Germline Genetic Testing to Predict Drug Response and Toxicity in Oncology—Reality or Fiction?

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Abstract

In addition to 6-mercaptopurine, 5-fluorouracil and irinotecan, the United States Food and Drug Administration (US FDA) has recently recommended label change for tamoxifen, to include pharmacogenetic information on treatment outcome. With the increasing availability of pharmacogenetic testing, on germline as well as somatic mutations, oncologists are now able to identify individuals at risk of severe treatment toxicity or poor treatment response. However, there are still knowledge gaps to fill before rationalised therapy based on pharmacogenetics can be fully integrated into clinical practice. This review provides an overview on the application of pharmacogenetic testing for germline mutations in oncology to predict response and toxicity.

Key words: Pharmacogenetics, Response, Toxicity

Introduction

There is an increasing interest in personalised medicine, perhaps none greater than in the field of oncology. The idea of making therapeutic decisions based on an individual’s genetic makeup, with the ability to predict tumour response, as well as minimise toxicity is extremely appealing to the oncologist. This is especially because many chemotherapeutic agents have a narrow therapeutic index. To date, there are at least 5 United States Food and Drug Administration (US FDA) drug labels with recommendations for germline genetic testing to be considered prior to administration of the drug or drug group for the treatment of cancer.1 This review aims to provide a brief insight on the genetic basis for inter-individual variations in therapeutic outcome relevant to key classes of anticancer agents and the potential application of genetic testing for treatment.

Variability in Response and Toxicity

Pharmacogenetics is defined as the study of inheritable genetic variation in drug response. Contrary to popular belief, it is not a new and modern concept. Observations of individual differences in response to food and drugs date back to as early as the 6th century BC. Pythagoras first made the observation that some individuals fall ill after ingesting uncooked fava beans, and disallowed his followers to eat them. It was not until the 1950s that the link between glucose-6-phosphate dehydrogenase deficiency, haemolytic anaemia, and fava beans was established.

With regards to the development of cytotoxic drugs with narrow therapeutic index, the assumption that the treatment response is proportional to dose is often made. The recommended dose for further clinical testing is usually defined as the dose at which less than one-third of the treated subjects developed severe toxicity.2 Hence, it is inevitable that for patients treated with any cytotoxic drug, there exist four categories of individuals: (i) Responders with tolerable toxicity, (ii) Responders with severe toxicity, (iii) Non-responders with tolerable toxicity, and lastly, and most undesirably, (iv) Non-responders with severe toxicity. Ideally, if we could predict which category a patient belongs to, we will be able to make appropriate treatment decisions.

To some extent, individualised therapy is already being practised on a daily basis. Chemotherapy is dosed based on an individual’s morphometric (e.g. body surface area), physiologic (e.g. liver, renal function), and demographic

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(e.g. age) characteristics. With the introduction of genetic testing, another parameter is now available to guide individualised cancer therapy. Genetic testing for the majority of chemotherapeutics is still largely under investigation. There are however, a few agents that have set the stage for others to follow.

**6-Mecaptopurine (6-MP)**

6-MP is one of the first chemotherapeutic agents recommended by the US FDA to consider genetic testing prior to commencement of therapy. It is used predominantly for the treatment of childhood acute lymphocytic leukemia (ALL). Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyses the methylation of aromatic and heterocyclic sulphhydryl compounds. The substrates of TPMT include 6-MP, 6-thioguanine and azathioprine. Although there are 2 other pathways competing with TPMT for the metabolism of 6-MP, the activity of the competing pathways in haematopoietic tissues is negligible and TPMT is the major inactivating enzyme for 6-MP in these tissues.3

TPMT activity has a tri-modal distribution and is inherited in an autosomal codominant pattern.4 Genetic polymorphisms in TPMT have been associated with 6-MP toxicity and therapeutic efficacy.3 Patients with TPMT deficiency require dose reduction to prevent life-threatening toxicity. 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 (normal) allele.6 Patients homozygous for the wild-type allele are less likely to have severe treatment toxicity but may be at higher risk of disease relapse.7

To date, at least 21 TPMT polymorphisms have been identified, 17 of which were shown to have reduced TPMT activity. Approximately 1 in 300 Caucasians has almost no detectable TPMT activity, and 1 in 10 has intermediate TPMT activity. TPMT*2 (238G>C, P240A), TPMT*3A (460G>A, A154C; 719A>G, C240Y), and TPMT*3C (719A>G, C240Y) account for over 90% of low or intermediate activity phenotypes in Caucasians, with reported allelic frequencies of 0.2%, 4.4%, and 0.4%, respectively.6 TPMT protein variants are prone to enzymatic degradation, resulting in lower catalytic activity.9

The mean calculated cost per life-year gained by TPMT genotyping in ALL patients was 2100€ (~SGD$3650), based on genotyping costs of 150€ (~SGD$260) per patient, and this is expected to further improve following wider use and decreasing cost due to availability of lower cost genotyping methods.10 With measurement of TPMT red blood cell activity being time-consuming and may be unreliable following blood transfusions, pretreatment genetic testing is an attractive alternative to screen for patients with TPMT deficiency.11 In 2003, a US FDA advisory committee made a landmark decision to recommend the addition of pharmacogenetic information on TPMT polymorphisms and treatment toxicity to the prescribing information for 6-MP.

However, several questions remain unanswered. There are substantial differences in the frequency of TPMT variants across various population groups. In Asian populations, TPMT*3C is the most common TPMT variant, with estimated allele frequency of TPMT*3C in the region of 2% to 4%.12 This difference means that the cost effectiveness of testing in specific populations is still unclear.

In addition, variable number tandem repeats (VNTR) have been found in the promoter region of TPMT. Although there is in vitro evidence to suggest that VNTR polymorphisms correlate negatively with TPMT activity, the importance of VNTR polymorphisms has not been clearly established in clinical studies.13

There is currently still no consensus regarding the optimal dose of chemotherapy for TMPT heterozygotes in all populations. Similarly, there has been no study to date to compare if dose individualisation for heterozygotes is superior to dose adjustment based on toxicity.

**Irinotecan (CPT-11)**

Irinotecan is a topoisomerase I inhibitor, and commonly used for the treatment of colorectal and lung cancers. It is converted to an active metabolite SN-38, by carboxylesterase 2, which is 100 to 1000 times more potent. UDP glucuronosyltransferase 1A1 (UGT1A1) is the major enzyme responsible for the glucuronidation of SN-38, rendering it more soluble for elimination via bile and urine.14

The UGT family of enzymes catalyses the glucuronidation of many lipophilic xenobiotics and endogenous substrates, including bilirubin. There are numerous polymorphisms of the UGT 1A1 gene, of which the most extensively studied being UGT 1A1*28 and UGT1A1*6.15

In Caucasian populations, UGT1A1*28 polymorphism is the most common variant associated with Gilbert syndrome.16 Approximately 5% to 15% of Caucasian and 12% to 27% of African populations have this polymorphism, but this is present in only 1.2% to 5% of South East Asian and Pacific populations.17,18 In East Asians, Gilbert syndrome has been linked to other functional polymorphisms, namely UGT1A1*6 (211G>A, G71R) and UGT1A1*60 (3279T>G), with reported allelic frequencies 13% to 23% and 13.6% respectively.18 Homozygotes for all the above polymorphisms, as well as double heterozygotes (*6/*28) are associated with an increased risk of toxicity from irinotecan due to decreased clearance of SN-38.

Up to 35% of patients receiving irinotecan for metastatic
colorectal cancer have been reported to experience dose limiting toxicities.\textsuperscript{9} In 2005, the US FDA amended the product label of irinotecan to include a precautionary note warning of increased neutropenia and toxicity for patients who are homozygous for UGT1A1*28.\textsuperscript{20} A meta-analysis of 9 studies in 2007 confirmed the association for doses greater than 150mg/m\textsuperscript{2} with increase neutropenia in patients homozygous for UGT1A1*28.\textsuperscript{21} However, more importantly, no association was seen at lower doses (100 to 125mg/m\textsuperscript{2}), which is the dose often used for weekly dosing.

In a large study of patients treated with irinotecan, 5-Fluorouracil (5-FU) and leucovorin (FOLFIRI), it was observed that there was an 8-fold increased risk of developing severe neutropenia during the first treatment cycle in patients with UGT1A1*28 polymorphisms.\textsuperscript{22} Interestingly, patients homozygous for UGT1A1*28 also seemed to have better tumour response, time to tumour progression, and a trend toward better survival. Clinical data from 2 Japanese studies had also demonstrated that increased risk of neutropenia is present even in heterozygous UGT1A1*6, with toxicity observed even in low dose irinotecan.\textsuperscript{23,24} This highlights the lack of evidence that dose reduction alone can reduce toxicity, and perhaps toxicity may even somehow represent a biomarker for response.

The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group recently concluded that the evidence is currently insufficient to recommend using genotyping of UGT1A1 to modify the dose of irinotecan.\textsuperscript{25} The recommendation came after the observation that although there was a significant association between the UGT1A1*28 homozygous genotype and severe neutropenia, there was no statistically significant difference in the frequency of severe diarrhoea. More importantly, there was suggestion that reducing irinotecan dose may result in patient harm due to decreased tumour response.

The US FDA approved the InvaderR UGT1A1 molecular assay (Third Wave Technologies) for the detection of UGT1A1*28 in 2005.\textsuperscript{1} Following a label revision in 2008 in Japan, similarly, the Japanese Ministry of Health, Labour and Welfare has approved the use of UGT1A1 genotyping for UGT1A1*28 and UGT1A1*6 variants to predict for toxicity.\textsuperscript{26} The variants are detected using invasive cleavage of oligonucleotide probes with cleavage enzyme and a fluorescence resonance energy transfer cassette.\textsuperscript{27} Several gaps remain to be filled, including knowledge pertaining to the ideal dose of irinotecan in heterozygotes. At present, the main usefulness of genotyping for UGT1A1 is in allowing clinicians to consider alternative treatment options if genetic testing reveals an unfavourable genotype, or to consider a weekly dosing schedule instead of the usual 2- or 3-weekly dose.

5-Fluorouracil (5-FU) has been a key chemotherapeutic agent in the treatment of colorectal cancer over the past few decades, and has activity in a variety of other cancers, including head and neck, gastric, and breast cancer. 5-FU is converted to its cytotoxic nucleotides, which in turn inhibit thymidylate synthase (TYMS) or incorporates into RNA and DNA. It is metabolised to its inactive form, 5, 6-dihydro-5-fluorouracil, by dihydropyrimidine dehydrogenase (DPD).\textsuperscript{28} DPD is the rate-limiting enzyme in the catabolism of pyrimidines such as uracil and thymidine, and the synthesis of β-alanine.\textsuperscript{29} Decreased DPD activity can lead to the accumulation of 5-FU and severe toxicities, including mucositis, neutropenia, neurological symptoms and death.\textsuperscript{30}

More than 40 different polymorphisms of DPYD (the gene coding for DPD) are reported to date, of which 17 mutations are found in patients with severe 5-FU toxicity.\textsuperscript{31} Approximately 3% to 5% of the population is heterozygous and 0.1% is homozygous for alleles with impaired DPD function.\textsuperscript{32} DPYD*2A is the most common DPYD polymorphism associated with impaired DPD activity. DPYD*2A is caused by a 5' splice site mutation at intron 14 G1A resulting in the formation of a truncated protein.\textsuperscript{33} It is estimated that about a quarter of patients suffering from severe 5-FU toxicity have DPYD*2A polymorphism.\textsuperscript{34,35} The allelic frequency of DPYD*2A is only about 1.8% in European Caucasians and less than 1% in Asian populations.\textsuperscript{36,37}

Up to two-thirds of patients who experienced treatment toxicity do not have a molecular basis for DPD deficiency.\textsuperscript{38} Other genetic or epigenetic variations may account for this, including variations in TYMS and 5, 10-methylenetetrahydrofolate reductase (MTHFR) which is an important enzyme that regulates folic acid and homocysteine homeostasis.

The TYMS gene contains 7 exons and a 5'-flanking untranslated enhancer region containing a 28-bp tandem repeat sequence.\textsuperscript{39} The number of tandem repeats varies from two (2R) to nine (9R) copies.\textsuperscript{40} The translational efficiency is correlated with the number of tandem repeats. In vitro, there is a 2.6- to 3.6-fold increase in TYMS expression with the 3R variant compared with the 2R variant.\textsuperscript{41} The distribution of tandem repeats in Caucasians is 16% 2R/2R, 51% to 55% 2R/3R, 29% to 32% 3R/3R and <1% for other variants.\textsuperscript{42,43} In patients with metastatic colorectal cancer, those homozygous for 3R had a poorer survival (12 months vs 16 months) and a lower response rate (9% vs 50%) to 5-FU, compared with those homozygous for 2R.\textsuperscript{44}

Another polymorphism, 3RC, alters the transcriptional activity of the TYMS gene.\textsuperscript{45} 3RC is a G→C single nucleotide polymorphism (SNP) located at the 12th nucleotide of the second tandem repeat of 3R. The 3RC allele occurs in 56%,
28% and 37% of all 3R alleles in Caucasians, African-Americans and notably, Singapore Chinese, respectively, and a 3RC variant has a lower TYMS expression level and is associated with better clinical outcome with 5-FU compared to the 3R variant. However, TYMS polymorphisms have relatively modest and inconsistent associations with toxicity, and several studies have failed to replicate the results, and further studies are indicated.

A large prospective German study of 683 patients looked to assess the predictive value of polymorphisms in DPYD, TYMS and MTHFR for severe toxicities related to fluorouracil (FU) treatment. The sensitivity of DPYD*2A genotyping for overall toxicity was 5.5% with a positive predictive value of only 46%. Inclusion of additional DPYD variants improved prediction only marginally. Interestingly, while women had a higher risk for toxicity, DYPD genotype was not a dependent factor.

Commercially available assays such as TheraGuide 5-FU™ are currently available to test for variations in DPYD and TYMS. However, testing should be indicted only if alternative therapy is a consideration, given the low sensitivity for testing (especially low sensitivity for women), infrequent prevalence and heterogeneity of functional DYPD polymorphism, and inconsistency of TYMS polymorphism association with toxicity.

Tamoxifen

Tamoxifen is used in the treatment of estrogen receptor—positive breast cancer and also for chemoprevention for patients at high risk for developing breast cancer. It is metabolised via the cytochrome P450 (CYP) pathway to form several metabolites, including endoxifen and 4-OH-tamoxifen which are the most potent. Endoxifen is present at a much higher plasma concentration than 4-OH-tamoxifen and is thought to be largely responsible for the therapeutic effect of tamoxifen.

CYP2D6 is the predominant CYP isoform that catalyses the formation of endoxifen. At least 88 allelic variants of CYP2D6 have been described, many of which are nonfunctional or have reduced catalytic activity, resulting in a tetra-modal distribution: poor, intermediate, extensive and ultrarapid. It is estimated that 5% to 10% of Caucasians have nonfunctional variants. The most common nonfunctional variants, CYP2D6*3, CYP2D6*4, CYP2D6*5, and CYP2D6*6, constitute approximately 97% of the poor-metaboliser phenotype, resulting in up to a 4-fold difference in endoxifen concentration in homozygotes for these variants, compared to wild-types. Ultrarapid metabolisers carry gene duplications or multiple duplications of functional alleles resulting in increased enzyme activity. These genotypes are rare in Caucasians and Asians but are common in Ethiopians and Saudi Arabsians.

Initial studies suggest that women with decreased CYP2D6 activity had poorer clinical outcomes when treated with tamoxifen, both in the adjuvant and chemoprevention settings. For example, in a large retrospective analysis of German and US patients, Schroth et al showed that the recurrence rates were 14.9% for extensive metabolisers, 20.9% for heterozygous extensive/intermediate metabolisers, and 29.0% for poor metabolisers. Ultrarapid metabolisers, and possibly extensive metabolisers, may potentially benefit from extended tamoxifen use (up to 5 years) before switching to AI. However, retrospective analyses of 2 large adjuvant breast cancer trials, ATAC and BIG1-98, were presented recently, and they failed to establish a relationship between CYP2D6 polymorphisms and treatment outcome in patients treated with tamoxifen. Although only in abstract form, these results highlight the uncertainty surrounding genetic testing for CYP2D6 to guide tamoxifen therapy currently.

Administration of CYP2D6 inhibitors may affect tamoxifen-related breast cancer outcomes by converting an extensive metaboliser to a phenotypic poor metaboliser and should be avoided. CYP2D6 inhibitors include antidepressants such as fluoxetine and paroxetine, whereas moderate/weak inhibitors include cimetidine, amiodarone, ticlopidine and haloperidol.

An FDA approved microarray-based pharmacogenetic test, the AmpliChip CYP450R test, is available for pretreatment testing of CYP2D6. It can detect 27 variant alleles of CYP2D6 and three variants of CYP2C19 using an array of over 15,000 probes. The variants tested include nonfunctional variants (CYP2D6*3, CYP2D6*4, CYP2D6*5 and CYP2D6*6), and those that are more commonly found in East Asians (CYP2D6*10), African Americans (CYP2D6*17), and Middle Eastern and North African populations (CYP2D6*2XN).

At this point, expert opinion is that there is currently no strong evidence to routinely recommend CYP2D6 testing prior to tamoxifen prescription, and more studies are required. While CYP2D6 is important, it may not be the only determinant of tamoxifen outcome. Other determinants may include other metabolising enzymes, complex interplay of different active metabolites in addition to endoxifen, and possibly concomitant medications. It is still recommended that patients who are on tamoxifen avoid potent CYP2D6 inhibitors.

Conclusion

Pharmacogenetic testing is now widely available, and not merely fiction. The ability to predict outcome even before commencement of treatment is now a reality. However, widespread implementation is hampered by knowledge gaps
in optimal drug dosing. The relevance in specific patient populations, especially where the frequency of the allele is low, is also continually under question. Certainly, genetic testing is not for everyone, but it promises the potential for cost saving, reduced toxicity and improved efficacy in the near future.

To keep pace with the wealth of information generated from preclinical pharmacogenetic studies and the increasing accessibility of pharmacogenetic testing, there is an urgent need for more validation studies, especially adequately powered prospective trials to facilitate the translation of pharmacogenetic knowledge into clinical practice.

REFERENCES


