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Companion diagnostics and personalized medicine: A review of molecular diagnostic applications

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ABSTRACT

Personalized medicine is the customization of treatment based on a patient's hereditary or somatic genetics and holds the promise of revolutionizing healthcare. Companion diagnostics, many of which are molecular genetics assays, are critical tools in the implementation of personalized medicine. Information derived from these tests provides for customizing specific therapies based on the genetics of the disease. While the benefits are clear, the path to a successful companion diagnostic has required a forging of new alliances between drug and diagnostic developers, clinical laboratories, physicians, pathologists, and healthcare providers. Molecular genetic companion diagnostic assays are becoming more relevant and important in an environment of increased regulatory guidance in their development and application. Here, we review key molecular genetics companion diagnostic tests and their applications in personalized medicine.

KEYWORDS: personalized medicine, genetic, molecular, companion diagnostics

INTRODUCTION

The concept of personalized medicine, whereby disease diagnosis, treatment and prevention are customized to one's genetic composition, is now well established [1, 2]. The advantages of approaching medicine in this way are theoretically clear; personalized medicine has the potential to more efficiently, effectively, and safely direct health care than traditional non-targeted approaches. While the rate of progress has clearly increased, there are still significant technical and regulatory hurdles to overcome. Several guidance documents from regulatory organizations worldwide have attempted to address these challenges, and no doubt more will be presented in the near future [3].

Companion diagnostics are increasingly relied upon to ensure the effective, safe development and use of a personalized therapeutic. Multiple liaisons and partnerships between key stakeholders are needed in this complex, dynamic process. Many successful companion diagnostics are genetic tests - particularly molecular diagnostics - and this speaks to their high impact and relevance in the field of personalized medicine.

Personalized medicine and the "new" genomics

"Personalized medicine" is a phrase first coined in the 1990s, although the concept pre-dated this [4, 5]. Achieved successfully, personalized medicine harnesses power from innate biological information to direct appropriate therapies for appropriate patients - a goal that maximizes key components of effectiveness, efficiency, and safety.

Independent of the Human Genome Project, personalized medicine efforts initially began with a consortium of the world's largest pharmaceutical

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companies and scientists, created to identify natural genetic differences between people [4]. The goal was to correlate informative genetic biomarkers with disease symptoms, or serious side effects to certain medications. Drug developers then hoped to develop more effective, safer drugs to target these patient populations.

More recently, the interest in personalized medicine has increased substantially; based on PubMed searches on the term 'personalized medicine', a 2011 publication found that the number of scientific publications on the subject has shown an exponential growth in the period from 1999 to 2010 [6]. Kongkaew *et al.* [7] estimated that more than 5% of hospital admissions are associated with adverse reactions to prescribed drugs. Many of these are due to individual genetic differences that render one hypersensitive to the drug, or unable to metabolize it properly [8].

Challenges in the drug development process

Responses from the larger pharmaceutical and biotechnology companies to create personalized therapeutics have been lower than expected, based on interest level. Success rates in bringing these drugs to market have also been low. A number of scientific, strategic, commercial, and regulatory factors have been attributed to this [9].

Jorgensen *et al*. [6] argued that the initial "one drug for one disease" model does not fit the clinical reality of heterogeneous disease mechanisms at the molecular level. As a result, some diseases have not been as amenable to personalized medicine, as was initially postulated. Scientifically, it has also been more difficult to identify and validate biomarkers in as timely a fashion than the industry initially expected. While the drug development and regulatory process is well known and understood, the development of a successful biomarker requires an understanding of several success factors including biomarker availability, robust technical assay validation, the importance of demonstrating clinical utility, and the ability to bring an investment-positive commercial value proposition to the table [3]. This is extremely difficult to find without partnering with several organizations, introducing several logistical challenges to the process.

Early strategic partnerships are necessary to create personalized therapeutics. Few pharmaceutical companies have depth of experience in the diagnostics arena. Partnerships can address experimental design, assay discovery, assay validation, marketing and commercialization [3]. However, some of these partnerships are not part of current pharmaceutical company outsourcing practices. In addition, early alliances are difficult because the value of the drug and its diagnostic are difficult to predict [3]. These and other factors bring about several business challenges.

Commercially, there are several challenges for a companion diagnostic. The total market for the therapeutic needs to be large enough to not only justify the development cost of the therapeutic itself, but also now the cost of development for the associated companion diagnostic. In addition, one has to market the value of both the therapeutic and the diagnostic. A companion diagnostic has the potential to reduce the market size for a therapeutic by limiting the patient population. Similarly, in an extreme case the companion diagnostic may leave the physician without a viable therapeutic treatment. These issues can make pharmaceutical companies less commercially motivated to pursue a personalized therapeutic.

Regulatory factors can also bring challenges, as there are inefficiencies in the current drug development process. The co-submission of a therapeutic and diagnostic complicates the regulatory submission process and can lead to increased costs and delays.

Historically, there were few regulatory guidance documents to manage drug and companion diagnostic co-development. More recently, regulatory agencies have responded with guidance documents, attempting to inform best practices, and to provide clarity and consistency in assay development and marketing approval [3].

Regulatory responses about companion diagnostics

Regulatory agencies are quickly recognizing that companion diagnostics can be the key to a safe, successful personalized therapeutic. In draft guidance from July 2011, the FDA indicated that, "in most circumstances, if use of an *in vitro*

companion diagnostic device (IVD companion diagnostic device) is essential for the safe and effective use of a therapeutic product, (it and its) therapeutic product should be approved or cleared contemporaneously by FDA for the use indicated in the therapeutic product labeling" [10]. The guidance also stated that, "the results of the IVD companion diagnostic device will be *essential* for the safe and effective use of the therapeutic product, and its use will be stipulated in the labeling of the therapeutic product." Because the IVD companion diagnostic was identified as *essential* for this purpose, it was noted that, "with some exceptions FDA does not believe it may approve a novel therapeutic product or new therapeutic product indication for use with an IVD companion diagnostic if the IVD companion diagnostic is not approved or cleared for that indication" [10]. Guidelines for the development of IVDs also exist in the European Union (EU) [11].

Regulatory guidances put an increased focus and relevance on the development of companion diagnostics, many of which are genetic tests. Gene-based and molecular diagnostics testing is growing at a 30-50% rate, and it has been estimated that as many as 1,500 genes and 5,000 proteins may be candidates for new molecular test targets [12, 13]. It has also been recommended that companion diagnostics be used at an early stage in the drug development process [9]. From a financial perspective, molecular diagnostics within the USA alone was valued at approximately $2.7 billion in 2006, and was expected to reach $5 billion by 2010 (AGR 15%) [14]. Oncology molecular diagnostics was the fastest growing sector at that time and was predicted to increase by 30% each year, tripling from its 2005 level of $315 million to more than $1.35 billion by 2010 [2, 14].

Personalized medicine in the oncology sector

There has been significant progress for companion diagnostics and personalized medicine in the oncology sector. For example, the use of pre-symptomatic genetic testing and "targeted therapies" tailored to genetic profiles of tumors is part of a recommended evaluation for cancers of the colon, lung, breast and other sites [1]. These companion diagnostics typically identify somatic mutations identified in tumor cells, which help direct use of an appropriate therapeutic (Table 1).

Recent successful genetic companion diagnostic tests in oncology

Crizotinib and non-small cell lung cancer

Recently, rearrangements of the anaplastic lymphoma kinase (ALK) gene were reported in non-small cell lung cancer (NSCLC) [16, 17]. Within three years, studies of ALK inhibition yielding dramatic response rates in patients with advanced NSCLC containing ALK rearrangements were reported [16, 18, 19]. In pretreated patients that generally have a 10% response rate to conventional chemotherapy, treatment with the oral ALK inhibitor crizotinib (Xalkori®) yielded an overall response rate of 55% and an estimated six-month, progression-free survival rate of 72% [16].

Significantly, the mechanism of resistance was associated with ALK kinase domain mutations, substantiating that ALK was indeed the genetic target of the personalized therapy [16]. This also reinforced that appropriate clinical application of ALK-targeted therapy was absolutely dependent upon a companion diagnostic to identify patients most likely to respond. The FDA has since approved the drug, and requires use of its companion diagnostic; this is indicated in product labeling.

Vemurafenib and metastatic malignant melanoma

The B-Raf proto-oncogene serine/threonine-protein kinase (BRAF) gene is critical in the development of melanoma [20, 21]. Melanoma tumor cells with *BRAF* mutations contain distinctive characteristics, such as unique morphological variants, an age at diagnosis often before 55 years, and others [21]. A multi-centric study reported that the treatment of metastatic melanomas carrying the V600E mutation in *BRAF* with a selective small molecule inhibitor PLX4032 (vemurafenib) resulted in complete or partial regression of disease in most patients [20, 22]. From clinical trial studies, patients with

Table 1. Oncology companion diagnostics required by the FDA.

Genetic biomarker	Required companion diagnostic	Drug	FDA approved	Drug manufacturer
t(15;17) chromosome translocation or *PML*/*RARα* gene expression in acute promyelocytic leukemia	*PML*/*RARα*	Vesanoid® (tretinoin), Trisenox® (arsenic trioxide)	1995, 2000	Roche, Cell Therapeutics, Inc.
Overexpression of *HER-2* in metastatic breast cancer tumor cells	HercepTest™ (1998)	Herceptin (trastuzumab), Tykerb (lapatinib)	1998, 2007	Genentech, GlaxoSmithKline
Philadelphia chromosome positive [*BCR-ABL*+] chronic myeloid leukemia in chronic phase	*BCR-ABL*	Gleevec® (imatinib)	2001	Novartis
Platelet-derived growth factor receptor (PDGFR) gene rearrangements in myelodysplastic/myeloproliferative diseases	*PDGFR*	Gleevec® (imatinib)	2001, (2006 this indication)	Novartis
Hodgkin's lymphoma cells expressing CD20 antigen	CD20	Bexxar® (tositumomab)	2003	Corixa
High expression of *EGFR* (more likely to respond)	DakoCytomation *EGFR* pharmDx™ test kit	Erbitux® (cetuximab), Vectibix® (panitumumab)	2004, 2006	Eli Lilly, Amgen
Chromosome 5q deletion associated with transfusion-dependent anemia due to low- or intermediate-1-risk MDS, with/without additional cytogenetic abnormalities	5q deletion	Revlimid® (lenalidomide)	2005	Celgene Corporation
Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to prior therapy	*BCR-ABL*	Sprycel® (dasatinib)	2006	Bristol-Myers Squibb
Expression of CD25 component of IL-2 receptor in persistent or recurrent cutaneous T-cell lymphoma	CD25	Ontak® (denileukin diftitox)	2008	Eisai Medical Research
ELM4-ALK translocation-positive advanced or metastatic non-small cell lung cancer	*ELM4-ALK* translocation	Xalkori® (crizotinib)	2011	Pfizer
BRAF V600E mutation in unresectable or metastatic melanoma	*BRAF* V600E mutation	Zelboraf™ (vemurafenib)	2011	Genentech (Roche)

Sources: [6, 9, 15].

BRAF V600E mutation-positive melanoma receiving vemurafenib (Zelboraf™) showed improved rates of overall and progression-free survival, as compared to those receiving conventional therapy. This highlighted the importance of a molecular disease model focusing on specific biomarkers, identified by companion diagnostics, as *bona fide* targets that could benefit melanoma patients [21]. The FDA subsequently approved the drug and requires use of the companion diagnostic prior to its administration; this is indicated in product labeling.

Key companion diagnostics outside the oncology sector

Genetic biomarkers identify risk for life-threatening drug side effects

Companion diagnostics for hereditary mutations are also becoming more widespread (Table 2). Heterozygosity for the human leukocyte antigen *HLA-B*1502* allele, found almost exclusively in individuals from some parts of Asia, placed one at increased risk of life-threatening reactions to carbamazepine, a commonly prescribed anti-convulsant in those geographical regions [25]. Once the risk for these serious reactions in those with the *HLA-B*1502* allele became known, the FDA issued an alert, indicating that if an individual tests positive for the allele … "carbamazepine should not be started unless the expected benefit clearly outweighs the increased risk of serious skin reactions." (http://www.fda.gov.login.ezproxy.library.ualberta.ca/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm124718.html). The FDA then recommended an *HLA-B*1502* companion diagnostic to be performed prior to prescribing carbamazepine to those from at-risk populations. A 2011 letter to the *New England Journal of Medicine* augmented this warning by stating, "Given the availability of other elective therapeutic choices, it may be prudent to advise *HLA-B*1502* carriers to avoid not only carbamazepine but also other structurally related anticonvulsants, such as phenytoin, oxcarbazepine, and possibly lamotrigine." [26, 27].

Drug metabolism variances due to hereditary mutations

Other successful companion diagnostics are associated with hereditary mutations that affect drug metabolism. Metabolism in the liver by cytochrome P450s represents the most common route of drug breakdown. Fast- and slow-metabolizing variants due to mutations in these enzymes can lead to under- and over-dosing of drugs [8, 28]. The FDA approved Roche's AmpliChip™ microarray-based assay to identify 29 variants in the two most common drug-metabolizing P450s: CYP2D6 and CYP2C19. Known to mediate the metabolism of almost 25% of drugs, adverse events with nearly 30 drugs are known to be related to drug accumulation in patients carrying variants in these two enzymes [8, 29].

Recently, findings were published on the prediction of dose selection for warfarin after correlation with genomic data and a pharmacogenetic algorithm [8, 30]. Under- or over-dosing with warfarin is the worldwide leading cause of hospitalization related to adverse events. Variants in CYP2D9 and VKORC1 are known to influence the biologic breakdown of warfarin. Study results have shown that the prediction of dose selection with a pharmacogenetic algorithm correlated well with empirically determined maintenance doses. In fact, this outperformed clinical prediction and standard dose estimates. This was particularly true in the outlier population; patients with common variants of the metabolizing enzymes fell within the range of standard dosing [8, 30]. The FDA has been updating drug labels to include such genetic information where compelling data exist [8].

New alliances are needed to ensure successful companion diagnostics

The pathway to a successful companion diagnostic is complex, and is best achieved through a closely coordinated interaction between diagnostic manufacturers, drug companies and regulatory agencies [11]. More specifically, this requires alliances between drug and diagnostic developers, clinical laboratories, physicians, pathologists, healthcare providers, and others (Figure 1).

Although companion diagnostics have been developed and approved in some therapeutic areas, the regulatory process itself is still evolving. Each companion diagnostic case is unique and requires early interactions and planning to ensure an efficient path to market [11].

Table 2. Companion diagnostics outside the oncology sector required or recommended by the FDA.

Drug	Manufacturer	Indication(s)	Companion diagnostic
Selzentry® (maraviroc)	Pfizer	Treatment of only CCR5-tropic human immunodeficiency virus (HIV) in adults, in combination with other antiretroviral agents.	Trofile™ (Monogram Biosciences) tropism assay for CCR5 receptor - required by the FDA
Depakote® (valproic acid)	Abbott Laboratories	Treatment of bipolar disorder, depression, seizures, autism, chronic pain with neuropathy, migraine headaches. Hyperammonemic encephalopathy (sometimes fatal) has been reported following initiation of valproic acid therapy in patients with urea cycle disorders, particularly ornithine transcarbamylase deficiency (OTC).	Metabolic and/or molecular testing for urea cycle disorders - recommended by the FDA
Coumadin® (warfarin)	Various	Anticoagulant used to reduce the risk for or treat thrombosis, or as secondary prophylaxis to reduce the risk for embolism. Those with certain mutations in CYP2C9 and VKORC1 P450 enzymes metabolize warfarin differently.	*CYP2C9* and *VKORC1* genotyping - recommended by the FDA
Lipitor® (atorvastatin)	Pfizer	Treatment of hypercholesterolemia (familial and non-familial). Those with homozygous and heterozygous familial hypercholesterolemia need to receive specific doses.	*LDLR*, *APOB*, *PCSK9* mutation analysis (for autosomal dominant forms) - recommended by the FDA
Tegretol® (carbamazepine)	Various	Treatment of seizures. Severe dermatologic reactions are associated with *HLA-B*1502* allele, found in some Asian patients.	*HLA-B*1502* genotyping - recommended by the FDA
Pegasys® (peginterferon alfa-2a)	Genentech	Treatment of chronic hepatitis C infection with compensated liver disease. A single nucleotide polymorphism (SNP) near the gene encoding interferon-lambda-3 (*IL28B*) is associated with variable sustained virological response rates.	*IL28B* genotyping - recommended by the FDA
Ziagen® (abacavir)	GlaxoSmithKline	Antiretroviral treatment of HIV. Patients who carry the *HLA-B*5701* allele are at high risk for experiencing a hypersensitivity reaction to abacavir.	*HLA-B*5701* genotyping - recommended by the FDA
Aralen® (chloroquine)	Various	Antiparasitic treatment of malaria; can also be used to treat lupus. Increased risk of hemolysis when administered to patients with glucose-6 phosphate dehydrogenase (G6PD) deficiency.	Metabolic and/or molecular testing for G6PD deficiency - recommended by the FDA

Sources: [23, 24].

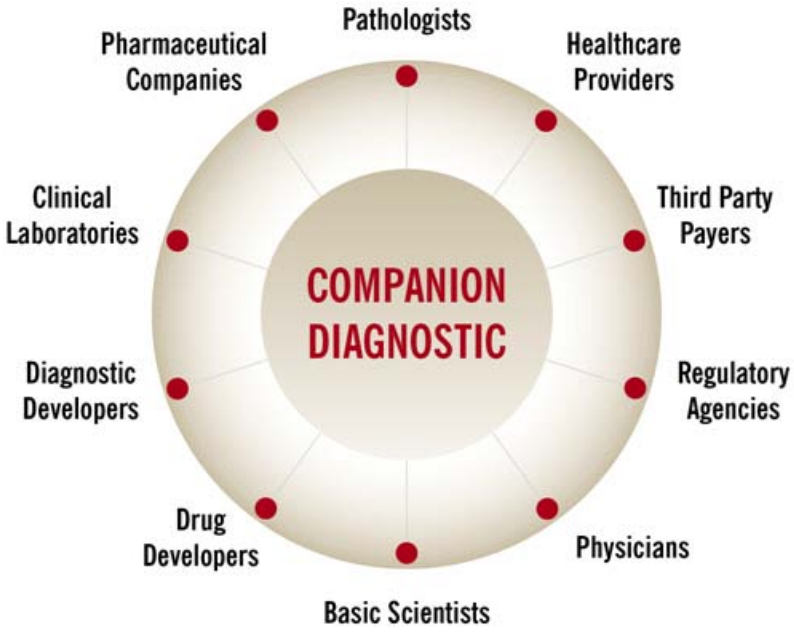

Figure 1. Necessary alliances to develop a successful companion diagnostic.

CONCLUSION

The field of molecular diagnostics has advanced rapidly since the first widespread introduction in the early 1980s, and has exhibited continual technological development. Thirty years into the age of molecular diagnostics, the stunning pace of advancement has shown no signs of abating, with the next generation of technologies rapidly becoming the method of choice for drug developers looking to include a companion diagnostic into their drug development cycle. While drug development companies are becoming increasingly receptive to adding a diagnostic to their drug development cycle, many large instructions are challenged by the implementation. Current development relies heavily on in-house technology, intellectual property, and expertise. However, development of a companion diagnostic requires a complex and symbiotic collaboration between public and private enterprise, research institutions, diagnostics, drug development, clinicians, patients and regulatory authorities. No one organization currently has the capabilities to combine all of the required pieces, and only such partnerships have the financial, scientific and technical capabilities necessary to complete this complex development cycle.

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