

# Special Report: Pharmacogenomics of Cancer–Candidate Genes



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## Executive Summary

### Background

Pharmacogenomics describes the relationship between variation in the human genome, such as differences in DNA sequence, copy number, or transcriptional perturbations, and individual variation in response to or adverse effects from drug therapy. The benefits and harms of drug therapy for cancer may be influenced by pharmacodynamic variability (genetic variability in drug target effector molecules or downstream products) or pharmacokinetic variability (genetic variability in molecular pathways involved in drug uptake, distribution, and metabolism). In cancer, pharmacodynamic variability often involves somatic (non-germline) genetic changes in tumor tissue that are characteristic of tumor type or stage; or variability in gene expression reflecting alterations in genetic regulation, gene copy number, or tumor interactions with local tissue. Pharmacokinetic variability generally reflects inherited (germline) changes in gene coding, for example, enzymes that metabolize drugs.

The goal of pharmacogenetic research is “personalized medicine,” or the ability to detect key genetic variation in individual patients in order to predict the most effective treatment, or avoid severe adverse effects. Such individualized therapy could help select the best treatment or dose early, avoiding trial and error management based on averages from clinical trials in large populations. Drug safety is an important concern, and an additional hope of individualized medicine is to identify patients with a high likelihood of severe reaction to a particular drug, and either modify the initial dose or choose an alternative treatment, avoiding extended monitoring and the extensive medical support required for severe toxicity reactions.

Relevant genes for pharmacogenomic study may be chosen via two different methods. The first is selecting “candidate” genes, based on known molecular interactions associated with the drug. The second involves large microarray analysis to define genetic marker patterns that correlate with drug response or adverse events. Either way, the goal is to develop genetic tests for clinical use in predicting response to therapy, or adverse reactions prior to treatment initiation. Genetic tests are already commercially available that purport to aid in selecting therapies that are most effective or that avoid adverse events.

### Objective

The objective of this Special Report is to catalog genetic tests and measures of gene activity that are currently under study for pharmacogenomic applications. Pharmacogenomics will be broadly interpreted to include detecting the specific molecular targets of the newer, “targeted” therapies to select patients likely to respond, as well as detecting other pharmacodynamic markers that influence targeted therapy response. Molecular indicators of variability in response or toxicity to standard chemotherapy drugs will also be reviewed. This Report will also provide a more detailed summary

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of the supporting data for a few illustrative examples. The tests identified in this Report are not comprehensive, but rather reflect the greatest research activity and in most cases are commercially available. Further, only tests developed from candidate genes will be examined; microarray pattern analysis and the development of predictive panels composed of many genetic markers are beyond the scope of this Report.

### **Search Strategy**

A MEDLINE search of relevant review articles was completed for 2006 and 2007 (through June). The search strategy included the text words “pharmacogenetics OR pharmacogenomics” combined with the MeSH® term “neoplasms.” The bibliographies of these review articles were also examined for other relevant articles.

### **Selection Criteria**

Articles were reviewed and a list was constructed of pharmacogenomic tests that were most often described and that were recommended for active use in clinical situations. The test menus of several laboratories known to offer molecular tests were also reviewed and a list of tests constructed. A final list of tests to be described in this report was limited primarily to those recommended in several articles for active use and available from CLIA-licensed clinical laboratories.

In addition to information culled on each test from the review articles, separate searches were conducted for articles on each test. These searches consisted of the term for the marker detected by the test, combined with terms such as “therapy,” “chemotherapy,” “response,” “toxicity,” or “adverse effects” [MeSH®], depending on the intent of the test. Relevant articles that provided examples or supportive data on test clinical use were selected for review.

## **Discussion**

### **Targeted Therapy**

Ideal drugs would interfere with a molecular target that is critical to and restricted to specific cancer types; presence of the target in the tumors of individual patients would vastly increase the likelihood of a meaningful clinical response and thus would determine eligibility for the targeted treatment. Such targets may be normal proteins that are found in abundance in cancerous tissue (e.g., CD20 protein expressed on the surface of normal B cells and on B-cell non-Hodgkin’s lymphoma cells); or may be the expression of somatically acquired genetic variants found only in malignant cells (e.g., BCR/ABL gene rearrangement [Philadelphia chromosome] characteristic of most chronic lymphocytic leukemias). This section describes types of targeted therapies, discusses a particular example (Trastuzumab [Herceptin®]), describes the ideal co-development of targeted drug and target assay, and the impact of multiple targets. A table lists several examples of targeted therapy and specific laboratory tests that may (or may not) be used to determine eligibility for therapy.

### **Targeted Therapy: Pharmacodynamic Predictors of Response**

Detection of the target molecule of a targeted drug is the first step in determining the likelihood of a response to therapy. However, in some cases presence of the target may not be sufficient; additional genetic alterations that differ among patients may affect response. Detection of these additional genetic changes may be important for selecting initial therapy to obtain the optimal response. In addition, initial response to a targeted drug may not last due to the emergence of drug-resistant clones. The table in this section lists some examples of genetic changes that are under study as additional determinants of response and/or resistance to targeted therapies. Genetic testing for imatinib resistance in chronic myelogenous leukemia and in gastrointestinal stromal tumors is discussed in greater detail.

### **Predicting Response to Non-“Targeted” Chemotherapy**

Chemotherapy drugs that were not originally developed to “target” a specific molecule and favorably modify disease have historically been tested, dosed, and incorporated into treatment protocols based on “trial and error” approaches resulting in a single or a range of recommended dosages based on studies of populations. However, germline interindividual variability in rates of metabolism of these drugs results in sometimes large interpatient differences in systemic

exposure, leading to toxicity for some, lack of efficacy for others, and a satisfactory response mainly for those close to population average metabolism. Chemotherapy drugs commonly have a narrow therapeutic index that may overlap with the range of systemic exposure, resulting in severe toxicity for some patients.

Many studies have been published describing associations between germline genetic variants of metabolizing enzymes and the toxicity of or the response to various chemotherapy drugs. The table in this section provides limited detail on a few examples, most of which have commercially available genetic tests. In addition, two examples are discussed in more detail: dihydropyrimidine dehydrogenase deficiency and its importance in predicting 5-fluorouracil and capecitabine toxicity; and tamoxifen efficacy in cytochrome p450 2D6 poor metabolizers.

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## Objective

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The objective of this Special Report is to catalog genetic tests and measures of gene activity that are currently under study for pharmacogenomic oncology applications. Pharmacogenomics will be broadly interpreted to include detecting the specific molecular targets of the newer, “targeted” therapies to select patients likely to respond, as well as detecting other pharmacodynamic markers that influence targeted therapy response. Molecular indicators of variability in response or toxicity to standard chemotherapy drugs will also be reviewed. This Report will also provide a more detailed summary of the supporting data for a few illustrative examples. The tests identified in this Report are not comprehensive, but rather reflect the greatest research activity and in most cases are commercially available. Further, only tests developed from candidate genes will be examined; microarray pattern analysis and the development of predictive panels composed of many genetic markers are beyond the scope of this Report.

## Introduction

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The promise of pharmacogenomics is particularly appealing in oncology, in which treatment failure usually results in death. Moreover, the dose range for effective treatment may be narrow and overlap with the dose range in which severe toxicity may be experienced. Successful pharmacogenomic screening methods would direct clinicians to the best individualized treatment selection for optimal response. Accounting for individual genetic variability could help fine tune dose to achieve tumor exposure to the active drug within the therapeutic window, and avoid severe toxicity.

Regarding the targeted therapies, some were developed in concert with a test to detect the drug target. For example, detection of HER2 amplification in breast cancer cells was co-developed as a prerequisite for treatment of breast cancer with the HER2-specific monoclonal antibody (MAb) trastuzumab (Herceptin®). Most contemporary pharmacogenomic applications, however, are being developed after the fact, either for recently introduced drugs (targeted or otherwise), or for chemotherapy agents that have been in use for many years. Particularly in the latter case, it becomes challenging to develop a test that improves upon physicians’ experience and comfort with

historic trial-and-error drug dosing. Results of genetic testing may be difficult to interpret, requiring additional education in this area; and compelling data on improved outcomes are needed to change physician behavior.

For a few oncology drugs, the U.S. Food and Drug Administration (FDA) have approved label revisions to include information on genetic determinants of variable response and/or toxicity. Haga et al. (2006) discuss the examples 6-mercaptopurine (6-MP; Purinethol®) and irinotecan (Camptosar®), used to treat pediatric acute lymphocytic leukemia (ALL) and colorectal cancer (CRC), respectively. Inherited (germline) genetic variants in metabolizing enzymes predispose a minority of patients taking either of these drugs to unusually severe and potentially life-threatening toxicity. Currently, patients are monitored by blood counts and for symptoms of toxicity. Labels were revised to include information on detecting genetic variability and identifying the rare individuals with homozygous genetic variants (2 variant gene alleles) who have a high likelihood of severe reaction.

Missing from most label changes, however, is information on how to use the genetic test results to modify treatment; while dose reduction for homozygous genetic variants is recommended, the better dose is not specified. Moreover, during the deliberations there was concern regarding potential harm for heterozygotes (1 variant and 1 normal gene allele), who have some, but not complete, predisposition to toxicity: dose reduction could compromise efficacy. In the final revised label for each drug, no recommendation or requirement was made for pretreatment screening for genetic variants. As Haga et al. (2006) note, a major reason for label revisions that supply only information without recommendations is “the absence of substantial prospective data on clinical outcomes.” Lacking was information on “the predictive value of pharmacogenetic testing for adverse events and the genetically based dosing or treatment changes that would improve treatment outcomes.”

The components of genetic test validation that are likely to satisfy clinical needs have been carefully examined in a report funded by the Centers for Disease Control and Prevention. The ACCE (analytic validity, clinical validity, clinical utility and ethical, legal and social implications) Model System for Collecting,

Analyzing and Disseminating Information on Genetic Tests (available at <http://www.cdc.gov/genomics/activities/fbr.htm>) provides an evaluation framework that is applicable to a variety of genetic tests. The ongoing model project, Evaluation of Genomic Applications in Practice and Prevention (EGAPP), used the ACCE framework as a starting point and in the process of establishing and evaluating a systematic, evidence-based process for assessing genetic tests and other applications of genomic technology in transition from research to practice (<http://www.egappreviews.org>). The evaluation framework examines:

- **Analytic validity:** measures the specific genotyping test performance characteristics, i.e., whether the test accurately and reproducibly detects the gene marker(s) of interest.
- **Clinical validity:** refers to the associations of the test result(s) with patient outcomes of interest; may be expressed as clinical sensitivity, specificity, and predictive value for the outcome. Evidence is usually retrospective. Note that epidemiologic analyses of risk ratio or hazard ratio may indicate strong statistical associations between the test result and outcomes in populations, yet the test may not adequately discriminate between individual patients who do and do not have the outcome (Wald et al. 1999; Pepe et al. 2004; Ware 2006; for an example, see also Wang et al. 2006).
- **Clinical utility:** determines whether the use of genetic testing to modify management decisions improves patient outcomes. Best evidence is prospective, from randomized clinical trials of standard management procedures vs. genetic test-directed management. Evidence may also be derived using banked samples from already-completed clinical trials; or by constructing an indirect chain of evidence linking test result to clinical outcome.

Evidence of clinical validity is usually available in the published literature while evidence of clinical utility is often lacking. The Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS) was chartered to provide advice to the U.S. Secretary of Health and Human Services on policy issues surrounding the development and use of genetic technologies and their integration into clinical and public health practice. In its 2007 draft, “Realizing the Promise of Pharmacogenomics:

Opportunities and Challenges” ([http://www4.od.nih.gov/oba/sacghs/SACGHS\\_PGx\\_PCdraft.pdf](http://www4.od.nih.gov/oba/sacghs/SACGHS_PGx_PCdraft.pdf)), SACGHS reported that “In addition to demonstrating the clinical validity of new technologies such as PGx [pharmacogenomic] tests, there is a growing need to demonstrate improved clinical outcomes that result from the use of these technologies in actual practice.”

Although few pharmacogenetic tests currently meet these high standards, the field is gaining momentum from several federally supported efforts; a few examples follow. The FDA’s Critical Path Initiative “is FDA’s effort to stimulate and facilitate a national effort to modernize the scientific process through which a potential human drug, biological product, or medical device is transformed from a discovery or “proof of concept” into a medical product.” (<http://www.fda.gov/oc/initiatives/criticalpath/>) Among Critical Path projects initiated in 2006 are:

- Develop a Concept Paper on Biomarker Qualification
- Draft Drug-Diagnostic Co-Development Guidance
- Examine Genetic Basis of Adverse Events

The Oncology Biomarker Qualification Initiative (OBQI) is a collaboration involving the FDA, the National Cancer Institute (NCI), and the Centers for Medicare and Medicaid Services (CMS). The goal is “to qualify cancer biomarkers that can be useful in research, developing diagnostic tests, medical product quality assessment, and evidence-based decision-making. This collaboration focuses on four key areas: (1) cancer imaging; (2) molecular diagnostic assays and targeted therapies; (3) clinical trials; and (4) data mining.” (<http://www.fda.gov/bbs/topics/news/2006/NEW01316.html>)

Several institutes of the National Institutes of Health fund the Pharmacogenetics Research Network (PGRN), a nationwide collaboration of scientists studying the effect of genes on people’s responses to a wide variety of medicines (<http://www.nigms.nih.gov/Initiatives/PGRN/>), and PharmGKB, which curates information that establishes knowledge about the relationships among drugs, diseases and genes, including their variations and gene products (<http://www.pharmgkb.org/index.jsp>).

NCI’s Cancer Therapy Evaluation Program (CTEP) encourages the co-development of

drugs and qualifying diagnostics, and has developed guidelines for investigators and pharmaceutical/biotechnology companies concerning the conduct of pharmacogenetics protocols linked to CTEP-sponsored clinical trials ([http://dctd.cancer.gov/ProgramPages/CTEP-Resources\\_Online\\_resources\\_for\\_industry.htm](http://dctd.cancer.gov/ProgramPages/CTEP-Resources_Online_resources_for_industry.htm)).

This Report summarizes some of the progress that has been made in pharmacogenetics research in oncology and identifies commercially available genetic tests that may be of use in predicting cancer therapy response or adverse events. However, there is pressing need for gathering clear evidence of clinical validity and utility for these and other tests in well-defined cancer populations undergoing specific therapies. Where single-gene tests have insufficient power to discriminate among affected vs. unaffected patients, panels of multiple markers need to be identified and similarly validated. Finally, genetic variability is likely to be only one factor influencing treatment outcomes; prediction panels may need to incorporate parameters of tumor characteristics, patient co-morbidities and other patient characteristics that affect clinical response to therapy and outcomes. “Clinicians and patients will embrace pharmacogenomic tests when they provide information that fills an essential knowledge gap deemed clinically important to the diagnosis, prognosis, treatment and monitoring of patients with serious disease.” (Lesko 2007)

## Methods

### Search Methods

A MEDLINE search of relevant review articles was completed for 2006 and 2007 (through June). The search strategy included the text words “pharmacogenetics OR pharmacogenomics” combined with the MeSH® term “neoplasms.” The bibliographies of these review articles were also examined for other relevant articles.

### Study Selection

Articles were reviewed and a list was constructed of pharmacogenomic tests that were most often described and that were recommended for active use in clinical situations. The test menus of several laboratories known to offer molecular tests were also reviewed and a list of tests constructed. A final list of tests to be described in this report was limited

primarily to those recommended in several articles for active use and available from CLIA-licensed clinical laboratories.

In addition to information culled on each test from the review articles, separate searches were conducted for articles on each test. These searches consisted of the term for the marker detected by the test, combined with terms such as “therapy,” “chemotherapy,” “response,” “toxicity,” or “adverse effects” [MeSH®], depending on the intent of the test. Relevant articles that provided examples or supportive data on test clinical use were selected for review.

### Medical Advisory Panel Review

This Special Report was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on June 28, 2007. In order to maintain the timeliness of the scientific information in this Special Report, literature searches were performed subsequent to the Panel’s review (see “Search Methods”). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the tables and text where appropriate. There were no studies that would change the conclusions of this Special Report.

## Targeted Therapy

### What Is Targeted Therapy?

Cancer is a collection of many different diseases; developing drugs and identifying the best therapies for each cancer and stage has been predominantly an empirical task. On average, oncologic therapies are effective in only 25%

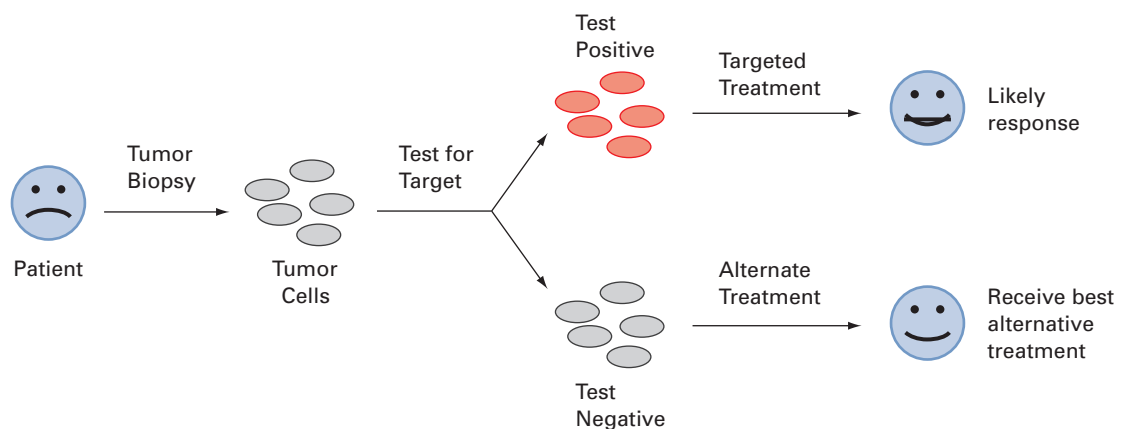
of patients due to heterogeneity between and within cancer types (IOM 2007). Ideal drugs would interfere with a molecular target that is critical to and restricted to specific cancer types; presence of the target in the tumors of individual patients would vastly increase the likelihood of a meaningful clinical response and thus would determine eligibility for the targeted treatment (Figure 1). Such targets may be normal proteins that are found in abundance in cancerous tissue; or may be the expression of somatically acquired genetic variants found only in malignant cells.

Choosing a target that is found disproportionately or solely in tumor tissue compared to normal tissue could contribute to disease-selective drug effects. In reality, however, the molecular mechanisms of cancer development and progression are only partly understood for any cancer, making it difficult to develop drugs that specifically target cancer types and subtypes with predictable success and minimal adverse effects. Furthermore, many putative targets are components of complex pathways that regulate cell behavior, often with built-in redundancies that minimize the consequences of inhibiting a single target.

### Types of Targeted Therapies

Nevertheless, some progress in targeted therapy has been made. Table 1, Part A, gives several examples of FDA-approved, targeted therapies for which eligibility is determined by detection of the molecular target in tumor tissue. In some cases, the presence of the target may be assumed due to historical evidence that the target is expressed in most tumors of the particular cancer type (Table 1, Part B).

**Figure 1.** Selection of Patients for Targeted Therapy



**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part A. Testing for Target Expression Required for Treatment Eligibility</b>				
Herceptin [Trastuzumab®] Mab	HER2-positive breast cancer	HER-2/neu (overexpression due to somatically acquired gene amplification in cancer cells)	IHC, EIA, FISH	Trastuzumab is indicated for the adjuvant treatment of patients with HER-2 overexpressing, node-positive breast cancer; and for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER-2 protein. Confirmation of HER-2 overexpression is required for treatment.
Lapatinib [Tykerb®] SMI	HER2-positive breast cancer	HER-2/neu (see above)	IHC, EIA, FISH	Lapatinib is indicated for women with advanced or metastatic breast cancer who have received prior treatment with trastuzumab and taxanes and anthracyclines.  Lapatinib is an epidermal growth factor receptor (EGFR) and HER2/neu (ErbB-2) dual tyrosine kinase inhibitor. Patients who have received prior treatment with Herceptin will be HER2-positive.
Gemtuzumab [Mylotarg®] Mab	CD33-positive acute myeloid leukemia (AML)	CD33 (product of a normal, inherited gene that is found on normal myelomonocytic cells and their progenitors as well as on myeloid leukemic cells)	Flow cytometry	Gemtuzumab ozogamicin is used for the treatment of patients with CD33-positive acute myeloid leukemia (AML) in first relapse who are 60 years of age or older and who are not considered candidates for other cytotoxic chemotherapy. Gemtuzumab is a monoclonal antibody conjugated to a toxin; the specificity of the antibody delivers the toxin to the tumor.  CD33 is positive in about 80% of AML cases and expression may be part of the initial characterization of the malignancy. While some studies did not detect a relationship between CD33 expression and response to gemtuzumab (Jilani et al. 2002; van der Heiden et al. 2006), others indicate a significant relationship between CD33 positivity and clinical response (Walter et al. 2007; Amadori et al. 2005) or in vitro cytotoxicity (Walter et al. 2005).  Phenotyping for CD33 prior to treatment may identify potential responders and spare likely nonresponders from possible severe toxicity (e.g., hepatic veno-occlusive disease).

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part A. Testing for Target Expression Required for Treatment Eligibility (cont'd)</b>				
Rituximab [Rituxan®] MAb	CD20-positive, B-cell non- Hodgkin's Lymphoma (NHL)	CD20 (product of a normal, inherited gene; found on normal B-cells and B-cell malignancies)	Flow cytometry, IHC	<p>Rituximab (anti-CD20 monoclonal antibody) has been approved for various indications related to CD20-positive, B-cell NHL.</p> <p>Because CD20 is found on &gt;90% of NHL cells and may be part of the initial characterization of the malignancy, CD20 testing specifically for treatment eligibility may not be required.</p> <p>In some patients who relapse after rituximab treatment, malignant cells may be CD20-negative (e.g., Chu et al. 2002); in some cases apparent CD20 negativity may be due to rituximab blockade. However, true CD20-negative relapsed cases may not respond to retreatment.</p>
Ibritumomab [Zevalin®] MAb	CD20-positive, B-cell non- Hodgkin's Lymphoma	CD20 (see above)	Flow cytometry, IHC	<p>Ibritumomab tiuxetan (anti-CD20 monoclonal antibody), as part of a specific therapeutic regimen, is used for the treatment of relapsed or refractory low-grade, follicular, or transformed B-cell NHL, including follicular NHL that is refractory to rituximab therapy.</p> <p>(See rituximab "Comments" for CD20 testing information.)</p>
Tositumomab [Bexxar®] MAb	CD20-positive, B-cell non- Hodgkin's Lymphoma	CD20 (see above)	Flow cytometry, IHC	<p>The BEXXAR therapeutic regimen (tositumomab and Iodine 131 tositumomab) is indicated for the treatment of patients with CD20 antigen-expressing relapsed or refractory, low grade, follicular, or transformed NHL, including patients with rituximab-refractory NHL.</p> <p>(See rituximab "Comments" for CD20 testing information.)</p>

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part A. Testing for Target Expression Required for Treatment Eligibility (cont'd)</b>				
Imatinib [Gleevec®] SMI	Ph+ acute lymphoid leukemia (ALL)	BCR/ABL (Philadelphia chromosome; gene rearrangement somatically acquired in malignant cells)	Chromosome analysis, FISH, PCR	Imatinib is indicated for adult patients with relapsed or refractory Ph+ ALL. In adult ALL, 20-30% of cases are Ph+ or BCR/ABL-positive. Testing for Ph or BCR/ABL, usually part of characterizing this malignancy, is necessary to determine eligibility for imatinib treatment in ALL patients.
	KIT-positive gastrointestinal stromal tumors (GIST)	KIT proto-oncogene receptor [also targets PDGFRs] (also called CD117; KIT is constitutively activated by somatically acquired KIT gene mutations in cancer cells)	IHC	Imatinib is also indicated for patients with KIT-overexpressing unresectable and/or metastatic malignant GIST.  Most GIST tumors harbor activating mutations in the KIT (CD117) receptor tyrosine kinase gene and testing for KIT overexpression is part of the diagnostic workup. KIT-negative GIST may have activating mutations in platelet-derived growth factor receptor-alpha (PDGFRA) that are sensitive to imatinib (an appropriate but technically off-label use).  Note: When the Dako c-Kit pharmDx™ manufactured assay is used to detect KIT overexpression, positivity for c-kit protein expression is defined as any specific cytoplasmic and/or membrane staining in the tumor cells. However, focal positivity or staining in less than 10% of tumor cells should be interpreted with caution.
	Myelodysplastic/ myeloproliferative diseases with platelet-derived growth factor receptor (PDGFR) gene rearrangements	PDGFR fusion genes (e.g., FIP1L1-PDGFRα; fusion genes are somatically acquired by malignant cells and result in PDGFR overexpression)	IHC; molecular methods to detect fusion genes	Constitutive activation of the PDGFRA or PDGFR-beta (PDGFRβ) gene as a result of fusion to a variety of partner genes is seen in some chronic myeloid malignancies that are negative for Ph. Malignancies that harbor PDGFRA fusion genes are responsive to imatinib.  In the specific cases of hypereosinophilic syndrome/chronic eosinophilic leukemia (HES/CEL) and aggressive systemic mastocytosis (ASM) associated with eosinophilia, patients with a demonstrated FIP1L1-PDGFRα fusion kinase may respond to a lower, starting dose of 100 mg/day. Those patients without a fusion gene may also respond, but are more likely to require dose escalation up to 400 mg/day.

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Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part A. Testing for Target Expression Required for Treatment Eligibility (cont'd)</b>				
Dasatinib [Sprycel®] SMI	Ph+ ALL	BCR/ABL Gene rearrangement (see above)	Chromosome analysis, FISH, PCR	Dasatinib inhibits BCR-ABL, SRC family, KIT, EPHA2, and PDGFR tyrosine kinases. Dasatinib is indicated for the treatment of adults with Ph+ ALL with resistance or intolerance to prior therapy. Eligibility testing recommendations are the same as those for imatinib.
Sunitinib [Sutent®] SMI	GIST	Multiple kinases including KIT, PDGFRs (like KIT, PDGFRs are constitutively activated by somatically acquired PDGFR mutations in cancer cells)	IHC	Sunitinib is indicated for the treatment of GIST that is progressing or intolerant to imatinib mesylate. Eligibility testing recommendations are the same as those for imatinib.
Denileukin diftitox [Ontak®] fusion protein	Cutaneous T-cell lymphoma (CTCL)	CD25 (product of the normal, inherited gene for IL-2; found on normal activated T cells and some leukemia and lymphoma cells)	Flow cytometry, IHC	Denileukin diftitox is a fusion protein, consisting of a fragment of diphtheria toxin genetically fused to Interleukin-2. Denileukin diftitox targets the alpha subunit of IL-2 receptors (CD25) on the surface of malignant cells and delivers cytotoxic toxin.  Denileukin diftitox is indicated for the treatment of patients with persistent or recurrent cutaneous T-cell lymphoma whose malignant cells express the CD25 subunit of the IL-2 receptor. According to the label, the patient's malignant cells should be tested for CD25 expression prior to denileukin diftitox administration.

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part B. Target Expression Assumed, Testing Not Needed</b>				
Imatinib [Gleevec®] SMI	Philadelphia chromosome- positive (Ph+) chronic myelocytic leukemia (CML)	BCR/ABL gene rearrangement (see above)	Chromosome analysis, FISH, PCR	Imatinib has several indications for Ph+ CLL in adult and pediatric patients. Most cases of CML are Ph+, if not by cytogenetic analysis, then by FISH or PCR analysis for the BCR/ABL fusion gene. Thus, specific testing for BCR/ABL to determine treatment eligibility may not be necessary. However, detection of BCR/ABL may be used to monitor clinical response.
Dasatinib [Sprycel®] SMI	CML	BCR/ABL gene rearrangement (see above)	Chromosome analysis, FISH, PCR	Dasatinib is indicated for the treatment of adults with chronic, accelerated, or myeloid or lymphoid blast phase CML with resistance or intolerance to prior therapy including imatinib.  Eligibility testing recommendations are the same as those for imatinib.
Alemtuzumab [Campath®] MAb	B-cell chronic lymphocytic leukemia (B-CLL)	CD52 (product of a normal, inherited gene; protein is found on the surface of a variety of normal leukocytes and some leukemias and lymphomas)	Flow cytometry	Campath is a humanized antibody targeted against CD52, an antigen that can be expressed at high density on the surface of malignant CLL cells. Binding of Campath to CD52 on the target cells is necessary for cell death and therapeutic response. Campath is indicated for the treatment of B-CLL in patients who have been treated with alkylating agents and who have failed fludarabine therapy.  The vast majority of low-grade B cell lymphoproliferative disorders, including B-CLL, express CD52 and thus CD52 expression testing is not necessary to determine treatment eligibility.
Cetuximab [Erbix®] MAb	Advanced squamous cell carcinoma of the head and neck	EGFR. (epidermal growth factor receptor; normal, inherited gene is expressed in epithelial cells; somatically acquired genetic abnor- malities resulting in EGFR overexpression found in several epithelial cancers)	IHC	Because expression of EGFR has been detected in nearly all patients with head and neck cancer, documentation of EGFR expression is considered unnecessary in these patients.  See note regarding EGFR testing in gefitinib "Comments."

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part C. Target Expression Testing of Uncertain or No Benefit</b>				
Gefitinib [Iressa®] SMI	Non-small cell lung cancer (NSCLC)	EGFR (see above)	IHC	<p>Gefitinib is indicated as monotherapy for the continued treatment of patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies who are benefiting or have benefited from gefitinib. The relationship between EGFR expression and clinical response is uncertain.</p> <p>Note: When the Dako EGFR pharmDx™ IHC kit is used to detect EGFR expression, positive staining is defined as any staining above background level.</p>
Erlotinib [Tarceva®] SMI	locally advanced or metastatic NSCLC; pancreatic cancer	EGFR (see above)	IHC	<p>Erlotinib is indicated for locally advanced or metastatic NSCLC after failure of at least one chemotherapy regimen; and, with gemcitabine, for first-line treatment of patients with locally advanced, unresectable or metastatic pancreatic cancer.</p> <p>The impact of EGFR expression status on clinical outcome is uncertain.</p> <p>See note regarding EGFR testing in gefitinib “Comments.”</p>
Cetuximab [Erbix®] MAb	Colorectal carcinoma (CRC) with EGFR expression	EGFR (see above)	IHC	<p>Cetuximab is indicated, alone or in combination with other antineoplastic agents, for the treatment of metastatic colorectal cancer in patients with tumors that express EGFR and who are refractory to irinotecan-based therapy.</p> <p>Patients enrolled in supportive clinical studies for colorectal cancer were required to have immunohistochemical evidence of EGFR expression; but response rates did not correlate with either the percentage of positive cells or the intensity of EGFR expression.</p> <p>See note regarding EGFR testing in gefitinib “Comments.”</p>

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part C. Target Expression Testing of Uncertain or No Benefit (cont'd)</b>				
Panitumumab [Vectibix®] MAb	EGFR-expressing, metastatic CRC with disease progression despite chemotherapy	EGFR (see above)	IHC	<p>Patients enrolled in the colorectal cancer clinical studies supporting FDA approval were required to have immunohistochemical evidence of EGFR expression. Exploratory analyses assessing the relationship between EGFR expression and PFS did not suggest that the PFS benefit differed as a function of EGFR staining intensity or percentage of EGFR-expressing tumor cells.</p> <p>See note regarding EGFR testing in gefitinib "Comments."</p>
Bevacizumab [Avastin®] MAb	Metastatic CRC; NSCLC	VEGF (vascular endothelial growth factor; inherited, normal gene expressed on blood vessel epithelial cells)	—	<p>Bevacizumab inhibits VEGF, a specific angiogenesis growth factor that binds to receptors on blood vessels and stimulates the proliferation of new ones. Bevacizumab blocks VEGF receptor binding and stops tumors from developing a blood supply, thus limiting growth.</p> <p>Bevacizumab, in combination with 5-fluorouracil-based chemotherapy, is indicated for treatment of patients with metastatic CRC; and, in combination with carboplatin and paclitaxel, is indicated for first line treatment of patients with NSCLC.</p> <p>Pretreatment VEGF levels do not appear to be predictive of response to anti-angiogenic therapy (Longo and Gasparini 2007).</p>
Sunitinib [Sutent®] SMI	Advanced renal cell carcinoma	Multiple kinases e.g., KIT, PDGFRs, VEGF receptors (see above)	—	<p>Sunitinib was developed for its potency against the antiangiogenic receptor tyrosine kinases. Unregulated growth of blood vessels that supply malignant tissue is a characteristic of kidney cancer.</p> <p>No eligibility testing is required.</p>

**Table 1.** Examples of FDA-Approved Targeted Drugs, Indications, and Tests Currently Used to Determine Eligibility for Therapy (cont'd)

Drug [Trade Name] MAb or SMI	FDA-Approved Targeted Indication <sup>1</sup>	Drug Target (Is relevant gene sequence inherited or somatically acquired?)	Test Method to Detect Target	Comment
<b>Part C. Target Expression Testing of Uncertain or No Benefit (cont'd)</b>				
Sorafenib [Nexavar®] SMI	Advanced renal cell carcinoma.	Multiple kinases e.g., KIT, VEGF receptors (see above)	—	Sorafenib is indicated for the treatment of patients with advanced renal cell carcinoma. Clinical efficacy is believed to be due to inhibition of angiogenesis and signaling in tumor cells.  No eligibility testing is required.
Temsirolimus [Torisel®]	Advanced renal cell carcinoma.	mTOR (mammalian target of rapamycin; cellular kinase is the product of a normal, inherited gene; mediates cell growth and proliferation)	—	Temsirolimus targets the cellular protein mTOR, which regulates the growth of tumor cells and blood vessels.  No eligibility testing is required.

<sup>1</sup> May not include all approved indications.

#### Abbreviations

MAb monoclonal antibody

SMI small molecule inhibitor

IHC immunohistochemistry

EIA enzyme immunoassay

FISH fluorescent in situ hybridization

PCR polymerase chain reaction

CD\_ cluster of differentiation (indicates a specific antigen targeted by commercially available antibodies; the presence of cell surface antigens, as detected by antibodies, is used to identify the cell type, stage of differentiation and activity of a cell).

#### Sources of Information

FDA-approved labels for listed biologics.

Chin et al. 2006.

National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology™, available at <http://www.nccn.org>.

In other cases, the importance of testing for the target for optimal response is uncertain (Table 1, Part C). Categories of targeted therapy drugs include monoclonal antibodies that are engineered to bind with high affinity to specific extracellular targets like the extracellular domains of cell membrane embedded receptors and interfere (in ways that are often not fully understood) with downstream signal transduction (e.g., trastuzumab [Herceptin<sup>®</sup>], see Table 1).

A second targeted drug category is the small molecule inhibitors that selectively bind to and inhibit the action of enzymes and growth factor receptors that are involved in cancer cell growth. Small molecule inhibitors may be taken orally, are cell-permeable and thus may affect intracellular processes. In some cases, the target of small molecule inhibitors may be the same as those bound by monoclonal antibody targeted drugs (e.g., lapatinib [Tykerb<sup>®</sup>] and trastuzumab [Herceptin<sup>®</sup>]); however, small molecule inhibitors are able to bind intracellular domains of the target molecule whereas monoclonal antibodies bind only to extracellular domains. Finally, some include antiangiogenic agents as a category of targeted therapy; however, perhaps because anti-angiogenic drugs are postulated to act on vascular endothelium, rather than directly on the tumor, tumor expression of the targets of these drugs has not been associated with response in these types of drugs.

Targets may represent the protein expression of normal, inherited genes (e.g., CD33 is found on normal myelomonocytic cells and on myeloid leukemic cells). In some cases additional copies of normal genes may be somatically acquired in tumor cells (gene amplification) resulting in detectable overexpression of the protein (e.g., HER2). In other cases, normal genes may acquire somatic mutations in the tumor cells resulting in abnormal expression (e.g., the KIT gene is constitutively activated by somatically acquired KIT gene mutations resulting in detectable overexpression in certain tumors). The specific, somatically acquired gene alteration in tumor cells may have to be detected to determine eligibility for targeted treatment; for example, the abnormal BCR-ABL fusion gene (Philadelphia chromosome) is acquired by acute lymphoid leukemia tumor cells; the test detects the fusion gene while the drug treatment (imatinib) targets the ABL portion of the BCR-ABL abnormal (and abnormally active)

protein product. Table 1 provides detail on the nature of the target for particular cancer types and drug treatments.

#### **Targeted Therapy: Trastuzumab (Herceptin<sup>®</sup>)**

A commonly cited example of successful targeted therapy development involves the human epidermal growth factor receptor 2 (HER2), a tyrosine kinase receptor that is normally involved in signal transduction pathways leading to cell growth and differentiation. HER2 is a normally inherited gene, but HER2 gene amplification is abnormal and specific to some breast cancers; thus, HER2 gene amplification is termed a somatically acquired, cancer-specific trait. When the HER2 gene is abnormally amplified, the HER2 protein is overexpressed and the protein becomes catalytically active in the absence of ligand binding; the downstream result is abnormally increased cell growth. HER2 is overexpressed in about 20% of breast cancer cases, and denotes a more aggressive cancer type that is more responsive to anthracycline-based therapy than to standard therapies like CMF (cyclophosphamide, methotrexate, and fluorouracil).

Trastuzumab (Herceptin<sup>®</sup>) is a recombinant DNA-derived humanized monoclonal antibody that binds to the extracellular portion of the HER2 protein. Trastuzumab is believed to reduce cancer cell proliferation by preventing ligand-independent HER2 activity and by tagging cancer cells for removal by immunologic mechanisms. An assay for the overexpression of HER2 on tumor cells was co-developed along with the drug and used to select patients for inclusion in clinical trials supporting FDA approval of the drug. While some controversy remains regarding best assay methods and interpretation, demonstrated presence of HER2 overexpression is required for treatment eligibility and ensures a higher likelihood of response than if trastuzumab treatment was administered more generally. In fact, mathematical models suggest that detecting treatment efficacy would have been difficult without an enriched population of responders (IOM 2006).

#### **Co-development of Targeted Drug and Target Assay**

Co-development of a targeted drug and an assay to screen for patients with a high likelihood of response is ideal. However, co-development appears not to be the norm. Developing a test is difficult; it must be very accurate (i.e., high negative predictive value) such

that patients denied eligibility truly would not benefit from the drug. In some cases, detectable presence of the drug target in tumor tissue does not appear to adequately (or at all) select for response due, perhaps, to downstream signaling pathway elements that are poorly understood. Thus, therapy that is initially developed as targeted may fail as such from a clinically functional perspective (but succeed as a treatment). For example, bevacizumab is a monoclonal antibody that is directed against vascular endothelial growth factor-A (VEGF-A or commonly VEGF), a proangiogenic factor (Table 1, part B). VEGF is a normally inherited gene that is abnormally upregulated in the tissue of most malignancies, where it promotes the formation of new vasculature that supports tumor growth. Increased tumor-associated VEGF levels tend to correlate positively with tumor microvascular density. However, the level of VEGF expression in tumor tissue does not appear to correlate with response to bevacizumab (Bergsland 2006; Jubb et al. 2006; Longo 2007), and the drug is administered empirically, based on clinical trial results, without ascertaining tumor VEGF expression.

#### **One Drug, Multiple Targets**

Some small molecule inhibitors interact with multiple targets. For example, imatinib, which inhibits the ABL tyrosine kinase (a normally inherited gene), was originally developed for its activity against the enzyme portion of the protein product encoded by the abnormal, somatically acquired BCR-ABL fusion gene. BCR-ABL is expressed in the malignant cells of patients with chronic myelogenous leukemia (CML; see Table 1). The fusion gene is created in most cases by a chromosomal translocation that fuses the ABL tyrosine kinase gene on chromosome 9 with the BCR region on chromosome 22 and results in the cytogenetically identifiable Philadelphia chromosome (Ph). In a minority of cases the BCR-ABL fusion gene is formed by other genetic changes; in these cases the Ph is not seen in cytogenetic analysis but the fusion gene is detectable by molecular methods. The product of BCR-ABL is also a tyrosine kinase; the kinase domain of the BCR-ABL protein is the same as the kinase domain of the normal ABL protein. However, the abnormal BCR-ABL protein is resistant to normal regulation. Instead, the enzyme is constitutively activated and drives unchecked cellular signal transduction resulting in excess cellular proliferation. Nearly all patients with CML have the BCR-ABL fusion gene; these

patients have a high response rate to imatinib and some achieve prolonged remissions. As a result, imatinib became the primary therapy for most patients with newly diagnosed CML.

In addition to inhibiting both normal ABL and fusion gene BCR-ABL tyrosine kinase products, imatinib also inhibits KIT kinase and platelet-derived growth factor receptors (PDGFR). KIT is a normally inherited receptor tyrosine kinase that plays a role in cell survival, proliferation, and differentiation. PDGFRs (also normally inherited) and their ligands, platelet-derived growth factors, play critical developmental roles in mesenchymal cell migration and proliferation. Constitutive activation of these genes appears to play a causative role in some malignancies. Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract. Most GIST cells have somatically acquired activating point mutations or deletions in either the KIT or PDGFR-alpha (PDGFRA) genes; these mutations appear to be the oncogenic event in these malignancies (Lasota and Miettinen 2006). Most GISTs characterized by either KIT or PDGFRA activating mutations are sensitive to imatinib.

#### **Targeted Therapy: Pharmacodynamic Predictors of Response**

Detection of the target is the first step in determining the likelihood of a response to targeted drug therapy. However, in some cases presence of the target may not be sufficient; additional genetic alterations that differ among patients may affect response. Detection of these additional genetic changes may be important for selecting initial therapy to obtain the optimal response. In addition, initial response to a targeted drug may not last due to the emergence of drug-resistant clones. Table 2 lists some examples of genetic changes that are under study as additional determinants of response and/or resistance to targeted therapies. Two examples are discussed in greater detail.

#### **Genetic Testing for EGFR-Targeted Therapies**

Many types of advanced cancer are treated with cytotoxic chemotherapy, usually with poor outcomes and significant toxicity. As a consequence, alternatives with improved outcomes have been sought, in particular targeted therapies that have potential to improve clinical outcomes with substantially reduced systemic

**Table 2.** Pharmacogenomic Applications to Predict Response to Targeted Drugs

Drug	Indication	Normal Protein; Gene	Normal Protein Function	Somatically Acquired Gene Variants	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Imatinib [Gleevec®] Dasatinib [Sprycel®]	Chronic myelogenous leukemia (Ph+)  Ph+ ALL	ABL	Abelson tyrosine kinase (ABL) is important in the regulation of cell growth and apoptosis	Primary and secondary mutations in the abnormal fusion gene, BCR-ABL	Associated with resistance to imatinib (see text for additional detail)  Associated with resistance to imatinib	Yes	Provisional recommendations for BCR-ABL transcript monitoring and mutation analysis to anticipate resistance and select therapy have been published (see text for additional detail).  Data on BCR-ABL mutations in Ph+ ALL are more limited than in CML (Pfeifer et al. 2007).  Mutations have been detected in about 80% of patients (Soverini et al. 2006) and the TKI-resistant mutation T315I is common (Soverini et al. 2007).  Ph+ ALL with other imatinib-resistant mutations may respond to dasatinib (Ottmann et al. 2007).
Imatinib [Gleevec®]	Gastrointestinal stromal tumors (GIST)  Systemic mastocytosis (SM)	KIT	The KIT proto-oncogene encodes a transmembrane tyrosine kinase that regulates a variety of biological responses including cell proliferation  The wild type KIT receptor tyrosine kinase plays a role in normal mast cell development.	Primary and secondary mutations in KIT  D816V KIT mutation present in most patients with disease	Activating (primary) mutations play a causal role in disease. Some primary and secondary mutations are associated with resistance to imatinib (see text for additional detail)  Activating mutations deregulate mast cell proliferation and play a causal role in disease.  In vitro studies suggest patients with D816V-mutated KIT may be resistant to imatinib.	Yes  Yes	Predictors of response, such as kinase mutation genotype, other biomarkers, or gene expression profiles, are currently under study (see text for additional detail).  Efficacy studies have shown conflicting results. Droogendijk et al. (2006) report partial response to imatinib in 11 patients with D816V mutation. Efficacy of other tyrosine kinase inhibitors is under study.  PGx application: test may help distinguish between SM and SM-HEL and predict response.

**Table 2.** Pharmacogenomic Applications to Predict Response to Targeted Drugs (cont'd)

Drug	Indication	Normal Protein; Gene	Normal Protein Function	Somatically Acquired Gene Variants	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Imatinib [Gleevec®]	Systemic mastocytosis plus hyper-eosinophilic syndrome (SM-HES); chronic eosinophilic leukemia (CEL)	PDGFRA	PDGFRs and their ligands, platelet-derived growth factors, play critical developmental roles in mesenchymal cell migration and proliferation	Abnormal FIP1L1-PDGFR fusion gene	FIP1L1-PDGFR is a constitutively activated tyrosine kinase that transforms hematopoietic cells. Associated with response to imatinib	Yes	SM-HES patients likely to respond well to imatinib (Valent et al. 2005; Droogendijk et al. 2006).  FIP1L1-PDGFR+ CEL patients treated with low-dose imatinib achieve hematological and cytogenetic remission, and the majority of patients also achieve a molecular remission (Jovanovic et al. 2007; Cools J 2003; Cools J 2005). Response has also been seen with low-dose sorafenib (Lierman et al. 2006).  [Note: presence of mutation also distinguishes CEL from HES.]
EGFR inhibitors: gefitinib, erlotinib, cetuximab	Lung cancer (NSCLC), colorectal cancer	Epidermal growth factor receptor (EGFR)	Type 1 tyrosine kinase; induces proliferation	EGFR over-expression or mutations	Associated with treatment response in lung cancer	Yes	Retrospective studies and phase II prospective studies suggest association between EGFR TK mutations and response of advanced NSCLC to TKI therapy (see text on EGFR Targeted Therapy for details).  Cetuximab proven efficacious alone or in combination with chemotherapy in metastatic CRC, but response not associated with EGFR protein expression.
EGFR inhibitors: gefitinib, erlotinib, cetuximab	Lung cancer (NSCLC), colorectal cancer	K-ras	Wild-type protein is a guanine nucleotide-binding protein that acts as a self-inactivating signal transducer	Point mutations	K-ras oncogene has point mutations causing constitutive activation but reduced activity;  Mutations are associated with resistance to targeted treatment;  Mutations in K-ras are mutually exclusive with EGFR mutations	Yes	K-ras mutations appear to confer primary resistance to TKIs in NSCLC (Pao et al. 2005).  Because K-ras and EGFR mutations tend to be mutually exclusive, patients with EGFR TK mutations may respond to TKI therapy while those with K-ras will not. Those negative for both are indeterminate, individuals positive for K-ras and negative for EGFR mutation also will not respond.  K-ras positivity appears to confer resistance to cetuximab in metastatic CRC (Lievre et al. 2006).

**Table 2.** Pharmacogenomic Applications to Predict Response to Targeted Drugs (cont'd)

Drug	Indication	Normal Protein; Gene	Normal Protein Function	Somatically Acquired Gene Variants	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
EGFR/HER2 inhibitors: gefitinib, trastuzumab	Lung cancer (NSCLC); breast cancer	PIK3CA/ PTEN	Heterodimeric lipid kinase that regulates cellular growth, transformation, and other functions central to normal cell function as well as tumorigenesis	Expression	PIK3CA and PTEN expression associated with longer OS in gefitinib-treated NSCLC;  Trastuzumab treatment more successful in cells with elevated PTEN expression	?None identified	Oncogenic PIK3CA mutations have been reported in 15% of all human cancer types studied (Karakas et al. 2006). Other data suggest a frequent and early role of PI3-kinase/ Akt pathway in lung carcinogenesis (Massion et al. 2004). Genomic amplification of PIK3CA and pAkt overexpression may represent biomarkers of tumor development.  The PI3-kinase is a possible target for specific therapy, but due to its wide range of intracellular functions, a broad spectrum kinase inhibitor could be very toxic. More specific agents are needed to target this entity, but data are scant.
Rituximab	Follicular non-Hodgkin's lymphoma (NHL)	Fc gamma receptor 3A (FCGR3A)	One postulated mechanism of rituximab action is immune cell Fc gamma receptor binding of the tumor-bound rituximab MAb with subsequent tumor cell clearance	Single nucleotide polymorphism: valine (V) vs. phenylalanine (F) at locus 158	Gene variation is correlated with monoclonal antibody binding affinity to immune effector cells, which may determine efficiency of tumor cell clearance	In commercial development	A small number of studies of NHL patients administered rituximab indicate statistically significant differences in response based on FCGR3A V/F genotype (e.g., Kim et al. 2006; Cartron et al. 2002). In the study by Kim et al. (2006), the response rate to rituximab +CHOP1 for the least responsive group was similar to that for CHOP alone; genotype did not predict survival.  Response rates to rituximab do not appear to differ by FCGR3A genotype in patients with chronic lymphocytic leukemia (Farang et al. 2004).
Erlotinib [Tarceva®]  (off-label application)	Polycythemia vera (PV), idiopathic myelofibrosis (IMF), essential thrombocythemia (ET)	JAK2	A tyrosine kinase linked to cytokine receptors for hematopoietic growth factors	Mutations in the JAK2 tyrosine kinase, e.g., V617F	V617F mutation is an activating mutation associated with oncogenesis and hematopoietic growth factor hypersensitivity.  With analogy to the BCR-ABL model, mutations may predict response to tyrosine kinase inhibitor therapy	Yes  (currently intended for applications in diagnosis and prognosis)	One preliminary study shows that erlotinib is a potent inhibitor of JAK2 in an in vitro colony culture assay (Li et al. 2007). No published studies report treatment response in patients, with or without respect to activating mutations.

<sup>1</sup> CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone.

toxicities. Epidermal growth factor receptor (EGFR) -targeted drugs are an example of this approach for several reasons (Dei Tos and Ellis, 2005; Giaccone and Rodriguez, 2005; Fruehauf, 2006; Metro et al. 2006; Sharma et al. 2007; Toschi and Cappuzzo, 2007). First, EGFR is a protein kinase involved in the maintenance and regulation of key cellular processes that include growth, differentiation, apoptosis, and morphogenesis. Second, EGFR was identified as an oncogene (a mutated form of a normal cellular gene), with high levels of the receptor and its ligands found in premalignant lesions of the oral cavity, lung, cervix, and prostate, and commonly overexpressed on the surface of cells in a variety of human epithelial cancers including NSCLC and colorectal cancer (CRC). Finally, aberrant EGFR expression in tumors has been associated with more aggressive disease, resistance to chemo- and radiotherapy, angiogenesis, increased propensity for metastasis, and decreased survival.

EGFR overexpression in cancer tissue at the genetic level involves abnormal, somatically acquired transcriptional or translational modification, gene amplification, or mutations in the EGFR gene that result in constitutive activation of downstream signaling. This discovery led to the commercial development of two main classes of anti-EGFR agents, SMIs gefitinib and erlotinib and monoclonal antibodies (MAbs) cetuximab and panitumumab, that target these processes in non-small-cell lung cancer (NSCLC), colorectal cancer (CRC), breast cancer, pancreatic cancer, and squamous cell head and neck cancer (Bunn et al. 2006; Heymach et al. 2006; Riely et al. 2006; Rosell et al. 2006; Thomas et al. 2006) (Table 1). Another Assessment (Volume 22, Number 6, “Epidermal Growth Factor Receptor Mutations and Tyrosine Kinase Inhibitor Therapy in Advanced Non-Small-Cell Lung Cancer”) will examine the evidence for EGFR mutation testing in the treatment of non-small-cell cancer.

A corollary to identification of anti-EGFR drug mechanisms of action at the genome level is that this also permits testing to predict sensitivity of individual patients’ tumors to these agents. A separate TEC Assessment will review the use of pharmacogenetic tests as a means to guide anti-EGFR therapy, primarily emerging pharmacogenetic approaches to predict

response of advanced NSCLC to TKI therapy, and EGFR expression analysis to determine eligibility of CRC patients to receive EGFR inhibitory treatment. The ultimate goal of pharmacogenomics in this setting is to select individuals who have increased probability of benefiting from EGFR inhibitory therapy and, if sufficiently predictive, to exclude individuals from such therapy who are highly unlikely to benefit from treatment.

#### Genetic Testing for Imatinib Resistance

Due to high response rates and good tolerability, imatinib has become first-line therapy for a number of malignancies. However, imatinib treatment does not usually result in complete eradication (by molecular analysis) of malignant cells. Malignant clones resistant to imatinib may be acquired or selected during treatment (secondary resistance), resulting in disease relapse. In addition, a small fraction of malignancies that express the target do not respond to treatment, indicating intrinsic or primary resistance. Resistance to imatinib and genetic tests to detect resistant clones will be described for CML and GIST. Additional examples are listed in Table 2.

**Chronic Myelogenous Leukemia.** Imatinib specifically inhibits the abnormal, tumor cell-specific BCR-ABL fusion gene product by interacting with the ATP<sup>1</sup> binding site of the ABL tyrosine kinase in its inactive conformation, preventing activation and downstream signal transduction. However, creation of the abnormal BCR-ABL gene is associated with genomic instability resulting most often in point mutations within the ABL gene kinase domain. Rarely, other acquired cytogenetic abnormalities such as BCR-ABL gene amplification and protein overexpression have also been reported (Walz and Sattler 2006). These mutations may result in resistance to imatinib; other non-BCR-ABL dependent resistant mechanisms may also be present or develop, such as altered cellular drug efflux or activation of alternative or downstream signaling pathways.

Primary resistance to imatinib is uncommon in newly diagnosed chronic phase patients, but more common in the accelerated phase of the disease (Ritchie and Nichols 2006). Secondary resistance is more clinically frequent, developing after an initial clinical response to the drug.

<sup>1</sup> ATP or adenosine triphosphate is a cellular source of energy and plays a necessary role in signal transduction between effector molecules.

Secondary resistance may be due to expansion of pre-existing tumor cell clones harboring a resistant mutation, or mutations may be acquired de novo; molecular mechanisms of resistance are under study. Residual disease at the molecular level can be detected by monitoring BCR-ABL transcripts in peripheral blood leukocytes (includes circulating tumor cells); rising levels are associated with clinical resistance and with molecular detection of BCR-ABL mutations (Martinelli et al. 2006).

BCR-ABL kinase domain point mutations are believed to be the most common cause of secondary resistance (Ritchie and Nichols 2006). At least 58 different point mutations have been identified (Mughal and Goldman 2007). The degree of resistance depends on the position of the mutation within the kinase domain of the protein. Some mutations (e.g., the methionine to threonine substitution at position 351, designated M351T) are associated with moderate resistance and are responsive to higher doses of imatinib (Mughal and Goldman 2007), while other mutations may not be clinically significant (Willis et al. 2005). Two mutations, T315I (threonine to isoleucine at position 315) and E255K (glutamic acid to lysine at position 255) are consistently associated with resistance to imatinib at all usual doses. The T315I mutation is relatively common at frequencies ranging from 4–19%, depending on the patient population; it is more common in patients with advanced-phase CML and in Ph-positive ALL patients than in patients with early chronic phase CML (Nicolini et al. 2006; Soverini et al. 2006; Jabbour et al. 2006).

Resistance not explained by point mutations may be due to abnormal BCR-ABL overexpression in tumor cells with or without gene amplification. This can be detected by fluorescent in-situ hybridization (FISH) specific for BCR and ABL genes. Imatinib-resistant patients with BCR-ABL overexpression are likely to respond to dose escalation (Walz and Sattler 2006).

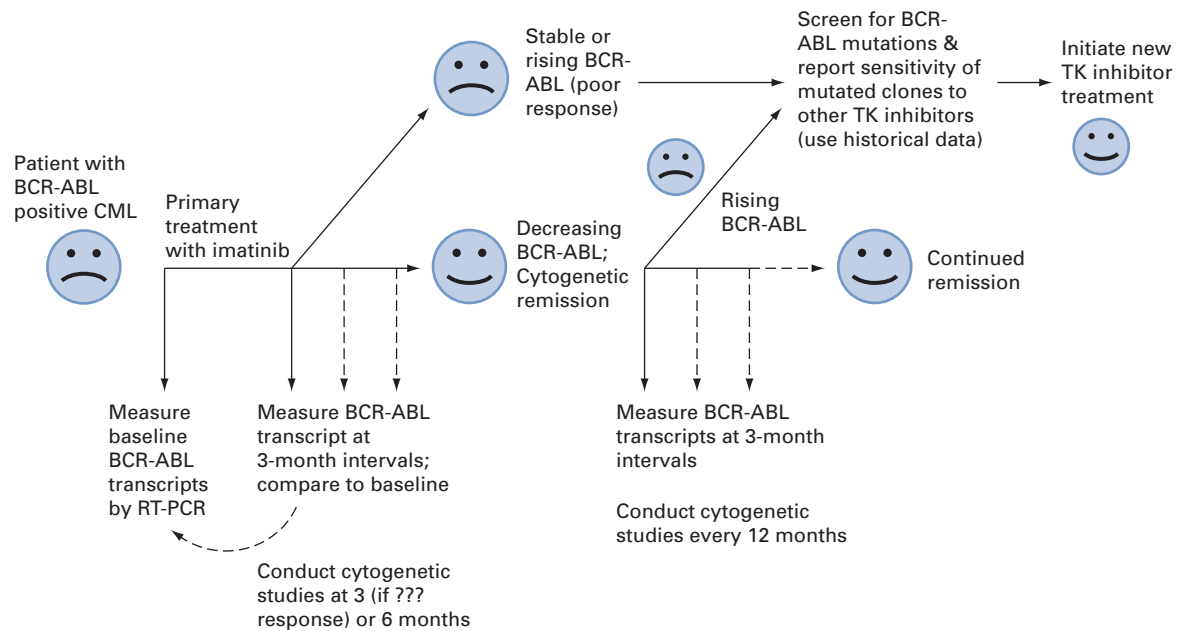
Structural studies of the ABL-imatinib complex have resulted in the design of novel ABL inhibitors, including dasatinib [Sprycel<sup>®</sup>] and nilotinib [Tasigna<sup>®</sup>].<sup>2</sup> These agents appear to be more potent than imatinib and/or more effective against several imatinib-resistant BCR-ABL-

positive tumor cell clones based on in vitro (e.g., Bradeen et al. 2006) and in vivo studies (e.g., Hochhaus et al. 2007b). Compared to imatinib, fewer mutations are associated with resistance to dasatinib or nilotinib (von Bubnoff 2006; Piccaluga et al. 2006). For example, Guilhot et al. (2007) and Cortes et al. (2007) studied the use of dasatinib in imatinib-resistant CML patients in the accelerated phase and in blast crisis, respectively, and found that dasatinib response rates did not vary by the presence or absence of baseline tumor cell BCR-ABL mutations. However, neither dasatinib nor nilotinib are effective against resistant clones with the T315I mutation (Guilhot et al. 2007; Mughal and Goldman 2007) and new agents and treatment strategies are in development for patients with T315I resistance.

Because expansion of a Ph-positive tumor cell clone with an ABL kinase domain mutation is likely to be associated with developing resistance to imatinib, and alternative treatment is available, early detection of rising levels of BCR-ABL transcripts and, subsequently, testing for ABL mutations has been recommended to “aid in risk stratification and molecular-based treatment decisions” (Hochhaus et al. 2007a). In October 2005 a number of investigators in this area participated in a meeting held by the National Institutes of Health. Based on the results of the meeting, provisional recommendations for laboratory diagnosis and monitoring of CML patients have been published (Hughes et al. 2006). As the authors note, “recommendations are based on incomplete clinical data that emanate from a very rapidly evolving field. They will necessarily be subject to frequent review.” Nevertheless, the recommendations represent an attempt to standardize laboratory measurements and reporting, and to suggest reasonable monitoring frequencies. Recommendations for monitoring patients are diagrammed in Figure 2.

**Gastrointestinal Stromal Tumors.** Activating, somatically acquired mutations in the KIT gene resulting in constitutive KIT receptor tyrosine kinase overexpression and positive immunohistochemical staining are found in 85–90% of GIST cells (Hornick and Fletcher 2007). A subset of KIT mutation-negative GISTs harbor somatically acquired activating mutations in

<sup>2</sup> Nilotinib was designated an orphan drug by the FDA on March 20, 2007 for the treatment of gastrointestinal stromal tumors. Novartis has filed an application with the FDA for nilotinib as a therapy for adult patients with chronic or accelerated phase Ph+ CML with intolerance or resistance to Gleevec<sup>®</sup> (imatinib).

**Figure 2.** Recommendations for Use of BCR-ABL Transcript Level Monitoring and Mutation Testing (Hughes et al. 2006)

the PDGFRA gene; some of these show less or nondetectable KIT immunohistochemical staining. KIT and PDGFRA oncogenic mutations are mutually exclusive. A small proportion of GISTs have neither KIT nor PDGFRA-activating mutations and are negative for KIT immunohistochemistry (IHC). Where KIT IHC is negative in suspected GIST cases, KIT and PDGFRA mutational analyses can be used to confirm the diagnosis (PDGFRA IHC is currently problematic; Hornick and Fletcher 2007).

The detection of KIT overexpression does not necessarily predict KIT-activation mutations that are sensitive to imatinib. However, because of the high prevalence of KIT (or PDGFRA) activating mutations in GIST, sensitivity to imatinib (currently the recommended first-line treatment) is highly likely and pretreatment KIT (or PDGFRA) mutation analysis is not consistently recommended (Hornick and Fletcher 2007; Skarlos et al. 2007). While KIT mutation analysis is readily available at commercial laboratories, PDGFRA mutation analysis appears to be much less available.

Variable responses to the standard initial dose of imatinib are likely due to the position of the activating mutation (Lasota and Miettinen

2006; Tarn and Godwin 2006). Cases with the most common KIT exon 11 mutations (70% of all GIST cases) show the most benefit from imatinib whereas those with the most common PDGFRA mutation (D842V; 4% of all GIST cases) show no response (Hornick and Fletcher 2007). Some mutations confer partial primary resistance to imatinib but respond to dose escalation.

Secondary resistance to imatinib can arise as a result of additional (sometimes multiple) somatic mutations in KIT or PDGFRA genes (e.g., Debiec-Rychter et al. 2005). As with primary resistance, the degree of secondary resistance depends on the mutation type and location (Weisberg et al. 2006; Lasota and Miettinen 2006); some confer partial resistance that is responsive to dose escalation while others, such as the common V654A, are intrinsically resistant to imatinib (Fletcher and Rubin 2007; Roberts et al. 2007). Some imatinib-resistant mutations are sensitive to sunitinib, also labeled for this use; sunitinib is recommended for GIST that is insensitive to standard and increased imatinib dose (Hornick and Fletcher 2007). Nilotinib has also been studied, but neither nilotinib nor sunitinib appear to be effective against PDGFRA mutation D842V-associated GIST (Weisberg et al. 2006).

Both activating and secondary mutations are highly heterogeneous and include insertions and deletions of various sizes as well as point mutations that result in amino acid substitutions (Roberts et al. 2007; Lasota and Miettinen 2006). KIT or PDGFRA mutation genotype may have variable impact on downstream signaling pathways, impacting GIST phenotype and possibly response to treatment. Predictors of response, such as kinase mutation genotype, other biomarkers, or gene expression profiles, are currently under study (Tarn and Godwin 2006).

### Tumor Heterogeneity

Inherent in the logic of targeted therapy is the assumption that the target is expressed with relative homogeneity throughout the tumor, such that therapy will have significant positive effects. As noted, changes in the target can allow the tumor to develop resistance to treatment. Lack of homogeneously expressed target throughout the tumor might also result in resistance to targeted therapy.

HER2 expression in breast cancer has been extensively studied. A majority of patients with HER2-primary tumors have HER2-positive metastases (77-92% in 2 studies: Tapia et al. 2007; Simon et al. 2001) and some discordance may be due to problems with interpretation of the HER2 assay results (Tapia et al. 2007). Within the primary tumor, a variable minority show within tumor heterogeneity using routine IHC or FISH (Hanna et al. 2007; Shin et al. 2006; Ooi et al. 2004), although some studies have reported discordance rates as high as 36% using reverse transcriptase polymerase chain reaction (RT-PCR) detection techniques (Glockner et al. 2002). It is of concern, though not conclusively shown, that heterogeneity of HER2 gene amplification within a given tumor or between primary and metastatic cancer foci may affect response to treatment, and explain why some apparently HER2-positive tumors do not respond to trastuzumab.

Other markers of targeted therapy have also been studied for within tumor heterogeneity. For example, significant heterogeneity in EGFR gene expression and amplification has been demonstrated in esophageal and gastric carcinoma (Personeni 2006; Kimura et al. 2005). The same appears to be true for BCR-ABL mutations in CML (Ma et al. 2006; Tauchi and Ohyashiki 2004), suggesting that a single inhibitor may not be able to block all tumor clones. The pattern of KIT IHC staining has

been reported as heterogeneous across whole sections of small cell lung cancers, and that core biopsy is not always representative of conventional tumor sections (Donati et al. 2004). Marked differences in KIT immunoreactivity across multifocal lesions in GIST may make a firm diagnosis difficult (Urbanczyk et al. 2005). Whether and to what degree within tumor heterogeneity affects response to targeted treatment is not well understood.

### Predicting Response to Non-“Targeted” Chemotherapy

Chemotherapy drugs that were not originally developed to “target” a specific molecule and favorably modify disease have historically been tested, dosed, and incorporated into treatment protocols based on “trial and error” approaches resulting in a single or a range of recommended doses based on studies of populations. However, inherited interindividual variability in rates of metabolism of these drugs results in sometimes large interpatient differences in systemic exposure, resulting in toxicity for some, lack of efficacy for others, and a satisfactory response mainly for those close to population average metabolism. Chemotherapy drugs commonly have a narrow therapeutic index that may overlap with the range of systemic exposure that results in severe toxicity.

Inherited polymorphisms in the genes coding for key molecules involved in the metabolism of chemotherapy drugs may result in increased drug metabolism and elimination, reducing exposure and possibly reducing efficacy, or conversely may result in reduced drug metabolism and elimination, increasing systemic exposure and predisposing to toxicity. These genetic variants affect the pharmacokinetic PK characteristics of the drug. In a few cases, inherited genetic variants may result in a modified drug target, as in those drugs discussed above as “targeted” therapy, and thus affect the pharmacodynamic (PD) characteristics of the drug (an example is thymidylate synthase, the target molecule of 5-fluorouracil). The actual utility of detecting a genetic variant in either pharmacokinetic or pharmacodynamic pathways depends on the contribution of the variant gene product to the response, the availability of alternative pathways that may alleviate the impact of the polymorphism, and the prevalence of the least-common variant allele. However, when dominant PK or PD pathways

are known, and variants in key PK or PD genes strongly influence response to treatment or toxic reactions, initial genotyping may favorably influence choice of drug and/or dose to improve outcomes (Figure 3).

Many studies have been published describing associations between various inherited genetic variants and the toxicity of or the response to various chemotherapy drugs. Table 3 provides limited detail on a few examples, most of which have commercially available genetic tests. In addition, two examples are discussed in more detail: dihydropyrimidine dehydrogenase deficiency and its importance in predicting 5-fluorouracil and capecitabine toxicity; and tamoxifen efficacy in cytochrome p450 2D6 poor metabolizers.

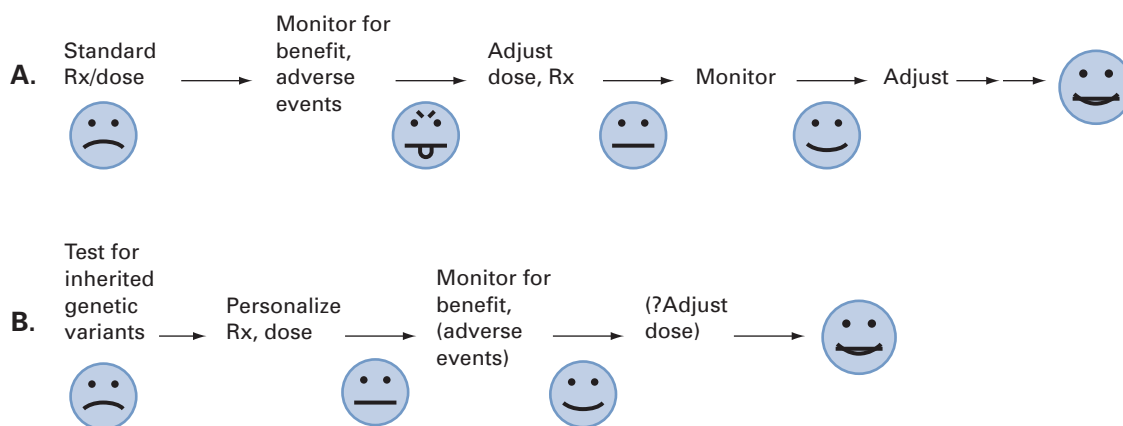
### 5-Fluorouracil, Capecitabine, and Dihydropyrimidine Dehydrogenase Deficiency

5-Fluorouracil (5-FU) is one of the most commonly prescribed components in chemotherapy regimens for a variety of cancer types such as colorectal, breast, and head and neck cancers. 5-FU must first be anabolized to the nucleotide level where it interferes with the synthesis of DNA and RNA, essential to the growth of the tumor. 5-FU can also be administered in the form of the prodrug capecitabine, which is given orally, is safer, and is more effective (Seck et al. 2005). Capecitabine is preferentially converted within the tumor site by thymidine phosphorylase (TP) into 5-FU (van Kuilenburg 2004).

Opposing 5-FU anabolism is 5-FU catabolism by dihydropyrimidine dehydrogenase (DPD); approximately 80% of administered 5-FU is rapidly degraded to inactive compounds (van Kuilenburg 2004). 5-FU has a narrow therapeutic window; in 5-FU excess, anabolic products accumulate and may cause World Health Organization (WHO) grade 3 to 4 hematologic toxicity (mainly neutropenia). Gastrointestinal toxicity and neurotoxicity may also occur. Examination of 5-FU pharmacokinetic parameters has shown that the drug concentration required for tumor response is within the general range where toxicity occurs (Ploylearmsaeng et al. 2006). The estimated threshold for severe toxicity is similar across different 5-FU-based treatment regimens (van Kuilenburg 2004). In a meta-analysis of about 1,200 patients with advanced colorectal cancer treated only with 5-FU, severe grade 3-4 hematologic toxicity was more likely with bolus delivery rather than by continuous infusion (31% vs. 4%, respectively) whereas grade 3-4 non-hematologic toxicity was similar at 13-14% (Meta-Analysis Group in Cancer, 1998).

Systemic low DPD activity, resulting in reduced 5-FU clearance, is associated with an increased risk of severe 5-FU toxicity. DPD activity may be low due to severely impaired liver function, patient age, elapsed time during 5-FU infusion (Etienne et al. 1998) or due to the presence of inherited DPD genetic variants. The DPD gene (DPYD) is large and complex, with 23 exons;

**Figure 3.** Standard Drug and Dose Followed by Monitoring and Empirical Drug and/or Dose Adjustment (A) vs. Initial Genotyping for PK or PD Variants to Guide Initial Drug and Dose Choice Followed by Monitoring and Empirical Dose Adjustment if Needed (B)



over 30 sequence variations have been identified, some of which have been associated with severe 5-FU toxicity. In particular, a G to A splice site substitution (IVS14+1G>A, designated DPYD\*2A) results in a nonfunctional protein product. In one study, the DPYD\*2A mutation was found in 24% of 5-FU-treated patients with severe WHO grade 3 or 4 toxicity; among those with the mutation, all had grade 4 myelosuppression (Raida et al. 2001). In another study, 50% of patients with 5-FU-associated grade 4 neutropenia had the DPYD\*2A mutation (Van Kuilenburg et al. 2002).

General population allele frequencies of the DPYD\*2A mutation range from 0 to nearly 3 percent in limited studies of different ethnic populations (van Kuilenburg 2004). The frequency of all known inactivating mutations in the general population is in the range of 3-5%; however, the prevalence of homozygosity for inactivating mutations, resulting in no DPD activity and placing patients at highest risk for severe and possibly lethal toxicity (van Kuilenburg et al. 2001), is only about 0.1% (Miller and McLeod 2007).

The FDA-approved labels for both 5-FU and the 5-FU prodrug capecitabine contain the following warning: “Rarely, unexpected, severe toxicity (e.g., stomatitis, diarrhea, neutropenia, and neurotoxicity) associated with 5-fluorouracil has been attributed to deficiency of dihydropyrimidine dehydrogenase activity.” In addition, the capecitabine label contains the following statements; “XELODA is contraindicated in patients with known dihydropyrimidine dehydrogenase

(DPD) deficiency.” However, the labels are silent regarding testing for DPD deficiency in advance of treatment.

van Kuilenburg et al. (2004) attempted to quantify the reduction in the frequency of severe toxicity if patients prescribed 5-FU treatment were screened for the DPYD\*2A mutation, and those positive for the mutation were treated with a non-5-FU regimen. Using Bayes’ theorem and various assumptions from the published literature, the frequency of grade 3–4 hematologic toxicity was predicted to drop from 31% (Meta-Analysis Group in Cancer, 1998) to 25%. Across the large treatment population, this could be a significant reduction. However, this assumed the much higher rate of hematologic toxicity associated with bolus delivery. The assumptions and the corresponding reductions in frequency of severe hematologic toxicity assuming continuous infusion and for severe non-hematologic toxicity are shown in Table 3, and indicate a much smaller population effect of pre-treatment DPD genetic testing.

Other genetic or epigenetic variability may also help explain some cases of severe 5-FU toxicity. An inherited inactivating mutation in dihydropyrimidinase, the second enzyme after DPD in the 5-FU catabolic pathway, has been reported in a breast cancer patient with severe 5-FU toxicity (van Kuilenburg et al. 2005). A particular inherited polymorphism in the gene coding for thymidylate synthase (TS), the target enzyme inhibited by 5-FU anabolic products, may predict toxicity (Lecomte et al. 2004). Methylation of the DPYD promoter region may

**Table 3.** Estimated Reduction in Frequency of Severe (WHO grade 3–4) Toxicity after DPYD\*2A Mutation Screening (Calculations per van Kuilenburg et al. 2004).

	Bolus	Continuous Infusion
Probability of DPYD*2A in patients with severe grade 3–4 toxicity	0.28	0.28
Probability of DPYD*2A in patients without severe toxicity (same as prevalence of mutation in the normal population)	0.018	0.018
Overall probability of severe 5-FU hematologic toxicity	0.31	0.04
<b>Probability of toxicity in patients with DPYD*2A mutation</b>	87%	47%
<b>Reduction in frequency of severe toxicity</b>	31% → 25%	4% → 3%
Overall probability of severe 5-FU non-hematologic toxicity		14%
<b>Probability of toxicity in patients with DPYD*2A mutation</b>		76%
<b>Reduction in frequency of severe toxicity</b>		14% → 11%

also cause down regulation of DPD activity and 5-FU toxicity (Ezzeldin et al. 2005). Clinical parameters such as performance status and age have been reported to predict toxicity (Meta-Analysis Group in Cancer, 1998) but have not been analyzed in combination with genetic mutations.

Avoidance of severe 5-FU toxicity is highly desirable; DPYD\*2A heterozygotes have a high risk of toxicity and for the rare DPYD\*2A homozygotes, severe and possibly life-threatening toxicity is almost certain. However, screening all patients prescribed 5-FU chemotherapy for DPYD mutations has not been routinely implemented for several reasons:

- Only DPYD\*2A is relatively prevalent; other mutations in DPYD have been reported but each is relatively rare and the clinical relevance has not been determined for all.
- Mutation frequencies in different ethnic populations have not been well characterized; continued discovery of new mutations is likely.
- Mutations in genes coding for other 5-FU catabolic enzymes as well as epigenetic modifications of DPD (and other genes) may be important in predicting toxicity.
- Panels of genetic markers for predicting risk of toxicity have not been tested.
- Genotype alone may not predict all toxicity.
- Testing for DPYD\*2A alone could be accomplished in an efficient and automated format; however, determination of all known mutations would greatly increase test complexity (Yen and McLeod 2007). Because undiscovered mutations are likely, not all would be found at testing.
- The number needed to test (NNT) to detect one patient heterozygous for a DPYD\*2A mutation is nearly 100; to detect one patient that is homozygous for DPYD\*2A, the NNT is 1000 or more.

In their recent update of recommendations for tumor marker use in gastrointestinal cancers, the American Society of Clinical Oncology (Locker et al. 2006) found “Little empirical evidence supports DPD alone as a prognostic marker.” Data were judged insufficient for use in patient management.

#### **Tamoxifen and CYP2D6 Poor Metabolizers**

Tamoxifen undergoes extensive primary and secondary metabolism, and the concentrations of tamoxifen and its metabolites vary widely.

Although 4-hydroxytamoxifen (4-OH tamoxifen) represents less than 10% of tamoxifen primary oxidation, it has been considered to play an important role in tamoxifen’s anticancer effect given its 100-fold greater affinity for the estrogen receptor and its 30- to 100-fold greater potency in suppressing estrogen-dependent cell proliferation when compared with the parent drug.

Recent studies strongly suggest that another tamoxifen metabolite, 4-hydroxy-*N*-desmethyl tamoxifen (endoxifen) is more important than 4-OH tamoxifen in terms of the relative contribution to the overall anticancer effect of tamoxifen and thus to inter-individual variability in response to the drug (Desta et al. 2004). Endoxifen has identical properties and potency compared with 4-OH tamoxifen in terms of its binding affinity to ERs, suppression of estradiol-stimulated cell proliferation, and gene expression. Furthermore, steady-state plasma endoxifen concentrations are 5- to 10-fold higher than 4-OH tamoxifen.

Although the metabolism of tamoxifen to 4-OH tamoxifen is catalyzed by multiple enzymes, endoxifen is formed predominantly by the CYP2D6-mediated oxidation of *N*-desmethyl tamoxifen, the most abundant tamoxifen metabolite. Inherited polymorphisms in the CYP2D6 gene that are present in some individuals result in reduced or no enzyme activity; the most common nonfunctional polymorphism is designated CYP2D6\*4 (12-23% allele frequency in Caucasians; lower frequency in Black Africans and Asians). Individuals with two \*4 alleles are referred to as poor metabolizers (PM) and individuals with one \*4 allele have reduced enzyme activity and are designated intermediate metabolizers (IM). Concomitant medications that inhibit wild-type CYP2D6 (wt or CYP2D6\*1) activity (e.g., some selective serotonin reuptake inhibitor [SSRI] antidepressants, often co-prescribed with tamoxifen) may also result in a PM phenotype.

Studies have suggested that CYP2D6 PM phenotype is associated with a higher risk of breast cancer recurrence in post-menopausal women administered tamoxifen for 5 years. The supporting data are summarized in Table 4. Only one retrospective study of banked samples from a prospective randomized controlled trial (Goetz et al. 2007; an update of Goetz et al. 2005) evaluates clinical outcomes of tamoxifen treatment by CYP2D6 polymorphism status and

**Table 4.** Studies of Tamoxifen and CYP2D6 Genotype in Postmenopausal Breast Cancer Patients Administered Tamoxifen

Study	n	Patients	Gene	Comparison	Results
<b>Association of Genotype with Tamoxifen and/or Metabolite Concentration</b>					
Stearns 2003	12	Breast cancer pts taking TAM and paroxetine (SSRI; CYP2D6 inhibitor)	CYP2D6	Compare levels of tamoxifen metabolites (e.g., endoxifen) by genotype	Baseline endoxifen concentrations lower in *4 carriers than in wt/wt (p=0.002)
Borges 2006	158	Breast cancer patients taking TAM	CYP2D6	Genotype vs. tamoxifen metabolite ratio of NDM/endoxifen <sup>1</sup>	Ratios significantly different for CYP2D6 PMs vs. IMs vs. EMs, p<0.001; EMs taking concomitant potent CYP2D6 inhibitors had lower endoxifen concentrations compared to pts not taking inhibitors
Grabinski 2006 [ABSTRACT]	299	Breast cancer pts taking TAM	CYP2C9 CYP2D6 SULT1A1 <sup>2</sup> ER alpha	Test association of genotype with tamoxifen metabolite levels	4-hydroxyTAM varied significantly among CYP2D6 PM, IM, and EM genotypes (p=0.0002)  ER alpha genotypes associated with TAM levels (p=0.02) Ethnicity (Caucasian vs. Hispanic) associated with TAM and 4-hydroxyTAM
<b>Association of Genotype with Clinical Outcome; Concomitant Inhibitor Medications not Considered in Analysis</b>					
Goetz 2005	223	Archived blocks from NCCTG RCT (89-30-52) of women with ER+ resected Breast cancer who received TAM	CYP2D6	Relapse-free time and survival of women with *4/*4 genotype (n=13) vs. *4/wt or wt/wt;  Moderate/severe hot flashes in *4/*4 vs. *4/wt or wt/wt	Relapse-free time: HR 1.85, p=0.176 Disease-free survival: HR 1.86, p=0.089 Overall survival: HR 1.12, p=0.78 — adjusted for nodal status and tumor size  0% vs. 20%, p=0.064
Wegman 2005	226	Breast cancer patients in trial of adjuvant TAM	CYP2D6 SULT1A1 <sup>2</sup>	Risk of recurrence in pts carrying CYP2D6*4 allele (n=24) and/or SULT1A1*1/*1 vs. not	CYP2D6*4: RR=0.28, 95% CI 0.11-0.74, p=0.009 SULT1A1*1/*1: RR=0.48, 95% CI 0.21-1.12, p=0.074 CYP2D6*4+SULT1A1*1/*1: RR=0.38, 95% CI 0.19-0.74, p=0.0041
Nowell 2005	162 175	Breast cancer pts taking TAM Breast cancer pts not taking TAM	CYP2D6 SULT1A1 <sup>2</sup> UGT2B15 <sup>2</sup>	Overall survival by genotype using Cox modeling, adjusting for age, race, stage, hormone-receptor status	CYP2D6: no significant association + or - TAM UGT2B15: high activity genotype associated with increased risk of recurrence and poorer survival in TAM-treated pts  UGT2B15+SULT1A1: pts with 2 variant genotypes and taking TAM had increased risk of recurrence and poorer survival

**Table 4.** Studies of Tamoxifen and CYP2D6 Genotype in Postmenopausal Breast Cancer Patients Administered Tamoxifen (cont'd)

Study	n	Patients	Gene	Comparison	Results
<b>Effect of Genotype + Concomitant Enzyme Inhibitors</b>					
Stearns 2003	12	Breast cancer pts taking TAM and paroxetine (SSRI; CYP2D6 inhibitor)	CYP2D6	Compare levels of tamoxifen metabolites before and after paroxetine, by genotype	Endoxifen concentration decreased 64% (95% CI, 39-89%) in wt/wt vs. 24% (95% CI, 23-71%) in *4 carriers
Jin 2005	80	Breast cancer pts taking TAM ± CYP2D6 inhibitors	CYP2D6	Compare levels of tamoxifen metabolites by genotype and inhibitor med	After 4 mos TAM, endoxifen levels lower in pts carrying a variant genotype compared to wt/wt (p=0.003); among wt/wt, endoxifen 58% lower in those taking inhibitor med (n=24) than those not taking inhibitor (p=0.0025)
Borges 2006	158	Breast cancer patients taking TAM	CYP2D6	Genotype vs. tamoxifen metabolite ratio of NDM/ endoxifen <sup>1</sup>	EMs taking concomitant potent CYP2D6 inhibitors had lower endoxifen concentrations compared to pts not taking inhibitors
Goetz 2007	190	Archived blocks from NCCTG RCT (89-30-52) of women with ER+ resected breast cancer who received TAM	CYP2D6*4	Evaluate combined effect of CYP2D6 genetic variation and concomitant use of CYP2D6 inhibitory medications on breast cancer progression-free and overall survival	6% of patients were co-prescribed a CYP2D6 inhibitor In multivariate analysis, patients with reduced CYP2D6 metabolism (either by presence of one *4 allele or by inhibitory concomitant medication) had a significantly shorter time to recurrence in multivariate analysis: HR = 1.91; 95% CI 1.05-3.45; p=0.034  CYP2D6 poor metabolizers compared to extensive metabolizers had the most significant risk of breast cancer relapse: HR = 3.12, p=0.007 No significant effect on survival.

<sup>1</sup> N-Desmethyltamoxifen (NDM) is a major primary metabolite of TAM and is hydroxylated by CYP2D6 to yield endoxifen, which has high antiestrogenic potency

<sup>2</sup> Sulfotransferase 1A1 and UDP-glucuronosyltransferase 2B15 are phase II (detoxifying) enzymes that metabolizes 4-hydroxyTAM

takes concomitant inhibitory medications into account. Other studies of CYP2D6 polymorphisms and clinical outcomes do not consider inhibitory medications, but generally support the conclusions of Goetz et al. (2007), which are that patients with reduced CYP2D6 metabolism had a significantly shorter time to recurrence in multivariate analysis (HR = 1.91; 95% CI 1.05-3.45;  $p=0.034$ ).

On October 18, 2006, the FDA Advisory Committee for Pharmaceutical Science, Clinical Pharmacology Subcommittee, met to discuss possible tamoxifen label changes regarding CYP2D6 low activity and potential clinical consequences of treatment. The committee recommended label changes to include relevant information, but did not agree on whether to recommend pretreatment CYP2D6 genotyping. As of October 19, 2007, a revised label had not been released by the FDA.

Additional studies, such as outcomes of increased dose tamoxifen or alternatives to tamoxifen (aromatase inhibitors or AIs) evaluated by CYP2D6 patient genotype, in addition to cost-effectiveness analyses would help determine the utility of pretreatment CYP2D6 genotyping. A recent review article mentioned that the Breast Intergroup “has tentatively approved a randomized study to further evaluate the role of CYP2D6 status and treatment with an AI upfront versus tamoxifen followed by an AI in postmenopausal women (Hartman and Helft 2007).

### Multigene Profiles

With only a few, low-prevalence exceptions (e.g., TPMT, DPD homozygous nonfunctional variants), inherited polymorphisms in single candidate genes are unlikely to have strong and reliable associations with toxicity or response in the majority of patients treated. Many single-gene associations have only borderline statistical significance and questionable clinical significance because determinants of response and toxicity to single drugs are likely multigenic. This complexity is multiplied by the use of combination chemotherapy regimens, and further by the influence of non-genetic confounders (e.g., tumor stage and

molecular characteristics, age, comorbidity, concomitant medication). Thus, some investigators are evaluating panels of candidate genes to improve the accuracy of prediction.

For example, Salonga et al. (2000) evaluated the intratumoral gene expression of TS, TP, and DPD in colorectal cancer with respect to response to 5-FU and reported that all responding tumors (11 of 33) had low expression values of all three of the genes, whereas in each of the nonresponding tumors, at least one gene had high expression. Matsuyama et al. (2006) constructed a “response index” from 3 candidate genes (not included in Table 5) for 5-FU treatment of colorectal liver metastases and tested the index in 22 patients. Among 11 cases with positive index values, 9 achieved a reduction in liver metastases whereas in 11 cases with negative index values, only 1 responded to chemotherapy. Goekkurt et al. (2006) evaluated GSTP1 and TYMS genotypes (see Table 5) in 52 advanced gastric cancer patients treated with 5-FU. Overall survival was longer (11 months) in patients with lower activity (GSTP1) and/or low gene expression (TYMS) alleles, and shorter (6 months) in patients with both higher activity and high gene expression for the respective genes. Others have similarly tested different gene combination panels in small studies (e.g., Martinez-Balibrea et al. 2007; Stoehlmacher et al. 2004), but additional validation in large treatment populations is needed for the most promising panels. Davies (2006) has proposed the following steps “toward truly personalized medicine”:

- identification of significant genes,
- integration of multiple genes into a profile,
- demonstration that use of the profile in management decisions improves outcomes, and
- profile use is accepted on a population-wide basis.

However, these steps assume a candidate gene approach. Parallel efforts to discover predictors of response and toxicity involve the use of large-scale arrays and pattern analysis for predicting outcomes. While this topic is beyond

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
6-mercaptopurine [Purinethol]  (Also azathioprine for RA, IBD, prevention of transplant rejection, etc.)	Pediatric acute lymphocytic leukemia (ALL); acute myelocytic leukemia (AML), acute myelomonocytic leukemia (AMML), acute promyelocytic leukemia (APML)	Toxicity currently monitored by WBC count, LFT	<b>Protein:</b> thiopurine methyltransferase (TPMT);  <b>Gene:</b> TPMT	Catalyzes S-methylation of thiopurines, the principal mechanism of thiopurine inactivation	*2, *3A, *3C account for 95% of abnormal, reduced function variants	Severe myelosuppression possible, can be life-threatening  <b>Gene variant (var) frequencies:</b>  <b>89% *1/*1 (wild type)</b>  <b>11% *1/*var:</b> reduced TPMT activity, high efficacy at standard dose but moderate to severe toxicity may require modest dose reduction for some patients  <b>0.36% *var/*var:</b> TPMT deficient; severe, possibly fatal toxicity at standard dose; require ~90% dose reduction	Yes  for 3 most common variants; 5% of variants will be missed	FDA initiated label changes to include information about TPMT genetics and testing, including: "If a patient has clinical or laboratory evidence of severe toxicity, particularly myelosuppression, TPMT testing should be considered.... Substantial dose reductions are generally required for homozygous-TPMT deficiency patients... The optimal starting dose for homozygous deficient patients has not been established."  At the Advisory Panel review on July 15, 2003, the Panel did not agree to recommend or mandate pretreatment TPMT testing for all patients. Information is lacking on toxicity in heterozygotes and on best doses to retain efficacy but avoid toxicity for each genetic group; however, testing could avoid life-threatening toxicity in rare homozygotes and indicate whether toxicity symptoms in any patient are likely due to 6-MP or to other concurrently administered drugs.  At a 1.4% overall frequency of severe myelosuppression, pretreatment testing could reduce frequency to 1%; if overall severe myelosuppression frequency is 5%, testing could reduce to 3.5% <sup>2</sup>

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Various, including cyclophosphamide [Cytoxan®], oxaliplatin [Eloxatin®]	Various	Tx response	<b>Protein:</b> glutathione S-transferases (GSTs);  <b>Genes:</b> GSTP1, GSTM1, GSTT1	Involved in the detoxification of various exogenous and endogenous reactive species including cyclophosphamide reactive metabolites and platinum drugs	GSTP1*B allele (A>G, I105V) substantially reduces activity	Low activity or null alleles have been associated with increased response or lower risk of relapse.	Yes	Reduced detoxification capacity due to low or null activity alleles potentially enhances effectiveness of cytotoxic drugs.
		Toxicity						For example, in one study of pediatric ALL, GSTP1*B, and GSTM and GSTT1 null alleles were associated with reduced risk of relapse (Stanulla et al. 2000).
								Stoehlmacher et al. (2002) reported that the GSTP1 G low activity allele (but neither GSTM1 nor GSTT1 null alleles) in advanced colorectal cancer patients treated with 5-FU and oxaliplatin was independently and significantly associated with survival in a dose-dependent fashion.
								Lecomte et al. (2006) studied peripheral neuropathy due to oxaliplatin treatment in patients with GI solid tumors and reported that grade 3 neuropathy was significantly more frequent and survival poorer in patients homozygous for the GSTP1 A (normal activity ) allele.

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Irinotecan [Camptosar®]	Colorectal	Toxicity  currently monitored by WBC count	Uridine diphosphate glucuronosyl-transferase 1A1 (UGT1A1; hepatic)	Inactivates SN-38, the product of irinotecan hydrolysis and a potent topoisomerase inhibitor and determinant of antitumor activity	UGT1A1 promoter TA repeat variants: *1 = 6 TA repeat *28 = 7 TA repeat *6 – 211G>A (G71R)  Other UGT1A1 variants: SNP at base -3156	Increased TA repeats result in decreased UGT1A1 expression and reduced irinotecan detoxification; risk of severe neutropenia and diarrhea higher in *28/*28 compared to *1/*1 and *1/*28.  ?better predictor of toxicity than TA repeats	Yes	The CDC-funded Evaluation of Genomic Applications in Practice and Prevention (EGAPP) commissioned a systematic review: “UGT1A1 testing in colorectal cancer patients treated with Irinotecan,” which should be publicly available in Q4 2007 ( <a href="http://www.egappreviews.org/">http://www.egappreviews.org/</a> ).
Methotrexate	Leukemia, lymphoma, other	Toxicity	<b>Protein:</b> 5, 10-methylenetetrahydrofolate reductase (MTHFR)  <b>Gene:</b> MTHFR	Pivotal to folate homeostasis, which is required for protein and nucleic acid synthesis	C>T; A226V variant protein has about 30% of wild type activity; heterozygotes have about 60% of wild type activity	Reduced activity; possibly increased risk of hepato- and bone marrow toxicity	Yes	Association of TT genotype with toxicity in some studies, not confirmed in others (summarized in Bomgaars and McLeod 2005).  Studies were of low-dose methotrexate treatment without folinic acid (leucovorin) rescue, now standard treatment.
	Pediatric ALL	Tx response	<b>Protein:</b> Folate transporter 1  <b>Gene:</b> SLC19A1	Transports methotrexate and natural folates into cells	G >A; R27H	The ability of leukemia cells to accumulate methotrexate is an important determinant of treatment success.  The A variant is associated with altered folate levels and higher methotrexate plasma concentrations	?	In one study, children with an A variant allele had higher levels of plasma methotrexate and worse prognoses than patients with the GG genotype (p=0.04; Laverdiere et al. 2002).

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
5-Fluorouracil (5FU) or 5FU prodrug e.g., capecitabine, doxifluridine	Colorectal, breast, others	Tx response	<b>Protein:</b> thymidylate synthase (TS);	Catalyzes methylation of deoxyuridine monophosphate, a DNA building block; Inhibited by primary active 5FU metabolite 5-FdUMP; Inhibition leads to interference with DNA synthesis and repair	Intratumoral gene expression  TSER*2 and TSER*3 variants have 2 and 3 28-base pair tandem repeat sequences; variants differentially affect regulation of TYMS expression  G>C exchange in the second tandem repeat of the TSER*3 allele  6-bp insertion/deletion in 3' UTR	Low tumor TS expression may predict longer survival  TSER*2/*2 patients express less TS than TSER*3/*3 and are more likely to respond to 5FU treatment; TSER*2/*2 may also have an increased risk of severe toxicity  Modifies TSER variants; C allele associated with reduced expression  del6/del6 associated with lower TS levels	Yes	Popat et al. (2004) conducted a meta-analysis of TS protein expression and survival in CRC; low TS expression was significantly associated with better survival, but heterogeneity and possible bias prevented firm conclusions.
			<b>Gene:</b> TYMS  TYMS 5' promoter-enhancer region: TSER					TSER polymorphism currently being tested as a predictor in a prospective study of pharmacogenetics-guided 5-FU therapy of rectal carcinoma (Miller and McLeod 2007).  Patients with combinations of TYMS low-expression variants have significantly better survival than those with high-expression variants (Toffoli and Cecchin 2007; Salgado et al. 2007).  Limited data suggest that patients with low TYMS expression are more responsive to irinotecan (Noda et al. 2006).
		Toxicity	<b>Protein:</b> dihydro-pyrimidine dehydrogenase (DPD);	DPD accounts for ~80% of FU catabolism and is rate-limiting step; DPD activity varies more than 20-fold among patients	>30 inactivating mutations reported; ~50% are IVS14+1G→A (DPYD*2A)	DPD deficiency and resulting excess anabolic products are associated with severe GI, hematologic, and neurologic toxicity from FU; in rare cases toxicity can be fatal	Yes	DPYD*2A heterozygotes have a high risk of toxicity and for the rare DPYD*2A homozygotes, severe and possibly life-threatening toxicity is almost certain. However, screening all patients prescribed 5-FU chemotherapy for DPYD mutations has not been routinely implemented.  (See text for detail.)

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
5-Fluorouracil (5FU) or 5FU prodrug e.g., capecitabine, doxifluridine	Colorectal, breast, others	Toxicity	<b>Protein:</b> dihydropyrimidinase (DHP)  <b>Gene:</b> DPYS	Also part of 5-FU catabolic pathway	Missense mutation G>A (G278D)	May be associated with severe toxicity	?	Associated with severe toxicity in one case report (van Kuilenburg et al. 2003).
Capecitabine [Xeloda®]; 5FU prodrug	Colorectal	Tx response	<b>Protein:</b> thymidine phosphorylase (TP);  (also platelet-derived endothelial cell growth factor)  <b>Gene:</b> ECGF1	Mediates final step in conversion of 5-FU prodrug to 5-FU;	Gene over-expression by immunohistochemistry (IHC) or RT-PCR	Overexpression in tumor tissue may predict clinical response	?	TP is expressed in higher concentrations in some tumors than in normal tissues; 5-FU activity may be concentrated in high TP-expressing tumors.  In a cohort of 67 patients, a pre-planned biomarker analysis found that TP overexpression by IHC in either primary or metastatic tumors was significantly correlated with overall survival and (in primary tumors only) response to treatment (Meropol et al. 2006).
Tamoxifen [Nolvadex®]	Breast	Tx response	CYP2D6	Converts TAM into active metabolite 4-OH-TAM (endoxifen)	CYP2D6*3, *4, *5, or *6 nonfunctional variants	Homozygous variants are PMs, associated with shorter relapse-free survival  Use of CYP2D6 inhibitor drugs (e.g., SSRIs) regardless of genotype could also reduce TAM efficacy	Yes	Only one retrospective study of banked samples from a prospective randomized controlled trial (Goetz et al. 2007) evaluates clinical outcomes of TAM treatment by CYP2D6 polymorphism status and takes concomitant inhibitory medications into account. (See text for additional detail.)
		Tx response	SULT1A1	Sulfotransferase; sulfurylation of TAM leads to excretion	SULT1A1*2 (G>A; R213H)	Common polymorphism associated with reduced enzyme activity and increased risk of recurrence	?	Study results correlating SULT1A1*2 with higher recurrence risk are difficult to interpret as reduced activity should lead to longer half-life of active TAM metabolites (Choi et al. 2006). (See Table 4 for studies of SULT1A1, CYP2D6, and TAM treatment.)

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Oxaliplatin [Eloxatin®], cisplatin, carboplatin	Colorectal, lung	Tx response	Excision repair cross complementing group 1 (ERCC1)	Part of nucleotide excision DNA repair pathway, which removes DNA adducts produced by oxaliplatin	C>T; N118N variant	Lower ERCC1 levels and lower gene expression in tumor tissue associated with longer survival  Presence of T/T allele associated with poorer response rate	Yes	Association of low ERCC1 expression levels with longer survival documented in several studies, reviewed in Garcia-Campelo et al. (2005).  ERCC1 and RRM1 pretreatment tumor expression results were used to help choose among different chemotherapy regimens for patients with advanced NSCLC in a small proof of principle trial (Simon et al. 2007). Overall survival and progression-free survival were 59% and 14% at 12 months. There were no controls for comparison.
		Tx response	ERCC2 (also known as XPD)	Part of nucleotide excision DNA repair pathway, which removes DNA adducts produced by oxaliplatin	A>C, K751Q variant	Lower expression associated with longer survival.  Presence of C/C allele associated with poorer response rate	Yes	Expression correlated with ERCC1 and RRM1 expression (Garcia-Campelo et al. 2005).
		Tx response	X-ray cross complementing group 1 (XRCC1)	Part of nucleotide excision DNA repair pathway, which removes DNA adducts produced by oxaliplatin	G>A, R399Q variant	Presence of A/A allele associated with poorer response rate	Yes	In combination with other markers, XRCC1 may help in predicting response to platinum-based treatment (e.g., Gurubhagavatula et al. 2004; Stoeckl 2004).

**Table 5.** Selected Pharmacokinetic Effects of Genetic Variants on Drug Metabolism and Clinical Outcomes (cont'd)

Drug	Cancers Studied for PGx Application	PGx issue	Protein; Gene	Protein Function	Gene Variants <sup>1</sup>	Consequences of Having a Functional Gene Variant	Commercial Lab Test Available?	Comment
Anthracyclines	Breast	Tx response	Topoisomerase II alpha (TOP2A)	Type II DNA topoisomerase vital for DNA replication, chromosome segregation, and maintenance of chromosome structure	Gene amplification or overexpression	HER-2 amplification + TOP2A amplification correlated with sensitivity to anthracyclines	?	<p>Several studies of patients with breast cancer have suggested that TOP2A gene amplification or protein overexpression is associated with increased sensitivity to anthracycline-based chemotherapy. However, other studies are contradictory.</p> <p>It has been hypothesized that the interaction between HER2 and anthracycline efficacy may depend on TOP2A (Di Leo and Isola 2003). A recent study that evaluated TOP2A only in tumors with HER2 amplification found that co-amplification of these two genes was associated with better relapse-free survival (Scandinavian Breast Group Trial 9401 2006).</p>
Gemcitabine [Gemzar]	Non-small cell lung cancer	Tx response	RRM1	Crucial gene for nucleotide metabolism and the molecular target of gemcitabine	Gene expression	Gene expression inversely correlated with response to gemcitabine	?	(see “Comment” for oxaliplatin and ERCC1)

<sup>1</sup> Polymorphisms are indicated by the DNA nucleotide substitution (e.g., G>A) and the single letter codes for the amino acid change at the mRNA codon number (e.g., G>A; R213H means that when the G is present in the DNA sequence, codon 213 results in arginine but when the A is present in the DNA sequence, codon 213 results in histidine). In some cases, especially in more complicated polymorphisms, simplified nomenclature is created and defined e.g., gene\*1 for wild type and gene\*2, \*3, etc. as additional polymorphisms are discovered.

<sup>2</sup> Bayes' Theorem and assumptions from van den Akker-van Marle et al. 2006: Probability of a mutation in patients with severe myelosuppression, 32%; probability of a mutation in patients without myelosuppression (assumed to be the allele frequency in the general population), 11%. Calculation as for DPD deficiency (see text).

the scope of this Report, array-developed predictor panels, because they typically involve many more genes than candidate gene panels, may be even more specific to cancer type and stage, treatment protocol, and other nongenetic confounders, greatly increasing the complexity of panel design and validation. Thus, as Davies (2006) counsels,

“... the field will benefit from a cautious approach to describing applications that are still in the future; “personalized medicine for all” is not on the immediate horizon, more a distant goal.”

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