

Personalized Medicine—Some Things To Consider

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Abstract

The promise of individualized therapy has resonated with scientists, healthcare providers, and patients since the inception of the Human Genome Project. The ability to use designer drugs, such as novel monoclonal antibodies and molecularly targeted tyrosine kinase inhibitors, has created a new hope in the management of the cancer patient by individualizing therapy based on the unique molecular mutations in the cancer and the patient's germline DNA. The nuances of implementing germline and somatic genomic testing in a clinical setting are discussed and the future role of pharmacogenomics testing in personalized cancer treatment is examined.

Keywords

Personalized medicine, pharmacogenomics, molecular profiling, targeted therapy, cancer management

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Before the inception and subsequent reporting of the Human Genome Project data, novel paradigms of more personalized therapeutics were forecast as a benchmark for the success of the project. Along with better diagnostic and prognostic applications arising from the sequencing of the human genome, the promise of novel and more specific molecularly targeted therapeutics based on genomic information is coming to fruition and changing both diagnostic and therapeutic management algorithms. These advances in molecular therapeutics are resulting in more sophisticated and focused clinical trial designs and study protocols for matching specific patients to specific therapies. These advances underscore the potential for more specialized and successful therapies for cancer patients.

The concept of an individual variation in drug handling and response is not novel and those practitioners who have emphasized the principles of therapeutics have led the way for personalized medicine. In 510 BC, Pythagoras recognized that some individuals, but not all those exposed, developed hemolytic anemia with fava bean consumption.¹ Hemolytic anemia due to fava bean consumption was later determined to occur in glucose-6-phosphate dehydrogenase (G-6 PD) deficient individuals.^{2,3} In 1902, Garrod recognized the potential hereditary nature of a metabolic defect associated with alkaptonuria and associated this with specific enzymes that detoxify xenobiotics so that they may be excreted.⁴ He subsequently made the observation that some people lacking these enzymes experience significant adverse effects.⁵ In 1959, Fredrich Vogel was the first to coin the term pharmacogenetics, which he defined as genetic variation found in an individual that resulted in a varied response to therapeutic drugs.⁶ The realization that differences between individuals, both genetic and environmental, result in success or failure

of a therapy holds the promise that future therapies can be tailored to an individual's genetic make-up. For the cancer patient, additional tumor mutational analysis could be used to individualize anticancer therapies.⁷

Pharmacogenetics—Drug Metabolism versus Molecularly Targeted

The impact of genomic testing to inform therapeutic drug selection and dosing in oncology has occurred at a record pace and includes two facets of pharmacology. The first is drug metabolism pharmacogenetics (PGXm) and corresponds to genetic variants that determine drug disposition (i.e. the processes of absorption, distribution, metabolism, and excretion) also referred to as drug pharmacokinetics.⁸ The second is molecularly targeted pharmacogenetics (PGXt), which relates to the selection of a therapeutic moiety based on the presence or absence of a specific drug target or pathway in the tumor.⁹ Unlike PGXm, where the genetic variants are typically present in the germline, for PGXt applications the variants and/or mutations are typically somatic and necessitate analysis of tumor tissue.

Examples of classic PGXm include the conversion of a parent compound to an inactive metabolite or a metabolite that is more easily excreted or the conversion of a prodrug to its active metabolite. Enzymes associated with drug metabolism have been well characterized, as have their genes and their polymorphic variants. Many of the genetic variants found in the metabolic enzyme genes are not associated with disease yet can lead to an individual being characterized as a poor, intermediate, extensive, or ultrarapid metabolizer, and this is best exemplified by the cytochrome P450 enzyme, CYP2D6. This classification of an individual patient's drug metabolism status can be critical to both selection and dosing of a particular therapy.

Figure 1: Schematic Showing the Metabolic Pathway of Tamoxifen

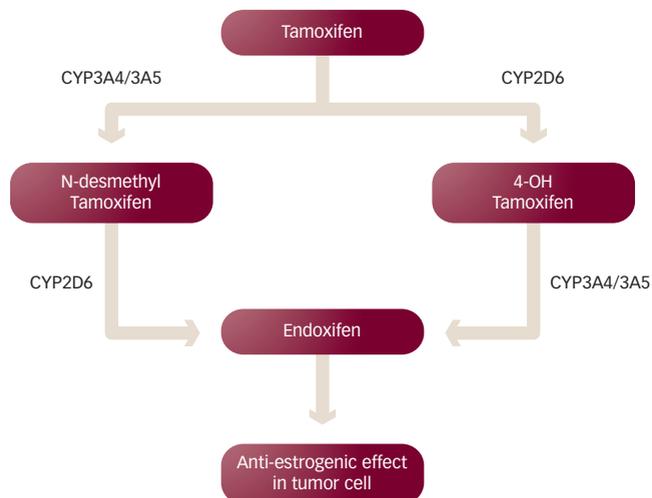
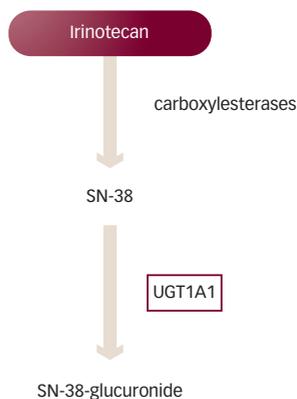


Figure 2: Schematic Showing the Metabolic Pathway of Irinotecan



An example of drug-metabolizing gene variants effecting response to a commonly used therapeutic is seen in breast cancer. Estrogen receptor (ER)-positive breast cancers are treated with hormonal therapies that are estrogen antagonists. One such moiety is tamoxifen, which itself has been shown to have altered metabolism due to CYP450 genetic polymorphisms. Tamoxifen and its metabolites compete with estradiol for occupancy of the ER, and in doing so inhibit estrogen-mediated cellular proliferation. Conversion of tamoxifen to its active metabolites occurs predominantly through the CYP450 system and its primary and secondary metabolites are important because they have a greater affinity for the ER than tamoxifen itself (specifically endoxifen has approximately 100 times greater affinity for the ER). Activation of tamoxifen to endoxifen is primarily due to the action of CYP2D6 (see Figure 1). Therefore, patients with defective CYP2D6 alleles potentially derive less benefit from tamoxifen therapy than patients with functional copies of CYP2D6. The most common null allele among Caucasians is CYP2D6*4, a splice site mutation (G1934A) resulting in loss of enzyme activity, and, therefore, lack of conversion of tamoxifen to its most active metabolite endoxifen. This

could result in significantly decreased response to anti-estrogen therapy. While several retrospective studies have suggested that individuals with loss of function CYP2D6 alleles (ex. CYP2D6*4) have greater rates of tumor recurrence and shorter relapse-free survival; however, other studies have not corroborated these findings.¹⁰

The ongoing debate as to whether CYP2D6 genotype impacts outcomes with tamoxifen was addressed in a large trial from the Breast International Group (BIG) I-98 and Arimidex, Tamoxifen, Alone or in Combination (ATAC) studies where the investigators resolved that CYP2D6 genotyping has no effect.^{11,12} Unfortunately, these studies have come under intense scrutiny due to the departure from Hardy-Weinberg equilibrium of the results.¹³ This is thought to be due to errors in the genotyping performed in those studies. More specifically, those studies are said to be biased in terms of genotyping that occurred in tumor tissue DNA instead of germline DNA. The discordant genotype frequencies could most likely be due to known loss of heterozygosity of the CYP2D6 locus in breast tumor tissue and/or the detection of nearby pseudogenes. Accumulating data will need to be re-analyzed and re-assessed in terms of this potential PGXm application.

Another common example of PGXm is seen with the use of Irinotecan (Camptosar®), a prodrug, that is initially metabolized by carboxylesterases to its active compound, SN-38. SN-38 is inactivated by glucuronidation via the UGT1A1 enzyme to its metabolite SN-38-glucuronide, which is then excreted in the urine (see Figure 2). A dinucleotide (TA) repeat polymorphism has been identified in the promoter region of the UGT1A1 gene. Individuals with normal UGT1A1 activity contain six copies of this TA repeat in the promoter region, referred to as the UGT1A1 *1 allele. Individuals who harbor seven copies of the repeat (referred to as the *28 allele) have decreased UGT1A1 activity, which results in reduced conversion of the active metabolite, SN-38 to its inactive glucuronide. The frequency of this polymorphism varies among ethnic groups and in Caucasians 9–17 % are homozygous while 28–36 % are heterozygous. In African Americans, 17–33 % are homozygous and 38–50 % are heterozygous for the *28 allele.⁹ This enzyme variant has been associated with increased frequency and severity of irinotecan-related adverse events including severe diarrhea and myelosuppression. Testing for UGT1A1 polymorphisms can identify those patients at risk for severe toxicity and allow the treating physician to adjust irinotecan dosing prospectively.

In 2005, the US Food and Drug Administration (FDA) issued a black box warning making providers aware of this genetic variant, its prevalence, and association with these adverse events. The FDA stopped short of recommending or requiring genetic testing for this genetic variant. Because UGT1A1 is also responsible for glucuronidation of bilirubin, many practicing oncologists use the bilirubin as a surrogate marker for UGT1A1 activity in caucasian patients and adjust irinotecan dose based on this, rather than performing the genotyping test.

PGXt refers to targeted pharmacogenetics and the more recent advances in cancer therapeutics that target those specific proteins in cellular pathways that are pivotal for the survival and proliferation of cancer cells. Genetic variants in the genes for the proteins involved in these pathways have now been shown to influence response to therapies targeting that pathway. Unlike PGXm, PGXt variants can be associated with disease

development as well as response to therapy. Personalization of targeted therapy is warranted due to the low therapeutic index (TD50/ED50) of most cytotoxic chemotherapeutic agents and the numerous adverse events associated with them (see *Table 1*).

One of the first modern examples of targeted therapy is to be found in the treatment of breast cancer. The human epidermal growth factor-2 (HER2) oncogene encodes a transmembrane receptor, which has been shown to be amplified in up to 35 % of all breast cancers and is associated with a poor prognosis. With such a significant incidence of gene amplification in a common cancer, the HER2 receptor became a focus for therapeutic development. Trastuzumab (Herceptin™) became the first targeted monoclonal antibody therapy in a human solid tumor. Cardiotoxicity was identified as the most severe side effect of this drug and thus breast tumors needed to be tested for HER2 gene amplification and/or overexpression to determine likelihood of response and thus appropriate use of the drug.¹⁴ The FDA approved an immunohistochemistry (IHC) assay for detecting the HER2 receptor in formalin fixed, paraffin embedded (FFPE) tissue sections and subsequently a fluorescence *in situ* hybridization (FISH) assay for detecting gene amplification (see *Figure 3*).

More recently, targeted therapies in lung cancer, colon cancer, and melanoma have been successfully developed based on several tumor cell proliferative/survival pathways and their known genetic variation in targeted pathway proteins.^{15–18} The epidermal growth factor receptor (EGFR) pathway has been implicated in many human cancers and the EGFR gene contains both sensitizing (activating) mutations and resistance mutations (see *Figure 4*). Anti-EGFR therapies include the monoclonal antibodies cetuximab and panitumumab as well as the small molecule tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib. Two common activating mutations have been identified in exons 19 and 21 of the EGFR gene, which sensitize non-small cell lung cancer cells (NSCLCs) to the anti-EGFR TKIs.¹⁵ In metastatic colorectal cancer, anti-EGFR monoclonal antibodies have been shown to selectively inhibit downstream EGFR signal transduction pathways. Somatic mutations in the KRAS oncogene, mainly codons 12 and 13, drive tumor unresponsiveness to these therapies⁷. Thus, KRAS status, in particular normal or wild-type sequence, is associated with a favorable response to cetuximab or panitumumab.^{19,20} Interestingly, tumors harboring KRAS mutations in codons 12 and 13 were initially thought to trump the EGFR mutation status as a downstream collateral mechanism for resistance. Some data now suggests that tumors with a KRAS codon 13 (G13D) mutation may have partial response to the addition of cetuximab to first-line chemotherapy.²¹ Similarly, the MAP kinase pathway is being targeted in melanoma with inhibitors to BRAF and MEK.^{22,23}

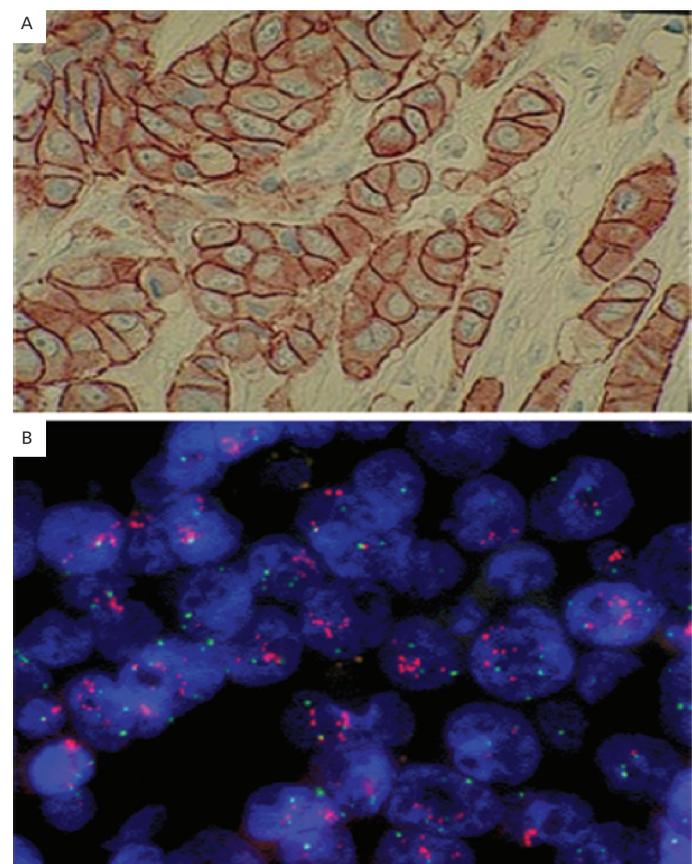
Therapeutic Targeting in Human Cancers

While cancer is considered a complex, polygenic, and multifactorial disease, there are hallmarks that are similar between most cancer cells. These characteristics include the ability to enable uncontrolled cell replication, induction of angiogenesis, genome instability, and resistance to apoptosis.²⁴ The initiating events for many of these characteristics more commonly include somatic mutations to several genes and in some cases of hereditary cancers, germline mutations to other single genes. Germline variants can be easily detected in DNA isolated from blood, buccal swabs, or saliva. Identification of variants associated with targeted therapy response and eligibility are more often somatic and typically

Table 1: Adverse Effects of Cytotoxic Chemotherapy

Immediate (hours–days)	Early (days–weeks)	Delayed (weeks–months)	Late (months–years)
Extravasation	Myelosuppression	Cardiotoxicity	Secondary cancer
Emesis	Mucositis	Lung fibrosis	Cognitive Impairment
Hypersensitivity	Alopecia	Peripheral neuropathy	Sterility
Tumor lysis syndrome	Cystitis	Hepatotoxicity	Teratogenicity

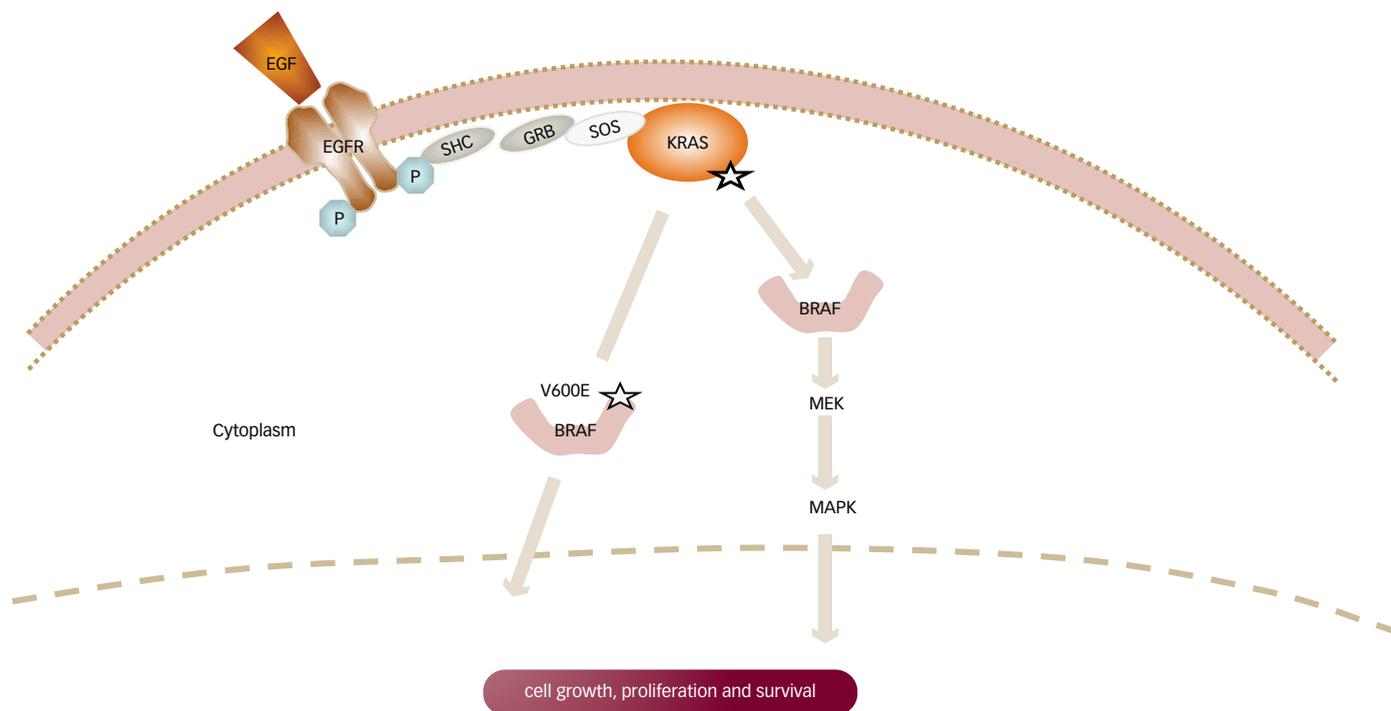
Figure 3: (A) Immunohistochemical Staining for the HER2 receptor; (B) Fluorescence *in situ* Hybridization Analysis for the HER2 Gene Amplification (Orange Dots)



detected in DNA extracted from tumor cells in a diagnostic specimen such as a cytology fine needle aspirate, biopsy, or resected tumor tissue. In most cases, the tissue has been processed for histology and exists as FFPE tissue blocks.

FFPE specimens are used routinely for molecular genetic analysis and do not usually pose a problem for such testing. However, the type and amount

Figure 4: Schematic Showing the Epidermal Growth Factor Receptor Pathway



of the tissue as well as tissue-processing techniques can impact the quality and quantity of nucleic acids that can be obtained for ex vivo testing. Paraffin is also known to contain inhibitors of enzymatic molecular reactions such as the polymerase chain reaction (PCR) and good laboratory quality control practices are needed to determine when this may be a problem.

Isolating nucleic acids from neoplastic tissues introduces the matter of tumor heterogeneity. Tissue heterogeneity refers to the numerous cell types that may be present in a section of tumor tissue and include stromal cells, inflammatory cells, and normal parenchymal cells. It is important to determine and document the percent tumor cell content (tumor cellularity) in the specimen being used for analysis and how this may influence the genetic assay being run. Tumor heterogeneity, on the other hand, refers to the variability seen within each individual tumor. This can be described as inter- and intra-tumor heterogeneity. Inter-tumor heterogeneity refers to the differences in genetic variation seen in similar tumors from different patients. Intra-tumor heterogeneity refers to the genetic differences observed in tumor cells from the same individual.²⁵ In a given specimen, there is the potential to have a heterogeneous population of tumor cells, some of which may or may not harbor the same mutation profile as the rest of the tumor. Both tissue and tumor heterogeneity can impact the result of genetic analyses.^{26,27}

As PGxT applications continue to evolve, several interesting clinical dilemmas arise. First, should the primary tumor or the metastatic tumor be tested for genetic variants? It is possible that the genetic variants are present in only the primary or only the metastatic tumor, or in both. For instance, EGFR overexpression in colon cancer has been shown to exhibit dramatic discordance between primary and metastatic tumor, reflecting the ongoing genetic mutations in a single disease.²⁸ These variations have

implications for testing, prognosis, and potential treatment decisions. Second, what criteria are appropriate to define a test being positive for a genetic variant in order to impact clinical management? Currently these test results are reported as qualitative, positive, or negative results. We know from FISH-based assays that it is common for tumors to have a variant present in only a small percentage of cancer cells represented in the section used for genetic analysis. It is possible that the reason for lack of clinical response to a targeted therapy maybe because of the absence of that target in the majority of the tumor but detectable only in a small proportion of tumor cells.

Technologic Advances

Clearly the discussion of personalized medicine would not be occurring if it were not for the rapid advances in genomic technologies.^{29,30} Initially, probe-based techniques, such as the Southern blot transfer, identified genes of interest and allowed for semiquantification, but required fresh tissue and large amounts of high-molecular-weight DNA. The introduction of PCR allowed for the rapid analysis of gene targets and in DNA or RNA isolated from FFPE tissues. Advances in realtime PCR made multiplex analysis and mutation detection possible within clinically relevant turnaround times.

The newest major technologic advance is on us and includes the routine use of microarrays and next-generation sequencing (NGS).²⁹ Microarrays are a chip-based technology whereby millions of probes for various genomic sequences are densely packed onto a microchip to which the patients DNA is hybridized. This technology results in a large amount of data, but allows for the simultaneous testing of large numbers of genetic variants. NGS or massively parallel sequencing represents the newest technology to be introduced into the clinical laboratory.³¹ Unlike traditional

dideoxy or Sanger sequencing, NGS utilizes novel chemistries to rapidly sequence millions of fragments of DNA isolated from the specimen. Alignment and sequence determination are then performed by various software packages. For oncology, the ability to sequence a targeted number of genes whose mutations are known to be therapeutically important will allow the laboratory to provide massive amounts of critical information from which the oncologist can then design a therapeutic management approach (see *Figure 5*).

The more traditional model of a single biomarker test result being used to select oncology treatments, monitor the effects of those therapies, and switch therapies as treatment resistance emerges is no longer optimal. Reflex testing from one gene or panel of mutations to the next as part of a pathway analysis is becoming cost prohibitive and results in delayed turnaround times. In order to predict whether a certain drug/monoclonal will be effective in a particular tumor setting requires a more complex analysis so that the results taken as a whole can more accurately predict therapeutic outcomes.

The Need For Multi-targeted Therapies

As a complex yet common disease, cancer is amongst the main causes of morbidity and mortality worldwide, but especially so in developed countries. Our understanding of the various metabolic and signaling pathways that are altered during the tumorigenic process has led to the identification of multiple potential targets of novel therapies such as monoclonal antibodies, TKIs and multi-targeted TKIs (MTKIs).^{32,33} A biological characteristic of human cancers is the constant potential to mutate various genes and thus activate or inactivate various pathways needed for proliferation and or survival. Therefore, as we treat patients with particular targeted therapies, the tumor cell response in the form of resistance occurs via mutation of other genes either downstream in the current pathway or in other pathways.^{34,35} An approach to managing tumor cell resistance could be similar to that which was successfully employed in the management of HIV-1 infected patients in the late 1990s. By using combinations of anti-retroviral therapies, physicians and researchers were able to reduce the viral load to below the limits of detection of clinical assays. Similarly, it may be possible to include more novel targeted therapies in combination with cytotoxic chemotherapy or other targeted therapies to improve the efficacy of cancer treatment. In addition, therapies which modulate multiple targets such as some MTKIs could be beneficial if used in the right patient at the right dose and right time.³⁶

Companion Diagnostics

Mutation analysis as a precursor to implementation of targeted therapy has become the standard of care for certain tumor types. The FDA has recognized the need for and importance of the ‘companion’ diagnostic. Recently, the agency has been forthcoming with FDA-approved assays that can select patients for treatment with certain targeted therapies.³⁷

In 2005, the FDA approved the Third Wave Technologies Invader assay for the detection of the TA polymorphism in the UGT1A1 gene promoter associated with irinotecan toxicity. Previously, the FDA had approved an IHC and FISH assay for detection of HER2 gene overexpression and amplification associated with eligibility for trastuzumab (Herceptin) treatment, indicating the likelihood of response.¹⁴ In 2011, the FDA approved a KRAS mutation assay to predict unresponsiveness to the anti-EGFR therapies—cetuximab and panitumumab. More recently, the FDA granted approvals for two

Figure 5: Ion AmpliSeq Cancer Panel

KRAS	BRAF	EGFR	TP53	PIK3CA	CSF1R	JAK2
NRAS	PTPN11	ERBB2	SRC	FGFR3	NPM1	CDKN2A
RET	HNF1A	SMAD4	GNAS	PDGFRA	MPL	ABL1
PTEN	FLT3	STK11	SMARCB1	KIT	MET	NOTCH1
FGFR2	RB1	JAK3	VHL	KDR	SMO	
HRAS	AKT1	ALK	MLH1	FBXW7	ERBB4	
ATM	CDH1	IDH1	CTNNB1	APC	FGFR1	

Table 2: Some Assays Used in Clinical Laboratories Cancer Treatment Selection

Genomic Marker	Assay Type	Therapy	Tumor Type
ALK rearrangement	LDT and IVD	Crizotinib	NSCLC
BCR-ABL	LDT and IVD	Imatinib, dasatinib, nilotinib	Chronic myelogenous leukemia
BRAF	LDT and IVD	Vemurafenib	Melanoma
EGFR	LDT	Gefitinib, erlotinib	NSCLC
HER2	IVD	Trastuzumab	Breast
KRAS	LDT and IVD	Cetuximab, panitumumab	Colorectal

IVD = in vitro diagnostic; LDT = laboratory-developed test; NSCLC = non-small cell lung cancer.

drugs and companion diagnostic testing. These included approval for BRAF V600E mutation testing via realtime PCR in patients with metastatic melanoma as prerequisite to receiving vemurafenib and approval of ALK gene rearrangement testing via FISH in patients with advanced-stage NSCLC in order to receive crizotinib. In addition to the many FDA approved or cleared assays, clinical laboratories have developed and validated their own laboratory developed test (LDT) for use as companion diagnostics for other targeted therapies (See *Table 2*).

Future Directions

Recent genomic advances have been welcomed by the oncology community as the potential of bringing cancer drugs to market more rapidly, improving patient outcomes, and making personalized cancer therapy is realized. It can be argued that these changes, mark the transition from the ‘Dark Ages’ to the ‘Renaissance’ in oncology. It is anticipated that laboratory analysis of human cancers will become more complex, within which will come the information needed to more effectively treat cancer patients with the true hope of eradicating the disease. ■

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