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Oncologic Drugs

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Pharmacogenomics is of particular importance in oncology, a medical subspecialty characterized by rapidly lethal diseases and drugs with narrow therapeutic indices and significant toxicities. Identification of individuals likely to respond to or experience toxicity from a given chemotherapeutic agent, will have significant impact on outcomes, particularly in the field of oncology. Several models currently exist for discovery of pharmacogenomic markers in oncology. Phenotypic variations may range from variability in response as measured by survival or time to progression, to variability in toxicity in individuals treated with a particular agent. Measurements of toxicity can be a challenge to quantify in individuals because of interobserver variability. Lymphoblastoid cell lines (LCLs) and the NCI60 bank of tumor cell lines have been used as models for clinical phenotypes. To date, there are several examples of germline polymorphisms and somatic mutations that predict likelihood of response and/or toxicity from chemotherapeutic agents. A pattern of interethnic variability in response and toxicity has been observed for some chemotherapeutic agents, and the associated field of pharmacoethnicity is likely to contribute to our understanding of pharmacogenomics.

Pharmacogenomics has found extensive application in the field of oncology and is likely to remain an important tool in the race toward personalized medicine. Individualization of therapies is of particular importance in oncology because of several unique features of cancer treatment. First, most oncologic therapies have potential for organ toxicity and typically give rise to an array of potential life-threatening side effects. For example, taxanes are highly efficacious against malignancies of the lung, breast, ovary, and head and neck, but are also associated with significant toxicities such as myelosuppression and peripheral neuropathy. Identification of individuals unlikely to respond to taxane therapy a priori will be tremendously important in therapeutic decision making, because alternative therapies can be considered, thereby reducing the likelihood of unnecessary toxicity. Second, many oncologic diseases progress rapidly and are generally lethal in the absence of effective therapy. Consequently, prompt diagnosis and early institution of efficacious therapies is of paramount importance. In the absence of knowledge about predictors of response, individuals could be subjected to therapies to which their tumors might not respond, resulting in further disease progression. With more advanced disease and organ dysfunction, some therapies may no longer be given safely and may only serve a palliative rather than a curative role. For example, a five-year period of adjuvant tamoxifen therapy following successful treatment of early-stage estrogen receptor (ER)-positive breast cancer in a premenopausal woman is associated with a reduction in the rate of disease recurrence and mortality. A poor metabolizer phenotype results in insufficient conversion of tamoxifen to endoxifen and an increased risk of disease relapse and progression (1). Affected individuals may be better served by alternative antiestrogen maneuvers such as the combination of ovarian ablation and aromatase inhibitor therapy. Third, most chemotherapeutic agents have a fairly narrow therapeutic index (see Figure 10.1). The therapeutic index of a drug compares the dose that produces toxicity with the dose that produces the desired effect and, as such, provides a measure of the drug's safety. Given the significant likelihood of an adverse effect even within the therapeutic window, treatment with a particular agent is best reserved for individuals likely to respond, with the careful weighing of risks and benefits and informed decision making on the part of the patient. Finally, the expenses associated with oncologic therapies necessitate avoidance of therapy-related morbidity that further increases the likelihood of hospitalization and overall cost of care. For example, trastuzumab is an agent used in the treatment of HER2neu-positive breast cancer, is typically infused on a three-weekly schedule for at least one year, and may cost as much as $70,000 for a full course of therapy (2). Given all the aforementioned, it is not surprising that current pharmacogenomic research

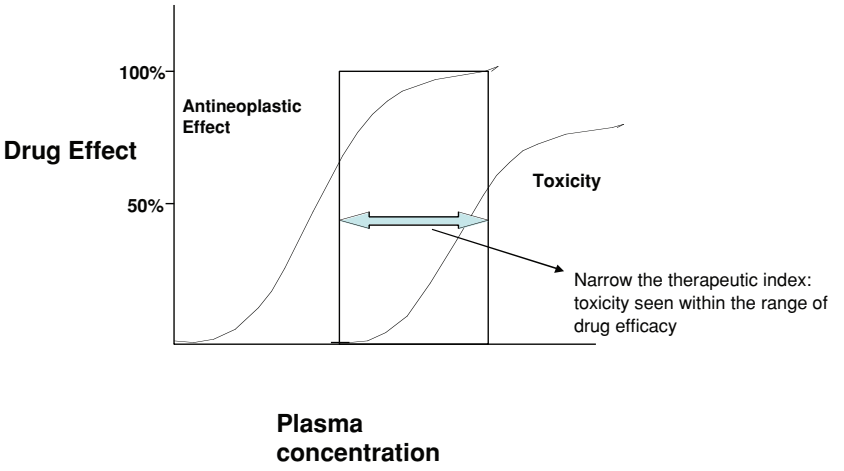


Figure 10.1. Illustration of therapeutic index.

is dominated by investigation of variability in response to, and toxicity from, oncologic therapies (3).

PHARMACOGENOMIC DISCOVERY IN ONCOLOGY

Germline Polymorphisms and Somatic Mutations

Unlike pharmacogenomic research in other medical subspecialties, variability in response to chemotherapeutic agents may be studied for association with variants in germline DNA, somatic DNA, or both. Neoplastic cells are characterized by malignant transformation, a process that involves significant alteration in the cellular genetic material, including changes in oncogene and tumor suppressor gene expression (4). Downstream effects include alteration in cellular signaling, proliferation, and apoptosis. These somatic mutations cause a change in the genotype of the resultant cancer cell, and are not present in nonmalignant cells. In contrast, germline variants are found in all cells, may be passed on to offspring, and can be expected to be identical in all cells of the same individual (see Figure 10.2).

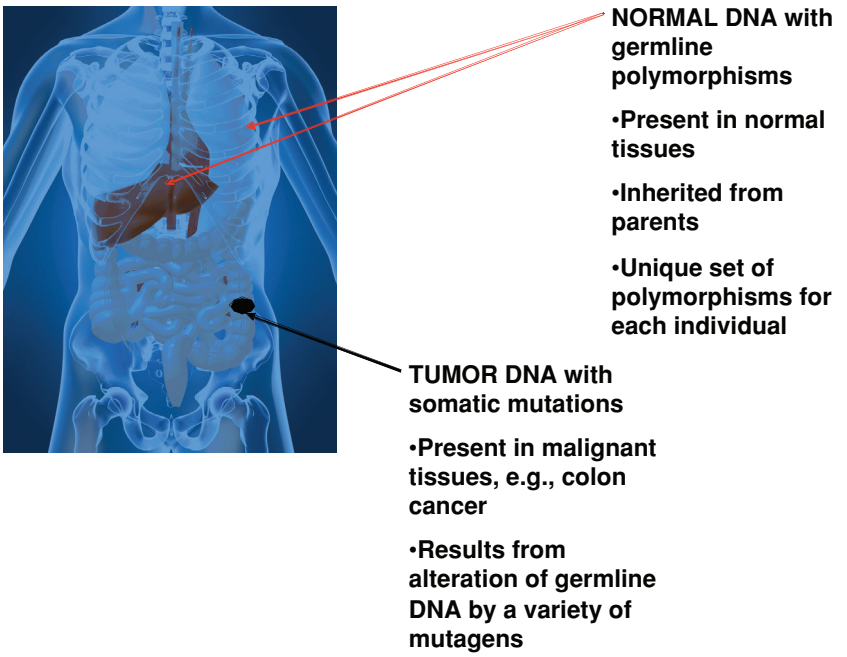


Figure 10.2. Distinguishing features of somatic mutations and germline polymorphisms.

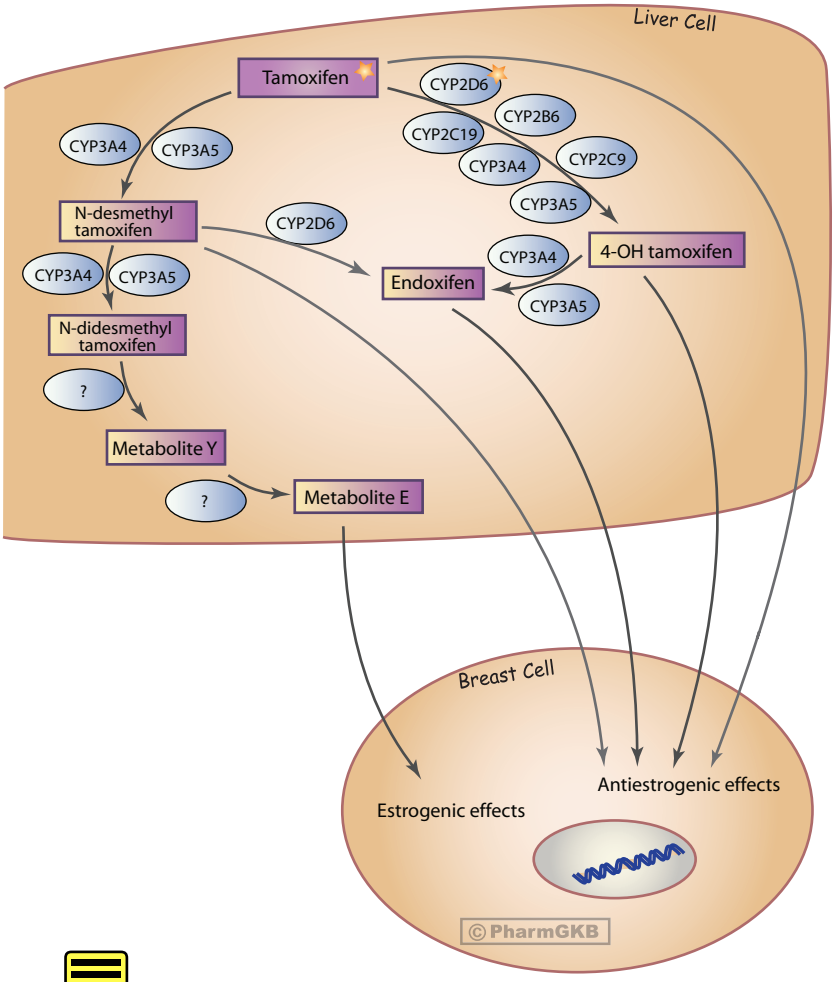


Figure 10.2. EGFR signaling pathway. Reproduced with permission from PharmGKB and Stanford University (68).

Somatic and germline mutations occurring in the same gene may give rise to different phenotypes. For example, the *TP53* gene is a tumor suppressor gene involved in several human malignancies. Germline mutations give rise to the Li-Fraumeni syndrome, which is characterized by predisposition to early-onset cancers such as breast carcinoma, sarcomas, brain tumors, and adrenocortical carcinomas. On the other hand, somatic mutations in *TP53* occur in almost every type of human cancer at rates as high as 38 percent to 50 percent, and tend to be more frequent when the cancer is of advanced stage or aggressive behavior (5). In addition, it has been demonstrated that somatic mutations can be highly variable even within the same tumor (6) and change as the tumor evolves (7). Because the efficacy of an antineoplastic agent is related to its ability to exert an effect on malignant tissues, studies of the variability in intended drug effect have historically relied on the analysis of somatic DNA and gene expression within the tumor (8). For example, mutations in the epidermal growth factor receptor (EGFR) gene have been found to correlate with response to EGFR tyrosine kinase inhibitors (Figure 10.3). In contrast, toxicity results from the effects on normal tissues and therefore is most likely predicted by germline polymorphisms and their effects on drug pharmacokinetics or pharmacodynamics.

Although germline polymorphisms in general are thought to affect drug toxicity, there is growing evidence that germline polymorphisms may also predict chemotherapeutic response (9). For example, host genetic variations are associated with treatment response for childhood acute lymphoblastic leukemia (ALL), with polymorphisms related to leukemia cell biology and host drug disposition associated with the lower risk of residual disease (10). In addition, genotypes in ethnic Asian patients with non-small cell lung cancer (NSCLC) are

predictive of response to gemcitabine (11). Although variation exists, germline DNA and matched somatic DNA may also have significant concordance in variants of pharmacogenetic genes (12), a feature that supports a possibility of correlation between germline polymorphisms and intended drug effects. Given the relative ease with which germline DNA may be collected from healthy individuals and that it remains the same throughout an individual's life, there is great value in determining germline variants contributing to either response or toxicity.

Phenotypes in Pharmacogenomic Studies of Chemotherapeutic Agents

A wide range of phenotypes may be studied for chemotherapeutic agents both in the clinical and preclinical setting. Clearly, cancer patients are the optimal system for identifying genetic variants contributing to chemotherapeutic drug response and toxicity. The problem with human studies in oncology is the rarity in which a homogeneous patient population receives the same dose of a single chemotherapeutic agent. For measurements of chemotherapeutic toxicity, interobserver variability and difficulty in obtaining quantitative measurements is another factor. To get around these issues and avoid confounders such as comorbidities, concomitant medications, and diet, preclinical cell-based models have been developed that provide a useful discovery tool. Critical to the process is the validation of these markers in a clinical setting.

Clinical Phenotypes

Clinical phenotypes may be binary, such as death and survival, or continuous, such as bone marrow suppression. Because side effects may be subjective, a toxicity-grading system is often used. The Common Terminology Criteria for Adverse Events was developed by the National Cancer Institute and is the predominant system used to describe the severity of adverse events in oncology clinical trials (13). A myriad of toxicities may be associated with oncologic therapies and range from relatively benign to severe and life threatening. Examples of benign side effects include nausea, vomiting, alopecia, fatigue, and anorexia, all of which may be seen with most chemotherapeutic agents. Such side effects are often easy to control with symptomatic therapies like antiemetics, and typically do not have a major influence on the choice of chemotherapeutic agents. On the other hand, examples of severe toxicities include peripheral neuropathy from taxanes and epothilones, cardiomyopathy from anthracyclines and HER2-targeted agents, profound myelosuppression from several agents, central nervous system toxicity from ifosfamide and 5-fluorouracil (5-FU), and hemorrhagic cystitis from cyclophosphamide. The suppression of bone marrow cells can be considered a quantitative phenotype; however, the degree of suppression often depends on the frequency at which the measurement is taken. Peripheral neuropathy may be debilitating by affecting an individual's ability to perform activities of daily living, whereas cardiomyopathy and central nervous system toxicity may be life threatening. The latter category of side effects is of significant clinical importance, because their occurrence typically necessitates a change of therapy.

The measurement of the response to chemotherapy may take several forms, giving rise to a range of clinical response phenotypes for use in pharmacogenomic studies. For solid tumors, the response to therapy may take the form of a decrease in the size of a tumor mass (partial response) or the complete disappearance of a lesion (complete response). In addition, the lack of change in a lesion may signify stable disease. For standardization, the Response Evaluation Criteria in Solid Tumors guidelines are most commonly used, and use unidimensional measurements of target lesions before and after therapy to evaluate for a complete response, partial response, or progressive disease (14).

For hematologic malignancies, the response to therapy is assessed differently because such neoplasms are associated with the presence of aberrant cells in the blood and/or bone marrow rather than with distinct masses evident on radiologic imaging. For example, chronic myelogenous leukemia (CML) is characterized by the presence of large numbers of neoplastic myeloid cells bearing the Philadelphia chromosome or t(9;22) translocation that gives rise to the *BCR-ABL1* chimeric gene (15). Imatinib is standard therapy for CML and induces a high rate of hematologic and cytogenetic response. A complete hematologic response is defined as the attainment of a white blood cell count of $<10{,}000/\mu$L, no immature granulocytes and <5 percent basophils, and a platelet count of $<450{,}000/\mu$L with a nonpalpable spleen. In contrast, a complete cytogenetic response is defined by the complete disappearance of Philadelphia chromosome positive cells. A major molecular response is present when the ratio of BCR-ABL transcript to housekeeping genes is ≤ 0.1 percent on an international scale (16). Additional parameters such as rate of generation of metabolites and overall drug exposure, as measured by the area under the drug pharmacokinetic curve, are related to drug pharmacokinetics and may also be used as phenotypes in clinical pharmacogenomic studies (17).

Preclinical Cellular Phenotypes

The selection of molecular phenotypes in cell lines that accurately reflect clinical drug response is a major challenge. The appropriate phenotype usually depends on the mechanism of action of the drug, and on the clinical phenotype of interest (18). For example, anticancer drugs are intended to cause growth inhibition, cell death,

or apoptosis; therefore, measuring cellular apoptosis or cell growth inhibition across a range of drug dosages is generally performed (19). Another phenotype to consider is measurement of the conversion of parent drug to active metabolite. This has been effectively analyzed in the case of methotrexate glutamation (20) and the chemotherapeutic drug cytarabine, in which the amount of active metabolite (AraCTP) was associated with a specific genotype within an important drug-metabolizing gene (21).

There are a number of advantages to using cell lines derived from individuals for pharmacogenomic discovery. Cells can be grown under identical conditions, allowing the genetic contributions toward a specific phenotype to be tested in a well-controlled, isolated system without the confounders present in vivo. Cell lines offer ease of experimental manipulation and unlimited resources to study pharmacodynamic effects that would be considered unsafe in healthy volunteers. Despite the advantages of this ex vivo system, there are limitations to using cell lines to identify pharmacogenetic effects. These include the following: (1) Few cell lines are available from nonmalignant tissue, making it difficult to find an appropriate ex vivo system to study toxicities such as neurotoxicity, cardiovascular toxicity, and nephrotoxicity, to name a few. (2) Most cell lines do not have a cytochrome P450 system, making it difficult to study the pharmacokinetics of drugs that require metabolic conversion. (3) Transformation of lymphoblasts into LCLs or tumors into tumor cell lines could introduce phenotypic changes, which may result in expression differences with regard to the phenotype of study.

Approaches in Chemotherapy Pharmacogenomic Research

Whether using cell lines, animals, or humans for pharmacogenomic studies, there are primarily two approaches, the candidate gene approach, in which a gene or pathway is identified as potentially important based on literature evidence and then subjected to further study, and a hypothesis generating approach, in which the whole genome is considered and no assumptions are made about what genes are important. The sequencing of the human genome and the genetic resource provided by The International HapMap Project have allowed researchers to greatly expand the focus of pharmacogenomic studies to more routinely perform genome-wide studies. A major advantage to the genome-wide approach is the enormous amount of information gained; however, along with that information comes false-positive findings as a result of multiple testing on a large scale. A major advantage of the genome-wide approach is that it opens up the possibility of identifying previously unknown genetic variants that contribute to chemotherapy-induced cytotoxicity.

Candidate Gene Approach in Oncology

In this method, a single gene or genes within a pathway known to be important in the pharmacokinetics or pharmacodynamics of a particular drug are examined for genetic variability and compared with phenotypic variation. Such an approach has met with success in elucidating the pharmacogenetics of chemotherapeutic agents (Table 10.1). Some successful examples of candidate gene studies include (1) genetic variations in thiopurine methyltransferase (*TPMT*) associated with increased risk for severe myelosuppression after 6-mercaptopurine (6-MP) treatment (22); (2) *UGT1A1*28* associated with a decrease in UGT1A1 expression and increased risk of severe neutropenia when irinotecan is administered (23); and (3) lack of response to tamoxifen in *CYP2D6* poor metabolizers (24). Importantly, the candidate gene approach depends on the presence of a small number of alleles seen in a significant fraction of the general population that gives rise to a major alteration in drug effectiveness. In addition, the approach is most successful if the gene involves a key step in the drug metabolic pathway. Because such a scenario is not applicable to all chemotherapeutic agents, and because chemotherapy-induced response and toxicity are most likely multigenic traits, a broader approach is important in accurately elucidating genetic predictors of response and toxicity (25).

Genome-Wide Association Studies in Oncology

A genome-wide approach (genome-wide association study, or GWAS) takes the whole genome into consideration and uses an unbiased method to generate candidate genes that may be further subjected to functional evaluation and validation (26, 27). GWAS has been facilitated by completion of the sequencing of the human genome and the International HapMap projects. The Human Genome Project was launched in 1990 and completed in 2004, and served to provide an accurate sequence of the human genome as a foundation for genetic studies of disease and response to drugs (28). The International HapMap Project was initiated in 2002 to characterize common variations in DNA sequence among four different populations and to construct haplotype maps (29) but has been extended to study eleven additional populations.

Genome-wide studies of germline DNA have been used in generating genomic predictors of response to a variety of chemotherapeutic agents. As a result of the large number of polymorphisms studied for association, GWAS may result in a high false-discovery rate. This important limitation may be curtailed by validation of findings in an independent set of similarly treated cells or patients. Nevertheless, a few GWAS findings have been correlated with results obtained by using a candidate gene approach. For example, cytarabine is a chemotherapeutic

Table 10.1. Pharmacogenomics, of Chemotherapeutic Agents Derived by a Candidate Gene Approach

Tumor Type	*Chemotherapeutic Agent(s)*	*Molecular Target*	*Pharmacogenomic Issues of Importance*	*Clinical Significance*
Breast cancer	Tamoxifen	ER	At least seventy CYP2D6 allelic variants exist and give rise to poor, intermediate, extensive, and ultrarapid metabolizers with progressively higher concentrations of the active metabolite, endoxifen.	Breast cancer patients homozygous for the null allele, CYP2D6*4 have shorter time to relapse and worse disease-free survival than those with CYP2D6*4/*1 or CYP2D6*1/*1 genotypes. No CYP2D6*4/*4 patients experienced moderate or severe hot flashes, whereas approximately 20% of women with CYP2D6*4/*1 or CYP2D6*1/*1 do experience hot flashes.
Colorectal cancer	Irinotecan Monoclonal antibodies against EGFR (EGFR-I), e.g., cetuximab, panitumumab	Topoisomerase I EGFR	The UGT1A1*28 allele, characterized by seven TA repeats in the promoter region, results in reduced activity of the UGT1A1 enzyme, with consequent accumulation of the toxic metabolite, SN-38. Gain-of-function mutations in the KRAS gene involved in downstream signaling result in bypassing of the EGFR signaling pathway.	Several studies show that individuals homozygous for the UGT1A1*28 allele are more predisposed to late irinotecan toxicity manifesting as diarrhea, neutropenia, or both. Patients with KRAS-mutated tumors are resistant to therapy with EGFR-I agents.
NSCLC	EGFR-tyrosine kinase inhibitors (EGFR-TKI), e.g., gefitinib and erlotinib	EGFR-tyrosine kinase	Somatic mutations in EGFR gene result in altered function of the associated tyrosine kinase.	Somatic mutations in EGFR in NSCLC are highly correlated with response to gefitinib and erlotinib, particularly among nonsmoking Asian females.
Multiple malignancies	5-FU	Dihydropyrimidine dehydrogenase Thymidylate synthetase	Several sequence variations in the dihydropyrimidine dehydrogenase gene (DPYD) have been described; however, only the relatively infrequent DPYD*2A and DPYD*13 alleles have been consistently associated with DPD deficiency. Variants such as a 6 base pair insertion and deletion polymorphism in the 3′-untranslated region, and a variable number of tandem repeats in the promoter-enhancer region, lead to increased expression of the thymidylate synthetase gene (*TS*).	Mutations resulting in DPD deficiency result in increased likelihood of potentially life-threatening toxicity from 5-FU. High *TS* gene expression variants are associated with decreased survival in colorectal cancer patients treated with 5-FU.
A variety of hematologic malignancies, e.g., childhood and adult ALL, childhood AML, childhood non-Hodgkin's lymphoma	6-MP and 6-thioguanine (6-TG)	Purine analogs, antagonists to endogenous purines required for DNA synthesis in the S-phase of the cell cycle	Azathioprine is converted to 6-MP. Both 6-MP and 6-TG are catabolized by thiopurine methyltransferase (TPMT). Seventeen mutant TPMT alleles have been described, some of which give rise to intermediate or low enzyme activity.	Low TPMT activity results in failure to catabolize purine analogs, with consequent life-threatening toxicity, including myelosuppression.

agent used in the treatment of hematologic malignancies such as adult acute myeloid leukemia (AML). The rate-limiting step in cytarabine catabolism is catalyzed by the enzyme deoxycytidine kinase (DCK). Earlier research demonstrated that low levels of DCK mRNA in blast cells correlated with poorer outcome as shown by a shorter disease-free and overall survival (30). In addition, clinical studies showed that low intracellular levels of cytarabine in leukemia cells resulted in similarly poor outcomes (31, 32). Consistent with clinical findings, a GWAS study using LCLs showed that single-nucleotide polymorphisms (SNPs) within the gene *DCK* resulted in increased enzyme levels and heightened sensitivity to cytarabine (21).

Advances in molecular biology and bioinformatics coupled with the development of methods for high-throughput analysis have made it feasible to study large numbers of individuals in GWAS. Such studies may be conducted clinically by genotyping individuals with a variable drug response; however, there are important considerations in clinical GWAS. First, because variation in response to most clinically administered drugs depends on the combined contribution of multiple genes, one must consider sample size for a study to have adequate statistical power. Clinical GWAS studies are expensive and time consuming, and require large numbers of patients and infrastructure to obtain reliable clinical phenotype data. Establishing a prospective cohort can take years because of the time required to meet regulatory requirements, to accrue a population of sufficient size, and for follow-up analysis. Although samples from retrospective clinical trials require fewer resources, in general, they are not powered to answer specific pharmacogenetic questions. This problem is further compounded by the need for multiple large patient cohorts to enable both discovery and replication studies. In addition, confounding factors such as comorbidities, dosage, timing of drug administration, and diet are difficult to standardize and cannot be easily separated from genomic contributions to variation in drug response. Uncontrolled confounders, including population stratification or admixture, can bias measured effect estimates of genotype-phenotype relationships. Finally, pharmacogenetic discovery for highly toxic drugs, such as chemotherapeutics and certain antiviral agents, poses additional challenges because these drugs cannot be administered to healthy individuals for classical genetic studies. For the reasons just mentioned, some researchers have turned to the use of human cell-based models for pharmacogenetic discovery and validation studies (18).

Cell-Based Models in Chemotherapy Pharmacogenomic Discovery

For oncologic research, the examples of cell lines used include those from healthy individuals, and those derived from tumors from humans. As described earlier, the DNA and certainly expression of genes can vary considerably between normal tissue and tumor. Lymphoblastoid cell lines and the NCI60 bank of tumor cell lines are the most frequently used cell-based models for pharmacogenomic discovery. The NCI60 bank of cancer cell lines is derived from multiple human tumors and contains somatic DNA. As part of the International HapMap Project, LCLs were collected in phases from distinct world populations, including whites from Utah (CEU), Yorubas from Nigeria (YRI), Chinese from Beijing (CHB), and Japanese from Tokyo (JPT). LCLs are commercially available, genotyped (with many being sequenced through the 1000 Genome Project), and, for the CEU samples, also are part of large pedigrees. The NCI60 bank of tumor cell lines are derived from diverse human malignancies including those of the brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate, and skin. For cell-based models, phenotypes such as growth inhibition, cell death through apoptosis, generation of an active metabolite, and biochemical activities have been used (18).

LCL Model

Use of the LCL model allows pharmacogenomic research to be conducted with cells from healthy, related individuals for whom inclusion in chemotherapeutic drug studies would not be feasible because of ethical considerations. Cell lines in culture may be treated with a range of drug concentrations for a set period of time to obtain cellular growth rate inhibition or apoptosis. In addition, parameters such as concentration at which 50 percent growth inhibition occurs (IC_{50}) or area under the percentage survival-concentration curve (AUC) can be used as a single value representing the degree of cellular sensitivity to the drug. Lymphoblastoid cell lines are prepared by Epstein-Barr virus (EBV) transformation of peripheral blood mononuclear cells, which results in the immortalization of B-lymphocytes and the ability to proliferate indefinitely (33). In particular, during the past few years, GWAS using the EBV-transformed LCLs (e.g., the HapMap samples) have demonstrated the feasibility of integrating genotypic data (e.g., >3.1 million SNPs) with cytotoxicities of anticancer agents; for example, 5-FU, docetaxel, etoposide, cisplatin, daunorubicin, carboplatin, cytarabine, and gemcitabine (21, 34–37). Table 10.2 lists some of the discoveries made by using this cell-based model. One of the main criticisms of the use of LCLs for pharmacogenomic discovery of variants contributing to chemotherapeutic toxicity is the effect of confounders (variation in cellular growth rate, baseline EBV copy number, and ATP levels) on phenotypes measured or cellular growth rate (38). Although studies have shown that baseline ATP or EBV copy number was not significantly correlated with

Table 10.2. Examples of Pharmacogenomic Discoveries Using LCLs

Chemotherapeutic Agent	*Examples of Related Gene(s)*	*Significant SNPs and Loci*	*Pharmacogenomic Discovery*	*Reference*
5-FU	TYMS	rs2847153 and rs2853533 9q13-q22	2 SNPs in TYMS, the gene encoding thymidylate synthetase, are significantly associated with 5-FU cytotoxicity in HapMap CEPH LCLs. Genome-wide linkage analyses demonstrate a quantitative trait locus (QTL) at 9q13-q22 which influences 5-FU cytotoxicity in HapMap LCLs.	(59), (35)
Docetaxel	N/A	5q11-21, 9q13-22	Genome-wide linkage analyses demonstrates 2 QTLs on chromosomes 5q11-21 and 9q13-q22 which influence docetaxel cytotoxicity in HapMap LCLs.	(35)
Etoposide	UVRAG, SEMA5A, SCL7A6, PRMT7	22 SNPs with 15 located within introns of the aforementioned genes (rs10079862, rs571826, rs16882871, rs10213926, rs2135071, rs3777359, rs369459, rs446732, rs421548, rs442173, rs486947, and rs268478 in SEMA5A; rs7116263 in UVRAG; rs11644360 in SLC7A6; and rs3785125 in PRMT7) and 1 (rs1127773) located in a 3′-untranslated region of SLC7A6	Linkage-directed association demonstrates 22 SNPs that are significantly associated with etoposide cytotoxicity at one or more treatment concentrations in HapMap CEPH LCLs.	(60)
Cisplatin	CDH13, ZNF659, LRRC3B, PITX2, LARP2	20 SNPs including 10 located within the 5 aforementioned genes (rs17758876 in CDH13; rs17041972, rs17624452, rs17041968, and rs2278782 in PITX2; rs4834232 in LARP2; rs1026686 and rs3860575 in SNF659; and rs17018468 and rs7652737 in LRRC3B), and 10 nongenic SNPs (rs7131224, rs7113868, rs7119153, rs7949504, rs11944754, rs1028074, rs12795809, rs10510534, rs7683488, and rs6848982)	Linkage-directed association analysis demonstrates that 20 SNPs are associated with cisplatin cytotoxicity in HapMap CEPH LCLs, with 4 of those explaining 10% of variation in apoptosis.	(61)
Daunorubicin	HNRPD, CYP1B1	rs1551315, rs12052523, rs2195830, rs623360, rs10083335, rs3750518.	2 SNPs (rs120525235 and rs3750518) were significant predictors of transformed daunorubicin IC_{50} in a validation set of HapMap CEPH LCLs, whereas 6 SNPs predicted 29% of variation of transformed daunorubicin IC_{50}. rs3750518 acts by altering HNRPD gene expression. Additionally, rs10932125 genotype was associated with CYP1B1 expression and transformed daunorubicin IC_{50}.	(62)

Chemotherapeutic Agent	*Examples of Related Gene(s)*	*Significant SNPs and Loci*	*Pharmacogenomic Discovery*	*Reference*
Carboplatin	GPC5	rs1031324 and rs1993034	2 SNPs are significantly associated with carboplatin cytotoxic phenotypes at all concentrations and IC_{50} through an effect on GPC5 gene expression in HapMap LCLs from Yoruba individuals probably by a distant-acting effect because the SNPs are not located within the GPC5 gene.	(63)
Cytarabine	G1T1, SCL25A37, P2RX1, CCDC24, RPS6KA2, SSH2, LOC399491, ANPEP, SOD3	rs17808412, rs2775139, rs17795186, rs368182 in Caucasian LCLs; rs938562, rs10906723, rs2430853, rs10181725, rs10193059 in Yoruba LCLs	4 SNPs explain 51% of variability in sensitivity to cytarabine in HapMap cell lines from white individuals, while 5 SNPs explain 58% of variation in HapMap cell lines from Yoruba individuals, by affecting expression of the aforementioned target genes.	(21)
Gemcitabine and cytarabine	IQGAP2 and TGM3	rs3797418 and rs6082527	A SNP in IQGAP2 (rs3797418) is significantly associated with variation in multiple gene expression as well as both gemcitabine and cytarabine IC_{50} in ethnically defined "Human Variation Panel" LCLs. A second SNP in TGM3 (rs6082527) is associated with gene expression and gemcitabine IC_{50}.	Li L, et al. 2009 (43)

cellular growth rate or drug-induced cytotoxicity, cellular growth rate and drug-induced cytotoxicity were significantly, directly related for a number of chemotherapeutic agents. Importantly, cellular growth rate is under appreciable genetic influence ($h^2 = 0.30$–0.39). Not surprisingly, a percentage of SNPs that significantly associate with drug-induced cytotoxicity also associate with cellular growth rate ($P \leq 0.0001$). Studies using LCLs for pharmacologic outcomes should therefore consider that a portion of the genetic variation explaining drug-induced cytotoxicity is mediated via heritable effects on growth rate (39).

NCI60 Cell Lines

The NCI60 is a bank of sixty human cancer cell lines derived from several malignant tissues including those in the brain, blood and bone marrow, breast, colon, kidney, lung, ovary, prostate, and skin. NCI60 cell lines have been screened for the cytotoxic effect of more than 40,000 compounds, and results are publicly available (40). In addition, they have been used to study genetic predictors of response to several chemotherapeutic agents. For example, the pharmacogenomics of gemcitabine has been studied in such a model (41). A number of studies have correlated baseline gene expression with sensitivity to specific compounds in the NCI60 panel (42). Table 10.3 lists some of the discoveries made using the NCI60 cell lines. An important limitation in the use of cancer cell lines for pharmacogenomic research lies in the small sample size that is available and that the sixty cell lines comprises nine different tissue types. Nevertheless, tumor cell lines may be used for functional studies of predictive genes/loci derived from clinical studies or studied in LCLs. For example, RNA interference technology may be used to upregulate or downregulate gene function in relevant tumor cell lines in order to study variability in drug response resulting from altered gene expression (43).

PHARMACOGENOMICS OF IMPORTANCE IN ONCOLOGY

Germline Polymorphisms

Historically, such variants have been found to predominantly affect pharmacokinetic pathways resulting in altered levels of active drugs and/or metabolites.

Table 10.3. Examples of Pharmacogenomic Discoveries Using NCI60 Cell Lines

Class(es) of Chemotherapeutic Agents Studied	*Related Gene(s)*	*Significant SNPs*	*Pharmacogenomic Discovery*	*Reference*
Taxanes, topoisomerase inhibitors, antimetabolites, N7 alkylating agents.	ERCC2, ERCC5, and GSTP1	rs13181, rs17655, rs1695	Cytotoxicity of taxanes is markedly dependent on ERCC2 genotype; ERCC5 genotype is important only for taxanes; and GSTP1 polymorphisms are relevant for other drug classes.	(64)
Gemcitabine	CDC5L, EPC2, POLS, and PARP1	rs525043, rs2279653, rs6739555, rs2293464	SNPs in 4 genes are significantly associated with gemcitabine sensitivity.	(41)
Erlotinib, geldanamycin, topoisomerase I and II inhibitors, alkylating agents	EGFR	rs2227983 EGFR-216G>T	rs2227983 is associated with lower sensitivity to alkylating agents, whereas -216G>T variants are associated with increased sensitivity to erlotinib and reduced sensitivity to geldanamycin, topoisomerase I inhibitors, and alkylating agents.	(65)
Antimetabolites	MTHFR	MTHFR-A1298C	Cells homozygous for the mutant allele (CC at MTHFR-A1298C) are more sensitive to cyclocytidine, cytarabine, and floxuridine than those with AA or AC.	(66)
Alkylating agents and topoisomerase I inhibitors	MDM2	SNP309	SNP309 is significantly associated with increased sensitivity to alkylating agents and topoisomerase I inhibitors in cells with wild-type TP53 gene.	(67)

Germline polymorphisms have typically predicted toxicity from chemotherapeutic agents, although there is emerging evidence that such variants may also predict response. A few clinically relevant examples are detailed here.

CYP2D6 Genotype and Tamoxifen

Tamoxifen is a selective estrogen receptor modulator (SERM) that exerts agonist effects on uterine endometrium and antagonist effects on breast tissues (44). It is the most widely used antiestrogen therapy in adjuvant treatment of ER-positive breast cancer in premenopausal women, and also has efficacy in preventing invasive and noninvasive breast cancer in women at highest risk. The National Surgical Adjuvant Breast and Bowel Project P1 trial (45) demonstrated a 49 percent reduction in the incidence of invasive breast cancer among high-risk women treated with a five-year course of tamoxifen. As a prodrug, the activity of tamoxifen depends on conversion to a number of active metabolites. In hepatocytes, it is metabolized by cytochrome P450 enzymes to *N*-desmethyltamoxifen and 4-hydroxytamoxifen (see Figure 10.4). Oxidation of both metabolites results in the synthesis of 4-hydroxy-*N*-desmethyltamoxifen (endoxifen). The antiestrogenic effects of tamoxifen and its metabolites depend on interaction with the ERs. Although both 4-hydroxytamoxifen and endoxifen have significant affinity for ER, endoxifen plasma concentrations are five- to tenfold higher, and so it is believed to be the more important metabolite (1).

CYP2D6 is one of the cytochrome enzymes involved in tamoxifen metabolism, and it is encoded by the *CYP2D6* gene on chromosome 22q13.1. Several polymorphisms are known to be present in the gene, and more than seventy-five variant alleles have been reported (46). Null alleles such as CYP2D6*4 and *6 are particularly important, because homozygosity for them results in a poor metabolizer phenotype, a condition in which CYP2D6 enzyme activity is negligible. Clinical trials have demonstrated that affected patients have lower endoxifen levels and poorer outcomes as shown by worse relapse-free time and disease-free survival in comparison with patients lacking null alleles (24). Concurrently,

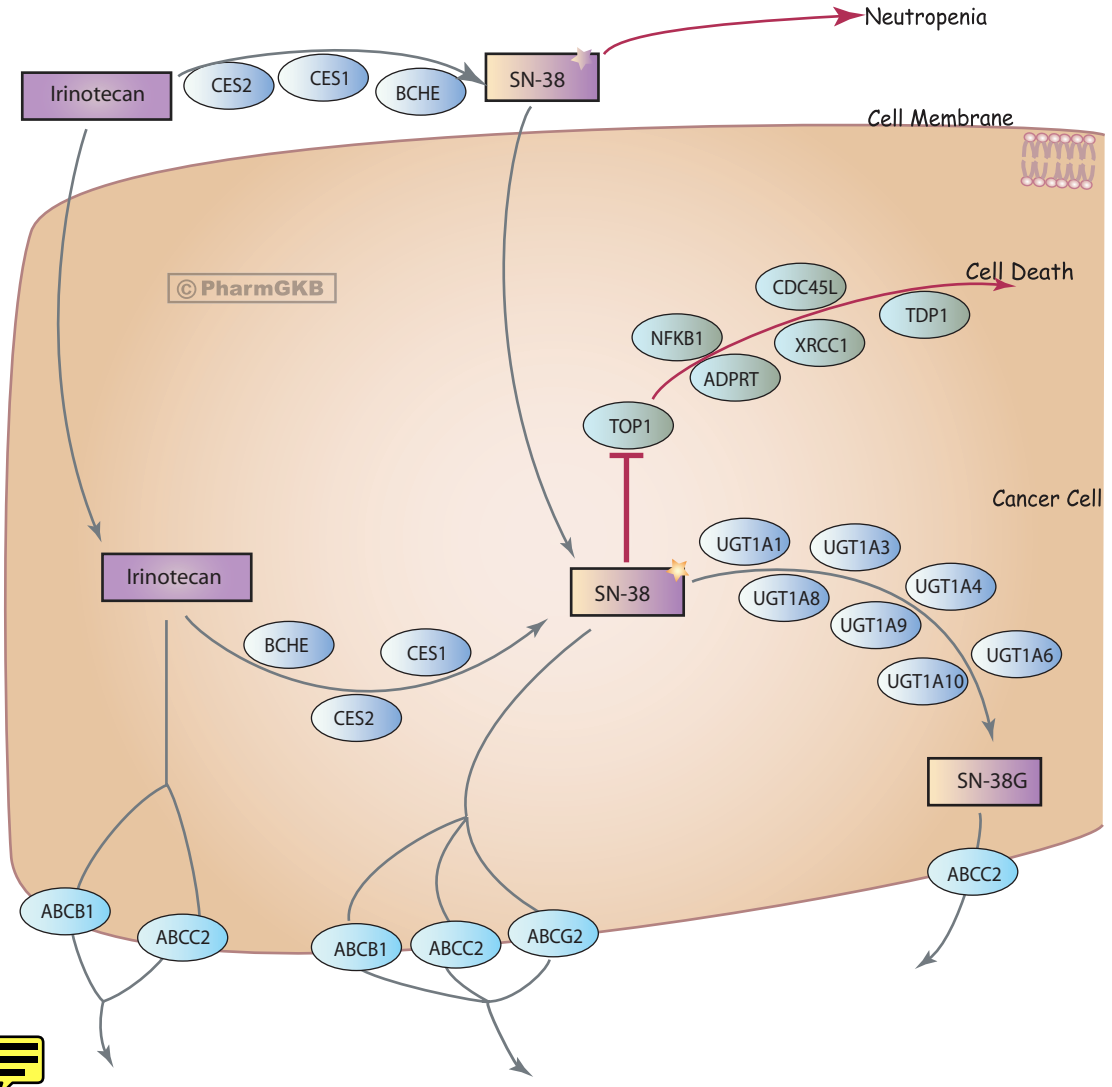


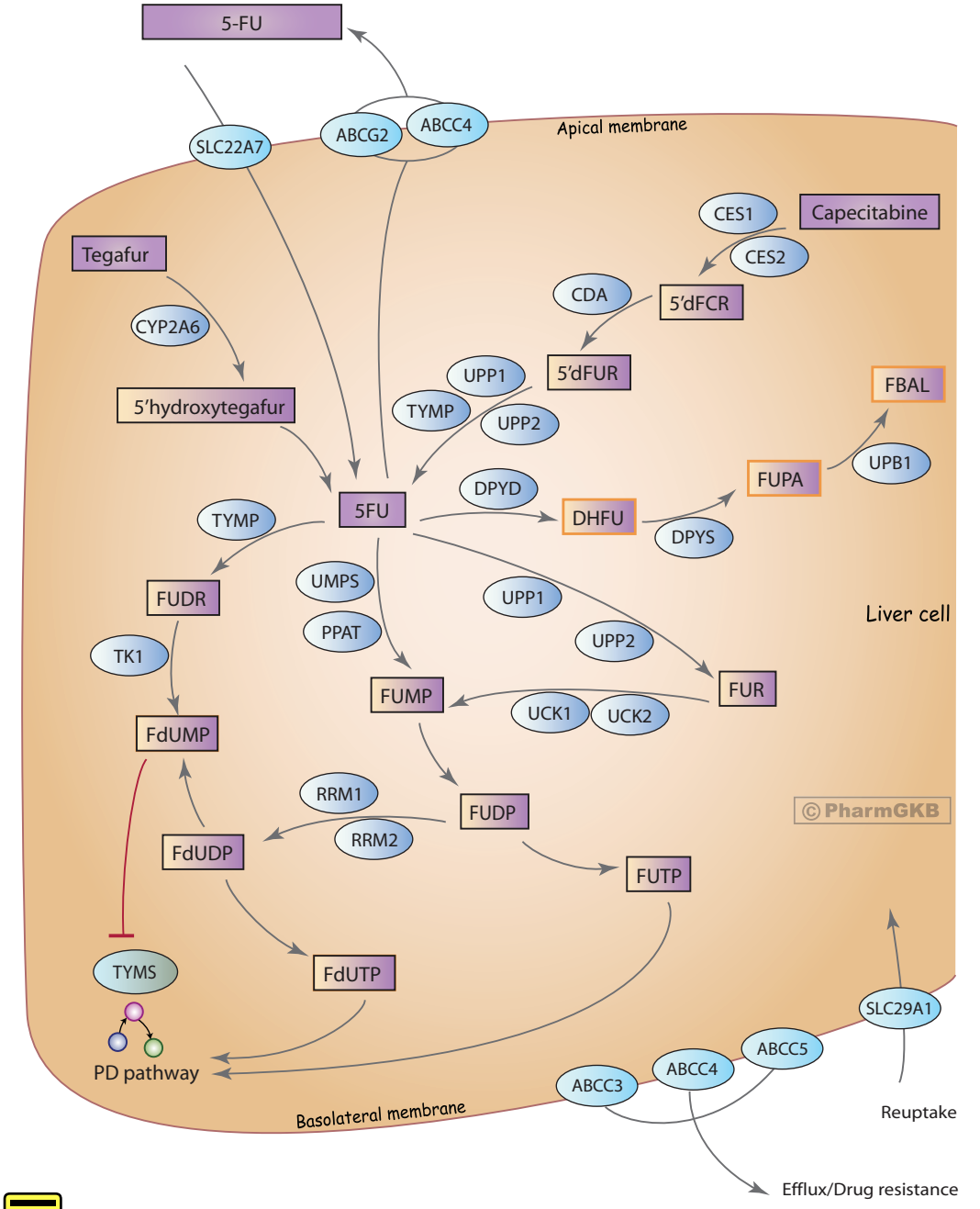
Figure 10.4. Tamoxifen pharmacokinetics and pharmacodynamics. Reproduced with permission from PharmGKB and Stanford University (68).

patients with homozygosity for the null alleles are less likely to experience hot flashes, a common side effect of tamoxifen. CYP2D6 genotyping is clinically available and is listed in the U.S. Food and Drug Administration (FDA) table of valid genomic biomarkers (47).

UGT1A1 and Irinotecan Therapy

Irinotecan is a topoisomerase I inhibitor and acts by binding reversibly to a topoisomerase I-DNA complex to induce double-strand DNA breaks that lead to cell death in the S-phase of the cell cycle (48). Irinotecan is indicated for treatment of metastatic colorectal carcinoma in combination with 5-FU and leucovorin, and may also be used in combination with cetuximab (49). Conversion of irinotecan to a more potent metabolite, SN-38, results from the activity of serum carboxylesterases. Degradation of SN-38 is mediated by uridine diphosphate-glucuronyltransferase 1A1 (UGT1A1), resulting in the formation of the glucuronide conjugate, SN-38-glucuronide (Figure 10.5). SN-38 is largely responsible for toxicities of irinotecan such as neutropenia and diarrhea (50).

Inadequate UGT1A1 activity results in accumulation of SN-38 and increased likelihood of irinotecan toxicity (51). Several polymorphisms in the *UGT1A1* gene have been reported to date (52). The homozygous genotype of *UGT1A1*28* has a frequency as high as 10 percent to 20 percent in some ethnic groups and has correlated with a high frequency of delayed irinotecan toxicity in clinical trials (53). Based on those findings, the irinotecan label was modified to incorporate the role of UGT1A1*28



e 10.5. Irinotecan pharmacokinetics. Reproduced with permission from PharmGKB and Stanford University (68).

polymorphism in predicting severe neutropenia with irinotecan therapy.

Dihydropyrimidine Dehydrogenase (DPYD) and Thymidylate Synthetase (TS) Polymorphisms and 5-FU Therapy

An important drug in the treatment of colorectal cancer, 5-FU, is a pyrimidine antimetabolite that acts by inhibition of thymidylate synthetase, an enzyme involved in the synthesis of dTMP in the DNA synthetic pathway. 5-FU has efficacy in the treatment of other solid malignancies, most notably those of the breast and head and neck. An oral prodrug formulation of 5-FU, capecitabine, is also in clinical use. Common side effects of 5-FU include myelosuppression, diarrhea, stomatitis, and hand-and-foot syndrome.

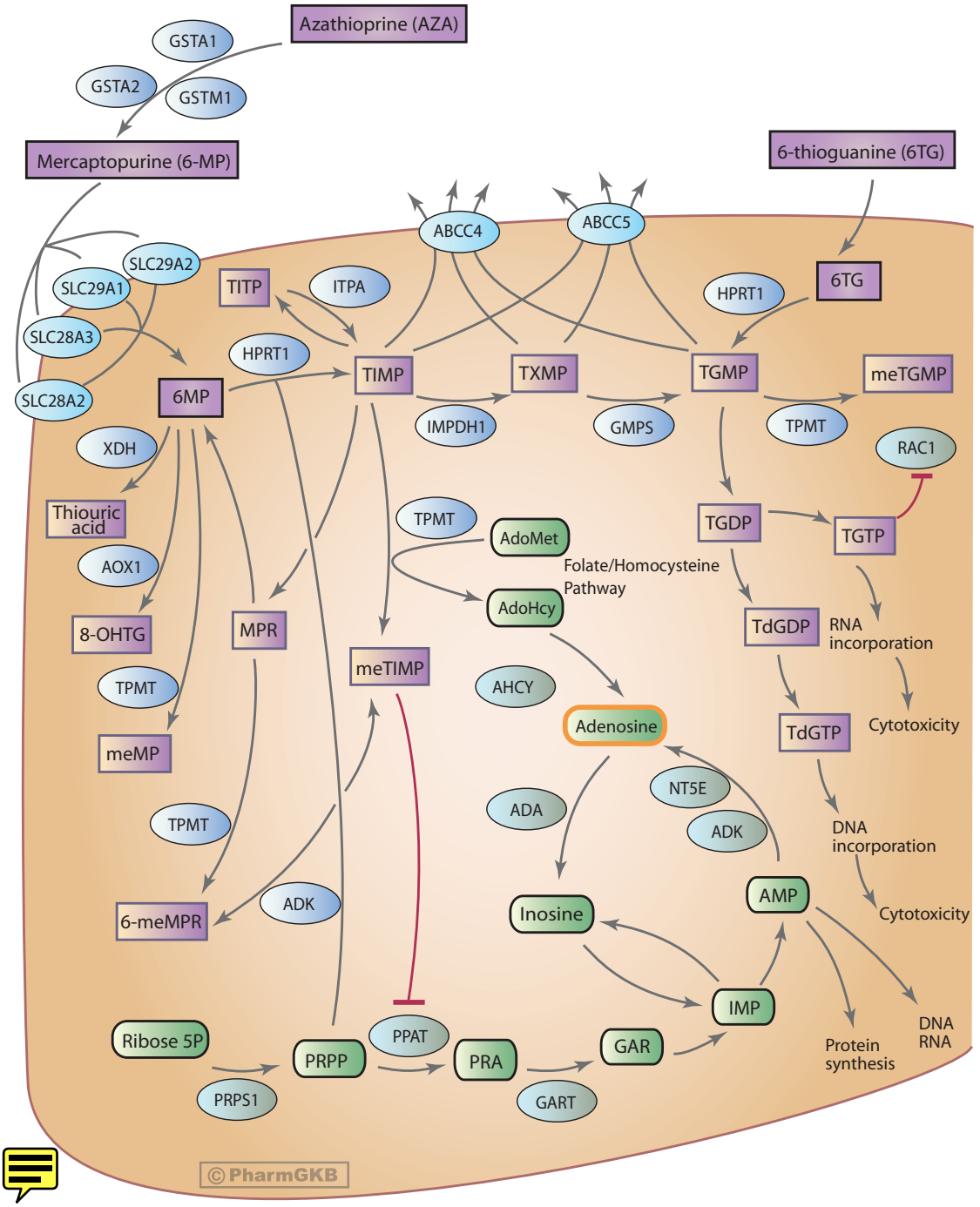
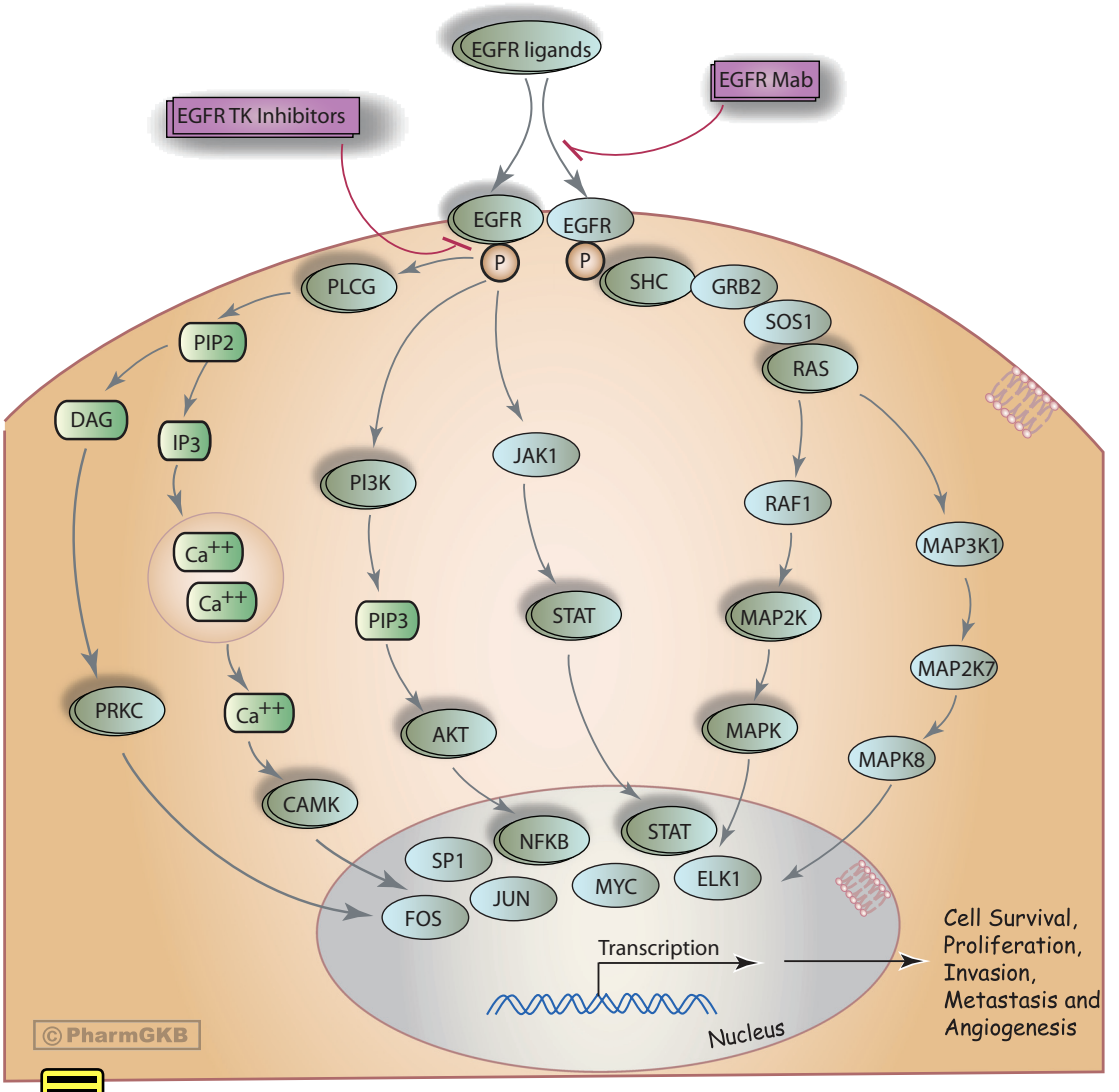


Figure 10.6. 5-FU pharmacokinetics. Reproduced with permission from PharmGKB and Stanford University (68).

The catabolic pathway of 5-FU involves the activity of DPD, an enzyme encoded by the *DPYD* gene on chromosome 1p22 (Figure 10.6). More than 80 percent of 5-FU is metabolized by DPD, and levels of the enzyme show significant interindividual variability. It has been estimated that 3 percent to 5 percent of the population is partially DPD deficient, whereas 0.2 percent is completely deficient (54). Several polymorphisms of uncertain significance have been reported in the *DPYD* gene; however, the *DPYD*2A* variant has been seen in 40 percent to 50 percent of individuals with partial or complete DPD deficiency (55). In pediatric oncology,



Figu Purine analog pharmacokinetics. Reproduced with permission from PharmGKB and Stanford University (68).

severe neurologic toxicity has been seen with complete DPD deficiency (56). Although such a state is almost invariably associated with a heightened risk of 5-FU toxicity, studies have shown that *DPYD* mutations do not always have an effect on DPD enzyme activity (55). As a result, decreased levels of DPD, rather than *DPYD* mutations, have been included in the FDA table of valid genomic biomarkers.

In addition to DPD, genetic variability in *TS*, the gene encoding thymidylate synthetase, is associated with outcomes in patients with colorectal cancer treated with 5-FU. Variants such as a six base pair insertion and deletion polymorphism in the 3′-untranslated region, and variable number tandem repeat polymorphisms in the promoter-enhancer region, lead to increased *TS* gene expression. In clinical studies, these high-expression variants have correlated with decreased survival in patients treated with 5-FU (55).

TPMT and Purine Analogs

Thiopurines such as 6-MP and 6-thioguanine (6-TG) are used in treatment of hematologic malignancies such as AML, ALL, and non-Hodgkin's lymphoma. Both drugs require activation by hypoxanthine-guanine phosphoribosyl transferase to exert cytotoxic effects by inhibiting DNA synthesis in the S-phase of the cell cycle. As analogs of the naturally occurring purines, they act as antagonists, thereby blocking DNA and RNA synthesis. A significant catabolic pathway for both 6-MP and 6-TG

involves conversion by TPMT to inactive metabolites, 6-methyl-MP and 6-methyl-TG (Figure 10.7). In the absence of TPMT activity, the active agents accumulate, increasing the likelihood of toxicity. Like many other chemotherapeutic agents, thiopurines have a narrow therapeutic index. The most concerning side effect seen is myelosuppression with increased risks of infection and bleeding. Several low-activity alleles are known to exist at the *TPMT* gene locus on chromosome 6p22.3, and may give rise to a heterozygous state with intermediate activity (10 percent of white individuals) or a homozygous state with negligible (0.3 percent of whites) activity (57). Examples of such low-activity alleles include *TPMT*2, TPMT*3A*, and *TPMT*3C*, which together account for 80 percent to 95 percent of cases of intermediate or low enzyme activity. Affected individuals are at heightened risk for potentially life-threatening myelosuppression as a consequence of thiopurine therapy. Furthermore, thiopurines and TPMT activity provide a successful example of genotype-driven chemotherapy dosing with recommendations for lower doses (30 percent to 50 percent) in patients with intermediate TPMT levels (57).

PHARMACOGENOMICS AND PHARMACOETHNICITY

Pharmacogenomics challenges the "one size fits all" approach that has dominated medical oncology care for decades. Of particular importance and relevance to global oncology practice is a pattern of interethnic differences in response to chemotherapeutic agents, as exemplified by the heightened sensitivity to EGFR-TKI seen among young, Asian, nonsmoking females with NSCLC. Although such differences are multifactorial, the evidence for a significant genetic component is growing. This area of study has been referred to as pharmacoethnicity and may be defined as ethnic diversity in drug response or toxicity (58). The objective of pharmacogenomics is to identify individuals most susceptible to a particular drug; however, the populations most sensitive to specific drugs may be enriched in the genetic variants associated with drug sensitivity. In low-resource settings, pharmacoethnicity will be of particular importance because sparse resources may be preferentially channeled toward purchase and administration of specific chemotherapeutic agents.

CASE PRESENTATIONS

Case 1: CYP2D6 Genotype and Response to Tamoxifen Therapy

A thirty-year-old postmenopausal woman sought medical care for a lump in the left breast. A mammogram revealed a suspicious left breast mass, and a core biopsy revealed malignant cells. Breast magnetic resonance imaging showed a single 2.2 × 2.6 cm mass in the upper outer quadrant of the left breast. She underwent lumpectomy and sentinel lymph node biopsy. Pathologic analysis of the resected specimen revealed a grade III infiltrating ductal carcinoma measuring 2.6 cm in greatest diameter. The tumor had estrogen (ER) and progesterone receptor (PR) expression, as well as gene amplification. None of four sentinel lymph nodes had metastatic disease. An Oncotype DX assay revealed a high recurrence score indicating a high likelihood of disease recurrence. As a result, a decision was made to treat with adjuvant chemotherapy in addition to antiestrogen therapy. She received four cycles of docetaxel and cyclophosphamide followed by local breast irradiation. Afterward, she was started on letrozole, an aromatase inhibitor. Unfortunately, she experienced severe joint aches that persisted even after letrozole was replaced by exemestane. After learning that tamoxifen was another option, the patient requested CYP2D6 genotyping to exclude the presence of a null allele.

Case 2: UGT1A1 Genotype and Irinotecan Toxicity

A seventy-six-year-old man was evaluated for abdominal cramping and a lower gastrointestinal bleed. Colonoscopy revealed a cecal mass that was biopsied with a finding of moderately differentiated adenocarcinoma of the colon. He underwent laparotomy and hemicolectomy. Pathologic analysis revealed a moderately differentiated colonic adenocarcinoma measuring 3 cm. The tumor penetrated the colon wall and extended into pericolic fat. Fourteen of fifty-seven pericolic lymph nodes had metastatic disease. Subsequently, staging computed tomography (CT) scans demonstrated multiple liver metastases. He was started on a combination chemotherapy with 5-FU, leucovorin, oxiliplatin, and bevacizumab. He received a total of six cycles and had a complete response with no further metastatic lesions evident on a repeat CT scan. Six months later, CT scans revealed new hepatic lesions and pathologic retroperitoneal adenopathy. Because his initial therapy had been administered less than twelve months earlier, he was started on a different regimen consisting of 5-FU, leucovorin, and irinotecan. After the first cycle, he developed profuse diarrhea, dehydration, and neutropenia that required inpatient management. Studies ruled out an infectious etiology. The same happened with the second and third cycles, even though the dose of irinotecan had been reduced by 25 percent for the latter. Genetic testing revealed homozygosity for the UGT1A1*28 polymorphism.

SUMMARY POINTS

- Chemotherapeutic agents are associated with significant toxicities and narrow therapeutic indices, and, for some drugs, high costs, making the field of pharmacogenomics of anticancer agents extremely important.
- A distinguishing feature of pharmacogenomic research in oncology is the consideration of both somatic DNA and germline DNA.
- Both candidate gene and genome-wide approaches have been used successfully in the study of pharmacogenetics of oncologic therapies.
- Although germline polymorphisms and somatic mutations have historically correlated with toxicity and response, respectively, there is emerging evidence to support a role for germline variants in predicting response.
- Pharmacoethnicity is an area that focuses on interethnic variability in drug responses and is likely to be an important focus in future chemotherapy pharmacogenomic research.

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