (Kim

JC, 2011). Regardless of the methodology, stringent statistics must be used to answer *a priori* hypotheses.

Ethical Considerations

The increasing availability of genetic information has led to several meetings designed specifically to address ethical considerations. In a 2005 review of the Nuffield Council on Bioethics, experts highlighted several concerns regarding informed consent in pharmacogenomics trials. An important concern noted was one with social and economic implications, suggesting that stratification of the population into genetic subgroups may mean that the costs of developing new medicines for small populations could be prohibitively expensive for pharmaceutical companies, with the result that effective therapies might not be developed for certain “orphan” groups (Corrigan, 2005). Though this has not come to pass in the seven years since this report was written, the possibility for such stratification exists and is something that will have to be keenly avoided.

Concerns about privacy are also paramount with genetic testing. The US has recently implemented federal mandates prohibiting insurance discrimination based on the knowledge obtained from genetic testing. Especially in regards to pharmacogenetic research, what happens with patient DNA and how pharmacogenetic data is de-identified from patient information is paramount to maintaining patient privacy. Current informed consent practices pertaining to pharmacogenetic trials have not been sufficient. As most pharmacogenetic trials are not therapeutic, the benefit to the patient in real time is negligible, though the potential benefit for future understanding of drug susceptibility and toxicity may be great. How this is conveyed in the consent process needs particular focus going forward (Corrigan, 2005). It is our recommendation that individuals enrolled in prospective randomized trials investigating new approaches for cancer treatment be properly consented for DNA collection and analysis in keeping with the Genetic Information Nondiscrimination Act (GINA).

Conclusions

In the past three decades, there has been significant progress in the treatment of breast cancer with the development of more targeted therapies, including hormonal therapies such as AIs and biologic therapies such as trastuzumab. Alongside these developments, there has been extreme interest in the underlying causes of variation between individual responses to a variety of breast cancer therapeutics. Consideration of the role that genetic variation may play in differences in drug metabolism and breast cancer outcomes continues to spark great interest.

Though we have made substantial progress towards understanding the role of pharmacogenetics in drug safety and efficacy, many questions remain unanswered and large scale trials designed to address pharmacogenetic questions are still lacking. Several recent publications have called into question the significance of pharmacogenetic effects on outcomes, but we must keep in mind that most studies reported to date were not designed to address pharmacogenetic questions, and have been predominantly retrospective with conflicting conclusions. Validation of the current hypotheses by prospective data collection remains important in order to truly assess clinical utility. Although some commercial tests are available to assess for genetic polymorphisms that have been suggested to have pharmacogenetic significance, the current body of knowledge is not yet sufficient to recommend testing in current practice. We await the results of ongoing trials designed to answer these questions and continue to assess data regarding breast cancer pharmacogenetics with a critical eye.

Acknowledgments

Financial support: Supported in part by Susan G. Komen for the Cure, by P30 CA006973, and by Grant #5T32CA009071-32, Molecular Targets for Cancer Detection and Treatment.

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Table 1

Candidate Genes that May Influence Breast Cancer-Related Outcomes

Agent	Variant Genes	Proposed Outcome
Tamoxifen	CYP2D6 Poor Metabolizers	Reduced survival Decreased time to progression Decreased hot flashes
	CYP2D6 Extensive Metabolizers	Increased likelihood of tamoxifen discontinuation
	<i>UGT2B15</i>	Increased risk of recurrence
	<i>SULT1A1</i>	Increased risk of recurrence Inferior survival
	<i>ABCB1</i>	Shorter time to recurrence
	<i>ESR1</i>	Increased risk of relapse
	<i>ESR2</i>	Decreased hot flashes
Aromatase Inhibitors	<i>CYP19</i>	Improved time to progression Poor response to letrozole
	<i>TCL1A</i>	Increased musculoskeletal adverse events
	<i>ESR1</i>	Increased risk of discontinuation due to toxicity
	<i>CYP19A1</i>	Increased risk of discontinuation due to toxicity
	<i>NCOR1</i>	Decreased risk of discontinuation of letrozole
Trastuzumab	Fc gamma RIIIIa-158 V/V	Improved objective response rate & progression-free survival
Bevacizumab	<i>VEGF-A 936C > T</i> <i>VEGF-2578 AA</i> <i>VEGF-634CC; VEGF-1498TT</i>	Shorter time to progression Improved median overall survival Less grade 3/4 hypertension
Capecitabine	<i>DPYD</i>	Increased toxicity
	<i>TS</i>	Increased toxicity Shorter duration of response
Gemcitabine	<i>RRM1</i>	Improved response rate
Paclitaxel	<i>CYP2C*8</i>	Increased clinical complete response Increased rates of neuropathy
	<i>CYP1B1*3</i>	Longer progression free survival
	<i>ABCB1</i>	Increased efficacy
	<i>FANCD2</i>	Increased risk of peripheral neuropathy
Docetaxel	<i>SLCO1B3</i>	Increased myelosuppression
Doxorubicin	<i>CBR3 11A</i>	Greater reduction of tumor burden Increased neutropenia
	<i>ABCB1 C3534T</i>	Increased clinical complete response Shorter time to progression and overall survival
	<i>MPO</i>	Improved survival
	<i>NOS</i>	Shorter time to progression
Epirubicin	<i>UGT2B7-His268T</i>	Increased risk of breast cancer recurrence
Cyclophosphamide	<i>CYP2B6</i>	Shorter time to progression
	<i>GSTP1</i>	Poor pathological CR Reduced risk of hematologic toxicity
	<i>ALDH1A1</i>	Poorer breast cancer outcomes

Table 2

Ongoing Studies Assessing CYP2D6 activity in Tamoxifen-Treated Patients

Trial PI and Number	Study Title
Goetz NCT00667121	Tamoxifen in women with breast cancer and in women at high-risk of breast cancer who are receiving venlafaxine, citalopram, escitalopram, gabapentin, or sertraline
Lee NCT00973037	CYP2D6 genotype on the clinical effect of tamoxifen (ASTRRA-CYP2D6)
NCT01075802	Study of Tamoxifen Dose Escalation in Breast Cancer Patients With CYP2D6 Polymorphisms (TADE)
Gauducheau NCT01220076	Biological response to tamoxifen in patients with non-metastatic hormone receptor-positive breast cancer
Stearns NCT01124695	Tamoxifen citrate in treating patients with metastatic or recurrent breast cancer