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Clinically relevant cancer biomarkers and pharmacogenetic assays

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Abstract

Background: The number of pharmacogenetic assays available is continuously expanding as more molecularly targeted anticancer drugs are under clinical development. While the literature regarding drug–gene associations and therapeutic implications is often robust, reviews regarding clinical assay availability and profiling methodologies of commonly used cancer biomarkers are often lacking.

Objective: To concisely identify and describe cancer biomarkers and their respective pharmacogenetic assays currently available in clinical practice.

Discussion: Analysis of germ-line DNA mutations can often help to predict pharmacokinetic and pharmacodynamic responses, whereas somatic DNA mutations are particularly useful in predicting tumor response. Molecular profiling and pre-emptive identification of cancer biomarkers can help to predict disease prognosis as well as response to anticancer therapy. Dozens of pharmacogenetic assays, utilizing several common methodologies, are currently available in clinical practice. It is essential for clinicians to understand the molecular pathways for anticancer drugs, the therapeutic implications of mutations within these pathways, the clinical assay(s) available to test for pharmacogenetic differences, and the common profiling methodology employed.

Conclusion: As research continues to unveil more drug–gene and disease–gene associations, it is critical that clinicians understand which pharmacogenetic assays are available to identify inter-individual differences that predict safety and efficacy of anticancer drugs as we move toward the concept of personalized medicine.

Keywords

Pharmacogenetic, cancer, biomarker, clinical, assay

Introduction

Considerable advancements have been made regarding the way human tumors are characterized. Our knowledge of cancer at the molecular level has dramatically changed the way we treat cancer and has caused a shift from characterizing tumors solely based on their location and histology to consideration of their molecular profile.[1] Given the biological heterogeneity known to exist from patient to patient, cancer biomarkers are

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being increasingly used to personalize cancer therapy. Both somatic mutations, variations found within the tumor, and germ-line mutations, heritable variations found within the host, may influence disease outcome and/or response to therapy. These mutations, or cancer biomarkers, can be broadly classified as prognostic, those mainly associated with disease outcome, or predictive, those associated with drug response.[1] This review aims to highlight important differences between classes of cancer biomarkers and molecular profiling methodologies, and elucidate the availability of pharmacogenetic assays currently used in clinical practice. Table 1 outlines the clinically relevant cancer biomarkers, therapeutic implications, pharmacogenetic assays used to identify the biomarker of interest, and the respective method of detection.

Somatic versus germ-line DNA mutations

DNA analysis for pharmacogenetic purposes is generally performed with germ-line DNA. However, for anticancer therapy, DNA is frequently analyzed within the tumor tissue and is referred to as somatic DNA. These mutations can be distinguished by the origin of the mutated cell. Germ-line DNA mutations are found within germ cells (the sperm or egg), and are therefore inherited and transmitted to the offspring, whereas somatic mutations occur within any other cell (i.e. tumor cell) after conception and subsequently are not passed on to progeny.[23]An additional difference is the sampling methodology. Germ-line DNA is readily obtained by peripheral blood samples. Subsequently, germ-line DNA mutations, if present, will occur homogenously throughout any randomly drawn blood sample within the same individual. Somatic DNA, however, must be obtained by tumor biopsy and is therefore subject to sample selection. Common practice is to obtain one biopsy from the primary tumor for molecular analysis, as it was assumed that tumors were homogenous and the section sampled accurately represented the complete tumor composition. However, recent evidence suggests that intratumor heterogeneity exists within cancer, which may lead to the underestimation of the genomic characterization of a tumor from the use of a single biopsy for molecular diagnosis.[24]

Clinically relevant germ-line mutations that have been shown to impact drug response include cytochrome P450 2D6 (CYP2D6), uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1), and thiopurine-S-methyl transferase (TPMT). CYP2D6 is necessary for tamoxifen activation and metabolism to the active metabolite, endoxifen.[5] Mutations in CYP2D6 may therefore result in altered concentrations of endoxifen in the serum. The clinical ramifications of decreased CYP2D6 enzymatic activity include increased breast cancer recurrence rates in poor and intermediate metabolizers compared to extensive metabolizers.[5] UGT1A1 is responsible for conjugating activated irinotecan, SN-38, to a glucuronide inactive metabolite, SN-38G. Mutations in UGT1A1 can result in significant reductions in glucuronidation, leading to increased exposure of SN-38 and an increased risk of toxicity, namely severe neutropenia.[25] TPMT is expressed throughout the human body and catalyzes the S-methylation of thiopurines, such as 6-mercaptopurine (6-MP) and thioguanine, into inactive compounds. Certain genetic polymorphisms result in deficient TPMT activity, which affects the ability of patients to tolerate standard, full dose 6-MP by potentially exposing them to higher drug concentrations (approximately 10-fold) and a subsequent increased risk of severe neutropenia.[12]

While germ-line DNA can often help predict pharmacokinetic and pharmacodynamic responses, somatic DNA is particularly useful in evaluating pharmacodynamic effects, and ultimately tumor response.[23] A well-known example is the mutation of the tyrosine kinase domain at codons 12 and 13 of the KRAS gene, conferring constitutive downstream tumor growth signaling. KRAS mutations are significantly associated with resistance to the epidermal growth factor receptor (EGFR) inhibitors, cetuximab and panitumumab, in metastatic colorectal cancer patients. Somatic mutations in the kinase domain of EGFR, seen in tumor tissue of non-small-cell lung cancers (NSCLC), are strongly associated with a favorable response to the small molecule tyrosine kinase inhibitor, erlotinib.[23] In fact, many insurance companies require genetic testing before coverage of the respective targeted therapy is attainable in clinical practice (i.e. BRAF and vemurafenib, anaplastic lymphoma kinase (ALK) and crizotinib, and KRAS and cetuximab/panitumumab). Knowledge of where a mutation occurs will be vital in understanding cancer prognosis, as well as predicting drug response.

Prognostic versus predictive biomarkers

A prognostic biomarker is a single trait or combination of traits found in certain individuals with respect to the outcome of interest, regardless of treatment.[26] Notable examples of prognostic biomarkers include the 21-gene recurrence score, Oncotype DX®, and the 70-gene profile, MammaPrint, for estrogen/progesterone receptor (ER/PR) positive and lymph node-negative breast cancer. These gene expression assays help to determine the risk of recurrence in women with early-stage breast cancer in order to drive treatment recommendations based upon recurrence score.[26] The use of Oncotype

Table 1. Clinically relevant biomarkers and clinical assay availability.

Tumor	Biomarker	Therapeutic implication	Clinical assay(s) available	Molecular profiling methodology	Frequency (%)[b]
Breast	ER/PR	Sensitivity to hormonal therapy	ER/PR pharmDx[a]; TargetPrint	IHC, quantitative microarray gene analysis	55–75[2]
Breast	HER2 (ERBB2)	Sensitivity to trastuzumab, lapatinib, or pertuzumab	HercepTest[a]; Ventana PATHWAY anti-HER-2/neu[a]; Insite[a]; PathVysion[a]; SPOT-Light[a]	IHC, FISH, and CISH	20[3,4]
Breast	CYP 2D6	Altered tamoxifen metabolism	AmpliChip Cytochrome P450 Genotyping test[a]; xTAG CYP2D6; P450-Glo	PCR followed by microarray analysis	*1–77.8 *4–18[5]
Colorectal	TYMS	Sensitivity to 5-fluorouracil	Theraguide 5-FU	RT-PCR	*3–54[6]
Colorectal	MSI	Sensitivity to 5-fluorouracil-based chemotherapy	Molecular MSI testing; MLH1/MSH2/MSH6 mutation test	IHC and RT-PCR	15[7]
Colorectal	KRAS	Resistance to cetuximab and panitumumab	Therascreen KRAS; RGQ PCR Kit[a]; DxS KRAS mutation test kit; Genzyme's KRAS mutation analysis	RT-PCR	35–40[8]
Colorectal, leukemia (CML)	UGT1A1*28	Adverse response to nilotinib and irinotecan	Invader UGT1A1 molecular assay[a]; INFINITI UGT1A1 assay; UGT1A1 Pyro	RT-PCR, microarray analysis	39[9]
Colorectal, breast, ovarian, and cervical	PIK3CA	Sensitivity to PIK3CA, mTOR, or AKT inhibitors	PIK3CA mutation analysis	RT-PCR	15–30 (tumor specific)[10]
Gastrointestinal stromal tumor	c-KIT	Sensitivity to imatinibmesylate	c-Kit pharmDx[a]	IHC	85[11]
Leukemia (ALL)	TPMT	Increased risk of 6-mercaptopurine induced neutropenia	TPMT enzyme diagnostic test; Prometheus TPMT genetic test	Enzyme assay and RT-PCR	~10[12]
Leukemia (CML and ALL)	BCR–ABL	Sensitivity to imatinibmesylate, dasatinib, and nilotinib	BCR–ABL mutation test	Sanger sequencing	>95 (CML)[13] ~25 (ALL; adults) ~3 (ALL; children)[14]
Leukemia (APL)	PML-RAR alpha	Sensitivity to all-trans-retinoic acid and arsenic trioxide	PML/RARA t(15;17) quantitative RT-PCR; FISH AML M3, PML/RARA translocation 15;17	RT-PCR and FISH	~100 (APL)[15]
NSCLC	ALK	Sensitivity to crizotinib	Vysis ALK Break Apart FISH probe kit[a]	FISH	5–7[16]

(continued)

Table 1. Continued.

Tumor	Biomarker	Therapeutic implication	Clinical assay(s) available	Molecular profiling methodology	Frequency (%)[b]
Lung	EGFR	Sensitivity to erlotinib	EGFR pharmDx[a]; EGFR mutation assay (Cobas 4800 System)[a]	IHC, Sanger sequencing, and RT-PCR	15[17,18]
Melanoma	BRAF (V600E)	Sensitivity to vemurafenib	Cobas 4800 V600E[a]	RT-PCR	>50[19]
Multiple	DPYD	Adverse response to 5-fluorouracilor capecitabine	DPD enzyme assay; Theraguide 5-FU	RT-PCR and enzyme assay	<1[20]
Myelofibrosis and other myeloproliferative disorders	JAK2	Sensitivity to ruxolitinib	JAK2 V617F mutation detection assay; HTScan JAK2 Kinase Assay Kit	RT-PCR and kinase activity assay	50–60[21]
Solid pediatric tumors	TPMT	Increased risk of cisplatin-induced otoxicity	TPMT enzyme diagnostic test; Prometheus TPMT genetic test	Enzyme assay and RT-PCR	10–15[22]

FDA: Food and Drug Administration; ER: estrogen receptor; PR: progesterone receptor; IHC: immunohistochemistry; HER2: human epidermal growth factor receptor; CISH: chromogenic in situ hybridization; FISH: fluorescence in situ hybridization; CYP2D6: cytochrome P450 2D6; RT-PCR: real-time polymerase chain reaction; TYMS: thymidylate synthase; MSI: microsatellite instability; EGFR: epithelial growth factor receptor; UGT: uridine-diphosphateglucuronosyltransferase; PIK: phosphatidylinositol 3-kinase; mTOR: mammalian target of rapamycin; TPMT: thiopurine-S-methyl transferase; CML: chronic myelogenous leukemia; ALL: acute lymphoblastic leukemia; APL: acute promyelocytic leukemia; NSCLC: non-small-cell lung cancer; ALK: anaplastic lymphoma kinase; DPYD: dihydropyrimidine dehydrogenase; JAK: janus kinase.

[a]FDA-cleared assay.

[b]Representative of the overall Caucasian population.

DX® is included in the 2007 American Society of Clinical Oncology guidelines, and has remarkably changed nearly a third of medical oncologists' recommendations regarding chemotherapy.[27]

A predictive biomarker is a single trait or combination of traits found in certain individuals with respect to the outcome of interest from specific therapies. Predictive biomarkers can be used to prospectively identify individuals who are likely to benefit from treatment and/or those pre-disposed to toxicity.[28] Notable positive predictive biomarkers involving somatic DNA mutations include BCR–ABL in chronic myelogenous leukemia (CML) and the tyrosine kinase inhibitors (imatinib, nilotinib, and dasatinib), EGFR in NSCLC and erlotinib, BRAF in metastatic melanoma and vemurafenib, and EML4/ALK in NSCLC and crizotinib, among others. Predictive biomarkers may also include mutations in germ-line DNA, such as TPMT, CYP2D6, and UGT1A1, which help to determine the exposure of active drug, or pharmacokinetics, and ultimately drug response and/or toxicity.[29]Alternatively, some biomarkers such as the human EGFR 2-neu (HER2) and ER/PR can be classified as both prognostic and predictive. In the absence of treatment, HER2 over-expression results in worse prognosis; however, after the development of therapies targeting HER2, such as trastuzumab, lapatinib, and pertuzumab, the predictive value of a positive response has greatly increased.[30] Similarly, ER/PR status can be used to measure disease prognosis utilizing the 21-gene recurrence score, while ER/PR-positive breast tumors are also 60% more likely to respond to hormonal therapy, compared to a 5–10% response in ER/PR-negative tumors.[2]

The clinical utility of these biomarkers are more than ever urgent, particularly in oncology as many anticancer drugs have a narrow therapeutic index, low overall response rate, rapid and severe systemic toxicity, and unpredictable efficacy. Table 1 identifies the clinically relevant drug–gene associations, their respective therapeutic implications, clinical assay availability, and the frequency of mutations across the Caucasian population.

Common molecular profiling methodologies

Common methodologies employed in tumor molecular profiling include DNA sequencing, real-time polymerase chain reaction (RT-PCR) amplification, fluorescent in situ hybridization (FISH), and immunohistochemistry (IHC). Two methods to detect the presence or absence of a specific genetic mutation are DNA sequencing and RT-PCR.

The most common method of DNA sequencing was developed by Sanger, Nicklen, and Coulson in 1977.[31] Sanger sequencing results in the synthesis of a specific DNA sequence complementary to the template strand of interest. The process is time and labor intensive and has a lower sensitivity, but allows for the analysis of all potential mutations in the target region as the patient's exact nucleotide sequence is generated.[32] Clinical applications for Sanger sequencing include sequencing for mutations in exons corresponding to the tyrosine kinase domain of the EGFR gene.

Alternatively, RT-PCR is based upon the generation of a fluorescent signal during the PCR process, which is detected during cycling and reflects the amount of PCR product synthesized during the reaction.[33] RT-PCR has better sensitivity than Sanger sequencing but only identifies mutations that the sequence specific primer is designed to detect.[32] PCR-based methods are also utilized to detect candidate genes as a research tool in validating genes discovered through genome-wide association studies.[32] Clinical applications of RT-PCR include identification of melanoma patients harboring the BRAF mutation V600E for treatment with vemurafenib and testing for the most common mutations in codons 12 and 13 of the KRAS gene.

More recently, single nucleotide polymorphism microarrays have be used to identify the genetic basis of traits and disease susceptibilities, as well as measure DNA polymorphisms, capturing the majority of common genetic variations found within the human population.[34] However, common variants may only have minimal effects on a certain phenotype or variable penetrance secondary to epigenetic influences. Furthermore, copy number variations and rare variants cannot be analyzed readily using existing genotyping microarray technologies.[35] Whole genome sequencing offers a potential solution and has become a popular research tool as it provides the most comprehensive collection of a patient's genetic variations, including rare variants and structural variation.[35] Although the use of whole genome sequencing is currently limited by high costs, a paradigm shift is likely to be seen in the near future secondary to dramatically falling costs and higher throughput.[35]

Molecular profiling may not always involve DNA sequencing. In many cases, phenotypic analysis can provide useful information via gene amplification or protein expression. FISH utilizes fluorescently tagged nucleic acid probes to assess intact cells for genetic alterations, including gene amplification, gene deletion, or translocations.[36] Clinical applications of FISH include assessment of HER2 gene copy amplification to predict clinical benefit from trastuzumab in metastatic and adjuvant breast cancer, qualitative detection of the presence of the BCR–ABL gene translocation which is diagnostic of CML, and identification of the ALK rearrangement to predict response to crizotinib in NSCLC.

IHC staining utilizes labeled fluorescent antibodies as specific reagents to detect and visualize antibody–antigen interactions that occur.[37] IHC is applied as a semi-quantitative tool with a scoring system reflective of intensity of staining to the number of protein or receptors on the surface of the cell.[37] While FISH detects the over-expression of gene copies, IHC detects the over-expression of proteins and, ultimately, the translational product of the gene. Clinical applications of IHC include assessment of ER/PR status as well as HER2 receptor over-expression to predict clinical benefit from hormonal and trastuzumab therapy, respectively, in metastatic and adjuvant breast cancer.[37]

While there are several methods available to detect HER2 status, FISH and IHC are the two most commonly employed methods. FISH is considered the gold standard because of its higher sensitivity and specificity; however, it requires an expensive fluorescence microscope and is often more time-consuming than IHC.[38] Studies have shown high concordance rates between FISH and IHC when IHC reported low expression (0 or 1+) or high expression (3+).[38] However, IHC 2+ cases were highly discordant with FISH.[38] As a result, common practice is to measure IHC initially and subsequently confirm HER2 status with FISH if the tumor is IHC 2+.

Each methodology for molecular analysis of tumors is associated with its own advantages and disadvantages in the analysis and interpretation of results, as previously mentioned. Additional factors to consider include turnaround time, assay cost, and whether the assay is cleared and/or approved by the Food and Drug Administration (FDA) (which may in turn affect reimbursement costs and confidence in quality control measures). While the majority of clinical assays are not FDA-approved, a number have been cleared by the FDA (FDA-clearance is offered through a pre-market notification (510(k)) submission).[39] Table 1 highlights these pharmacogenetic assays currently available in practice. The manufacturers of these assays vary from Dako (ER/PR pharmDx, HercepTest, c-kit pharmDx, and EGFR pharmDx), Roche (Cobas 4800 BRAF V600E, Amplichip CYP450, and EGFR mutation assay) to Abbott (Vysis ALK Break Apart FISH probe kit, and PathyVysion Her-2), amongst others. To acquire FDA-clearance, the applicant must indicate that the clinical assay is 'substantially equivalent' to a device that is currently legally marketed for the same use. For a clinical assay to gain FDA-approval, the applicant must provide reasonable assurance of the device's safety and effectiveness. Most pharmacogenetic and genetic device submissions are pre-market notification (510(k)) submissions for FDA-clearance.[39] Those assays that are not approved nor cleared by the FDA are still required to abide by Clinical Laboratory Improvement Amendments (CLIA), passed by Congress in 1998 and administered by the Center for Medicare and Medicaid Services. CLIA establishes quality standards for all laboratory testing to insure reliability and accuracy of test results regardless of where the test is performed. It is important to note that this oversight relates to the quality of performance of laboratory testing and does not evaluate the clinical utility of a given assay.[40]

Conclusion

The goal of molecular analysis is to elucidate the genetic basis for inter-individual differences in drug response and to use this information to predict safety, toxicity, and efficacy of anticancer drugs. Guidelines describing the drug–gene association and pharmacological management based on genetic results will be particularly valuable and may incline clinicians to adopt genotype guided therapy. The Clinical Pharmacogenetics Implementation Consortium (CPIC) of the National Institutes of Health's Pharmacogenomics Research Network and the Pharmacogenomics Knowledge Base (www.pharmgkb.com) was devised to provide peer-reviewed, updated, evidence-based, and freely accessible guidelines to assist clinicians in translating assay results into actionable prescribing decisions.[41]

As presented in Table 1, several pharmacogenetic assays are available, many of which are cleared by the FDA. While clinicians may be aware of relevant cancer biomarkers and therapeutic implications, the availability of assays and knowledge of molecular profiling methodologies are not always as evident. As pharmacogenetic studies continue to rise and more targeted therapies become available, the rapid translation of pharmacogenetic testing into clinical practice will be indispensable. In order to prepare for the future of personalized medicine, there is an unprecedented urgency for clinicians, including both physicians and pharmacists, to become trained on the availability of pharmacogenetic assays, understand the underlying method of detection of cancer biomarkers, and ultimately, how to interpret results and therapeutic implications to optimize cancer therapeutics.

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Conflict of interest

The authors declare that there is no conflict of interest.

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