MINI-REVIEW

Genomics and Pharmacogenomics of Breast Cancer: Current Knowledge and Trends

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Abstract

The impact of genomics and pharmacogenomics in the current arena of clinical oncology is well-established. In breast cancer, mutations in the BRCA1 and BRCA2 genes have been well-characterized to carry a high risk of the disease during a woman's lifespan. However, these high risk genes contribute to only a small proportion of the familial cases of breast cancer. Hence, further efforts aimed to study the contribution of genetic mutations in other genes, including the estrogen receptor gene, TP53, CYP19, and mismatch repair genes to further investigate the genetic component of breast cancer. Multiple pharmacogenomic studies have previously linked genetic variants in known pathways with treatment response in cancer patients. Currently, polymorphisms in drug metabolizing enzymes, efflux transporters, as well as, drug targets have shown correlations to variations in response and toxicity to commonly prescribed chemotherapeutic treatments of breast cancer. CYP2D6 variants have been correlated with tamoxifen response and interindividual variability seen. An emerging application of cancer genetics and pharmacogenetics involves the use of inherited or acquired genetic abnormalities to predict treatment toxicity or outcomes. Recently, methods that involve the scanning of entire genomes for common variants have begun to influence studies of cancer causation. Currently, treatment individualization for breast cancer can take place on the basis of few molecular targets including the estrogen receptor and the overexpression of the HER2 receptor. Overall, the current review summarizes the recent findings in the genetic and pharmacogenetic research of breast cancer and the advances made in personalization of treatment.

Keywords: Breast cancer - pharmacogenetics - efflux transporters - drug targets - personalized therapy

Asian Pacific J Cancer Prev, **12**, 1127-1140

Introduction

Cancer is clearly a genetic disease (Workman, 2003). A neoplasm is caused by the mutation, amplification, deletion or abnormal expression of key genes that represent critical factors in the regulation of cell fate. Cancer-inducing genetic abnormalities may be inherited, or produced in somatic cells by carcinogenic insults. Understanding the genetic basis of cancers is a major component of current cancer research (Workman, 2003). Such understanding of the genetic component of neoplasms can lead to the definition of the molecular pathways that are associated with malignancy and to the identification and validation of new molecular targets for therapeutic intervention. Luckily, a range of novel high-throughput technologies are being used now to accelerate the pace of gene discovery and the development of innovative therapies. These technologies include genomics, proteomics, high-throughput screening, combinatorial chemistry, and structural biology (Workman, 2003).

In medical oncology, interindividual differences in tumor response and normal tissue toxicities are consistently observed with most chemotherapeutic agents (Lee et al., 2005). Currently, it is well-established that inherited variations in drug disposition genes and drug target genes contribute to the observed variability in cancer treatment outcome (Lee et al., 2005). Pharmacogenetics and pharmacogenomics involve the study of the role played by inheritance in individual variation in drug response phenotypes such as disease outcomes and toxicity from drugs (Yan and Beckman, 2005; Freedman et al., 2010). Pharmacogenetics typically refers to effects involving a limited number of genes whereas pharmacogenomics involves the study of complex multigene patterns within the genome (Yan and Beckman, 2005). The overall goal of pharmacogenomic studies is to elucidate the genetic bases for interindividual differences and to use such genetic information to predict the safety, toxicity, and efficacy of drugs (Lee et al., 2005). Genetic polymorphisms are variants in individual genomes and remain constant throughout a person's lifetime. There are estimated 1.4 million single nucleotide polymorphisms (SNPs) identified in the human genome, and many of them contribute to variability in drug pharmacokinetic and pharmacodynamic processes (Yan and Beckman, 2005). Beckman, 2005). Such genetic variants can affect drug transport and metabolism as well as, cellular targets, signaling pathways, and cellular responses to treatment

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(Yan and Beckman, 2005). Recently, several clinically relevant examples of the utility of pharmacogenomics that associate specific genetic polymorphisms in drug metabolizing enzymes, drug transporters, and drug target enzymes with clinical outcomes in patients treated with commonly prescribed chemotherapy drugs have been established (Lee et al., 2005, Freedman et al., 2010). The ultimate goal for genetic and pharmacogenomic studies is the development of personalized medicine, facilitating prescription of drugs based on a patient's individual genetic profile (Yan and Beckman, 2005). In oncology, pharmacogenetics and pharmacogenomics have already been applied to predict cancer susceptibility, tumor progression and recurrence, patient survival, and response and adverse effects associated with traditional chemotherapy treatments (Yan and Beckman, 2005).

Recent advances in genetic technology, combined with new discoveries in pharmacogenomics, have emphasized the substantial role of genomic factors to predict drug response and clinical outcomes in cancer treatment. This review discusses the recent genetic findings in breast cancer research and summarizes current applications of pharmacogenomics in breast cancer therapy and its substantial role in personalized treatment.

Cancer and Carcinogenesis - "The Story Continues"

As an illustration of the enigma that cancer has presented to physicians and scientists over the years, it was not until the 1920s that meaningful attempts were made to define cancer (Pitot and Loeb, 2002). Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth, local tissue invasion, and distant metastases (Dipiro et al., 2005). The origin of the word cancer is credited to the Greek physician Hippocrates, the "Father of Medicine". Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors. In Greek, these words refer to a crab, most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab. The Roman physician, Celsus, later translated the Greek term into cancer, the Latin word for crab. Galen, another Roman physician, used the word oncos to describe tumors. Although the crab analogy of Hippocrates and Celsus is still used to describe malignant tumors, Galen's term is now used as a part of the name for cancer specialists, oncologists (American cancer society).

The mechanism by which cancers occur is incompletely understood. A cancer, or neoplasm, is thought to develop from a cell in which the normal mechanisms for control of growth and proliferation are altered (Dipiro et al., 2005). Progression from normal tissue to invasive cancer is influenced by hereditary genetic factors as well as somatic genetic changes (Devita et al., 2001). Current evidence supports the concept of carcinogenesis as a multistage process that is genetically regulated (Dipiro et al, 2005). The first step in this process is initiation, which requires exposure of normal cells to carcinogenic substances. These carcinogens produce genetic damage that, if not repaired, results in irreversible cellular mutations. This mutated cell has an altered response to its environment and a selective growth advantage, giving it the potential to develop into a clonal population of neoplastic cells. During the second phase, known as promotion, carcinogens or other factors alter the environment to favor growth of the mutated cell population over normal cells. At some point, however, the mutated cell becomes cancerous (conversion or transformation). Progression is the final stage of neoplastic growth (Figure 1). Cancer progression is further driven by a series of accumulating genetic changes. Thus, while almost all of the evidence points to a genetic basis for the conversion of a normal to neoplastic cell, such a neoplastic change must require a number of other accompanying changes that may be genetic, environmental, or both (Pitot and Loeb, 2002).

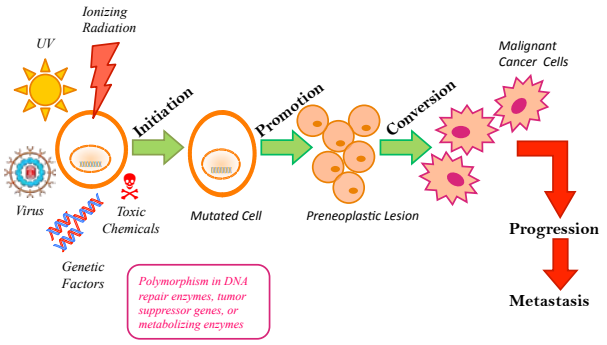

Figure 1. Steps of Carcinogenesis. Initiation requires the exposure of normal cells to carcinogenic factors. This produces genetic damage that, if not repaired, results in irreversible cellular mutations. Mutated cell has an altered response to its environment and a selective growth advantage. Carcinogenic effects result in irreversible cellular mutations leading to deregulations of oncogenes, cell cycle, and DNA transcription. These alterations mediate uncontrolled growth that can progress to tumor invasion into local tissues and the development of metastases.

Generally, genetic alterations involved in cancer can activate inductive processes (oncogenes) or block negative pathways (suppressor genes) (Devita et al., 2001). Early models of cancer genetics categorized cancer genes into oncogenes, which are growth inducing, and tumor suppressor genes, which are growth suppressing. Dominant oncogenes play a significant role in human cancers. Mutations in Ras, Ret, and Myc oncogenes are well-associated with plenty of human cancers. Similarly, amplification and overexpression of the HER2/*neu* receptor not only causes mammary malignancies, but is prognostic in human breast cancers. Although originally these oncogene abnormalities were thought to induce cancer primarily through unregulated growth, other cellular phenotypes such as enhanced survival and motility may be equally important contributors to the cancer state (Devita et al., 2001). Some oncogenes cause uncontrolled growth by activating persistent growth stimulatory signal transduction pathways. Other oncogenes cause uncontrolled growth by altering critical nodes in the cell cycle. In addition, uncontrolled growth can be caused by deregulation at the level of DNA transcription factors. Overall, the critical elements of this phase include tumor invasion into local tissues and the development of metastases (Devita et al., 2001; Dipiro et al., 2005).

Alternatively, tumor suppressor genes function mainly by inhibiting cell growth and blocking the emergence of tumor formation. p53 is a critical tumor suppressor protein playing a major role in DNA repair and apoptosis. A mutant p53 renders cells less likely to undergo apoptosis after cellular stress and enhances tumor formation (Devita et al., 2001). Thus, genetic instability may predispose the premalignant cell to generate malignant offspring. Chromosomal rearrangement can activate silent oncogenes or delete regions containing suppressor genes. Besides, mutations in cellular DNA can activate oncogenes or inactivate suppressor genes. Defects in DNA repair mechanisms contribute to the accumulation of genetic defects fueling cancer progression. Overall, the accumulation of such genetic defects is a major mechanism involved in tumorigenesis (Devita et al., 2001). The next part of this review will highlight the genetic component of breast cancer with a particular emphasize on the genetic defects associated with altered risk of breast cancer.

Genomics of Breast Cancer -"Who is at risk?"

Breast cancer is the most common site of cancer and is second only to lung cancer as a cause of cancer death in American women (Dipiro et al., 2005). Breast cancer that is confined to a localized breast lesion is often referred to as early, primary, localized, or curable. Unfortunately, breast cancer cells often spread by contiguity, lymph channels, and through the blood to distant sites. This often occurs early in the breast cancer growth, and deposits of tumor cells form in distant sites that cannot be detected with current diagnostic methods and equipment (micrometastases). When breast cancer cells can be detected clinically or radiologically in sites distant from the breast, the disease is referred to as advanced or metastatic breast cancer (Dipiro et al., 2005).

Both personal and family histories influence a woman's risk of developing breast cancer. The known risk factors for breast cancer including ionizing radiation, breast cancer in a first-degree relative, reproductive and hormonal factors, and alcohol consumption explain only a portion of the variability in breast cancer risk (Ambrosone, 2007). Of these risk factors, a family history of breast cancer is responsible for the greatest increase in risk, with women with a first-degree relative with breast cancer having twice the risk of those who do not (Ambrosone, 2007). In addition, twin studies indicated that up to 30% of breast cancer cases may be due to genetic factors (Lichtenstein et al., 2000).

In the last few years, several candidate genes for dominant breast cancer susceptibility have been discovered (Pitot and Loeb, 2002). It is likely that more than a single gene is involved in this inherited susceptibility, and breast cancer is still one of the most common genetic diseases in the world. In the early 1990s, pedigree analysis of twenty-three high-risk families for breast and ovarian cancer provided evidence for a rare autosomal dominant allele. From these families, a gene on the long arm of chromosome 17 was identified as abnormal in a large percentage of these hereditary breast cancer patients (Dipiro et al., 2005). This gene, the breast cancer susceptibility gene 1 (BRCA1) together with breast cancer susceptibility gene 2 (BRCA2) are the two major genes associated with breast and ovarian cancer risk (Hamilton, 2009). BRCA2 gene is located on chromosome 13 and mutations in either of these genes significantly increase individuals' risk for both breast and ovarian cancer across their lifespan (Hamilton, 2009). Everyone has a BRCA1 and BRCA2 gene (Hamilton, 2009). These genes are tumor suppressor genes that control cell growth (Hamilton, 2009). BRCA1 functions in a number of cellular processes, including chromatin remodeling, protein ubiquitination, DNA replication, DNA repair, regulation of transcription, cell cycle checkpoint control, and apoptosis (Yang and Xia, 2010). Disruption of any or all of these processes may contribute to the increased risk for carcinogenesis, as seen in carriers of germline BRCA1 mutations (Yang and Xia, 2010). Currently, it is well-established that mutations in BRCA1 gene confer a significantly elevated lifetime risk for breast and ovarian cancer. Although the loss of wild-type BRCA1 function is an important mechanism by which mutations confer increased cancer risk, multiple studies suggest mutant BRCA1 proteins may confer functions independent of the loss of wild-type BRCA1 through dominant negative inhibition of remaining wild-type BRCA1 protein, or through novel interactions and pathways. These functions impact various cellular processes and have the potential to significantly influence cancer initiation and progression (Linger and Kruk, 2010). Most breast cancers that occur in women with germline BRCA1 mutations are estrogen receptor-negative and typically lack expression of progesterone receptor and overexpression of human epidermal growth factor receptor (HER2), so-called 'triple-negative' breast cancers (Tung et al., 2010).

These BRCA1-associated estrogen receptor-negative tumors are most often high-grade invasive ductal carcinomas with a high mitotic rate that frequently exhibit other characteristic pathologic features including a prominent lymphocytic infiltrate, pushing or circumscribed margins, and geographic areas of necrosis or a central fibrotic focus. In addition, these tumors often express 'basal' biomarkers and most cluster within the 'basal-like' group in gene expression profiling studies (Tung et al., 2010). Interestingly, Jewish people of Eastern European decent (Ashkenazi Jews) have an unusually high carrier rate of germline mutations in BRCA1 and BRCA2 as compared to the rest of the United States population (Dipiro et al., 2005). To date, the most frequent single gene associated with hereditary breast cancer is the BRCA1 gene (Pitot and Loeb, 2002). Nevertheless, these 'high-risk' BRCA1 and BRCA2 genes, can explain 20–25% of familial breast cancer and only 5% of total breast cancer cases (Ambrosone, 2007). Genetic testing for deleterious mutations in BRCA1 and BRCA2 can provide key information to guide clinical decision making. Women who are heterozygous carriers of mutations in either gene have a 60–80% lifetime risk of breast cancer and a 10–40% lifetime risk of ovarian cancer (Domchek and Greenberg, 2009), reflecting a very high penetrance. In the clinic, genetic testing for BRCA1 and BRCA2 mutations is offered to women in high-risk families and yields one

of several possible results (Domchek and Greenberg, 2009). Upon the detection of such deleterious mutations, patients with such a mutation are counseled on risk reduction strategies such as breast MRI for early detection, chemoprevention, and prophylactic oophorectomy and mastectomy. In addition, therapies designed to exploit the DNA repair deficits in BRCA mutated cells are now entering the clinic (Domchek and Greenberg, 2009). In addition to the well-characterized risk harbored by the BRCA genes, the early age onset of breast cancer is a characteristic of the Li-Fraumeni syndrome, which is a clinically dominant disease in which gene carriers exhibit a high risk of childhood sarcomas, early onset of breast cancer, brain tumors, leukemia, and adrenocortical carcinoma (Pitot and Loeb, 2002). However, this is a relatively rare disease and is causative in far less than 1% of all breast cancers (Pitot and Loeb, 2002). Thus, there has been focused research on identification of additional genetic variants responsible for susceptibility to breast cancer. Unluckily, studies of familial breast cancer have failed to identify additional genes that infer high risk of breast cancer (Ambrosone, 2007). However, with the characterization of the human genome, as well as advances in technology to determine genetic variability across the genomes of populations, there has been focused effort on the identification of cancer susceptibility alleles through the use of genome-wide association studies (Ambrosone, 2007). These efforts have recently resulted in identification of a susceptibility locus for breast cancer by several groups, although the increases in risk are modest (Ambrosone, 2007). The following part will review some of the recent genetic variations in candidate genes associated with breast cancer risk.

Polymorphism of Estrogen Receptor Genes

Estrogen receptors are the first step along the path of signaling cell growth and development upon stimulation with estrogens (Cox et al., 2008). Estrogens act as growth factors in estrogen sensitive tissues, such as the breast, and this growth response to estrogens is mediated by estrogen receptors (Cox et al., 2008). Two estrogen receptor isoforms, ERα and ERβ exist, and are coded by two separate genes, the estrogen receptor 1 (ESR1) gene on chromosome 6 and the estrogen receptor 2 (ESR2) gene on chromosome 14 (Cox et al., 2008). In 2004, Gold et al. reported on estrogen receptor genotypes and haplotypes, describing haplotypes of ESR2 that may increase breast cancer risk among Ashkenazi Jewish women (Gold et al., 2004). In this study, 615 healthy subjects and 1011 individuals with histologically confirmed breast cancer were involved (Gold et al., 2004). Seventeen SNPs were analyzed in ESR1, and eight SNPs were reported in ESR2. Three common haplotypes in ESR1 were associated with a significantly decreased risk for breast cancer in the population studied ($P < 0.01$). These protective haplotypes (H4, H6, and H13) showed a statistically significant level of protection among overall research female subjects. In another study, the effect of two commonly studied SNPs at the 3' untraslated regions (UTRs) of ERβ on mRNA stability and translatability was investigated (Putnik et al., 2009). Five ERβ isoforms designated ERβ1-5, have been reported in humans (Putnik et al., 2009). Two SNPs in the ERβ gene have been studied for association with a number of diseases. They are referred to as rs4986938 and rs928554 (Putnik et al., 2009). rs4986938 is a G-A transition in exon 8, corresponding to ERβ1 3'UTR. rs928554 is a G-A transition in exon 9, corresponding to ERβ2 3'UTR (Putnik et al., 2009). The study demonstrated a significant difference in allelic expression of rs4986938, but not of rs928554, in breast tumor tissues from heterozygous individuals (Putnik et al., 2009). However, changes in mRNA expression and stability by these SNPs and the increase in disease susceptibility were suggested to be associated with a haplotype effect rather than the allelic effect of the individual SNPs per se (Putnik et al., 2009). To further investigate the effect of haplotypes of the estrogen receptor β (ESR2) gene and breast cancer risk, the National Cancer Institute Breast and Prostate Cancer Cohort Consortium has systematically selected haplotype tagging SNPs in genes along the steroid hormone synthesis, metabolism, and binding pathways, including the ESR2 gene (Cox et al., 2008). Four haplotype tagging SNPs tag the six major (> 5% frequency) haplotypes of the ESR2 gene. These polymorphisms have been genotyped in 5789 breast cancer cases and 7761 controls nested within the American Cancer Society Cancer Prevention Study II, European Prospective Investigation into Cancer and Nutrition, Multiethnic Cohort, Nurses' Health Study, and Women's Health Study Cohorts. The findings of this study showed that none of the SNPs were independently associated with breast cancer risk in the populations examined. Only one haplotype, hCCAC, of the ESR2 gene was significantly associated with breast cancer risk ($P = 0.0007$, odd ratio (OR) 1.17, 95% CI 1.07-1.28). Though, the inherited variants in ESR2, while possibly conferring a small increased risk of breast cancer, were not associated with appreciable changes in breast cancer risk among Caucasian women (Cox et al., 2008).

Polymorphism of DNA Repair Genes and

The ability to repair and faithfully replicate DNA is crucial and multiple mechanisms have evolved to maintain genomic integrity (Haiman et al., 2008). Recent evidence that some DNA-repair functions are haplo-insufficient adds weight to the idea that variants in DNA-repair genes contribute to cancer risk (Conde et al., 2009). In fact, higher levels of DNA damage and deficient DNA repair may predispose individuals to cancer (Conde et al., 2009). Commonly occurring SNPs in DNA repair genes have also been shown to incrementally contribute to cancer risk because of their critical role in maintaining genome integrity (Conde et al., 2009). The main mismatch repair (MMR) pathway is initiated by the recognition of a mismatch by the heterodimer consisting of the MSH2 and MSH6 proteins (also called MutSα) and the heterodimeric complexes of MSH2 and MSH3 (MutSβ) (Poplawski et al., 2005; Conde et al., 2009). MutSα is responsible for the recognition of base mismatches and insertion/deletions loops in mono- to tetranucleotide repeats (Conde et al., 2009). MutSβ mediates repair of small loops with 2–8 unpaired nucleotides (Poplawski et al., 2005). The MSH2 gene is central in mismatch recognition and there are

some studies reporting mutations and polymorphisms in several MSH2 variants. Indeed, several studies have shown that the most common mechanism inducing microsatellite instability in many cancers is the loss of the hMSH2 protein or genomic rearrangements found in the hMSH2 gene (Poplawski et al., 2005). Poplawski and colleagues investigated two polymorphisms of the hMSH2 gene, the Asn127Ser and the Gly322Asp, in breast cancer (Poplawski et al., 2005). All the patients and controls in this study were divided into three genotypes of hMSH2 Asn/127Ser: Asn/Asn, Asn/Ser, and Ser/Ser for the Asn127Ser polymorphism and Gly/Gly, Gly/Asp, and Asp/Asp for the Gly322Asp polymorphism (Poplawski et al., 2005). The results showed a strong association (OR 8.39; 95% CI 1.44–48.8) between the Gly/Gly genotype of the Gly322Asp polymorphism and breast cancer occurrence. However, no differences in the genotype distributions between cancer patients and control for the Asn127Ser polymorphism or for combined genotypes of both polymorphisms were reported (Poplawski et al., 2005). In addition, a case-control study in a Caucasian Portuguese population to estimate the potential modifying role of the MSH3, MSH4, MSH6, MLH1, MLH3, PMS1 and MUTYH gene polymorphisms on the individual susceptibility to breast cancer was conducted (Conde et al., 2009). Results showed that MSH3 1045Ala>Thr/MSH6 39Gly>Glu was associated with a decreased risk of breast cancer (P = 0.01); while MSH4 97Ala>Thr/MLH3 844Leu>Pro was associated with an increased risk for breast cancer (Conde et al., 2009). In another study, a multiethnic tagging strategy has been used to comprehensively capture the common genetic variations in coding and non-coding regions across 60 DNA repair-related pathway genes for association with breast cancer risk (Haiman et al., 2008). In five racial/ethnic populations, over 2600 SNPs were genotyped in each population and single- and multi-marker predictors of common alleles were selected to capture the linkage disequilibrium patterns specific to each group (Haiman et al., 2008).

The multiethnic analysis of African Americans, Native Hawaiians, Japanese Americans, Latinos, and Europeans Americans had revealed a variant in the Fanconi Anemia Complementation Group A (FANCA) gene (rs1061646, 0.15–0.68 frequency across populations) to be significantly associated with the risk of breast cancer amongst the five populations studied (Haiman et al., 2008). FANCA is part of a multi-subunit nuclear complex of Fanconi Anemia (FA) proteins that acts to repair blocks in DNA replication caused by cross-linking. This SNP yielded an 8% increase in breast cancer risk per allele (Haiman et al., 2008).

Polymorphism of MDM2 Gene

MDM2, an E3 ubiquitin ligase, is an important regulator of tumor development (Bouska and Eischen, 2009). MDM2 regulates p53 by controlling both the stability of the p53 protein and its activity as a transcription factor (Bouska and Eischen, 2009). Overall, MDM2 is considered a negative regulator of p53 tumor suppressor (Bouska and Eischen, 2009). Accordingly, overexpression of MDM2 oncoprotein may result in a higher risk of carcinogenesis and accelerated tumorigenesis by

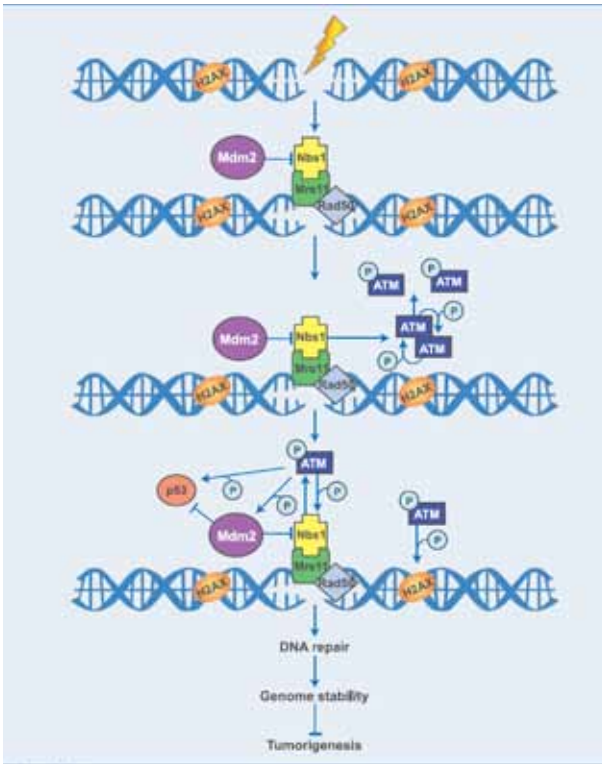

Figure 2. Model of Inhibition of DNA Break Repair by Mdm2. A DNA double-strand break induced by γ-irradiation is detected by the M/R/N complex, which localizes to the DNA break. The M/R/N complex recruits ATM to the DNA break and facilitates ATM dimer dissociation and activation (autophosphorylation). Activated ATM phosphorylates Nbs1, histone H2AX, p53, Mdm2, and numerous other proteins that are not pictured that are involved in the DNA break repair response. The presence of elevated levels of Mdm2 delays early phosphorylation events mediated by ATM that are necessary for a rapid DNA double-strand break repair response, resulting in inefficient repair of DNA breaks, genomic instability, and ultimately tumorigenesis (Reprinted with permission from "Bouska and Eischen. Mdm2 affects genome stability independent of p53. Cancer Res. 2009; 69:1697-701; Figure 2"14).

negatively regulating p53 tumor suppressor protein (Figure 2) (Bouska and Eischen, 2009, Sun et al., 2009). A functional SNP has been identified at position 309 within the first intron of the promoter region of the human MDM2 gene and hence designated SNP 309 (Sun et al., 2009). Conversion of the T allele to the G allele in this region causes a higher affinity for the Sp1 transcription activator and subsequently enhances the transcription of MDM2 gene (Sun et al., 2009). In one study, genomic DNA was obtained from the whole blood of 124 Taiwanese breast cancer patients and 97 cancer-free healthy women who were subjected to MDM2 SNP309 genotyping (Sun et al., 2009). Results showed that the frequencies of both heterozygous and homozygous genotypes were higher for breast cancer cases compared to the healthy controls (64.5% vs. 58% for TG, and 21% vs. 16% for GG). This observation suggested that the G allele in MDM2 SNP309 is associated with the risk of breast cancer in Taiwanese women. In addition, the GG and TG genotypes were significantly associated with an apparent increased risk of breast cancer compared to the TT genotype among the Taiwanese women examined and compared to the control

subjects (Sun et al., 2009). Furthermore, the average ages at diagnosis for breast cancer patients were 53.6, 52, and 47 years for those harboring TT, TG, and GG genotypes, respectively. Accordingly, it was concluded that MDM2 SNP309 GG genotype may be associated with both the increased risk and the earlier onset of breast cancer in Taiwanese women (Sun et al., 2009).

Polymorphism of TP53 Gene

TP53 is the gene encoding for the tumor suppressor protein p53 and it is one of the most extensively studied tumor suppressor genes (Hirshfield et al., 2010). TP53 is known to have a critical function in cell cycle regulation (Zhuo et al., 2009). In case of its mutation, this regulation could be lost, resulting in cell proliferation without control and development of cancer (Zhuo et al., 2009). Recently, much attention has been focused on possible associations of TP53 polymorphisms and cancer risks (Table 1). The most informative polymorphism in TP53 gene is located in exon 4 at codon 72, which encodes two distinct functional allelic forms arginine (Arg) and proline (Pro) because of a transversion G to C, resulting in different biochemical and biological protein features (Zhuo et al., 2009). Consequently, three distinct genotypes were created, namely, homozygous for arginine (Arg/Arg), homozygous for proline (Pro/Pro), and heterozygous (Arg/Pro) (Zhuo et al., 2009). Generally, TP53 mutations are considered of high penetrance, low frequency inherited variants. A recent meta-analysis was conducted with the goal to study the relationship between TP53 polymorhism and the risk of breast carcinoma (Zhuo et al., 2009). A search in the Medline, EMBASE, OVID, Sciencedirect, and Chinese National Knowledge Infrastructure (CNKI) without a language limitation, covering all papers published up to Jan 2009 had generated a total of seventeen case-control studies, including 12,226 cases and 10,782 controls, which were selected for the meta-analysis. Overall, no significant associations of TP53 codon 72 polymorphisms with breast carcinoma were observed (for Arg/Arg vs. Pro/Pro: OR = 1.20; 95%CI = 0.96–1.50). Moreover, in the subgroup analysis by ethnicity, statistically similar results were obtained when the data were stratified as Asians, Caucasians, and Africans (Zhuo et al., 2009). In another report, a haplotype-tagging approach was used to investigate the association of certain rare familial mutations in different genes including TP53 gene, and found no significant association of breast cancer with any individual or combination of tag SNPs (Baynes et al., 2007). Collectively, findings from these studies suggest that genetic variations of TP53 gene may not have a marked association with breast cancer risk.

Table 1. Genetic Loci Implicated in Hereditary, Familial, and Sporadic Breast Cancer Susceptibility
(Reprinted with permission from "Hirshfield et al, 2010".

High penetrance, low frequency	Low penetrance, low frequency	Low penetrance high frequency
BRCA1	CHEK2	FGFR2
BRCA2	ATM	LSP1
PTEN	PALB2	MAP3K1
p53	BRIP 1	TGFB1
STK11		TOX
		2q35
		8q

Polymorphism of CYP19A1 Gene

CYP19A1 encodes for aromatase, which irreversibly converts androgens to estrogens (Chen et al., 2008). Variation in this gene may affect individual susceptibility to breast cancer and other sex hormone-dependent outcomes (Chen et al., 2008). In a Chinese study, a set of CYP19A1 haplotype-tagging SNPs (htSNP) (rs1870049, rs1004982, rs28566535, rs936306, rs11636639, rs767199, rs4775936, rs11575899, rs10046, and rs4646), was examined in relation to risk of breast cancer and fibrocystic breast conditions in a case-control study conducted in Shanghai, China (Chen et al., 2008). Cases were diagnosed with breast cancer (n =614) or fibrocystic breast conditions (n =465) during 1989 to 2000. Controls were free of breast disease during the same period (n =879). None of the polymorphisms examined were associated with overall risk of breast cancer (Chen et al., 2008). In addition, haplotypes inferred using all polymorphisms were not associated with overall risk of either breast cancer or fibrocystic breast conditions (Chen et al., 2008).

Polymorphism of Minor Allele Genes

Overall, high penetrance germline mutation of the BRCA1 and BRCA2 genes account for up to 25% of the familial risk of breast cancer. (Frank et al., 2008). Hence, large research efforts tried to examine the possible association of breast cancer with many other genetic variants.

Nasim et al. conducted a comprehensive study to search for common low-penetrance susceptibility alleles to breast cancer in general (Mavaddat et al., 2009). Data on 710 SNPs in 120 candidate genes were available for analysis (Mavaddat et al., 2009). Genes that encode proteins in cellular pathways that are likely to be involved in breast carcinogenesis were chosen as candidates. The major pathways studied were steroid hormone metabolism and signaling, double-strand break DNA repair, oxidative damage repair, epigenetic modifiers, and cell cycle control. This association study included up to 4470 cases and 4560 controls (Mavaddat et al., 2009). Overall, no SNP was highly significant in effects analysis. Even the most significant association of CCND1 (gene encoding G1/S-specific cyclin D1) SNP rs3212879 with estrogen receptor–negative tumor types (P = 0.001) did not reach genome-wide significance levels ($P < 10^{-8}$) (Mavaddat et al., 2009). Additional minor allele genotyping studies for the minor alleles ERCC4 rs744154, TNF rs361525, CASP10 rs13010627, PGR rs1042838, and BID rs8190315 showed no overall association with breast cancer risk among women of European descent (Gaudet et al., 2009).

Pharmacogenetics of Breast Cancer -"The Kinetics and Dynamics"

This part will briefly review the pharmacogenetic factors that may affect the clinical implications for

commonly used treatment modalities in the management of breast cancer.

Tamoxifen

Tamoxifen has been the gold standard for the last 25 years for endocrine treatment of breast cancer (Schroth et al., 2009). Currently, tamoxifen is considered the treatment of choice for women with estrogen receptor-positive breast cancer (Goetz et al., 2008). Tamoxifen undergoes extensive hepatic oxidation by the CYP450 isoforms to several primary and secondary metabolites with variable potencies toward the estrogen receptor. Several lines of evidence indicated that most of the tamoxifen therapeutic effects in breast cancer are mediated by its metabolites 4-hydroxytamoxifen and endoxifen (Schroth et al., 2009) (see Figure 3). These metabolites exhibit significantly greater affinity for the estrogen receptor, and greater potency in suppressing cell proliferation compared to tamoxifen. CYP2D6 is the rate-limiting enzyme catalyzing the conversion of tamoxifen into its active metabolites (Goetz et al., 2008). The CYP2D6 gene is highly polymorphic, currently with 63 different major alleles known, many of which are associated with increased, decreased, or abolished function of the final gene product. The CYP2D6 phenotypes associated with these different alleles include poor, intermediate, extensive, and ultrarapid metabolizers (Goetz et al., 2008). Carriers of any two of approximately 20 known null alleles are phenotypic poor metabolizers. One of the most important functionally altered null variants, among others includes CYP2D6*4. In addition, important alleles associated with reduced enzyme activity include CYP2D6*10 and CYP2D6*17. Ultrametabolizers carry gene duplications and multiduplications of functional alleles, which lead to higher CYP2D6 expression and enzyme activity (Goetz et al., 2008). Recently, genetic variation in the metabolizing enzyme CYP2D6 has emerged as an important contributor to the interindividual variability in response after the administration of tamoxifen.

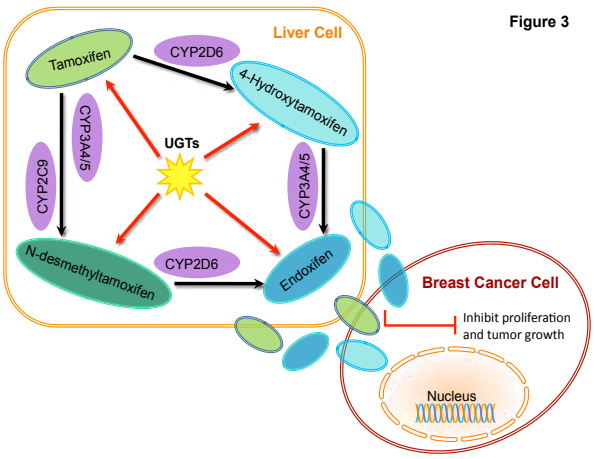

Figure 3. Major Metabolic Pathways of Tamoxifen. The inactivation of tamoxifen and its metabolites is mainly mediated by the enzymes UDP glucuronosyltransferase (UGTs). Tamoxifen and its active metabolites (4-hydroxytamoxifen and endoxifen) inhibit the proliferation and growth of breast cancer.

The effect of genetic variants of CYP2D6, CYP2C19, and three other cytochrome P450 enzymes on tamoxifen treatment outcome has been evaluate by Schroth and colleagues (Schroth et al., 2007). DNA from 206 patients receiving adjuvant tamoxifen monotherapy and from 280 patients not receiving tamoxifen therapy was genotyped for 16 polymorphisms of CYP2D6, CYP2C19, CYP2B6, CYP2C9, and CYP3A5 (Schroth et al., 2007). Data from this study showed that tamoxifen-treated patients carrying the CYP2D6 alleles *4, *5, *10, *41, all associated with impaired formation of antiestrogenic metabolites, had significantly more recurrences of breast cancer, shorter relapse-free periods (hazard ratio [HR], 2.24; 95% CI, 1.16 to 4.33; P =0.02), and worse event-free survival rates (HR, 1.89; 95% CI, 1.10 to 3.25; P =0.02) compared with carriers of functional alleles. Patients with the CYP2C19 high enzyme activity promoter variant *17 had a more favorable clinical outcome (HR, 0.45; 95% CI, 0.21 to 0.92; P =0.03) than carriers of *1, *2, and *3 alleles (Schroth et al., 2007).

Further studies by Schroth et al. had evaluated the correlation between the metabolic phenotypes of the enzyme CYP2D6 and the outcomes of tamoxifen treatment (Schroth et al., 2009). In this recent study, patients being treated with adjuvant tamoxifen therapy for early stage breast cancer who were estrogen-receptor positive, were grouped as extensive, extensive/intermediate, or poor CYP2D6 metabolizers (Schroth et al., 2009). A total of 1580 patients who were included in this study were assessed after the 9 year follow up period. Results showed that the risk of recurrence was higher in those who carried either the extensive/intermediate or poor metabolizer genotypes of the enzyme CYP2D6. In addition, these two genotypes showed worse event-free survival and disease-free survival when compared to extensive metabolizers. However, there was no significant difference in overall survival. The study concluded that among women with breast cancer treated with tamoxifen, there was an association between CYP2D6 variation and clinical outcomes, such that the presence of two functional CYP2D6 alleles was associated with better clinical outcomes and the presence of nonfunctional or reduced-function alleles with worse outcomes (Schroth et al., 2009). Additional studies were conducted to investigate relationships of polymorphisms in transporter genes and CYP2D6 to clinical outcome of patients receiving tamoxifen treatment (Kiyotani et al., 2010). Kiyotani and colleagues studied 282 patients with hormone receptor–positive, invasive breast cancer receiving tamoxifen monotherapy (Kiyotani et al., 2010). The effects of allelic variants of CYP2D6 and haplotype-tagging single nucleotide polymorphisms (tag-SNPs) of three adenosine triphosphate-binding cassette (ABC) transporters (ABCB1, ABCC2, and ABCG2) on recurrence-free survival in response to tamoxifen monotherapy were investigated. All of the decreased and null alleles of CYP2D6 (*4, *5, *10, *10-*10, *14, *21, *36-*36, and *41) were examined and denoted by the V allele (Kiyotani et al., 2010). Results indicated that the patients carrying one or two variant alleles (wt/V or V/V) had significantly shorter recurrence-free survival compared

with patients with homozygous wild-type alleles (wt/wt; P =0.0002). With respect to the ABC transporter genes, the investigators genotyped tag-SNPs in ABCB1, ABCC2, and ABCG2 which were suspected to be involved in the biliary excretion of tamoxifen or its metabolites (Kiyotani et al., 2010). Among 51 tag-SNPs in transporter genes, a significant association was found at rs3740065 in ABCC2 (P =0.00017) and was considered a risk allele. rs3740065 SNP or some other genetic variations linked to rs3740065 in ABCC2 may be associated with increased expression levels or transport activity of ABCC2 in breast cancer tissue, causing the lower exposure of breast cancer cells to endoxifen. Furthermore, the number of risk alleles of CYP2D6 and ABCC2 showed cumulative effects on recurrence-free survival (P =0.000000055). Such findings suggested that polymorphisms in CYP2D6 and ABCC2 are important predictors for the prognosis of patients with breast cancer treated with tamoxifen (Kiyotani et al., 2010).

The inactivation of tamoxifen and its metabolites is mainly mediated by the enzymes UDP glucuronosyltransferase (UGTs) (Lazarus et al., 2009). The most active hepatic UGT is UGT2B7 which can be also found in the gastrointestinal tract and breast tissue. O-glucuronidation of both trans-4-hydroxytamoxifen and trans-endoxifen was found to be lower in the UGT2B7268Tyr allele (Lazarus et al., 2009). Extrahepatic UGTs include UGT1A10 and UGT1A8. These two were found to have the highest activity in vitro on trans-4-hydroxytamoxifen and trans-endoxifen, and of the SNPs analyzed, there was no detectable glucuronidating activity in the UGT1A8277Tyr allele. Therefore, similar to what is described above for CYP2D6, functional SNPs in UGTs 2B7 and 1A8 could potentially affect overall patient response to tamoxifen therapy (Lazarus et al., 2009).

In addition to interpatient differences in the tamoxifen-metabolizing capacity, there is growing evidence that crosstalk between estrogen receptor and growth factor signaling contributes to tamoxifen resistance (Rokavec et al., 2008). TC21, also known as R-Ras2, participates in cell division, migration, adhesion, differentiation, and apoptosis. In a study by Rokavec et al., the influence of the TC21-582C>T promoter polymorphism on TC21 expression and treatment outcome was evaluated (Rokavec et al., 2008). In patients treated with adjuvant mono-tamoxifen therapy, the presence of high cytoplasmic TC21 expression or the 582T allele showed higher recurrence rates. This study used functional and patient-based approaches and found that prediction of tamoxifen treatment outcome in breast cancer was improved in the presence of TC21-582T polymorphism (Rokavec et al., 2008).

Letrozole

In postmenopausal women, aromatase is the main enzyme responsible for estrogen synthesis. Aromatase, CYP19, is carried by chromosome 15q21.1 (Garcia-Casado et al., 2010). Polymorphisms in the aromatase CYP19 gene are associated with altered aromatase activity in postmenopausal women (Colomer et al., 2008). The third-generation aromatase inhibitors anastrozole, exemestane and letrozole have found widespread use in breast cancer (Ingle, 2008). Aromatase inhibitors are highly specific, providing almost complete withdrawal of estrogen in postmenopausal women (Garcia-Casado et al., 2010). In addition, aromatase inhibitors found to be more effective than tamoxifen in breast cancer treatment of postmenopausal women (Garcia-Casado et al., 2010). In order to be a candidate for treatment with aromatase inhibitors, like letrozole, the patient must be both postmenopausal and have hormone receptor positive breast cancer (Colomer et al., 2008). Colomer et al. studied the effect of 3 SNPs in the CYP19 gene in order to determine if the analyzed SNPs played a role in letrozole efficacy in postmenopausal, hormone receptor-positive advanced breast carcinoma (Colomer et al., 2008). These SNPs are rs10046 and rs4646, located in the 3' UTR, and rs727479, located in the intron of the CYP19 gene. Postmenopausal patients (n=67) with hormone receptor-positive metastatic breast cancer were treated with the aromatase inhibitor letrozole (Colomer et al., 2008). Letrozole treatment was used until disease progression or unacceptable toxicity occurred. The primary endpoint was the time to progression (TTP). Data showed no association between TTP and rs10046 or rs727479 polymorphisms. However, it was found that TTP had significantly improved in patients with the rs4646 variant, compared with the wild-type gene (17.2 versus 6.4 months; P = 0.02). This study showed that the SNP rs4646 is associated with increased efficacy of letrozole in postmenopausal hormone receptor-positive breast cancer, and therefore screening for this SNP may be a useful predictive tool in treatment with aromatase inhibitors (Colomer et al., 2008). On the contrary, in the study conducted by Garcia-Casado and colleagues, the polymorphism in rs4646 in the 3'UTR in the aromatase CYP19 gene was associated with poor response after 4 months of treatment with letrozole, particularly in elderly patients (Garcia-Casado et al., 2010).

Pyrimidine Antagonists

Antimetabolites are structurally similar to naturally occurring nucleotides (Maring et al., 2005). They work by incorporation into DNA or RNA as false precursor or by inhibiting proteins involved in nucleotide metabolism. All pyrimidine antagonists are prodrugs and intracellular conversion into cytotoxic nucleosides and nucleotides is needed to produce cytotoxic metabolites. The most commonly used pyrimidine antagonists are 5-fluorouracil (5-FU), gemcitabine (dFdC), and cytarabine (ara-C) (Maring et al., 2005). Newer oral variants of 5-FU are capecitabine and tegafur (Maring et al., 2005). Polymorphisms in thymidylate synthase (TS), methylenetetrahydrofolate reductase (MTHFR), and dihydropyrimidine dehydrogenase (DPD) enzymes may influence the pharmacodynamics of fluoropyrimidines. In a prospective pilot study conducted by Largillier and colleagues, the effects of polymorphisms in these enzymes were assessed in response to toxicity and efficacy in patients receiving capecitabine monotherapy (Largillier et al., 2006). One hundred and five patients who are known cases of advanced breast cancer were

included in this study and were genotyped for a genetic polymorphism in the 5' regulatory region of the TS gene promoter consisting either of double (2R) or triple (3R) repeats of a 28 bp sequence (Largillier et al., 2006). This polymorphism has been shown to influence TS expression, with higher expression in 3R/3R tumors relative to 2R/2R (Largillier et al., 2006). Further experimental evidence had also shown that transcriptional regulation of TS is dependent on upstream stimulatory factor protein binding within the repeats. The presence of a G-C mutation in the 3R allele is associated with decreased transcriptional activation of TS gene. Besides, patients were genotyped for polymorphisms in the MTHFR gene focusing on the SNPs 677C>T and 1298A>C. With respect to DPD gene, polymorphisms studied in this perspective included IVS14 + 1G>A mutation, which is the most common functional mutation that leads to the skipping of the whole of exon 14 (165 bp) resulting in the complete loss of DPD enzyme activity in the event of homozygosity. The main results emerged from this study showed that patients homozygous for the TS 3RG allele, when compared to patients heterozygous or not carrying the TS 3RG allele had a trend toward higher toxicity rates upon capecitabine administration (50% versus 19% versus 13% respectively, P =0.064) (Largillier et al., 2006). Amongst the 105 patients screened, only one patient was found to be a carrier of the DPD IVS14 + 1G>A allele. This patient showed a decrease in hematologic toxicity associated with capecitabine treatment. In addition, duration of response was significantly shortened in patients homozygous for the 3RG allele compared with others (P =0.037). Overall, the results of the current study suggested that 3RG3RG breast cancer patients can be considered as poor candidates for capecitabine therapy (Largillier et al., 2006).

Doxorubicin and Cyclophosphamide

Doxorubicin and cyclophosphamide therapy is an effective treatment for early-stage breast cancer (Bray et al., 2010). Doxorubicin is a substrate for ABCB1 and the solute transporter SLC22A16. Cyclophosphamide is a prodrug that is converted to its active metabolite by the CYP450 oxidative enzymes. Cyclophosphamide is a substrate for the metabolizing enzymes CYP2B6, CYP2C9, CYP2C19, and CYP3A5. In a study by Bray and colleagues, variations in the genes encoding transporters and drug metabolizing enzymes relevant for the two drugs were investigated (Bray et al., 2010). Patients in this study were genotyped for SNPs in the ABCB1gene including 1236C>T, 2677G>T/A, and 3435C>T. SNPs investigated for the SLC2A16 included 146A>G, 312T>C, 755T>C, and 1226T>C. In addition, several SNPs were screened in the CYP450s metabolizing enzymes and these included CYP2B6*2, *8, *3, *4, *5, CYP2C*2, *3, CYP3A5*3, and CYP2C19*2. The results of the study showed that carriers of SLC2A16 A146G, T312C, and T755C had a lower frequency of dose delay (i.e. the timing of the drug administration cycle was delayed), indicating lower frequencies of toxicity. Carriers of SLC2A16 1226T>C and CYP2B6*2 and *5 showed higher frequencies of dose delay. Carriers of ABCB1 2677A, CYP2B6*2, CYP2B6*8, CYP2B6*9, CYP2B6*4 showed worse outcomes. Findings from this study indicated that polymorphisms in the ABCB1, SCL22A16, and CYP2B6 genes were associated with variations in cyclophosphamide response (Bray et al., 2010).

In addition to the CYP450 metabolizing enzymes, cyclophosphamide is also a substrate for the enzymes glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH) (Ekhart et al., 2008). In a study by Ekhart et al., SNPs in a number of metabolizing enzymes were evaluated for their effect on the pharmacokinetics of cyclophosphamide and its active metabolite, 4 hydroxycyclophosphamide (Ekhart et al., 2008). Sixteen polymorphisms were analyzed in nine genes (CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, GSTA1, GSTP1, ALDH1A1, and ALDH3A1) of putative relevance for cyclophosphamide and 4-hydroxycyclophosphamide disposition (Ekhart et al., 2008). Patients tested were receiving combination therapy of cyclophosphamide, thiotepa, and carboplatin. Findings in this study showed that none of the SNPs investigated were significantly associated with the interindividual variability in the pharmacokinetics of cyclophosphamide and its active metabolite in the population studied (Ekhart et al., 2008).

Carbonyl reductases (CBRs) are cytosolic enzymes that have many essential metabolizing roles (Lal et al., 2008). This group of enzymes is responsible for the reduction of xenobiotics, drug detoxification, signal transduction, apoptosis, mutagenesis, carcinogenesis, and drug resistance. Two isoforms are of interest, CBR1 and CBR3. In a study by Lal et al., polymorphisms in the CBR1 and CBR3 genes were evaluated for their effect on doxorubicin in Asians with breast cancer (Lal et al., 2008). Five polymorphisms were identified for each of the isoforms of interest. Data from this study found no significant association between polymorphisms in CBR3 and doxorubicin pharmacokinetics. However, results showed a significantly higher exposure level of doxorubicin in patients with CBR1 D2 diplotypes. There were no significant differences in individual CBR1 haplotypes on doxorubicin pharmacokinetics observed, so diplotypes were then considered. Two major haplotypes groups were identified, D1 and D2. Results of the study showed that CBR1 D2 diplotypes correlate with significantly higher exposure levels of doxorubicin, suggesting the possibility of lowered intracellular conversion to doxorubicinol in these patients (Lal et al., 2008).

Paclitaxel

Paclitaxel has a broad activity spectrum and is clinically used in combination with other chemotherapeutic agents in the treatment of solid tumors including the breast, ovarian, and lung cancers (Green, 2008). Paclitaxel treatment has been associated with a large interindividual variability in response and toxicity to the drug (Green, 2008). Genetic factors are now recognized to contribute, at least in part, to the differences observed in response to paclitaxel therapy (Green, 2008). Paclitaxel exerts its antitumor effects by inducing apoptosis indirectly by binding to β tubulin and stabilizing the microtubule. It has been suggested that mutations in β tubulin could be a factor in drug resistance.

However, studies have shown that the gene encoding the major isotype of β tubulin is highly conserved, and therefore mutations in this gene are unlikely to contribute significantly to paclitaxel's resistance or treatment failure (Green, 2008).

Paclitaxel is a substrate for P-glycoprotein (P-gp), an efflux pump transporter encoded by the gene ABCB1 (Chang et al., 2008). Polymorphisms in ABCB1 alter protein expression and function of the efflux pump. Earlier studies have found that the SNP 3435C>T in the ABCB1 gene to be associated with lower P-gp expression in the intestine. In a study by Chang et al., two common SNPs in ABCB1 gene, 3435C>T and 2677G>T/A were investigated for a possible effect in breast cancer treatment with paclitaxel (Chang et al., 2008). The results of the study showed that the 3435CT genotype, when compared to the CC genotype, is associated with significantly lower disease control rate (50% in CT, 84.9% in CC and 77.5% in TT: CT versus CC, P =0.025). In contrast, the ABCB1 2677 genotype and haplotypes did not correlate with response rate or disease control rate (Chang et al., 2008). In addition, the 3435CT genotype was associated with shorter overall survival when compared with the CC genotype. Currently, it is well-established that P-gp mediates, at least in part, the mechanism by which cancer cells develop resistance to chemotherapeutic drugs. In the same study by Chang et al., the 2677GG genotype, in comparison to other 2677 genotypes, had been associated with a higher frequency of anthracyline-refractory patients. No association was found with the genotypes 3435C>T and 2677/3435 haplotypes with regard to anthracycline or paclitaxel resistance. In this regard, data from cell culturing studies had showed dramatically higher levels of ABCB1 mRNA in cells resistant to both paclitaxel and doxorubicin treatments (Chang et al., 2008).

Oxidative paclitaxel metabolism occurs via the CYP450 pathway (Marsh et al., 2007). Significant variability (4- to 10-fold) in paclitaxel clearance may contribute to the unpredictability of clinical outcomes. Data from liver microsomes demonstrate that CYP2C8 and CYP3A4 are primarily responsible for paclitaxel metabolism, with CYP2C8 demonstrating a 2.3-fold greater metabolite production than CYP3A4. Variants in CYP3A4 could result in alternate metabolic pathways of paclitaxel. However, 3A4 variants are unlikely to be associated with total drug clearance (Marsh et al., 2007). Alternatively, the CYP2C8*3 allele has been investigated and association found with an altered turnover of paclitaxel. The CY2C8*3 variant allele has been associated with decreased paclitaxel 6 α-hydroxylase activity in human cell lines and human liver microsomes (Marsh et al., 2007).

Breast Cancer Management: Individualization of Therapy - "One Size Fits All?"

As more drug therapies are being applied to the management of disease and chronic illness, it has become apparent that the recommended doses as determined in clinical trials are not equally effective from individual to individual (Rakhra-Burris et al., 2010). Evidence now points to the fact that an individual's genetic makeup contributes to part of interindividual variation in response to treatment (Rakhra-Burris et al., 2010). In view of that, increased understanding of how variants of genes encoding metabolizing enzymes, transporters, and receptors affect drug efficacy and toxicity, in parallel with advances in genotyping means that clinical pharmacogenetics is drawing close to the reality (Rakhra-Burris et al., 2010). Current pharmacogenomic therapies under evaluation include small molecule, inhibitors of signal transduction, antibodies, and small interfering RNAs (siRNAs) (Bartlett, 2005). Such agents would also include those modifying tumor vasculature or other stromal elements to exert their effects (Bartlett, 2005). Hence, given the broad range of pharmacogenomic agents currently under evaluation for cancer therapy, it appears that a rapid extension of pharmacodiagnostic profiling will be required in the next 5-10 years if not sooner (Bartlett, 2005). If this to be successfully achieved, lessons learned in the past, particularly during the development of human epidermal growth factor receptor (HER2) testing for directing trastuzumab therapy in breast cancer, may provide a valuable framework for the development of future pharmacodiagnostic assays systems. Unluckily, breast cancer is characterized by a very heterogeneous clinical course which might complicate the process of prospective screening and individualization (Chang et al., 2009). More recently, a number of commercialized multigene prognostic and predictive tests have entered the complex and expanding landscape of breast cancer diagnostics (Ross et al., 2008). However, a major goal of recent studies is to evaluate whether such molecular diagnostic assays can accurately predict an individual's long-term potential for recurrence of breast cancer, so that appropriate treatment decisions can be made (Chang et al., 2009). These new technologies have been successfully applied to primary breast cancers and may eventually outperform currently used clinical parameters in predicting disease outcome and treatment selection. This part will summarize current state of genomic predictors and targets to personalize treatment for breast cancer patients.

Genomic Predictors of Outcome and Treatment Response

Historically, estrogen and progesterone receptor expression, HER2 overexpression and clinico-pathologic parameters have guided therapeutic decision making in breast cancer treatment (Dunn and Demichele, 2009). However, there are limits to the risk estimation provided by these parameters, leading to potential overtreatment of low-risk disease and under-treatment of poor-risk disease (Dunn and Demichele, 2009). In the past 10 years, the introduction of whole genome profiling technologies has greatly expanded the knowledge of the genes and genetic pathways associated with the development and progression of breast cancer (Ross et al., 2008). Genomic technologies now provide the opportunity to refine current therapeutic approaches by personalizing treatment to patients' individual tumor profiles (Dunn and Demichele, 2009). Gene profiles or signatures are groupings of genes that are differentially expressed between tumors,

reflecting differences in biologic behavior (Dunn and Demichele, 2009). Currently, there are three commercially available prognostic gene signatures: Oncotype DX™ (Genomic Health, Inc.), MammaPrint® (Agendia BV), and the HOXB13/IL17BR (H/I) ratio; (Theros H/ISM; bioTheranostics) (Dunn and Demichele, 2009). Gene signatures have the potential to transform breast cancer treatment as it becomes tailored to each patient's tumor expression profile and significantly improve the outcomes of this disease (Dunn and Demichele, 2009). Oncotype DX™ is a 21-gene profile that was developed to estimate the risk of recurrence in newly diagnosed patients with node-negative, estrogen receptor-positive, stage I or II breast cancer (Dunn and Demichele, 2009). Oncotype DX™ determines the 10-year risk for disease recurrence in patients with estrogen receptor-positive, lymph node-negative tumors (Ross et al., 2008). The cancer-related genes include a proliferation group (Ki-67 [MKI67], STK15 [AURKA], survivin [BIRC5], cyclin B1 [CCNB1], MYBL2), HER2 and its co-regulated gene GRB7, estrogen-related genes (ER, PGR, BCL2, and SCUBE2), a recurrence group (beta-actin [ACTB], GAPDH, RPLP0, GUS, and TFRC), invasion genes (stromelysin 3/matrix metalloproteinase 11 [MMP11] and cathepsin L2 [CTSL2]) and GSTM1, CD68, and BAG1. Expression levels of these genes were measured by RT-PCR and then placed in a quantitative algorithm to produce the recurrence score (RS), a number between 0 and 100. The RS is correlated with a continuous measure of recurrence risk, though three distinct risk categories have been developed: low (RS <18), intermediate (RS >18 but <30), or high (RS >30) (Dunn and Demichele, 2009). The MammaPrint® assay was the first fully commercialized microarray-based multigene assay for breast cancer (Ross et al., 2008). This assay is offered as a prognostic test for women under the age of 61 with either estrogen receptor-positive or estrogen receptor-negative, lymph node-negative breast cancer (Ross et al., 2008). The Theros H/ISM test is based on a 2-gene signature (HOXB13 and IL17B) developed for use in paraffin-embedded tissues (Dunn and Demichele, 2009). High expression of HOXB13 (the homeobox gene-B13) predicted recurrence, and high expression of IL17BR (the interleukin-17B receptor gene) predicted non-recurrence (Ross et al., 2008, Dunn and Demichele, 2009). A higher ratio of the two genes strongly predicted recurrence of breast cancer in this training set (Dunn and Demichele, 2009).

Treatment Individualization

Most chemotherapy drugs are administered to an individual based on a body surface area calculated from the patient's height and weight or, less often, area under the curve (Stearns et al., 2004).

Inherited variation in the activity of drug metabolizing enzymes that handle chemotherapeutic agents is well-recognized. There is increasing recognition that subtle changes in gene sequence, single nucleotide polymorphisms, may affect the ultimate function of the resulting product and that such variation may account for individual differences in efficacy and toxicity of treatment (Bao and Davidson, 2008). This variation may result in interindividual differences in pharmacokinetics of specific agents (Stearns et al., 2004). Currently, several lines of evidence support the utility of pharmacogenomics that associate specific genetic polymorphisms in drug metabolizing enzymes (e.g., TPMT, UGT1A1, DPD), drug transporters (ABCB1), and drug target enzymes (TS) with clinical outcomes in patients treated with commonly prescribed chemotherapy drugs, such as 5-FU and irinotecan (Lee et al., 2005). The ultimate goal of these genetic and pharmacogenetic studies is to enable the selection of the treatment that is most likely to provide benefit and minimal toxicity to patients (Table 2).

Until recently, only three individual biomarkers, estrogen receptor (ER), progesterone receptor (PGR), and HER2/ErbB2, were utilized in routine clinical care to guide treatment in breast cancer patients. ER and likely PGR expression are associated with a favorable prognosis and are highly predictive of benefit from endocrine treatment (Dunn and Demichele, 2009). Randomized trials have shown that tamoxifen delays recurrence and improves 10-year disease-free survival for younger and older women irrespective of nodal status. Aromatase inhibitors have been demonstrated to be an effective alternative endocrine treatment in postmenopausal women.

HER2/ErbB2 Pathway

The first example of the successful personalization of cancer treatment using a kinase-targeted therapy comes from therapeutic targeting of the ErbB family member, ErbB2 (HER2), a receptor tyrosine kinase overexpressed and/or amplified in 15% to 30% of breast cancers and that carries an adverse prognosis (McDermott and Settleman, 2009). Trastuzumab (Herceptin®), an antibody that targets the extracellular domain of HER2 was approved by the US

Table 2. Pharmacogenetic Polymorphisms with Known Impact on Breast Cancer Chemotherapeutic Agents

Drug	Gene	Possible consequences
Drug Target Pharmacogenetics		
Tamoxifen	*Estrogen receptor*	Treatment resistance
Aromatase inhibitors	*CYP19*	Treatment resistance Drug-related toxicity
5-Fluorouracil Capecitabine	*TS*	Worse outcomes
Metabolizing Enzyme Pharmacogenetics		
Tamoxifen	*CYP2D6*	Drug benefits/risks
Paclitaxel	*CYP2C8*	Decreased metabolism
5-Fluorouracil Capecitabine	*DPD*	Worse toxicity, especially neurotoxicity
Detoxification Enzymes		
Cyclophosphamide, Doxorubicin	*GST*	Improved outcomes due to lower enzyme activity greater drug availability
Drug Transporter Pharmacogenetics		
Doxorubicin Paclitaxel	*ABCB1*	Treatment resistance

Food and Drug Administration (FDA) in 1998 for use in the treatment of HER2-amplified metastatic breast cancer and has subsequently yielded clinical benefit when used in combination with cytotoxic chemotherapy as first-line or adjuvant therapy (McDermott and Settleman, 2009). On the basis of trastuzumab, lapatinib is a reversible tyrosine kinase inhibitor that potently inhibits both HER1and HER2 tyrosine kinase activities (Pruthi et al., 2007). Using *in vitro* cell-based assays, lapatinib treatment resulted in growth arrest and cell death in HER2- and EGFR-overexpressing breast cancer cell lines (Rusnak et al., 2001). Lapatinib also selectively inhibited breast tumor xenograft growth in a dose-dependent manner (Rusnak et al., 2001). In a pilot study conducted in patients with metastatic tumors, lapatinib treatment exhibited preliminary evidence of biologic and clinical activity in ErbB1- and/or ErbB2-overexpressing tumors (Spector et al., 2005). Additionally, lapatinib has undergone preclinical, phase 1, pharmacokinetic, and phase 2 and 3 evaluations in the setting of HER2-positive metastatic breast cancer, with impressive resulting data. Results of 3 phase 1 monotherapy studies in cancer patients showed that lapatinib was generally well-tolerated with most the common adverse events being diarrhea, nausea, anorexia, fatigue, and rash (Pruthi et al., 2007). A recent phase 1 dose-escalation trial was conducted to evaluate the safety and pharmacokinetics of lapatinib in Japanese patients with solid tumors that generally express ErbB1 and/or overexpress ErbB2 (Nakagawa et al., 2009). Overall, the majority of drug-related adverse events were mild and the most common events were diarrhea, rash, and dry skin (Nakagawa et al., 2009). Although both trastuzumab and lapatinib inhibit the same receptor, HER2; the combination is potentially attractive in breast cancer treatment because each agent targets a different part of the receptor (Pruthi et al., 2007). Trastuzumab targets the extracellular domain and lapatinib the intracellular domain. In addition, they appear to have different mechanisms of action, with trastuzumab activity at least in part due to increased internalization and degradation of ErbB2 and lapatinib inhibiting the ErbB2 tyrosine kinase.

Estrogen and Estrogen Receptor Pathway

More than 50% of primary breast cancers positively express the estrogen receptor and/or progesterone receptor. Generally, woman with hormone receptor-positive disease will be offered some form of hormonal intervention to treat the cancer. Most women with early breast cancer will likely receive adjuvant tamoxifen for 5 years. Postmenopausal women may be offered aromatase inhibitors instead of or following tamoxifen (Stearns et al., 2004). Interpatient variability exists in response to tamoxifen. The accepted dose of tamoxifen is 20 mg/day, but there is substantial variability with respect to levels of tamoxifen and its metabolites (Ingle, 2008). These data raise the question of whether one dose of tamoxifen 'fits' all patients. As discussed above, tamoxifen is metabolized in the liver by several cytochrome P450 enzymes. Emerging research has suggested inherited genetic variation in the CYP2D6 gene may be associated with a reduction in concentration of the active metabolite of tamoxifen, endoxifene (Bao and Davidson, 2008). This might in turn be associated with a poorer clinical benefit although the small studies completed to date have given mixed results on this question (Bao and Davidson, 2008). A commercially available test, AmpliChip CYP450 test® (Roche), provides comprehensive analysis of the CYP2D6 and CYP2C19 genes in a microarray-based assay (Bao and Davidson, 2008). In clinical settings, genotyping for CYP2D6 alleles *4, *5, *10, and *41 can identify patients who will have little benefit from adjuvant tamoxifen therapy. In addition to functional CYP2D6 alleles, the CYP2C19 *17 variant identifies patients likely to benefit from tamoxifen. Genetic polymorphisms in estrogen receptor may also influence tamoxifen-related toxicity or other benefits associated with the drug (Stearns et al., 2004). Pharmacogenetics may provide the needed tools to separate the women who are most likely to benefit from tamoxifen treatment, from those who are less likely to benefit from the drug, or even be harmed (Stearns et al., 2004).

Conclusion

Carcinoma of the breast remains the most prevalent cancer diagnosed among women in the world. Recent advances in genomic research have demonstrated a substantial role for gnomic factors in predicting response to cancer therapy. Genetic studies had shown a strong correlation between breast cancer risk during the lifetime of a woman and some genetic variants including the high risk BRCA1 and BRCA2 genes. Additional efforts trying to search the human genome looking for the possible contribution of many other genes to the higher risk of breast cancer in women were inconclusive.

Unfortunately, different genes were screened and genotyped, however strong correlations in the majority of clinical studies conducted were still lacked. Such disappointing results may be explained or expected on the basis of the heterogeneous nature of breast cancer and the different populations examined. In the same context, pharmacogenetic studies of treatments of breast cancer were drawing close to tangible health benefits. Recent findings in the pharmacogenetic studies of some metabolizing enzymes had successfully translated into real clinical practice. In this regard, genotyping patients for the CYP2D6 enzyme can help identifying candidates who will benefit more from tamoxifen treatment. In addition, understanding common polymorphisms in major metabolizing enzymes and drug transporters allowed better understanding of interindividual variations in response to cancer treatment. Examples include the CYP450 metabolizing enzymes, DHD, TPMT, and the ABC family of efflux transporters.

Thus, characterization of how variants in genes encoding metabolizing enzymes, transporters, and receptors affect drug toxicity and efficacy, in parallel with advances in genotyping and technology carry the potentials to personalize treatment and predict disease prognosis in breast cancer patients.

References

Ambrosone CB (2007). The promise and limitations of genome-wide association studies to elucidate the causes of breast cancer. *Breast Cancer Res*, **9**, 114.

Bao T, Davidson NE (2008). Gene expression profiling in breast cancer. *Adv Surg*, **42**, 249-60.

Bartlett JM (2005). Pharmacodiagnostic testing in breast cancer: focus on HER2 and trastuzumab therapy. *Am J Pharmacogenomics*, **5**, 303-15.

Baynes C, Healey CS, Pooley KA, et al (2007). Common variants in the ATM, BRCA1, BRCA2, CHEK2 and TP53 cancer susceptibility genes are unlikely to increase breast cancer risk. *Breast Cancer Res*, **9**, R27.

Bouska A, Eischen CM (2009). Mdm2 affects genome stability independent of p53. *Cancer Res*, **69**, 1697-701.

Bray J, Sludden J, Griffin MJ, et al (2010). Influence of pharmacogenetics on response and toxicity in breast cancer patients treated with doxorubicin and cyclophosphamide. *British Journal of Cancer*,**102**, 1003-9.

Chang H, Milne RL, Cox A, et al (2008). Association of the ABCB1 gene polymorphisms 2677G>T/A and 3435C>T with clinical outcomes of paclitaxel monotherapy in metastatic breast cancer patients. *Annals of Oncology*, **20**, 272-277.

Chang JC, Hilsenbeck SG, Fuqua SA (2009). Pharmacogenetics of breast cancer: toward the individualization of therapy. *Cancer Invest*, **27**,699-703.

Chen C, Sakoda LC, Doherty JA, et al (2008). Genetic variation in CYP19A1 and risk of breast cancer and fibrocystic breast conditions among women in Shanghai, China. *Cancer Epidemiol Biomarkers Prev*, **17**, 3457-66.

Colomer R, Monzo M, Tusquets I, et al (2008). A Single-Nucleotide Polymorphism in the Aromatase Gene is Associated with the Efficacy of the Aromatase Inhibitor Letrozole in Advanced Breast Carcinoma. *Clinical Cancer Research*, **14**, 811-6.

Conde J, Silva SN, Azevedo AP, et al (2009). Association of common variants in mismatch repair genes and breast cancer susceptibility: a multigene study. *BMC Cancer*, **9**, 344.

Cox DG, Bretsky P, Kraft P, et al (2008). Haplotypes of the estrogen receptor beta gene and breast cancer risk. *Int J Cancer*, **122**, 387-92.

Devita VT, Hellman S, Rosenberg SA (2001). Cancer: Principles and Practice of Oncology. 6th ed. Lippincott Williams & Wilkins.

Dipiro JT, Talbert RL, Yee GC, et al (2005). Pharmacotherapy: A Pathophysiologic Approach. 6th ed. McGraw-Hill.

Domchek SM, Greenberg RA (2009). Breast cancer gene variants: separating the harmful from the harmless. *J Clin Invest*, **119**, 2895-7.

Dunn L, Demichele A (2009). Genomic predictors of outcome and treatment response in breast cancer. *Mol Diagn Ther*, **13**, 73-90.

Ekhart C, Doodeman VD, Rodenhuis S, et al (2008). Influence of polymorphisms of drug metabolizing enzymes (CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP345, GSTA1, GSTP1, ALDH1A1, ALDH3A1) on the pharmacokinetics of cyclophosphamide and 4-hydroxycylcophosphamide. *Pharmacogenetics and Genomics*, **18**, 515-299.

Frank B, Wiestler M, Kropp S, et al (2008). Association of a common AKAP9 variant with breast cancer risk: a collaborative analysis. *J Natl Cancer Inst*, **100**, 437-42.

Freedman AN, Sansbury LB, Figg WD, et al (2010). Cancer pharmacogenomics and pharmacoepidemiology setting a research agenda to accelerate translation. *J Natl Cancer Inst*, **102**, 1698-705.

Garcia-Casado Z, Guerrero-Zotano A, Llombart-Cussac A, et al (2010). A Polymorphism at the 3'-UTR region of the aromatase gene defines a subgroups of postmenopausal breast cancer patients with poor response to neoadjuvant letrozole. *BMC Cancer*, **10**, 36.

Gaudet MM, Milne RL, Cox A, et al (2009). Five polymorphisms and breast cancer risk: results from the Breast Cancer Association Consortium. *Cancer Epidemiol Biomarkers Prev*, **18**, 1610-1616.

Goetz MP, Kamal A, Ames MM (2008). Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. *Clin Pharmacol Ther*, **83**, 160-6.

Gold B, Kalush F, Bergeron J, et al (2004). Estrogen receptor genotypes and haplotypes associated with breast cancer risk. *Cancer Res*, **64**, 8891-900.

Green H (2008). Pharmacogenomics of importance for paclitaxel chemotherapy. *Pharmacogenomics*, **9**, 671-674.

Haiman CA, Hsu C, de Bakker PI, et al (2008). Comprehensive association testing of common genetic variation in DNA repair pathway genes in relationship with breast cancer risk in multiple populations. *Hum Mol Genet*, **17**, 825-34.

Hamilton R (2009). Genetics: breast cancer as an exemplar. *Nurs Clin North Am*, **44**, 327-38.

Hirshfield KM, Rebbeck TR, Levine AJ (2010). Germline mutations and polymorphisms in the origins of cancers in women. *Journal of oncology*, **2010**, 2976-71.

http://www.cancer.org/Cancer/CancerBasics/TheHistoryofCancer/index

Ingle JN (2008). Pharmacogenetics and pharmacogenomics of endocrine agents for breast cancer. *Breast Cancer Res*, **10**, S17.

Kiyotani K, Mushiroda T, Imamura CK, et al (2010). Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer. *J Clin Oncol*, **28**, 1287-93.

Lal S, Sandanaraj E, Wong ZW, et al (2008). CBR1 and CBR3 pharmacogenetics and their influence on doxorubicin disposition in Asian breast cancer patients. *Cancer Science*, **99**, 2045-54.

Largillier R, Etienne-Grimaldi M, Formento J, et al (2006). Phamacogenetics of Capecitabine in Advanced Breast Cancer Patients. *Clinical Cancer Research*, **12**, 5496-502.

Lazarus P, Blevins-Primeau AS, Zheng Y, et al (2009). Potential Role of UGT Pharmacogenetics in Cancer Treatment and Prevention: focus on tamoxifen. *Annals of the New York Academy of Sciences*, **115 5**, 99-111.

Lee W, Lockhart AC, Kim RB, Rothenberg ML (2005). Cancer pharmacogenomics: Powerful tools in cancer chemotherapy and drug development. *Oncologist*, **10**, 104-11.

Lichtenstein P, Holm NV, Verkasalo PK (2000). Environmental and heritable factors in the causation of cancer. *N Engl J Med*, **343**, 78-85.

Linger RJ, Kruk PA (2010). BRCA1 16 years later: Risk-associated BRCA1 mutations and their functional implications. *FEBS J*, **277**, 3086-96.

Maring JG, Groen HJ, Wachters FM, et al (2005). Genetic factors influencing Pyrimidine-antagonist chemotherapy. *The Pharmacogenomics Journal*, **5**, 226-243.

Marsh S, Somlo G, Li X, et al (2007). Pharmacogenetic analysis of paclitaxel transport and metabolism genes in breast cancer. *The Pharmacogenomics Journal*, **7**, 362-365.

Mavaddat N, Dunning AM, Ponder BA, et al (2009). Common genetic variation in candidate genes and susceptibility to subtypes of breast cancer. *Cancer Epidemiol Biomarkers Prev*, **18**, 255-9.

McDermott U, Settleman J (2009). Personalized cancer therapy with selective kinase inhibitors: an emerging paradigm in

medical oncology. *J Clin Oncol*, **27**, 5650-9.
Nakagawa K, Minami H, Kanezaki M, et al (2009). Phase I Dose-escalation and Pharmacokinetic Trial of Lapatinib (GW572016), a Selective Oral Dual Inhibitor of ErbB-1 and -2 Tyrosine Kinases, in Japanese Patients with Solid Tumors. *Jpn J Clin Oncol*, **39**, 116-23.
Pitot HC, Loeb DD (2002). Fundamentals of Oncology. 4th ed. Marcel Dekker.
Poplawski T, Zadrozny M, Kolacinska A, et al (2005). Polymorphisms of the DNA mismatch repair gene HMSH2 in breast cancer occurrence and progression. *Breast Cancer Res Treat*, **94**, 199-204.
Pruthi S, Boughey JC, Brandt KR, et al (2007). A multidisciplinary approach to the management of breast cancer, part 2: therapeutic considerations. *Mayo Clin Proc*, **82**, 1131-40.
Putnik M, Zhao C, Gustafsson JA, et al (2009). Effects of two common polymorphisms in the 3' untranslated regions of estrogen receptor beta on mRNA stability and translatability. *BMC Genet*, **10**, 55.
Rakhra-Burris TK, Auman JT, Deverka P, et al (2010). Institutional profile. UNC Institute for Pharmacogenomics and Individualized Therapy: interdisciplinary research for individual care. *Pharmacogenomics*, **11**, 13-21.
Rokavec M, Schroth W, Amaral SM, et al (2008). A polymorphism in the TC21 promoter associates with an unfavorable tamoxifen treatment outcome in breast cancer. *Cancer Res*, **68**, 9799-808.
Ross JS, Hatzis C, Symmans WF, et al (2008). Commercialized multigene predictors of clinical outcome for breast cancer. *Oncologist*, **13**, 477-93.
Rusnak DW, Lackey K, Affleck K, et al (2001). The effect of the novel reversible epidermal growth factor receptor ErbB2 tyrosine kinase inhibitor GW2016 on the growth of human normal and tumor-derived cell lines in vitro and in vivo. *Mol Cancer Ther*, **1**, 85-94.
Schroth W, Antoniadou L, Fritz P, et al (2007). Breast Cancer Treatment Outcome With Adjuvant Tamoxifefn Relative to Patient CYP2D6 and CYP2C19 Genotypes. *Journal of Clinical Oncology*, **25**, 5187-93.
Schroth W, Goetz MP, Hamann U, et al (2009). Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA*, **302**, 1429-36.
Spector NL, Xia W, Burris H, et al (2005). Study of the Biologic Effects of Lapatinib, a Reversible inhibitor of ErbB1 and RebB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J Clin Oncol*, **23**, 2502-12.
Stearns V, Davidson NE, Flockhart DA (2004). Pharmacogenetics in the treatment of breast cancer. *Pharmacogenomics J*, **4**, 143-53.
Sun YF, Leu JD, Chen SM, et al (2009). Results based on 124 cases of breast cancer and 97 controls from Taiwan suggest that the single nucleotide polymorphism (SNP309) in the MDM2 gene promoter is associated with earlier onset and increased risk of breast cancer. *BMC Cancer*, **9**, 13.
Tung N, Wang Y, Collins LC, et al (2010). Estrogen receptor positive breast cancer in BRCA1 mutation carrier: clinical risk factors and pathologic features. *Breast Cancer Res*, **12**, R12.
Workman P (2002). The impact of genomic and proteomic technologies on the development of new cancer drugs. *Ann Oncol*, **13**, 115-24.
Yang ES, Xia F (2010). BRCA1 16 years later: DNA damage-induced BRCA1 shuttling. *FEBS J*, **277**, 3079-85.
Yan L, Beckman R (2005). Pharmacogenetics and pharmacogenomics in oncology therapeutic antibody development. *Biotechniques*, **39**, S565-8.
Zhuo W, Zhang Y, Xiang Z, Cai L, Chen Z (2009). Polymorphisms of TP53 codon 72 with breast carcinoma risk: evidence from 12226 cases and 10782 controls. *J Exp Clin Cancer Res*, **28**, 115.