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Pharmacogenomics of anticancer drugs: Personalising the choice and dose to manage drug response

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The field of pharmacogenomics has made great strides in oncology over the last 20 years and indeed a significant number of pre-emptive genetic tests are now routinely undertaken prior to anticancer drug administration. Many of these gene–drug interactions are the fruits of candidate gene and genome-wide association studies, which have largely focused on common genetic variants (allele frequency>1%). Examples where there is clinical utility include genotyping or phenotyping for *G6PD* to prevent rasburicase-induced RBC haemolysis, and *TPMT* to prevent thiopurine-induced bone marrow suppression. Other associations such as CYP2D6 status in determining the efficacy of tamoxifen are more controversial because of contradictory evidence from different sources, which has led to variability in the implementation of testing.

As genomic technology becomes ever cheaper and more accessible, we must look to the additional data our genome can provide to explain interindividual variability in anticancer drug response. Clearly genes do not act on their own and it is therefore important to investigate genetic factors in conjunction with clinical factors, interacting concomitant drug therapies and other factors such as the microbiome, which can all affect drug disposition. Taking account of all of these factors, in conjunction with the somatic genome, is more likely to provide better predictive accuracy in determining anticancer drug response, both efficacy and safety.

This review summarises the existing knowledge related to the pharmacogenomics of anticancer drugs and discusses areas of opportunity for further advances in personalisation of therapy in order to improve both drug safety and efficacy.

KEYWORDS
cancer, drug efficacy, drug safety, oncology, pharmacogenomics, personalised medicine

1 | INTRODUCTION

The fundamental aspects of pharmacogenomics can be traced back to 510BCE and the observations of Pythagoras who noted that some individuals became ill after eating fava beans (favism). We now know this intolerance of fava bean is due to glucose-6-phosphate dehydrogenase (G6PD) deficiency[1] caused by deleterious variants in the gene encoding the enzyme. Coincidentally, G6PD deficiency is now known to be important in oncology as it is associated with an increased risk of haemolysis in patients administered rasburicase for prevention of tumour lysis syndrome.[2]

Oncology is considered to be the field of medicine in which pharmacogenomics and personalised medicine is perhaps most established. Indeed, oncology indications represent 140/362 (39%) of

all Food and Drug Administration drug label warnings related to pharmacogenomic markers[3] (accessed 1August 2019). However, only 24 of these (20%) relate to germline, nontumour variants associated with interindividual variability in response (either safety or efficacy). Furthermore, just 21 of these drug label warnings report/describe an actual association (Table 1).

Several different approaches to the identification of predictive genetic biomarkers have been utilised in the previous 2 decades. Initially candidate genes studies analysing associations with variants in genes with a *priori* knowledge of impact on drug pharmacokinetics and pharmacodynamics were undertaken. However, as our understanding on population genetics, linkage disequilibrium (LD) and haplotype structure developed, genome-wide associations studies allowed us to conduct unbiased studies and thus identify novel loci associated with drug response. This understanding of the complexities of population LD has given us an understanding of differences in LD and allele frequencies in different ethnicities, evidenced by differences in drug responses between different ethnic groups.[36] It is important to note, however, that much of this work is based on associations with genetic variants that are common, often with a minor allele frequency >5%. Looking forward, as next-generation sequencing (NGS) becomes more routinely embedded within research studies, and eventually into clinical practice, the role of low penetrance, low frequency and even *personal* variants, will need to be evaluated in drug response, which will only be possible with large-scale population studies linked to electronic health record databases.

There are some important examples of oncology drugs where the level of evidence for gene–drug interactions is substantial and clinical validity/utility of pre-emptive testing is demonstrable to the extent that it is recommended or, in some instances, mandated. In this review, we provide an up-to-date analysis of gene–drug interactions in the field of oncology focusing on germline variants, rather than somatic variants. There are number of oncology drugs where genetic variation in genes encoding drug metabolising enzymes are associated with interindividual variability in outcome for efficacy and/or safety (Figure 1).

2 | ASSOCIATIONS WITH A HIGH LEVEL OF EVIDENCE

2.1 | *TPMT/NUDT15* and thiopurines

6-Mercaptopurine is used in the treatment of acute lymphoblastic leukaemia (ALL). It is metabolised by thiopurine methyltransferase (TPMT) to an inactive methylmercapturine resulting in less parent drug available for the formation of pharmacologically active, and potentially toxic, thioguanine nucleotide (TGN) metabolites. Variant alleles of *TPMT* are associated with low enzyme activity and consequently increased TGN levels leading to pronounced pharmacological effects. Indeed, individuals who inherit 2 loss-of-function alleles are at significantly increased risk of life-threatening myelosuppression as a result of increased TGN exposure.

Estimates suggest that between 5.8 and 15.5% of individuals carry an actionable TPMT low activity genotype (Table 2). Three key single nucleotide polymorphisms (SNPs), defined as variant alleles *2,*3A and *3C, lead to an unstable TPMT protein and enhanced protein degradation.[42] They account for >90% of low-activity phenotypes and have been demonstrated to be highly predictive of the low TPMT activity phenotype[43]

To reduce the risk of myelosuppression in mercaptopurine-treated individuals, clinical guidelines on dose optimisation guided by the *TPMT* genotype have been developed by the Clinical Pharmacogenetics Implementation Consortium (CPIC)[25] and Dutch Pharmacogenetics Working Group (DPWG)[44] which are based on pre-emptive TPMT activity genotyping of the 3 key low-activity variant alleles *(*2,*3A,*3C)*. It is, however, also important to note that there are phenotyping tests available for TPMT activity, which can in theory detect all variants in the TPMT genes (beyond the 3 alleles), and the phenotyping test is widely used. However, clinical phenotyping tests, based on enzyme activity, do have some limitations including not being reliable in patients post blood-transfusion.[45]

CPIC guidelines[25] recommend that where a starting dose of 75 mg/m^2 of mercaptopurine is used for treatment of ALL, a 50% dose reduction should be considered for individuals who are intermediate TPMT metabolisers (carriers of 1 functional and 1 nonfunctional allele). For poor metabolisers (carriers of 2 nonfunctional alleles) the recommended dose is 10%.

More recently, genome-wide association studies have identified variants in *NUDT15*[46] that strongly influence thiopurine intolerance in ALL patients. *NUDT15* encodes a nucleoside diphosphatase that catalyses the conversion of the cytotoxic thioguanine triphosphate (TGTP) metabolite to the less toxic thioguanine monophosphate. TGTP incorporates into DNA forming DNA-TG, the antileukaemic metabolite.[47]

Defective NUDT15–mediated catabolism results in elevated levels of TGTP and subsequently DNA-TG, leading to an increased risk of myelosuppression. The first NUDT15 SNP associated with thiopurine toxicity was rs116855232 (c.415C>T), which causes a p. R139C amino acid substitution resulting in almost complete loss of enzymatic activity and protein stability in vitro. Carriers of this allele have elevated DNA-TG levels[48] and severe myelosuppression. At standard maintenance doses of mercaptopurine in ALL, the risk of myelosuppression in carriers of the p. R139C variants is 14.5-fold higher than in wild-type individuals.[49] Indeed, in other paediatric ALL cohorts, individuals homozygous for p.R139C tolerated only 8% of the standard dose, while the figure was 63 and 85% for heterozygous and wild-type individuals, respectively.[46]

The p.R139C allele in NUDT15 is by far the most extensively studied and therefore provides the largest body of evidence for clinical implementation. However, there are many other variants of differing frequencies in the NUDT15, for many of which we have no data on functional activity. To overcome this limitation, a recent study[50] used the technique of saturation mutagenesis to identify 54 residues where variants led to a loss of protein stability, and

TABLE 1 Germline, nontumour pharmacogenomics markers of oncology-indicated drug-response (safety or efficacy) as reported by the Food and Drug Administration (FDA) biomarker list.[3] * indicates where an FDA drug labels reports no relationship between genotype and variable drug response. The existence of clinical guidelines for the clinical pharmacogenetics implementation consortium (CPIC), Royal Dutch Association for the advancement of pharmacy—Pharmacogenetics working group, (DWPG) Canadian pharmacogenomics Network for drug safety (CPNDS) and French joint working group comprising the National Pharmacogenetics Network (FNPGx) and the Group of Clinical Onco-pharmacology (GPCO-Unicancer; NPGCOP). ~indicates where clinical guidelines make no recommendation

| Gene	Drug	Safety	Efficacy	Ref	Included in FDA drug label	CPIC	DPWG	CPNDS	FNPGx
ACYP2	Cisplatin	Increased risk of ototoxicity in patients with brain tumours, osteosarcoma and other cancers who carry the rs1872328 A-allele.		4,5					
CEP72	Vincristine	Increased with of peripheral neuropathy in paediatric precursor cell lymphoblastic Leukaemia-lymphoma patients with rs924607 TT genotype.		6					
CYP2B6	Cyclophosphamide	*6 carriage associated with decreased risk of toxicity vs *1/*1 when treated with cyclophosphamide and fludarabine in chronic lymphocytic leukaemia		7					
CYP2D6	Gefitinib	CYP2D6 poor metabolisers have increased bioavailability and subsequent increased ADR risk (including rash and hepatotoxicity)		8,9	Y		Y~		
	Rucaparib		No CYP2D6 genetic effect on interindividual bioavailability.		Y*				
	Tamoxifen		CYP2D6 poor metabolisers associated with significantly lower endoxifen plasma concentrations and worse progression-free survival.	10	Y	Y	Y	Y	
CYP3A4	Paclitaxel	*8*20 and *22 carriers have increased risk of neuropathy		11,12					
CYP3A5	Paclitaxel	*1 allele carriage associated with increased risk of leukopenia/neutropenia and neurotoxicity vs *3.		13,14					
DPYD	Capecitabine/5-fluorouracil	Individuals with low or absent DPD activity at increased risk of severe ADRs (including mucositis, diarrhoea, neutropenia, and neurotoxicity)		15,16	Y	Y	Y		

(Continues)

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TABLE 1 (Continued)

Gene	Drug	Safety	Efficacy	Ref	Included in FDA drug label	Clinical guidelines			
						CPIC	DPWG	CPNDS	FNPGx
G6PD	Dabrafenib	Risk of haemolysis in G6PD-deficient individuals.		17	Y				
	Flutamide	Risk of haemolysis in G6PD-deficient individuals		18	Y				
	Rasburicase	Risk of haemolysis in G6PD-deficient individuals.		2	Y	Y			
	Trametinib	Patients with a known history of G6PD deficiency excluded from some clinical trials.		19	Y				
HLA-A	Ipilimumab		Trials enrolled only patients with HLA-A*0201 genotype which facilitates immune presentation of the investigational peptide.	20	Y				
HLA-B	Pazopanib	HLA-B*57:01 carriage associated with hepatotoxicity (alanine transaminase >3 × upper limit of normal)		21	Y				
HLA-DQA1, HLA-DRB1	Lapatinib	HLA-DQA1*02:01 and DRB1*07:01 carriage associated with hepatotoxicity (alanine transaminase >5 × upper limit of normal)		22,23	Y				
HLA-DRB1	Asparaginase	HLA-DRB1*07:01 associated with increased risk of hypersensitivity		24					
NUDT15	Mercaptopurine	Predisposition to myelosuppression and substantial dose reduction for individuals with homozygous NUDT15 deficiency. (Asian/Hispanic ethnicity)		25	Y	Y			
	Thioguanine	Predisposition to myelosuppression and reduce initial dose in individuals with homozygous NUDT15 deficiency. (Asian/Hispanic ethnicity)		25	Y	Y			
PNPLA3	Asparaginase, cyclophosphamide, daunorubicin, vincristine	Increased risk of hepatotoxicity carriers of rs735409 C-allele in paediatric patients with acute lymphoblastic leukaemia.		24					
RARG	Doxorubicin/daunorubicin			26				Y	

TABLE 1 (Continued)

Gene	Drug	Safety	Efficacy	Ref	Included in FDA drug label	Clinical guidelines			
						CPIC	DPWG	CPNDS	FNPGx
SLC28A3	Doxorubicin/daunorubicin	rs2229774 A-allele associated with increased risk of cardiotoxicity							
		rs7853758 A-allele associated with decreased risk of cardiotoxicity		26				Y	
SLCO1B1	Methotrexate	rs4149056 C-allele associated with decreased clearance in children with precursor cell lymphoblastic Leukaemia–lymphoma		27					
		Decreased risk of nephrotoxicity in rs4149056 (*5) CC carriers vs CT +TT.		28					
TPMT	Cisplatin	Increased risk of ototoxicity		29	Y				
	Mercaptopurine	Predisposition to myelosuppression and substantial dose reduction for individuals with homozygous TPMT deficiency.		25	Y	Y	Y		
	Thioguanine	Predisposition to myelosuppression and reduce initial dose in individuals with homozygous TPMT deficiency.		25	Y	Y	Y		
UGT1A1	Belinostat	Decreased clearance in *28/*28 individuals increase risk of dose-limiting toxicity		30	Y				
	Binimetinib	UGT1A1 genotype does not have a clinically important effect on binimetinib exposure.		31	Y*				
	Irinotecan	*28 allele carriage associated with increased risk of grade 3/4 diarrhoea and neutropenia.		32,33	Y		Y		
	Nilotinib	*28 homozygosity associated with increased risk of hyperbilirubinemia		34	Y				
	Pazopanib	*28 homozygosity associated with increased risk of hyperbilirubinemia		35	Y				Y
UGT1A6	Doxorubicin/daunorubicin	UGT1A6*4 *rs17863783 T-allele associated with increased risk of cardiotoxicity						Y	

ADR = adverse drug reaction.
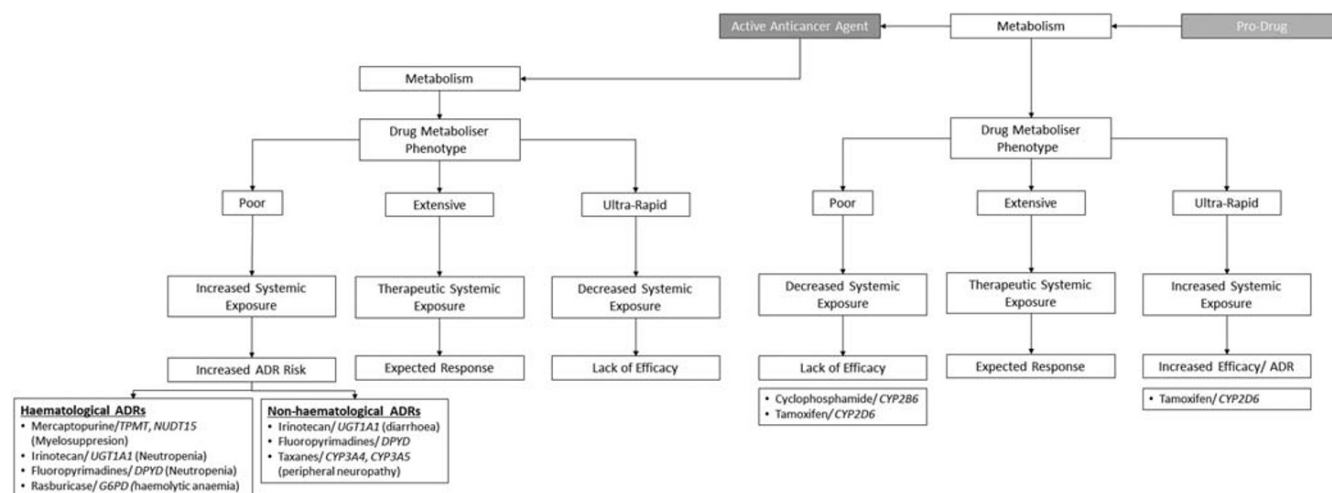
FIGURE 1 Schematic demonstration of variable outcomes of pharmacologically active oncology drugs and prodrugs as determined by metaboliser phenotype status for key drug metabolising enzymes. ADR = adverse drug reaction

TABLE 2 Studies reporting frequencies of actionable pharmacogenomic variants relevant to oncology drugs

		% population with actionable PGX variant				
	(n)	**UGT1A1 (PM)**	**TPMT (PM/IM)**	**DPYD (PM/IM)**	**G6PD deficiency**	**CYP2D6 (PM)**
		Irinotecan	Mercaptopurine	Capecitabine, fluorouracil	Rasburicase	Tamoxifen
Chanfreau-Coffinier et al 2019[37]	7 769 359	11.2	5.8	0.9	4.9	-
Bank et al 2019[38a]	n/a	-	15.5	-	-	5.0
Van Driest et al 2014[39]	9589	-	9.1	-	-	
Reisberg et al 2019[40]	44 000	12.3-13.1	6.4	0.9	-	4.1
Mostaf et al 2018[41]	5408	-	-	-	-	5.7 2.8

PM = poor metaboliser; IM = intermediate metaboliser; UR = ultra-rapid metaboliser.
[a]Estimates based on percentage actionable phenotypes for count incident prescriptions of specific drug.

another 17 residues where variants altered NUDT15 activity without affecting protein stability. As more patients have whole genome sequencing, the data generated by Yang and colleagues[50] will become valuable in taking into account all potential variants that may affect enzyme activity and the need to individualise dose. However, the complexity of dosing for individual patients should not be underestimated.

Whilst the influence of inherited *TPMT* dysfunction on the risk of thiopurine-induced intolerance is of greater importance in individuals of European or African ancestry, *NUDT15* risk alleles seem to be more important in those of Asian and Hispanic ethnicity. Reports of individuals who are intermediate metabolisers for both TPMT and NUDT15 have been reported (compound intermediate metabolisers). The 2 genes are independent of each other and the incidence of carriers of reduced function alleles of both will depend on population admixture. Therefore, in the individualisation of 6-mercaptopurine dose in the future, both genes should be evaluated irrespective of ethnicity as highlighted by the recent CPIC guideline.[25]

2.2 | *DPYD* and fluoropyrimidines

The fluoropyrimidines, 5-fluorouracil (5-FU) and its oral prodrugs, capecitabine and tegafur, are indicated for the treatment of colorectal cancer, breast cancer and other gastrointestinal tract cancers. 5-FU has a narrow therapeutic index and, although generally tolerated, 10–30% of patients develop severe (grade ≥3) toxicity that can result in prolonged hospitalisation, or death in 0.5–1% of patients.[51–53] Fluoropyrimidine adverse events include neutropenia, diarrhoea, stomatitis and hand–foot syndrome.[52]

The rate-limiting enzyme for 5-FU catabolism is dihydropyrimidine dehydrogenase (DPYD), encoded by a gene located on the short arm of chromosome 1, a phase I enzyme that metabolises ~80% of 5-FU into noncytotoxic dihydrofluorouracil.[54] Assays to determine DPYD enzymatic activity in peripheral blood mononuclear cells have been developed. DPYD phenotype can also be determined by the dihydrouracil/uracil (UH_2/U) plasma ratio or the uracil loading test.[15,54] DPYD activity follows a normal distribution[55]; approximately 3–8% of patients, depending on ethnicity, have partial DPYD

deficiency,[56] which increases 5-FU exposure by 1.5 times relative to normal DPYD activity.[57] Complete DPYD deficiency is rare with a prevalence of 0.1–0.2%, but can lead to fatal toxicities following exposure to standard doses of 5-FU.[53,56,58] DPYD activity is regulated by genomic, transcriptional (Sp1 and Sp3 transcription factors) and post-transcriptional (microRNAs-27a and -27b) factors.[59,60] For instance, hypermethylation of the promoter region of the DPYD gene has been identified,[61] but whether it affects expression and thereby predisposition to 5-FU toxicity is unclear.[62]

Genetic variation in the DPYD gene has been extensively investigated. Importantly, a meta-analysis identified 4 DPYD variants to be strongly associated with 5-FU-associated toxicity: DPYD*2A (rs3918290, c.1905+1G>A), DPYD*13 (rs55886062, p.I560S, c.1679T>G), rs67376798 (p.D949V, c.2846A>T), and rs75017182 (HapB3, c.1129–5923C>G).[53] In particular, genetic associations have been found for haematological and gastrointestinal toxicities, but not with hand–foot syndrome.[53] DPYD*2A leads to skipping of exon 14 and a nonfunctional DPYD protein, DPYD*13 and rs67376798 are missense variants, and rs75017182 (HapB3) in intron 10 introduces a cryptic splice site.[15] Patients with wild-type DPYD are assigned an activity score (AS) of 2. DPYD*2A and DPYD*13 have the most deleterious impact on DPYD activity and so heterozygotes are designated an AS of 1, and homozygotes/compound heterozygotes an AS of 0. Variants rs75017182 and rs67376798 are thought to moderately reduce DPYD activity and so heterozygotes are given an AS of 1.5.[56] In European populations, rs75017182 (HapB3) is the most common of these variants with a Minor Allele Frequency (MAF) of ~1–4%[63]; overall, ~7% of Europeans carry at least 1 reduced function DPYD variant.[15] The reduced function missense variant, rs115232898 (p. Y186C, c.557A>G), occurs in 1–4% of individuals of African ancestry.[64] A recent study in 1254 patients has also suggested DPYD*6 (rs1801160, p.V732I, c.2194G>A), whose MAF is 1–9% depending on the population, may be associated with 5-FU toxicity,[65] although replication is required. The majority of other recognised deleterious DPYD variants are rare.[15]

Fluoropyrimidine guidelines based on the 4 established DPYD variants have been developed by CPIC and DPWG.[15,56] These guidelines and their online updates are broadly similar, recommending a 50% reduction in starting dose in patients with a DPYD AS of 1–1.5 (heterozygous intermediate metabolisers) and avoiding fluoropyrimidine therapy when possible in those with an AS of 0–0.5 (poor metabolisers). Nevertheless, subtle differences exist between these guidelines. For example, the DPWG guideline contains recommendations for tegafur as well as 5-FU and capecitabine.[56]

Of interest, a smaller starting dose reduction of 25–50% was previously recommended for patients with an AS of 1.5 commencing 5-FU/capecitabine. However, the updated 50% dose reduction was advised following publication of a seminal real-world pharmacogenomics implementation study that enrolled 1181 patients from 17 hospitals in the Netherlands and prospectively genotyped them for DPYD*2A, *13, rs67376798, and rs56038477 (c.1236G>A, which is in perfect linkage disequilibrium with rs75017182[15]).[66] In this study, patients received an initial dose reduction of 50% (in DPYD*2A or *13

heterozygotes) or 25% (rs67376798 or rs56038477 heterozygotes), and were followed up for fluoropyrimidine-related grade ≥3 toxicity compared to a historical (noninterventional) cohort. The relative risk for toxicity (carriers vs DPYD wild-type patients) was 1.31 for genotype-guided dosing in the prospective study but 2.87 in the historical cohort for DPYD*2A carriers, no toxicity (in the 1 carrier) vs 4.30 in *13 carriers, 2.00 vs 3.11 in rs67376798 carriers, and 1.69 compared with 1.72 in rs56038477 carriers.[66] Moreover, rs67376798 carriers still had elevated 5-FU exposure compared to wild-type patients, and there was large variation in DPYD activity in rs56038477 carriers.[66] Thus, the initial dose reduction of 25% for rs67376798 or rs56038477 carriers was plausibly insufficient and larger initial reductions (50% starting dose) with individualised dose titration are now thought preferential.

Despite the strong associations with the above-mentioned DPYD variants, given the complexity of regulatory processes for the DPYD gene, and the occurrence of rare variants, genetic variation only explains up to 30% of the observed early onset 5-FU-associated toxicity.[52] DPYD phenotyping is an alternative or complementary strategy, and has been associated with 5-FU exposure and toxicity, albeit inconsistently.[55,67–69] DPYD phenotyping also has limitations; for instance, the correlation between hepatic and peripheral blood mononuclear cell DPYD activity is modest ($R^2 < .6$),[70] baseline UH2/U ratios probably reflect unsaturated DPYD and so may not always predict decreased DPYD activity,[68] technical expertise is required, and the assay is often only available in specialised centres. Furthermore, lack of assay standardisation and lack of clarity around cut-off levels, which denote risk, represent further limitations for widespread implementation of phenotyping assays.

Multiparametric assessments may be valuable here as shown by a recent nonrandomised multicentre prospective study in patients with colorectal cancer that used preprescription DPYD genotyping, UH_2/U phenotyping and demographic factors such as age and sex to determine risk.[71] Therapeutic drug monitoring (TDM, see later) was also used in patients with identified partial DPYD deficiency to provide further dose optimisation.[71] The study found that the frequency of early 5-FU-based grade 4–5 toxicity using the multiparametric intervention, compared to standard care, was significantly reduced from 4.2 to 1.2% ($P = .0019$).[71] Moreover, the intervention was associated with a borderline significant reduction in the proportion of patients with grade 3–5 toxicity from 17.6 to 10.8% ($P = .0497$).[71] However, this is a highly complex intervention, and whether it is cost-effective or whether it can be implemented more widely, is unclear.

2.3 | G6PD and rasburicase

Rasburicase is a recombinant urate oxidase enzyme administered intravenously and indicated for the prophylaxis and treatment of hyperuricaemia during chemotherapy in patients with haematological malignancy at risk of tumour lysis syndrome. Rasburicase is contraindicated in patients with glucose-6-phosphate dehydrogenase

(G6PD) deficiency, which is the most common enzyme deficiency in humans.[72,73]

G6PD, located on the X chromosome at Xq28 and is ubiquitously expressed.[73,74] It converts glucose-6-phosphate to 6-phosphogluconolactone, which is the first step in the pentose phosphate pathway (PPP), and this step concomitantly reduces nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH.[73] G6PD is the rate-limiting enzyme of the PPP and, in erythrocytes, the PPP is the only source of NADPH, which is required to maintain cellular levels of reduced glutathione.[73–75] Oxidative stress refers to an increase in reactive oxygen species (ROS) that can lead to structural cell damage. ROS are chemically reactive species containing oxygen such as superoxide and hydrogen peroxide and, importantly, reduced glutathione protects cells from oxidative stress by neutralising ROS.[76] However, steep increases in ROS overwhelm cellular antioxidant defences, including glutathione-mediated reduction, and erythrocytes are particularly susceptible to oxidative stress due to their role as oxygen carriers and reliance on G6PD.[75] Oxidative stress can be triggered by many factors including infections, foodstuffs (e.g. fava beans in favism) and specific drugs, for example, primaquine, nitrofurantoin and rasburicase.[77] The oxidation of uric acid to allantoin by rasburicase can lead to oxidative stress through production of hydrogen peroxide.[78]

The majority of reported genetic variants in *G6PD* are missense variants[74]; the lack of frameshift variants and large deletions is consistent with the observation that complete loss of G6PD is fatal *in utero*.[74,79] *G6PD* variants are classified by the World Health Organisation into 5 categories[80]: class I variants are very rare, usually associated with G6PD activity <10% of normal and occur in symptomatic patients with chronic nonspherocytic haemolytic anaemia (CNSHA); classes II and III have G6PD activities of <10% and 10–60%, respectively, but neither are associated with CNSHA and so individuals are asymptomatic most of the time; class IV variants have normal activity, and; class V is reserved for variants with increased activity,[80] although only 1 case has been reported.[73] Class II and III variants are responsible for the majority of G6PD deficiency. It is these asymptomatic patients that are susceptible to oxidative stress following rasburicase exposure and other triggers.[2] The 2 classically recognised variants are the Mediterranean (G6PD^Med, class II) and African American (G6PD^A−, class III) forms.[81]

Overall, around 400 million people are thought to have G6PD deficiency, and it is more common in Africa, Southeast Asia, the Mediterranean and the Middle East.[73] The prevalence of G6PD deficiency correlates with the worldwide distribution of malaria, leading to the hypothesis that G6PD deficiency evolved and is maintained due to selection pressure exerted from *Plasmodium* parasites, with an advantage being conferred to female heterozygotes.[82–85] The main clinical manifestation of G6PD deficiency is haemolytic anaemia, although other presentations include neonatal jaundice, methaemoglobinaemia and CNSHA.[86,87] In patients with G6PD deficiency, rasburicase is associated with haemolytic anaemia and, rarely, concomitant methaemoglobinaemia, which is due to oxidation of haemoglobin iron, leading to methaemoglobin and tissue hypoxia.[87,88]

The majority of affected individuals are male because G6PD deficiency is X-linked and so only 1 class I, II, or III variant is required (hemizygosity). However, females can rarely be homozygous or compound heterozygous for G6PD deficiency.[89] *G6PD* genotyping can be sufficient to establish the diagnosis of G6PD deficiency when specific variants of known functional consequence are detected.[2] However, the absence of specific variants does not preclude G6PD deficiency due to the presence of untested or unrecognised variants, and therefore G6PD phenotyping is often required to establish G6PD deficiency.[2] Moreover, in heterozygous females carrying 1 deleterious variant, G6PD activity is variable due to X-linked chromosome inactivation (lyonisation) giving rise to mosaicism,[90] and so enzyme phenotyping is needed because G6PD activity cannot be determined by genotype alone.[2] CPIC have produced a guideline to efficiently combine genotyping with G6PD phenotyping and, in those with G6PD deficiency in whom rasburicase is contraindicated, an alternative agent such as allopurinol is recommended.[2]

In cases of methaemoglobinaemia associated with G6PD deficiency, including after rasburicase, the main medicinal treatment, methylene blue, is contraindicated due to the risk of exacerbating oxidative stress, which can make management challenging. In this setting, the mainstays of treatment are high flow oxygen, ascorbic acid and blood transfusions.[87,88] Ultimately, as our understanding of the functional impact of *G6PD* variants increases, alongside *G6PD* sequencing or multi-*G6PD* variant panel testing, the contribution of genomics to establishing the diagnosis of G6PD deficiency is anticipated to grow.

2.4 | *UGT1A1* and irinotecan

Deficient expression of uridine 5′-diphospho-glucuronosyltransferase 1A1 (UGT1A1) is well-described in familial syndromes such as Crigler–Najjar (Type I) and Gilbert's syndrome.[91] In the latter, the majority of patients have a genetic variation in the promoter region of the UGT1A1 gene, termed *UGT1A1*28*, which reduces UGT function by about 70%. The frequency of the *28 allele is ∼29–45% in Caucasians, 42–51% in Africans and significantly lower (16%) in Asian populations.[92] Asian patients often have different polymorphisms in the *UGT1A1* gene, such as *UGT1A1*6*, which also have the same effect of reducing UGT1 activity.

Irinotecan is used in the treatment of colorectal and small cell lung cancer. It is a prodrug that is phase I metabolised to its pharmacologically active SN-38 form by carboxylesterases and subsequently glucuronidated to a hydrophilic conjugate by UGT1A1. A common variable nucleotide tandem repeat polymorphism in the *UGT1A1* gene promoter, known as the *28 allele, leads to reduced transcription of *UGT1A1* and lower hepatic enzyme expression. *UGT1A1*28* carriage is associated with impaired glucuronidation of irinotecan and elevated circulating SN-38 levels[32].

Significant evidence exists demonstrating that individuals homozygous for *UGT1A1*28* are predisposed to serious adverse drug reactions (ADRs; neutropenia and diarrhoea) with irinotecan.[93] A

meta-analysis of studies utilising *UGT1A1*28* genotyping in irinotecan-treated Caucasian patients[94] reported an increased risk of irinotecan-induced adverse events in **28/*28* individuals compared to **1*1* with neutropenia with an odds ratio (OR) = 4.79 (95% confidence interval [CI] 3.28–7.01; *n* = 1095) and diarrhoea, OR = 1.85 (95% CI 1.24–2.72; *n* = 1122). Because of this, the Food and Drug Administration amended the irinotecan label in 2004 to advocate dose reduction in **28/*28* carriers, and subsequently revised it to recommend **28* testing prior to irinotecan therapy in 2010. An analysis of the Japanese Biobank showed that UGT1A1**6/*6* genotype increased the risk of irinotecan-induced ADRs (OR = 6.59, 95% CI 2.33–18.6).[95]

As would be expected, dose is also important in predisposing to the serious adverse reactions associated with irinotecan. In a meta-analysis of 821 patients, the risk of toxicity was higher among patients carrying at least 1 *UGT1A1*28* allele when compared with *UGT1A1*1/*1* patients given medium and high doses of irinotecan, but not at lower doses (100–125 mg/m^2), which are in the commonly used therapeutic range.[96] Consistent with this, the French National Network of Pharmacogenetics has proposed no dose reduction in carriers of the *UGT1A1*28* allele when the dose given is <180 mg/m^2/wk, but with a dose reduction of 25–30% in **28/*28* patients when the dose is 180–230 mg/m^2 2–3 weekly and contraindicating use when the dose is ≥240 mg/m^2 2–3 weekly.[97] By contrast, the Dutch Pharmacogenetics Working Group guidelines for *UGT1A1* and irinotecan[98] recommend starting with 70% of the standard initial dose in **28/*28* patients irrespective of dose, but with no dose change in heterozygote patients, and with a dose increase if tolerated, guided by neutrophil count monitoring.

Given that irinotecan is currently largely used in combination therapies and at lower doses, the use of UGT1A1 genotyping is not common.

2.5 | *CYPD6* and tamoxifen

Cytochrome P450 2D6 (CYP2D6) is a key phase I drug metabolising enzyme, thought to metabolise ∼25% of all licensed drugs.[99,100] *CYP2D6* is a highly polymorphic gene and 5-10% of the population carry 2 nonfunctional alleles, and are referred to as CYP2D6 poor metaboliser (PM) while ∼1–30% of the population, depending on ethnicity, carry duplications of functional alleles and are referred to as ultrarapid metabolisers.

Tamoxifen is a selective oestrogen receptor modulator commonly used to both treat and prevent breast cancer. It is metabolised to its active form endoxifen by CYP2D6. For ultrarapid and normal metabolisers, therapeutic levels of endoxifen are typically achieved and these individuals are recommended to commence standard of care dosing (20 mg/d) avoiding concomitant administration of other drugs known to be moderate/strong CYP2D6 inhibitors.[101]

Individuals who are CYP2D6 PMs typically have lower circulating levels of endoxifen than those who are extensive metabolisers,[102] and

have been shown to have reduced efficacy and therefore a worse prognosis.[103] As such, guidelines have been developed that recommend that PMs receive an alternative therapy such as aromatase inhibitors or if contraindicated, an increase in tamoxifen dose to 40 mg/d should be considered.[101] There is, however, controversy due to the inconsistent evidence as to whether pre-emptive *CYP2D6* genotyping actually improves clinical outcomes with some large trials reporting conflicting results.[6]

Controversy also exists as to what therapeutic adjustment should be made in individuals who are normal or intermediate metabolisers since alleles such as *CYP2D6*10*, infer a nonfunctional enzyme rather than reduced function. As such the enzymatic activity score for IMs can vary substantially and subsequently so can the systemic endoxifen levels. Indeed, it has been suggested that therapeutic drug monitoring of endoxifen levels may represent a more accurate means by which to phenotype metaboliser status in order to individualise tamoxifen therapy.[104]

To attempt to address the controversies, the international tamoxifen pharmacogenetics consortium (ITPC)[105] undertook a meta-analysis of 4973 patients from 12 international sites. Using strict eligibility criteria (postmenopausal women with oestrogen receptor positive breast cancer receiving tamoxifen for 5 years), an association between CYP2D6 PM status and worse invasive disease-free survival was determined (hazard ratio = 1.25 (1.06–1.47); *P* = .009). However, the authors did point out that inclusion criteria were not defined *a priori* and so further prospective studies are needed to establish the utility of *CYP2D6* genotyping.

Although much work to standardise patient inclusion criteria, as well as disease and outcome phenotypes, has been undertaken in the intervening years, the clinical utility and benefit of *CYP2D6* genotyping prior to tamoxifen therapy remains contentious.

3 | ASSOCIATIONS WITH A LOWER LEVEL OF EVIDENCE

The drug–gene associations described above are considered to have a comparatively high level of evidence and clinical pharmacogenomic guidelines have been developed for each from at least 1 of the pharmacogenomic guideline writing consortia. In general, it is the body of supportive evidence rather than a pivotal trial that forms the basis of these guidelines in oncology. Nevertheless, several other genes have been associated with adverse reactions to specific oncology drugs, although the evidence is either currently restricted to 1 or a few studies, or is presently inconsistent. Many of these are highlighted in Table 1. Given the lack of space, we have not covered every association between pharmacogenomic variants and drug response, usually toxicity, associated with the individual drugs—readers are referred to the cited references for further detail. Some of these associations are described below to highlight the complexities of identifying clinically relevant associations, and we also hope that this may stimulate further research in these areas.

3.1 | *ABCB1* and chemotherapy toxicity

The adenosine triphosphate (ATP)-binding cassette (ABC) subfamily B (MDR/TAP) member 1 (*ABCB1*) gene encodes P-glycoprotein 1 (P-gp), which is a widely expressed membrane-associated ATP-dependent xenobiotic efflux pump with broad substrate specificity. Examples of P-gp oncology drug substrates include doxorubicin, docetaxel, paclitaxel and vincristine; doxorubicin and vincristine also induce P-gp.[106] Multidrug resistance (MDR) is a major cause of chemotherapy failure in metastatic cancer. It is a multifactorial and incompletely understood phenomenon, but basal and drug-induced P-gp overexpression in cancer cells has been associated with treatment failure in several cancer types,[107] highlighting the importance of P-gp to drug response. *ABCB1* is highly polymorphic, but studies to date have tended to focus on 1 or more of 3 common *ABCB1* variants and/or their haplotypes: c.1236C>T (rs1128503, a synonymous variant), c.2677G>T/A (rs2032582, a missense variant) and c.3435C>T (rs1045642, a synonymous variant). In addition, *ABCB1* c.1199G>A (rs2229109, a missense variant) has been shown to increase in vitro efflux transport of the tyrosine kinase inhibitors dasatinib, imatinib, nilotinib,[108] although its effect on predisposition to tyrosine kinase inhibitor-induced ADRs (particularly gastrointestinal toxicity) has yet to be demonstrated.

Genetic variation in *ABCB1* has been variably associated with cancer survival[109] and ADRs including anthracycline-induced cardiotoxicity,[26] paclitaxel-medicated peripheral neuropathy and neutropenia,[110] and vincristine neurotoxicity[111] in some, but not all studies.[109,112,113] Some of this variability may be attributable to small sample sizes and interethnic differences.[109] However, overall, the evidence is too inconsistent at present to support *ABCB1* genotyping.

3.2 | *CYP2B6* and cyclophosphamide

Cyclophosphamide is an alkylating agent indicated for a range of haematological and solid organ cancers including lymphoma and breast cancer, respectively. It is also used as an immunosuppressive in specific autoimmune diseases and bone marrow transplantation. It is a prodrug that is biotransformed to the intermediate metabolite, 4-hydroxy-cyclophosphamide, by hepatic CYP2B6 amongst other CYPs, which undergoes further nonenzymatic conversion to the therapeutically active metabolite, phosphoramide mustard.[114] In a genetic analysis of patients with chronic lymphocytic leukaemia within a randomised controlled trial, carrying the reduced function *CYP2B6*6* allele was associated with a lower likelihood of achieving a complete response and fewer adverse events in patients on fludarabine plus cyclophosphamide, but not in patients that received fludarabine or chlorambucil alone.[115] Notwithstanding the reduced adverse events, the inferior efficacy signal, if confirmed, suggests cyclophosphamide may be unsuitable in patients carrying *CYP2B6*6* and alternative chemotherapy advisable.

3.3 | *CYP3A7* and CYP3A-metabolised chemotherapeutics

The human CYP3A subfamily consists of CYP3A4, 3A5, 3A7 and 3A43. CYP3A7 is the main foetal hepatic CYP.[116] However, after birth, CYP3A7 expression is downregulated whilst CYP3A4 expression increases. Thus, CYP3A4 is the major adult CYP3A isoform, with adult levels reached around age 1 year.[117] Nevertheless, *CYP3A7* mRNA expression varies extensively and in ~10% of adult livers, CYP3A7 is detectable and contributes 9–36% of total CYP3A protein.[118] The allele, *CYP3A7*1C*, results from ~60 bp of its promoter region being replaced by the corresponding region of the *CYP3A4* adult promoter, and is thus associated with increased hepatic and intestinal CYP3A7 expression.[119,120] Interestingly, a putative interaction of borderline significance ($P_{interaction}$ = .06) has been found between carrying *CYP3A7*1C*, treatment with a CYP3A-substrate chemotherapeutic, and increased mortality in breast or lung cancer and disease progression in chronic lymphocytic leukaemia.[121] These findings are of particular interest because CYP3A metabolises approximately ~30% of clinically used drugs[122] yet, except for *CYP3A5*3* and potentially *CYP3A4*22* with tacrolimus,[123] pharmacogenomic associations within the *CYP3A* locus have proved elusive. This may be because CYP3A4 activity is modulated by multiple interacting genes and inhibition/induction via myriad of environmental factors.[124]

3.4 | *SLCO1B1* and methotrexate

Methotrexate is an antimetabolite used as an anticancer drug, notably in paediatric ALL, and as an immunosuppressant. The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene encodes the hepatic xenobiotic influx transporter, organic anion transporter polypeptide 1B1 (OATP1B1). Candidate gene studies[125–127] and a GWAS[27] in paediatric ALL have identified genetic variants in *SLCO1B1* that are associated with reduced methotrexate clearance. The most important variant is likely to be the reduced function *SLCO1B1* SNP, rs4149056 (c.521T>C, p.V174A), as several of the other identified *SLCO1B1* variants (e.g. rs4149081, rs11045879, rs11045821[125,126]) are in linkage disequilibrium with rs4149056.[27] It is notable that the *SLCO1B1* rs4149056 minor allele is also associated with increased exposure to most statins, and is considered an actionable pharmacogenomic variant for simvastatin myotoxicity.[128] It may also be associated with reduced risk of chemotherapy-induced amenorrhoea.[129]

Interestingly, a SNP–SNP interaction has been observed with methotrexate clearance between rs4149056 and gain-of-function rs2306283 (c.388A>G, p.N130D), which together define the most common *SLCO1B1* haplotypes (*1a, *1b, *5, *15).[27] Within each rs4149056 genotype group, the rs2306283 ancestral A allele is associated with even lower methotrexate clearance.[27] Importantly, methotrexate plasma concentrations have been correlated with increased global methotrexate toxicity.[126] Nevertheless, in adult haematological

malignancies, rs4149056 and rs2306283, have not been associated with methotrexate exposure,[28,130] and variably associated with toxicity.[28,130]

Intriguingly, 70–90% of methotrexate is eliminated in urine, yet OATP1B1 expression is limited to hepatocytes. *SLCO1B1* rs4149056 and rs2306283 variants significantly alter the abundance of specific metabolites in urine. Moreover, these metabolites are substrates for renal organic anion transporters (OATs) such as methotrexate, and half were associated with methotrexate toxicity.[28] This suggests that complex transporter–transporter interactions mediated by endogenous substrates may have a role in methotrexate clearance and toxicity.

4 | PHARMACOGENOMICS IMPLEMENTATION IN ONCOLOGY AND BROADER CLINICAL PRACTICE

Over the past decade, there has been slow but growing implementation of pharmacogenomics into real world practice. Many initiatives have implemented reactive single gene testing in individual healthcare institutions. For example, routine *DPYD* genotyping has been demonstrated to be acceptable and feasible, and to reduce the risk of severe fluoropyrimidine toxicity in implementation initiatives.[57,66,131] Moreover, single gene *DPYD* genotyping has been reported to be highly likely cost saving.[132]

Nevertheless, 99% of the population are estimated to carry at least 1 actionable pharmacogenomic variant within 13 pharmacogenes.[37] This observation has contributed to several initiatives utilising pharmacogene panel testing. For example, the European Ubiquitous Pharmacogenomics consortium has implemented genotyping 44 variants in 12 genes (including *CYP2D6*, *DPYD*, *TPMT* and *UGT1A1*) in a single test for patients starting 1 of 42 drugs and are recruited into the PREPARE implementation research study (ClinicalTrials.gov Identifier: NCT03093818).[133] Whilst patient recruitment and genotyping in PREPARE are reactive to the index drug prescription, the other results are available pre-emptively for future prescribing. Several other initiatives implementing pharmacogene testing have been set up, such as eMERGE,[134] IGNITE[135] PG4KDS[136] and ACCOuNT.[137] This pre-emptive approach is highly relevant in oncology because patients with cancer may have or develop indications for other actionable drug gene pairs: for example, nausea on chemotherapy (*CYP2D6*-ondansetron), pain (*CYP2D6*-codeine/tramadol), anxiety and depression (*CYP2D6* or *CYP2C19*-selective serotonin reuptake inhibitors), and concurrent or future cardiovascular risk prevention (*SLCO1B1*-simvastatin).

Beyond variant genotyping, NGS of specific pharmacogenes represents another strategy with the potential advantage of enabling patient pharmacogenomic results to keep pace with research progress in ascribing function to pharmacogene variants of uncertain significance, without the need for re-testing. Moreover, at least 14 countries have government-funded national

genomic medicine initiatives[138] and so a rapidly increasing number of patients will undergo whole exome and whole genome sequencing over the coming decade, accelerating the availability of pharmacogenomic results. For example, the UK 100 000 Genomes Project has a pilot programme to extract actionable *DPYD* variants from whole genome sequencing data in participants with cancer and make them available to clinicians via regional genomic medicine centres.[139]

Nevertheless, pharmacogenomic implementation remains arduous and complex with a need for multidisciplinary team working and stakeholder engagement to surmount the multiple barriers that include evidential, healthcare practitioner knowledge, financial and logistical.[140] However, the experiences learned from early adopter sites will help facilitate broader implementation.[141] One specific challenge is the inherent complexity of particular pharmacogenes, and *CYP2D6* in particular. *CYP2D6* can be affected by structural variations including gene deletion, multiplication, and tandem rearrangements or hybrid gene conversions with its upstream pseudogene, *CYP2D7*.[142] These structural variants impede accurate *CYP2D6* genotype-to-phenotype translation by conventional methods and standard short read sequencing.[143] However, long-read sequencing has been demonstrated to accurately genotype and phase *CYP2D6* and so offers a promising way forward.[142,144]

A second major challenge is the introduction of clinical decision support (CDS) systems, which are essential to support preemptive pharmacogenomic testing.[141] CDS can be passive, relying on the user to seek out the recommendations, or actively interrupt healthcare practitioners with automatic alerts. CDS can also either be integrated into existing information and communications technology (ICT) infrastructure, such as electronic healthcare records, or provided in separate programs such as web services, and patient-held healthcare *safety-code cards* or mobile applications.[145] An ideal system provides up-to-date recommendations when prescribing (or dispensing) a new drug to maximise uptake of recommendations, is user-friendly, a gateway to resources for impromptu user-directed learning, and recalls previous test results automatically when prescribing in future to avoid genetic re-testing. Thus active, interruptive CDS systems appears advantageous, providing alerts are judicious to mitigate alert fatigue. However, the heterogeneity of healthcare ICT systems and financial resources available for integration represent significant hurdles to broader adoption. It is expected that local solutions will be required, and hybrid models that variably implement through both central ICT infrastructure and patient-held devices might expedite implementation by decreasing reliance on any 1 system.

Lastly, the availability of consensus guidelines is paramount for successful implementation. Whilst CPIC and DPWG guidelines are an excellent resource and share a high degree of congruence, some differences exist between their recommendations.[146] Furthermore, existing guidelines offer little guidance on *when* to order genetic tests, may need translating, and potentially adapting to best fit a regional/national healthcare setting.

5 | MOVING BEYOND COMMON VARIANT AND SINGLE GENE PHARMACOGENOMICS

To date, the majority of ADR pharmacogenomic associations in oncology and other specialties relate to select, predominantly common, variants in a single germline gene. However, advances in technologies, sample sizes, and data processing mean that pharmacogenomics will be likely to evolve to encompass rare genomic variation, polygenic risk scores and pharmacomicrobiomics, and complement TDM.

5.1 | Rare variation

The first observations in the 20th century that ADRs could have a genetic basis were arguably in anaesthetics with malignant hyperthermia[147] and prolonged apnoea[148] following exposure to volatile anaesthetics or succinylcholine, respectively. These ADRs are rare and potentially life threatening. Subsequently, rare gain-of-function mutations in ryanodine receptor 1 (RYR1) or, to a lesser extent, the calcium voltage-gated channel subunit α1 S (CACNA1S) have been identified in individuals affected by malignant hyperthermia.[149,150] Pseudocholinesterase deficiency increases the risk of clinically relevant prolonged apnoea and can be acquired, or inherited in individuals that receive 2 reduced function butyrylcholinesterase (BChE) alleles.[151] Thus, these early examples highlight the importance of rare variants/genotypes in drug response.

Rare variants are generally defined as variants with a minor allele frequency of <1%.[152] Recent genetic epidemiological research has demonstrated that 93% of single nucleotide variants are rare in 146 pharmacogenes that influence drug pharmacokinetics.[153] Moreover, individuals of European and African ancestry harbour, on average, 101 and 121 single nucleotide variants within these 146 pharmacogenes, respectively.[153] Importantly, the contribution of rare and common variation to the putative function of individual pharmacogenes varies substantially between genes and overall, up to 30-40% of genetic-mediated functional variation in pharmacogenes might be attributable to rare variants.[153,154]

In oncology, germline rare variants in SLCO1B1 identified by deep resequencing have been associated with methotrexate clearance in paediatric ALL, in addition to common SLCO1B1 variation.[127] In total, a third of observed variability in methotrexate clearance in these ALL patients could be explained: 22.7% by clinical covariates and 10.7% by SLCO1B1 genotypes, of which about a fifth was attributable to rare variants.[127]

Deleterious germline rare variants in CYP3A4 have also been associated with increased frequency and severity of paclitaxel-induced peripheral neuropathy and increased treatment modifications due to peripheral neuropathy.[11] Specifically, whole-exome sequencing identified a CYP3A4*20 (premature stop codon) carrier and a novel CYP3A4*25 (deleterious missense variant) carrier from 8 patients with severe neuropathy; subsequent CYP3A4 variant screening by denaturing high-performance liquid chromatography in 228 paclitaxel-exposed patients found 3 more CYP3A4*20 carriers and a carrier of

each of CYP3A4*8 and CYP3A4*27 (deleterious missense variants).[11] Similarly, exome sequencing a patient who had suffered severe (grade 4) toxicity after the first cycle of 5-FU based adjuvant chemotherapy for colorectal cancer identified a novel splicing variant (c.321+2T>C) in DPYD.[155] As the patient was heterozygous, her 5-FU chemotherapy was restarted at a lower dose (30%) with subsequent titration, and she completed the whole chemotherapy course.[155]

As NGS is applied to larger and more ethnically diverse cohorts, the panoply of identified rare variants will continue to grow. However, functional characterisation of these variants remains challenging, with most being classified as variants of uncertain significance. Most computational tools for predicting the function of exonic variants were calibrated on variants associated with disease and rely on evolutionary conservatism, yet many pharmacogenes are poorly conserved.[156] Thus, a new optimised computational framework that integrates several algorithms has recently been developed and validated using experimental activity data from 337 variants in 43 pharmacogenes, and was shown to significantly outperform existing bioinformatics prediction algorithms.[156] Furthermore, state-of-the-art saturation mutagenesis and massively parallel functional assays has recently been applied to NUDT15, demonstrating the potential of high-throughput functional screening,[157] as outlined above. Briefly, a mutagenesis library of 3077 missense variants was constructed, representing 99.3% of all possible amino acid substitutions across the 163 residues of NUDT15. The in vitro functional effects of each variant on protein abundance and thiopurine toxicity were separately tested; overall, of the 2844 variants successfully analysed in both assays, 1103 variants were identified as damaging. In 2398 patients treated with thiopurines, 10 NUDT15 missense variants were identified, of which 6 were novel and rare. Importantly, the in vitro functional activity of these variants accurately predicted which alleles were associated with thiopurine toxicity with 100% sensitivity and specificity, in contrast to the relatively poor performance of conventional bioinformatic algorithms.[157]

These studies collectively demonstrate the abundance of rare variation in pharmacogenes, the enrichment of rare deleterious variants in patients with extreme phenotypes, and the novel approaches being developed to predict and empirically assess the functional effects of rare variants on gene products. It will be crucial to utilise the large scale genomic-medicine programmes active throughout the world,[138] with many incorporating NGS and patients with cancer, coupled to high throughput functional testing, to advance our understanding of rare variant pharmacogenomics in oncology.

5.2 | Polygenic risk scores

The identification of increasing numbers of variants of low effect size with common conditions has paved the way for polygenic risk scores (PRS) that combine variants, typically weighted on their effect size, to improve discriminative capability. A recent PRS in coronary artery disease, for example, consisted of 1.7 million variants and had higher concordance between model-based risk

estimates and observed incident events than any of 6 traditional cardiovascular risk factors.[158] In oncology, PRS have been recently developed for at least 12 different cancer traits including breast, prostate and skin, with the number of SNPs ranging from 5 to 313.[159,160] Such complex disease PRS appear to predict disease risk particularly accurately at the extremes of the risk distribution.[159]

Hitherto, there has been little investigation into pharmacogenomic PRS. This is likely to be due in part to the effect sizes for many single gene–drug associations being large compared to those of complex disorders, plausibly due to limited evolutionary selection pressure on these variants.[140] Thus, single gene/variant associations can be clinically actionable by themselves and directly adopted into guidelines.[146] It is also notable that cohort sizes for studying common disease genetics have rapidly grown, reflecting the recognised underlying genetic complexity of these diseases. Whilst such cohorts are well suited to development of disease PRS, the quality of drug utilisation and drug response phenotypic data in these cohorts is heterogeneous and can make pharmacogenomic investigations particularly challenging. The recent introduction of primary care data including medications into UK Biobank should help address this. Nevertheless, in cardiology, for example, a PRS of 61 common variants was a significant predictor of drug-induced torsade de pointes.[161] Moreover, in patients with advanced breast cancer in a clinical trial of paclitaxel, nab-paclitaxel and ixabepilone (microtubule targeting agents), a set of 13 variants increased the area under the receiver operating curve for progression-free survival from 0.64 to 0.81.[162] It has also been shown that the cumulative incidence of venous thromboembolism in patients with breast cancer is independently increased by chemotherapy and a PRS consisting of 9 genetic SNPs. Importantly, the influence of chemotherapy and high PRS (>95th percentile) were additive, and being in the older age stratum added further venous thromboembolism risk.[163] These examples demonstrate the potential of PRS to predict ADRs and drug effectiveness, and so their prominence in pharmacogenomics is likely to grow.

5.3 | Pharmacomicrobiomics

Commensal microorganisms (the microbiota) have evolved into a diverse array of specialised lineages that form microbial communities on all the surface barriers of our bodies.[164] The microbiota and its larger host represent a meta-organism, where crosstalk between the host's immune system and the microbiota have co-evolved multiple mechanisms for maintaining homeostasis.[165] The gut microbiome of the large intestine is particularly abundant and diverse. Importantly, there is growing recognition that gut microbiota can influence the efficacy and toxicity of drugs through several mechanisms including metabolism, immunomodulation, translocation, and reduction in microbiome diversity.[165]

Irinotecan can cause both acute and delayed (over 24 hours after administration) toxicity. Whilst acute diarrhoea is attributable to cholinergic stimulation, the gut microbiome is implicated in delayed-type irinotecan diarrhoea.[166] The major route of irinotecan excretion is via faeces. Interestingly, UGT1A1-glucuronidated SN-38 (SN-38G) can be deconjugated by secreted bacterial β-glucuronidase back to active SN-38 in the gut lumen. Free intestinal SN-38, derived from either intestinal deconjugation or direct biliary elimination of SN-38, is thought responsible for irinotecan delayed diarrhoea.[166] Bacterial β-glucuronidase inhibitors have been developed and shown to protect mice from irinotecan-induced colonic damage and diarrhoea without adversely affecting plasma SN-38 levels.[167,168] Furthermore, 2 distinct faecal metaboliser phenotypes (high vs low) have been identified from healthy volunteer stool samples, based on ex vivo incubation with SN-38G.[169] Subsequent clinical studies that correlate cancer patient faecal β-glucuronidase activity with irinotecan toxicity endpoints are now required.

The gut microbiome has also been associated in preclinical models with decreased methotrexate toxicity and increased oxaliplatin-induced peripheral neuropathy. Like irinotecan, methotrexate can cause severe gastrointestinal toxicity. Interestingly, genetic knockout of toll-like receptor 2, or microbiota depletion with antibiotics, resulted in more severe methotrexate-mediated intestinal mucositis in mice.[170] Toll-like receptor 2 stimulation in myeloid cells increased P-gp synthesis and drug-efflux activity,[170] and may reduce gastrointestinal toxicity by decreasing intracellular methotrexate accumulation. Germ-free mice, and temporary eradication of gut microbiota using antibiotics has also been associated in mice with decreased oxaliplatin-induced hyperalgesic pain.[171]

Immunotherapy using monoclonal antibody immune checkpoint inhibitors (ICIs) that overcome cancer-mediated immune suppression represent a pivotal breakthrough in cancer therapeutics. However, not all patients benefit from ICIs and some experience severe immune-related adverse events.[172] Thus, there is considerable interest in biomarker identification for treatment stratification. The gut microbiome has been implicated in both ICI efficacy and toxicity.[173] For example, in 26 patients with metastatic melanoma receiving ipilimumab, which targets cytotoxic T-lymphocyte–associated antigen 4, baseline microbiota enrichment in *Faecalibacterium* and other Firmicutes was associated with both longer survival and more frequent ipilimumab-induced colitis, compared to microbiota driven by Bacteroides.[174] A recent seminal case series of ICI-colitis successfully treated with faecal microbiota transplantation provides preliminary evidence that modulating the gut microbiome may overcome ICI-colitis.[175]

Overall, these examples highlight the growing need to characterise the microbiome of patients receiving chemotherapeutics to identify novel factors predictive of toxicity and gain greater mechanistic insight. These approaches should aid treatment stratification and/or development of novel interventions to mitigate chemotherapeutic toxicity. Given the significant gastrointestinal safety profile of many cancer drugs, this currently represents an area of significant unmet need.

5.4 | TDM

The exposure and response to most drugs is influenced by multiple factors. Clearly, the importance of genomics to drug efficacy and toxicity varies between drugs and outcomes, and so application of pharmacogenomics (or pharmacomicrobiomics) will not be feasible for several drugs. TDM is another strategy for medicines optimisation. TDM could complement preprescription pharmacogenomics recommendations through early dose refinement, or be used on its own where pharmacogenomic recommendations for a drug do not exist.

Drugs with extensive interindividual variation, narrow therapeutic window, severe ADRs, and where the majority of pharmacological activity is attributable to 1 analyte, are particularly well suited for therapeutic monitoring. In particular, CYP3A metabolic function varies 30–40-fold,[176] yet, as mentioned above, the major adult isoform, *CYP3A4*, is generally regarded to lack common genetic variants of large effect size, in contrast to other drug-metabolising CYPs such as CYP2D6, CYP2C9 and CYP2C19. Interestingly, the oral angiogenesis inhibitor, pazopanib, is partially eliminated by metabolism mainly by CYP3A4[177]; determination of pazopanib plasma concentrations in patients with renal cell carcinoma may help optimise systemic exposure for efficacy whilst decreasing the risk of specific ADRs including diarrhoea, hand–foot syndrome and stomatitis.[178] Other examples where therapeutic drug monitoring can improve the benefit-risk profile include the CYP3A4 substrate, imatinib,[179,180] high-dose methotrexate[181,182] and 5-FU therapy.[183,184] There are challenges for TDM, however, which include its lack of broad availability due to the need for specialised assays and equipment, and incompletely defined exposure–response relationships.

6 | CONCLUSION

Pharmacogenomic germline variation is common and can influence the response to anticancer drugs, both efficacy and safety. In particular, there are a number of pharmacogenomic variants that which have been associated with an increased risk of serious ADRs. Although the number of pharmacogenetic variants that have been implemented into clinical practice is small, as genomics data become more widely available, there will be an increasing need to consider pharmacogenetic variants, both common and rare, and whether they should be utilised to improve prescribing, both dose and choice of drug, in cancer treatment. Clearly this cannot be used in isolation, and must be used in combination with somatic genotypes, and clinical factors (such as age, renal function, hepatic function and concomitant drugs). Furthermore, additional technologies such as microbiomics and therapeutic drug monitoring, may also be of use with certain drugs. This inevitably makes the treatment of patients with cancers more complex—arguably this may not be a problem in oncology because most oncologists are already highly practiced in complex therapeutics. Nevertheless, computerised decision support systems will probably be needed in the future to reduce the problem of prescribing errors, and to aid the implementation of pharmacogenomics into clinical practice. It is important to point out that while oncology is regarded as the poster child for precision medicine, this has largely been based on improving efficacy. True precision medicine in oncology should address both efficacy and safety in the same patient.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

COMPETING INTERESTS

There are no competing interests to declare.

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