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PHARMACOGENOMICS MARKERS FOR PREDICTION RESPONSE AND TOXICITY IN CANCER THERAPY

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Absract: *In the oncology field, predictive markers allow physicians to improve the efficacy of cancer therapy, and prognostic markers allow patient selection with high risk of cancer recurrence for treatment, and those with low risk of recurrence for less intensive treatment or observation only.*

Genomic approaches for marker discovery now include genome-wide association studies and tumor DNA sequencing. The challenge is now to select markers for which there is enough evidence to transition them to the clinic.

In this review, we highlight the most recent genomic markers promises for both prognostic as well as predictive markers in cancer treatments.

Important barriers against implementation of routine clinical use of pharmacogenomic marker include the inherent low frequency of many of these markers, the lengthy validation process through trials, as well as legislative and low economic budgets.

Based on these actions, the oncologist will have a new features with which to make personalized treatment decision for their patients in order to maximize benefit and minimize toxicity.

KEY WORDS: *Pharmacogenetics, Chemotherapy, Predictive markers, Somatic mutation, Germline mutation.*

INTRODUCTION

Recent progresses have provided exceptional opportunities to identify prognostic and predictive markers of efficacy of cancer therapy. Genetic markers can be used to identify patients who will benefit from therapy, exclude patients at high risk of severe toxicity, and adjust dosing[1].

Pharmacogenomics and Pharmacogenetics (PGs) testing may support clinicians to identify patients who are less likely to benefit from expensive drugs, those who are susceptible to severe treatment related toxicities at standard doses, and also reduce the delay of the patient receiving perhaps the correct alternative treatment[2]. This is all more appealing in cancer therapy because many chemotherapeutic agents have a narrow therapeutic index and not uncommonly result in life threatening adverse events[3].

The utility of PGs extends beyond cancer therapy in the clinic. It has the potential to facilitate the identification of drug targets, accelerate the dis-

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covery and development of several drugs[4,5]. Neoplastic cells frequently acquire mutations in oncogenes, which can confer more sensitivity or resistance to drugs[6]. A better understanding of molecular processes and somatic mutations of tumors have led to an increasing number of targeted agents being discovered and developed[7]. The effective and appropriate use of expensive cytotoxic and targeted agents should ultimately translate into more cost effective treatments and eventually reduce overall healthcare costs. To evaluate the progress of PGs thus far, a simplistic classification of the most and examples are cited.

In particular, here we highlight the advances in the identification of both germline and somatic mutations, and the understanding of their predictive and prognostic values, in order to assess personalized treatment, a key goal of today's oncology[8].

Although, there still exist many challenges going forward: The pace of identifying such markers has not been harmonized by the speed of validation studies. Patient and physician education remains much to be improved upon[9]. Improvement in legislation and administrative processes is still ongoing. Nonetheless, the future for the development of PGs in cancer therapy remains promising.

MARKERS FOR PREDICTIVE RESPONSE TO CHEMOTHERAPY

The clinical application of PGs markers has been most successful in treatment response prediction. To date, there are several FDA approved anticancer drugs with validated predictive markers for treatment response (Table 1). These predictive markers are either acquired or somatic genomic alterations frequently characterized by DNA base mutations. In addition, neoplastic cells are often characterized by other genetic alteration as gene copy numbers changes, chromosomal rearrangement and epigenetic variations.

Intense clinical responses may be allowed when these tumor cells are treated with drugs targeting oncogenes to which tumors are dependent to for their growth, survival, and metastatic potential[10].

The most documented example is the epidermal growth factor receptor (EGFR) tyrosine kinase domain mutation and response to gefitinib and erlotinib in adenocarcinoma lung cancer. Differential responses and outcomes to targeted agents has led to the recognition of phenotypic characteristics (i.e. non-smokers, female), and the validation of genetic markers[11]. Somatic mutations in EGFR, including deletion mutations in exon 19 and substitution of Leucine to Arginine at codon 858 (L858R) in exon 21, have been identified for their ability to predict sensitivity to tyrosine kinase inhibitors (e.g. gefitinib or erlotinib)[12]. On the other hand, it has also been shown that the T790M mutation at exon 20 is the most commonly found mutation that confers resistance to therapy[12].

In contrast, the clinical utility of germline markers predicting for treatment responses are less well established. One of the most extensively studied examples is the relation between CYP2D6 activity and outcome[13]. CYP2D6 is responsible for the biotransformation of tamoxifen to its active metabolite, endoxifen. Decreased CYP2D6 activity, due to CYP2D6*10 polymorphism, was previously thought to be associated with poorer clinical outcomes in breast cancer patients treated with tamoxifen in the adjuvant setting[14]. However, the recent retrospective analyses of 2 large adjuvant breast cancer trials, ATAC and B1-98, failed to establish a relationship between CYP2D6 polymorphisms and treatment outcome of patients treated with tamoxifen[15]. Whether variation in the dose of tamoxifen would affect the outcome is also still not known. To complicate matters, rates of adherence to hormonal therapy may affect tamoxifen efficacy. In a prospective observational trial, CYP2D6 extensive metabolizers had higher discontinuation rates at 4 months. The extensive metabolizers who potentially may be more likely to benefit from tamoxifen were also puzzlingly more likely to stop therapy early[16,17].

Currently, it is still recommended that patients who are tacking tamoxifen avoid potent CYP2D6 inhibitors (i.e. fluoxetine, clopidrogel etc)[17]. Although the specific CYP2D6 test has been approved by the Food and Drug Administration (FDA) for detection individual metabolizer status. Although, the predictive value of CYP2D6 genotyping on tamoxifen outcome remains low, and more validation studies are needed.

MARKERS FOR PREDICTIVE TOXICITY TO CHEMOTHERAPY

There are many anti-cancer molecules with labels reporting germline pharmacogenetic markers of toxicity (Table 1). The majority of these polymorphisms were discovered by a candidate gene approach, where prior knowledge of pathophysiology, pharmacokinetics, pharmacodynamics and tumor biology is required. In recent years, the examination of population variation in all the annotated genes in the human genome has become possible[18]. Through statistical analyses and probability calculations, candidate genes can be identified without prior knowledge of the association

Table 1. Most common somatic and acquired mutation predictors for Response (R) and Toxicity (T) to anti-cancer drugs approved by the FDA. (www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm)

GENE	*Polymorphism (nucleotide translation)*	*Molecular effect*	*Drug*	*Effect Response (R) Toxicity (T)*	*Ref*
GERMLINE MUTATIONS:					
Cytochrome P450 family	Various SNP	Modify enzyme activity	Cyclophosfa mid Etoposide	Inter-individual variability in Pharmacokinetics	13
CYP2D6* 10		Decrease enzyme activity	Tamoxifen	(T) Poor metabolizer	14
TPMT*2, *3A, *3C	Various Polymorphism	Decrease enzyme activity	6-MP Thioguanine	(T) Hematopoietic Low expression	19
UGT1A *28 and *6	TA repeats in 5' promoter	Low expression	Irinotecan	(T) Severe Diarrhea Neutropenia	21, 22, 23
MDR1	(C3435T)	Low expression	Various	Drug resistance	49
TYMS	3 tandem repeats	High expression	5-FU, Metatrexate	Drug resistance	20
DPYD*2A	IVS14+1G	Decreease enzyme activity	Fluoropyrimidine	(T) Severe diarrhea neutropenia	41, 42
MTHFR	(C677T) and (A1298G)	Decreased enzyme activity	Metatrexate	(T) Hematopoietic	20
AQUIRED MUTATION:					
c-KIT	(T1982C) (T81421A)	Constitutive signal activation	Imatinib	Desensitizes activity in GIST	1
c-KIT	Codon D816V	ND	Imatinib Semaxinib	Good response in t(8;21)-positive AML	1
EGFR	Codon L858R Del(G719A/C/S)	Constitutive signal activation	Gefitinib Erlotinib	(R) Good response in NSCLC	12
EGFR	Codon T790M	ND	Gefitinib	Drug resistance	12
ABL	T(9;22) BCR/ABL fusion gene	Constitutive signal activation	Imatinib Dasatinib Nilotinib	Good response in CML	1
ABL	T315I M351T		Imatinib	Drug resistance	1
RARα	T(15;17) PML/RARα fusion gene	Block of maturation of Myeloid cells	All Trans Retinoic acid (ATRA)	(R) Good outcome in AML-M3 subtypes	1
K-RAS	Codon G12, G13		Cetuximab Panitumumab	(R) good outcome in wild type	37, 38, 39
B-RAF	Codon V600E	Constitutive signal	Vemurafenib	(R) good outcome in mutated V600	33
ALK	Fusion gene EML4/ALK[a]	Constitutive signal	Crizotinib	(R) Good outcome in NSCLC	28, 29

Abbreviations: TPMT = thiopurine methyltransferase; UGT1A1 = UDP-glucuronosyltransferase 1A1; MDR1 = multidrug resistance 1; TYMS = thymidylate synthase; DPYD = DihydroPyrimidine Dehidrogenase; MTHFR = 5,10-methylene tetra hydrofolate reductase; EGFR = Epidermal Grow Factor Receptor; 5-FU = 5-fluorouracil; 6-MP = 6-mercaptopurine; AML = Acute Myeloid Leukemia; NSCLC = Non-Small Cell Lung Cancer; CML = Chronic Myeloid Leukemia; EML4-ALK = Echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase.

Some of these may also affect efficacy, for example, thiopurine methyltransferase (TPMT) polymorphisms might affect 6-mercarptopurine (6-MP) response[19]. Even when treated at 10% of the standard dose of 6-MP, patients homozygous for TPMT variants have similar or superior survival compared with patients with at least one wild-type allele.

Even in patients who experience severe toxicity, the complex pharmacodynamic pathways may mean that the purported molecular marker identified may not be the only reason for the observed toxicity. Dihydropyrimidine dehydrogenase*2A (DPYD*2A) is the most common DPYD polymorphism associated with impaired DPD enzyme activity. Up to 25% of patients treated with Fluoropirimidine suffering from severe toxicity may have DPYD*2A polymorphism.

Although many polymorphisms for DPYD, 5,10-methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TYMS) have been identified

and studied, these polymorphisms have relatively modest or inconsistent associations with 5-fluorouracil toxicity, and several studies have failed to replicate the results. In order to assess the predictive value of polymorphisms in Fluoropirimidine based therapy a pharmacogenomics panel test were purposed on DPYD, TYMS and MTHFR for severe toxicities related to fluorouracil treatment[20]. The sensitivity of DPYD genotyping for overall toxicity was low with a positive predictive value of hardly half. The several proposed algorithm for 5-FU dosing is still theoretical without clinical utility.

Ethnic variation of drug response is an important factor that needs to be considered when a genetic testing model is attempted to be replicated across ethnic borders. The knowledge of the predominant polymorphisms and their respective frequencies should be borne to mind. In Caucasian populations, the UGT1A1*28 polymorphism is the most common variant but this is present in only 1.2-5% of South East Asian and Pacific populations[21,22]. In East Asians, the predominant functional polymorphism is UGT1A1*6, with a reported allelic frequency of 13-23%[23]. Indeed, the Japanese Ministry of Health and Welfare approved the use of testing for both UGT1A1*28 as well as UGT1A1*6[24]. The application of testing for UGT1A1*28 only would not be clinically relevant in the Japanese (and other Asian) population.

Although the associations between germline polymorphisms and treatment toxicities are well established[25], but they have not been used into routine clinical practice.

PROGNOSTIC MARKERS TO GUIDE THERAPY

Drug treatment directed at specific drug targets have created much enthusiasm in oncologic research, and have accelerated the development of several targeted anti-cancer molecules.

Under this new model, many confirmatory phase III trials are designed with some form of enrichment, in particular in tumors where somatic biomarkers for response was established proof of concepts, like lung, breast and colon (Berretta et Al 2011).

Many predictive markers in oncology such as EGFR mutation status, are found to have prognostic impact as well, aiding physicians in making clinical decisions for treatment or observation[26]. In recent years, Crizotinib, an anaplastic lymphoma kinase (ALK) inhibitor, has created much excitement for its unprecedented treatment response rate of greater than 70% in non-small cell lung cancer (NSCLC). However, the incidence of NSCLC harboring the echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK) fusion gene, the target for crizotinib, in the unscreened population is low, with an estimated incidence of 2-7%[27,28]. EML4-ALK in lung cancer is known to be more prevalent in females who are non-smokers and the adenocarcinoma subtype[29]. The knowledge that EML4-ALK and EGFR mutations are mutually exclusive has high significance[30]. Patients who are EGFR mutation negative with such phenotypic characteristics can be the target of randomized clinical trials for crizotinib, reducing the numbers needed to screen, and accelerating the development of crizotinib and increasing the chance of a successful trial. Vemurafenib has similar success with V600E BRAF mutation positive melanoma[31], and both drugs have transited with an accelerated pace from phase I trials directly to phase III[32,33].

Retrospective analyses of somatic mutations of completed prospective randomized trials have led to results that changed medical practice, for example, the addition of cetuximab and panitumumab to chemotherapy in patients who are KRAS wild type resulted in longer overall survival[34,35]. Prospective trials were designed thereafter with the aim to confirm the findings[36,37], although other studies have shown conflicting results[38,39]. The reasons for the discrepancies are not entirely clear.

In the retrospective analyses of previous trials for biomarker validation, it might be that not all the patients or samples may be available for analysis. It is pertinent though, that the available cohort of patients that are analyzed be representative of all the patients in the study, ideally a sizable number, or the validity of the analysis may fall short and be questioned.

EVALUATION COSTS OF PHARMACOGENOMICS

The finite nature of healthcare budget requires for treatments and biomarkers to be cost effective. Pharmacogenomics fields can potentially reduce healthcare cost by allowing the clinician identify patients that are most likely to benefit from treatment, thus reducing unnecessary treatment and minimize cost incurred during management of treatment related toxicities and hospitalizations[40].

The prevalence of a marker is an important factor that needs to be considered when validation trials are designed to determine clinical cost-effectiveness. Many pharmacogenetic markers have a low frequency in the population, making difficult their validation and clinical implementa-

tion. E relevant example is the allelic frequency of DPYD*2A is only about 1.8% in European Caucasians and less than 1% in Asian populations[41,42]. The majority, up to two-thirds, of patients who experienced severe treatment toxicity after 5-fluorouracil do not have a molecular basis for DPYD deficiency[43].

The clinical integration of PGs is often delayed by the cost of testing or lack of reimbursement from public or private insurers. Many countries especially developing ones, do not even have access to pharmacogenetic testing.

Several methods to assess the quality of cost-effectiveness, cost-utility and cost-benefit of PGs tests have become available. A relevant example is the National Institute for Health and Clinical Excellence (NICE). NICE forms a Diagnostic Advisory committee, which stimulates Pharma and Academic communities to produce a robust set of data, including the design and data source, for economic models of healthcare. In addition, NICE serve to better quantify the potential benefits of PGs testing in oncology[44]. However, limitations of individual economic evaluation models include not being able to capture important factors, such as, willingness of the patient to pay, psychological impact and patient preference.

Study focusing on the genotyping cost are low. It has been demonstrated that the mean calculated cost per life-year gained by TPMT genotyping in acute lymphoblastic leukemia patients treated with 6-MP was 2100,00 €, based on genotyping costs of 150€ per patient[45]. A more efficient PGs test is often not necessarily the cheapest test, but one that predicts more reliably the intended outcome, and allows for selection of the optimal treatment. With advances in technology, the cost and time of genotyping have dramatically decreased, with eventual realization of the "€20,00" per single polymorphisms[46]. In consideration of the dropping cost of genotyping, the incorporation of genomic scans in the patient evaluation becomes a dynamic and ongoing process, that should be constantly checked and updated by policy makers in accordance to the depreciating costs, to allow for more accessibility for genotyping and its benefits as more evidence becomes available.

CONCLUSIONS

The full application of PGs into clinical practice will require dramatic changes in regulations, legislative protection for privacy and reimbursement policies. Several recent regulatory policies, providing guidelines for genomic data management, pharmacogenetic testing, and designing of adaptive clinical trials, have been implemented to support genomic and personalized medicine[47,48].

There exist an acute lack of education of both the physicians and the patients regarding PGs and personalized care. The current knowledge of healthcare professionals regarding PGs is still low, and school curricula are only slowly including teaching of this subject in their courses[49,50]. Even when included, the depth of teaching may be limited[51]. PG knowledge is rapidly developing and changing, and it is imperative that healthcare professionals keep abreast of the advances and clinical indications.

Unfortunately, many have perceived notions that toxicity such as neutropenia can be easily managed, especially with advances in supportive care such as granulocyte colony stimulating factors. The large number of chemotherapeutic options available also means that physicians are often spoilt for choice, and have a low threshold to consider alternative therapies when toxicity becomes unmanageable. The need to evaluate the genetic basis for side effects becomes less clinically relevant in such circumstances.

However, it is often forgotten that genetic testing does not only predict for treatment related toxicity or allow for dose adjustment, and that it also determines response or lack thereof. It is frequently imperative that testing is done before treatment, as giving inappropriate treatment may result in an outcome poorer than the alternative. Patients who are EGFR wild types had a poorer outcome when treated with gefitinib[12]. A 'treat-and-see' approach has ethical and legal implications in this era where genetic testing is readily available, as it delays and even potentially deprives patients of appropriate treatment, and deterioration is often rapid without it.

These newer approaches serve as paradigmatic examples of the enrichment model, and this strategy is likely to be increasingly employed in this era of targeted and personalized medicine.

With increasing knowledge and understanding of the human genome, the clinical relevance of PGs in oncology will improve, especially with more validation studies and lowering costs of testing[52]. Several obstacles still exist before PGs can be fully adopted, for institutions, clinicians and patients. As more genetic and somatic information become easily accessible and available, we will be one step closer to making personalized medicine a reality.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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