



- The thymidylate synthase gene is polymorphic with respect to the number of tandem repeats of a 28 base-pair segment of DNA in the promoter region of its gene. It has 2 common variants, with differing rates of expression: the shorter version, labelled S, has 2 repeats, and the longer version, labelled L, has 3 repeats.⁹

TOXICITY OF CHEMOTHERAPY

Polymorphisms leading to variant expression of gene products can seriously affect the toxicity of chemotherapy.^{1,2} Below are examples of such variation with clinically significant frequency.

6-mercaptopurine

6-mercaptopurine (6-MP) is extensively used in maintenance chemotherapy for childhood acute lymphoblastic leukemia. It is a prodrug which is converted to 6-thioguanine in the body, and incorporated into DNA. Thiopyrimine methyltransferase (TPMT) can divert 6-MP from this pathway; the subsequent methylated version of 6-MP is inactive and not available for DNA incorporation. Thus, alteration in the function of TPMT can result in excessive amounts of 6-MP, with consequent gastrointestinal toxicity and myelosuppression.^{1,8,10,11} Relling et al have demonstrated that common polymorphisms of TPMT can engender wide variations (as much as 10-fold) in steady-state dosage of 6-MP.¹⁰ While 90% to 95% of patients have the wild-type gene, about 0.3% are homozygous for common variants leading to severely decreased expression.

5-FU and dihydropyrimidine dehydrogenase

The fluoropyrimidines, commonly used to treat a large variety of cancers, cause toxicities including myelosuppression, mucositis, skin changes and (rarely) cardiac and neurologic toxicity. The majority of these drugs are metabolized in the liver by dihydropyrimidine dehydrogenase (DPD).¹² 5-fluorouracil's (5-FU) mechanism of action probably occurs at multiple sites. The balance between its key activities as a pyrimidine analog or an inhibitor of thymidylate synthase can be modulated by method of administration (continuous initiation vs bolus) or concurrently administering reduced folates.¹³ Regardless of the mechanism of action, DPD regulates the available pool of drug. DPD is completely deficient in 0.1% of the general population and partially deficient in 3% to 5%.¹

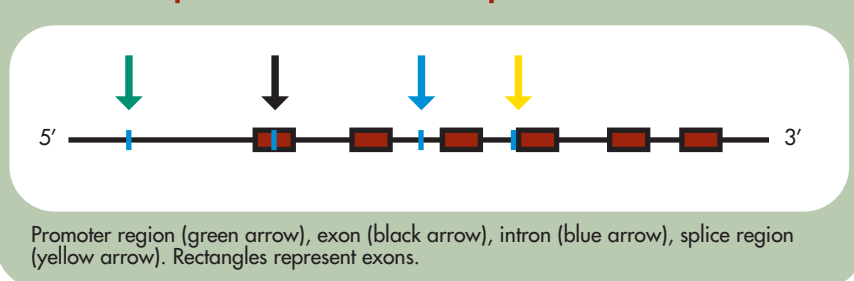
Inactivation of one allele of *DPYD* (the gene for DPD) can lead to clinical toxicity. The polymorphism *DPYD**2A is a SNP at a splice site — a guanine base between an intron and an exon

— which in this case is replaced by adenine at intron 14.¹² With the subsequent exon being ignored during transcription, the messenger RNA is truncated, resulting in deficient enzyme activity. Occurring in roughly 1% of the population, at least 1 study has demonstrated this abnormality in about half of patients with significant toxicity to 5-FU. Despite several attempts to provide predictive testing for such toxicity,^{12,14} no process is currently readily available in clinic. DPD deficiency resulting in severe gastrointestinal toxicity is life-threatening, and probably the most feared toxic effect of 5-FU.

Irinotecan and UGT1A1

Irinotecan is a semisynthetic analog of camptothecin with dose-limiting toxicities of myelosuppression and delayed diarrhea. A prodrug, its active metabolite SN-38 is eliminated via glucuronidation.¹⁵ The wild-type gene for uridine diphosphate glucuronyl transferase, UDP-glucuronyltransferase (*UGT1A1*), is polymorphic, but not for a SNP: this gene's expression varies inversely with the number of TA repeats in the promoter region. The wild-type gene has 6 repeats, and the common variant, *UGT1A1**28 — responsible for the majority of cases of the liver disorder Gilbert's syndrome — has 7. The subsequent reduction of activity leads to an increase of SN-38 passing into the gut, resulting in late-onset diarrhea.¹⁶⁻¹⁹ The homozygous and heterozygous states result in reductions of SN-38 glucuronidation of 50% and 25%, respectively. Homozygotes, who clinically have Gilbert's syndrome, are about 9% of the general population and are prone to periods

FIGURE 1: Representation of common positions of SNPs in DNA



of significant hyperbilirubinemia, e.g. at birth or during infection. Such patients can have significant toxicity with irinotecan. The nadirs of absolute neutrophil counts following irinotecan administration can be related directly to the heterozygous and homozygous states.¹⁹

Methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase (gene: *MTHFR*) regulates the pool of folates available for DNA and protein synthesis. A common SNP, which occurs in between 5% and 54% of the general population, reduces the enzyme activity to 35% of the wild type's level. Designated *C677T*, this substitution is responsible for some cases of profound neutropenia in patients receiving methotrexate and 5-FU.²⁰

RISK OF MALIGNANCY

As well as drug-host interactions (the focus of pharmacogenetics) pharmacogenomics also looks at the risk of malignancy in people with genetic variations when exposed to certain drugs or xenobiotics.

The folates again

The common SNP of *MTHFR* mentioned above (*C677T*) has been implicated as a risk factor contributing to excess incidence of premenopausal breast cancer. Semenza et al argue, "Because dietary folate deficiency has been suggested as a risk factor for breast cancer, it is possible that genetically controlled folate availability also modifies the risk." The stated hazard ratio for developing breast cancer of 3.08 for persons heterozygous or homozygous for this SNP compared to premenopausal controls suggests increased risk in this genetically defined group.²¹

Breast cancer and alcohol

The cytochrome P450 enzyme system is responsible for the metabolism of a large number of drugs and xenobiotics. Divided into subfamilies, several are well known because of being implicated in variation of response to medications and susceptibility to toxicity. *CYP2E1***C2*, a SNP (*C1053T*) in the 5' promoter

region of gene *CYP2E1* results in decreased enzyme activity. This variant is found in 20% of Asians and only 2% to 5% of Caucasians. Choi et al demonstrated an almost 2-fold risk of breast cancer in women with this SNP who consumed > 1 alcohol equivalent per month.²²

Tobacco and small cell lung cancer

Another cytochrome P450 enzyme system in the 3A subfamily is involved in tobacco carcinogen metabolism. *CYP3A4***1B* is a SNP (an adenine to guanine substitution 392 base pairs upstream from the gene, affecting transcription) previously implicated in prostate cancer. In a case control study, Dally et al showed significant relationships with the presence of this SNP in smokers vs non-smokers for small cell lung cancer. The authors suggest that the increased risk of this disease in smokers may be related to the increased activity of the *CYP3A***1B* variant, activating higher levels of carcinogens from tobacco smoke.²³

RESPONSE TO THERAPY

Certain genetic variations actually increase the activity of chemotherapy, some by heightened activity of the therapeutic drug, others by lower levels of metabolic targets.

Thymidylate synthase

Thymidylate synthase (TS) as a polymorphic gene, with long (L) or short (S) versions depending on the presence or absence of an extra 28 base-pair repeat in the promoter region. The long form has 2.6 times more activity than the short form. Phenotypic expression can be seen in the level of TS activity in tumours, and improved response of solid tumours to fluoropyrimidines has been demonstrated in patients with the S version of this gene.⁹ The presumed mechanism is that the complex of reduced folate and fluoropyrimidine more effectively inhibits TS in patients whose tumours express lower levels of TS.

Glutathione S-transferase

Glutathione S-transferase (GST) is a superfamily of enzymes important for

cellular defense. Five subclasses have been designated, originally using Greek letters. Only the pi version, GSTP1, directly participates in detoxification of platinum compounds. *A313G*, a common SNP resulting in a change of isoleucine to valine at amino acid 105, diminishes enzyme activity. Stoelmacher et al demonstrated increased survival in patients receiving 5-FU and oxaliplatin who were homozygous for this variant — slightly less than 10% of their patients.⁷ Recently, another SNP in this gene, resulting in an alanine to valine substitution at amino acid 114, improved median survival times by 5 months in patients with non-small cell lung cancer (NSCLC) receiving platinum-based treatment.²⁴

XPD and platinum

Xeroderma pigmentosa is associated with variants in the family of genes involved in nucleotide excision repair (NER). The *XPD* gene codes for helicase, a component of a transcription factor for NER. A relatively common mutation in this gene (*A751C* leading to Lys224Gln) may alter the ability to repair DNA damage from the drug oxaliplatin, resulting in impaired survival.²⁵

Breast cancer and cytochrome P450 enzymes

Two recent abstracts from the American Society of Clinical Oncology meeting highlight variations in response to treatment associated with the cytochrome P system of enzymes. Lloveras et al found that the *CYP19* aromatase gene can be affected by a SNP in the 3' untranslated region that occurs in 46% percent of the population and leads to longer time to progression for metastatic breast cancer patients treated with letrozole,²⁶ possibly due to increased sensitivity to aromatase inhibition. Stearns et al showed reduced endoxifen levels (an active metabolite of tamoxifen) in patients with 2 variants of *CYP2D6* (*4 and *6). They also demonstrated reduced endoxifen levels in patients taking the *CYP2D6* inhibitors sertraline and paroxetine, commonly used SSRIs, but not venlafaxine²⁷ (a SSRI


proposed for the treatment of “hot flashes”). The clinical implications of these findings have yet to be determined, but they are concrete demonstrations of how both common drugs and genetic variants can alter active drug levels.

Ondansetron and CYP2D6

Examples of response to therapy are not restricted to effects on the tumour, but may also be seen in the response of patients to medications used for supportive care. All 5-HT₃ inhibitors are metabolized through the cytochrome P450 family of enzymes. In particular, ondansetron is metabolized via the 2D6 and 3A4 subfamilies of enzymes, while tropisetron is metabolized by the former and granisetron by the latter. A small percentage of Caucasians, perhaps 2% to 4%, are true rapid metabolizers of ondansetron, with more than 2 active genes for CYP2D6 and up to 7-fold duplication. Such patients may gain less benefit by virtue of this rapid destruction and therefore may be more suitable candidates for granisetron.²⁸ Alternatively, patients with increased induction of the 3A4 subfamily by other medications may be more appropriate candidates for ondansetron.

THE RIGHT PATIENT WITH THE RIGHT TREATMENT

For years clinicians have noticed marked variations in the response to treatment and the rate of side effects experienced by their patients to medications prescribed. Often such variation has been deemed inexplicable. As we begin to discern some of the reasons underlying the observed differences, the practice of medicine seems a little less mystifying. Understanding pharmacogenomics — and its application to the complex interaction between drug, host and disease — will lead to better methods of selection as we improve our ability to predict toxicity, response to therapy and even risk of developing malignancy. The knowledge base will expand as interest grows. Currently most of these insights are only academic: development of standard testing processes

that will be available in the clinic is a key challenge for the next decade. 

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