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REVIEW

Germline oncopharmacogenetics, a promising field in cancer therapy

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Abstract Pharmacogenetics (PGx) is the study of the relationship between inter-individual genetic variation and drug responses. Germline variants of genes involved in drug metabolism, drug transport, and drug targets can affect individual response to medications. Cancer therapies are characterized by an intrinsically high toxicity; therefore, the application of pharmacogenetics to cancer patients is a particularly promising method for avoiding the use of inefficacious drugs and preventing the associated adverse effects. However, despite continuing efforts in this field, very few labels include information about germline genetic variants associated with drug responses. *DPYD*, *TPMT*, *UGT1A1*, *G6PD*, *CYP2D6*, and *HLA* are the sole loci for which the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) report specific information. This review highlights the germline PGx variants that have been approved to date for anticancer treatments, and also provides some insights about other germline variants with potential clinical applications. The continuous and rapid evolution of next-generation sequencing applications, together with the development of computational methods, should help to refine the implementation of personalized medicine. One day, clinicians may be able to prescribe the best treatment and the correct drug dosage based on each patient's genotype. This approach would improve treatment efficacy, reduce toxicity, and predict non-responders, thereby decreasing chemotherapy-associated morbidity and improving health benefits.

Keywords Germline variants · Oncology · Polymorphisms · Oncopharmacogenetics · Pharmacogenetics

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1 Introduction

Since the completion of the Human Genome Project, a great deal of research has focused on the impact of genetic polymorphisms on human health. Polymorphisms encompass many types of variation in DNA sequence, including SNPs (Single Nucleotide Polymorphisms), small insertion/deletions, inversions, mini- and microsatellite variations, and CNVs (Copy Number Variations). SNPs are the most common type of polymorphism, with approximately 10 million SNPs distributed throughout the human genome [1]. When SNPs are located within the transcribed portions of genes or in regulatory regions, they may affect the transcription or the function of encoded proteins and thereby impact health.

SNPs are being extensively investigated in multiple branches of human genetics, including linkage analyses, forensic tests, complex disease susceptibility, and individual drug responses. In particular, determining the genetic basis of drug response, a major challenge for personalized medicine, involves investigation of polymorphisms that could alter the activities of proteins involved in drug pharmacokinetics and pharmacodynamics. A great deal of information is available regarding the relationship of drug efficacy and toxicity to

polymorphisms in genes that encode molecular targets or proteins involved in drug metabolism. The study of inter-individual genetic variability related to the drug response is called pharmacogenetics (PGx); today, this type of investigation is considered essential for the optimization of personalized therapy.

PGx testing is particularly suitable for cancer treatments, with the goal of avoiding the use of drugs with low efficacy and preventing adverse effects. This is especially important because many cancer therapies are characterized by intrinsically high toxicity and low efficacy. Oncopharmacogenetics (onco-PGx) is the application of PGx in oncology. Using this approach, cancer treatments can be personalized and tailored for each patient, potentially leading to improved therapeutic benefits and less severe side effects. Onco-PGx is intimately connected with therapeutic drug monitoring (TDM), the measurement of administered drugs in biological samples taken from each patient at designated intervals. TDM aims to ensure that the concentration of the active principle in the bloodstream is maintained at constant levels, thereby optimizing the individual dosage regimen [2]. In daily clinical practice, the integration of TDM with the study of inter-individual variability in drug response, defined by onco-PGx testing, could play a pivotal role in facilitating the design of effective individualized therapies.

The integration of germline pharmacogenetic screening into drug development, TDM, and clinical practice is facilitated by the availability of high-throughput genotyping methods for genome-wide association studies (GWAS) and the development of computational methods [3, 4]. Ideally, by performing PGx tests, clinicians can prescribe the best treatment and the correct drug dosage to improve efficacy, reduce toxicity, and predict non-responders, ultimately leading to reductions in decreasing chemotherapy-associated morbidity and improving health benefits.

This review summarizes the progresses achieved in onco-PGx, focusing on the importance of the study of germline polymorphisms in the optimization of cancer therapy. In particular, we provide an overview of the few germline onco-PGx markers currently approved by institutions such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), and therefore currently assessed by physicians before prescribing drugs (e.g., polymorphisms of *DPYD* for fluoropyrimidine-based treatment, or TPMT variants for thiopurine treatment). Furthermore, we also describe some of the many potential germline onco-PGx variants with promising clinical applications that have not yet been approved by the public health community (e.g., *CYP2D6* for tamoxifen); due to conflicting or insufficient data about the clinical utility of these variants, additional proof will be required before they can be included in official recommendations for testing.

2 Overview and limitations of germline onco-PGx

The high toxicity of chemotherapeutic drugs and their unpredictable efficacy are often responsible for severe adverse events, treatment discontinuation, and therapy failure. Germline onco-PGx is a promising approach that opens new opportunities for the identification of inherited susceptibility to side effects or low treatment efficacy. However, despite continuing effort in this field, to date only a few labels include information about germline genetic variants associated with drug responses. In the case of targeted therapies, which are designed and developed on the basis of the presence of very precise molecular markers in a specific subset of cancers, somatic mutation identification is necessary to optimally prescribe targeted therapies. Indeed, recognition of these somatic alterations often represents the starting point for the oncologist's decision regarding whether to prescribe a targeted therapy. Examples include the monoclonal antibodies cetuximab and panitumumab, prescribed for colon cancers with wild-type KRAS that overexpress EGFR [5], and crizotinib, an inhibitor of rearranged ALK that is used to treat non–small cell lung cancer (NSCLC) [5]. These drugs are inefficient in patients whose cancer cells do not contain these mutations; therefore, molecular characterization is routinely performed for cancers for which such targeted therapies have been approved. A complete list of somatic mutations investigated for targeted therapy prescription has been compiled by EMA [5, 6]. In addition, both somatic genetic and epigenetic variants are currently used to predict drug efficacy, e.g., *MGMT* methylation is evaluated to predict the efficacy of temozolomide against glioblastoma [7].

Because the number of genetic markers useful for PGx is growing rapidly, it became necessary to create specific databases that must be continuously updated (Table 1). One of these databases, the Pharmacogenomics KnowledgeBase (PharmGKB) [8], is a comprehensive archive that is constantly updated by the NIH Pharmacogenomics Research Network. PharmGKB contains information not only from the EMA and US FDA, but also from other organizations and medicine agencies around the wordl that provide guidelines for gene testing, therapy, and clinical practice, such as CPIC (Clinical Pharmacogenetics Implementation) [9] and DPWG (Dutch Pharmacogenetics Working Group) [10].

Using the information compiled in PharmGKB, we selected the small number of anticancer drugs for which the EMA and US FDA have approved label indications related to germline genotyping of genomic loci associated with drug outcomes (Table 2). These validated onco-PGx genes are as follows: i) *DPYD*, dihydropyrimidine dehydrogenase (DPD), which is responsible for the degradation of 5-fluorouracil and its prodrugs, such as capecitabine [11]; ii) *TMPT*, thiopurine methyltransferase, which catalyzes the inactivation of 6-mercaptopurine or thioguanine via S-methylation [12]; iii)

Table 1 List of useful free websites for PGx and drug response investigations

Website	Information
www.pharmgkb.org	Database that contains all information for PGx variants. It collects data from US FDA and EMA and from other organizations (i.e.: CPIC and DPWG)
www.fda.gov	US FDA protects and promotes the public health by assessing the safety and efficacy of drugs, biological products, medical devices, and environmental components with an impact on health
www.ema.europa.eu	European Medicine Agency is responsible for the scientific evaluation of all drugs used in the Europe Union
www.ereasmumsc.nl/farmacogenetica	Netherland expertise centrum for PGx, linked to Erasmus MC University
www.aruplabs.com	Arup laboratories are reference laboratories in clinical and pathology from the University of Utah. The website contains information also about genetic tests and oncology treatments
www.drugsinteractions.com	Website useful to check multi-drug interactions including food and supplements
www.bioinformatics.charite.de/supercyp	Comprehensive database on Cytochrome P450 enzymes that up to now contains about 1,170 drugs, 2,785 Cytochrome-Drug interactions and about 1,200 alleles; including also a tool to predict CYP-drug interactions
www.cypalleles.ki.se	Human Cytochrome P450 (CYP) Allele Nomenclature Database
http://www.imh.liu.se/tpmtalleles	TPMT nomenclature committee

UGT1A1, uridine diphosphate glucoronosyltransferase 1A1, which promotes the conjugation of the highly active irinotecan metabolite SN-38 to the less active SN-38 glucuronide (SN-38G) [13] (the labels of pazopanib, erlotinib, nilotinib and regorafenib also contain information on *UGT1A1* genotype); iv) *G6PD*, glucose 6-phosphate dehydrogenase, genotyped for patients treated with dabrafenib and rasburicase; v) major histocompatibility complex MHC, which is investigated because germline polymorphisms in class II HLA alleles can promote hepatotoxicity and liver injury [14]; and vi) *CYP2D6*, which catalyzes the metabolism of approximately 25 % of all drugs currently used [15], but for which only the gefitinib label bears warnings.

Although the scientific literature is constantly being supplemented with data regarding altered drug responses in the presence of the genetic variants reported on labels, the EMA and US FDA require PGx tests for only two drugs, 6-mercaptopurine and rasburicase (Table 2). By contrast, for other drugs, information about altered drug responses is contained in label warnings, but no PGx testing is mandated. Furthermore, the efficacy of many other anticancer drugs is affected by inter-individual constitutional variability, but the difficulties in defining approved germline variants are often due to discrepancies in the results of studies aimed at assessing their impact on drug response [15]. The causes of these discrepancies may be related to the absence of standardized methods in PGx studies, which makes it challenging to replicate data. For example, the sample sizes of investigated populations, the lack of appropriate controls, the penetrance of variants, and the choice of biologic materials can influence the results of PGx association studies.

In particular, the genotyping of germline polymorphisms on DNAs from tumor tissues rather than constitutional ones (e.g., blood and saliva) can lead to inconclusive results, because DNA in cancer cells very frequently exhibits genomic instability, CNVs, rearrangements, and mutations; consequently, the germline genetic constitution is not fully maintained during oncogenic transformation.

Another important factor that influences the consistency of germline PGx studies is related to the intrinsically complex and multigenic nature of the personal response to oncologic drugs. Currently, genome-wide approaches based on next-generation sequencing (NGS) technologies enable us to pinpoint multiple loci frequently involved in drug responses [16]. However, these studies are not replicated very often, because the penetrance of each variant can be modified not only by the presence of other polymorphisms but also by environmental factors (e.g., chemical/drug interactions, diet, or lifestyle) that interfere with the activities of proteins involved in drug metabolism. For example, more than 100 polymorphisms have been identified for *DPYD*, but not all of them affect the functionality of the enzyme [17, 18]. The toxicity of 5-fluorouracil (5-FU) can also be attributed to the influence of *TYMS* and *MTHFR*, as well as the combination of many polymorphisms of these three genes [19, 20]. Nevertheless, in most cases, the toxicity of 5-FU remains unexplained. Alternative strategies, based on in vitro assessment of enzyme activity to directly evaluate protein activity when PGx is not informative [21, 22], or on plasma concentration of drug metabolites, can be used in some cases to predict drug response. In addition, the penetrance of germline polymorphisms may be conditioned by the presence of co-medications, which can obscure the real pharmacogenetic influence of the polymorphisms. For example, simultaneous administration of tamoxifen and *CYP2D6* inhibitors, such as the selective serotonin re-uptake inhibitors (SSRIs), can reduce the effectiveness of tamoxifen [23]. Moreover, genetic variations are present at different frequencies across ethnicities [24]; therefore, the population included in PGx studies represents another factor that can lead to contradictory findings when the studies are replicated in populations of a different origin.

Table 2 EMA and US FDA indications for germline oncoPGx genes

Drug	Type	Gene	EMA	FDA
Capecitabine	Anti-metabolite	*DPYD*	Mentioned[a]	Mentioned
5-fluoro uracil	Anti-metabolite	*DPYD*		Mentioned
Thioguanine	Anti-metabolite	*TPMT*		Mentioned
6-mercaptopurine	Anti-metabolite	*TPMT*	Mentioned	Recommended[b]
Cisplatin	Alkylating agent	*TPMT*		Mentioned
Irinotecan	Topoisomerase I inhibitor	*UGT1A1*		Mentioned
Pazopanib	TKI	*UGT1A1*	Mentioned	Mentioned
Erlotinib	TKI	*UGT1A1*	Mentioned	
Nilotinib	TKI	*UGT1A1*	Mentioned	Mentioned
Regorafenib	TKI	*UGT1A1*	Implied[d]	
Dabrafenib	BRAF V600E inhibitor	*G6PD*		Mentioned
Rasburicase	Recombinant urate-oxidase	*G6PD*	Mentioned	Required[c]
Gefitinib	TKI	*CYP2D6*	Mentioned	
Lapatinib	TKI	*HLA*	Mentioned	

[a] Mentioned: label does not imply any sort of test, but it contains information about possible changes in efficacy or toxicity related to such variants

[b] Recommended: label states or implies that some sort of test (on gene, chromosome or protein) is recommended before using this drug

[c] Required: label states or implies that some sort of test (on gene, chromosome or protein) should be conducted before using this drug

[d] Implied: label report information about gene/protein involved in drug metabolism, but it does not mention any genetic variants involved

All the information here reported are available on the PharmGKB database [58], EMA [6] or FDA [5]

In addition, personalized cancer therapy requires TDM, but this approach has rarely been included in daily clinical practice of cancer treatment. TDM is based on the theory that a strong relationship exists between plasma or blood drug concentration and drug response; this form of analysis is recommended for drugs with narrow therapeutic ranges, pharmacokinetic and pharmacodynamic,high interindividual variability and severe side effects. Information regarding the plasma or blood concentration of the drug is helpful in defining the optimal drug formulation for each patient to maximize treatment efficacy and minimize toxicity [2].

TDM is particularly suitable for cancer therapies, which are characterized by narrow therapeutic windows and strict dose–response relationships, also related to inter-individual genetic variability. However, the application of TDM is still uncommon in cancer care, especially for classical cytotoxic agents. This is mainly due to the paucity of published data from pharmacological trials (especially prospective studies) aimed at predicting therapeutic target ranges; the concomitant use of combination chemotherapies, leading to drug–drug interactions; and the absence of validated monitoring assays, especially in cases of anticancer prodrugs whose activated metabolites are unstable or only exist within cells [25, 26]. All of these factors complicate the measurement of the area under the curve (AUC) of plasma concentration versus time, which is considered the main pharmacokinetics parameter for determining the real systemic exposure to the administered drug. Moreover, oncologists are often reluctant to increase drug dosage in patients who are not exhibiting adverse effects; consequently, establishment of the effective dose is difficult because patients could be underexposed [27].

Recently, a European workshop organized by the French Society of Oncology Pharmacy (SEPO) in France has discussed the current progress and limitations of TDM in oncology care, and tried to define standardized guidelines to improve personalized therapies [25, 27, 28]. For the large majority of cytotoxic drugs, no relevant TDM data are available, with the exception of high-dose methotrexate, 5-fluorouracil infusion, mitotane, and a few other high-dose chemotherapeutic regimens. In those cases, individual dose adjustment guided by pharmacokinetics monitoring leads to a substantial improvement of the drug response, resulting in elevated drug benefits and reduced toxicities [25, 29–31]. Although these preliminary results are promising, the data are still insufficient to introduce TDM in an oncology setting.

For targeted agents, the situation is somewhat different. These orally administered therapies target specific onco-proteins, usually involved in signaling pathways, and are therefore characterized by lower toxicities than classical chemotherapies. Therefore, in the future, such agents could radically change cancers from deadly malignancies to chronically managed conditions.

Most of these drugs fulfill the main criteria for introducing TDM in clinical practice, as long-term administration, availability of TDM methods and inter-individual variability, both in terms of pharmacokinetics and pharmacodynamics, influenced not only by genetic features, but also by environmental factors (such as drug–drug interactions, diet, age, and

allergies) [28, 32]. Retrospective studies have demonstrated that the correlation between therapy outcome and targeted drug dosage (measured by AUC) is robust in several cancers. Good results have been obtained for imatinib [33], and new evidence is emerging for other TKIs such as nilotinib, dasatinib, erlotinib [34–36], and inhibitors of mammalian target of rapamycin (mTOR) [37]. Thus, TDM could be exploited for orally targeted drugs in cases of lack of response, severe side effects, or known drug–drug interactions and adherence issues. Finally, very few data are available regarding the application of TDM to monoclonal antibodies (mAbs). For rituximab and cetuximab, some promising evidence has been obtained that supports TDM approaches [38, 39].

These new treatments are highly expensive, for pharmaceutical companies during clinical trials as well as for patients. Therefore, it is clear that the establishment of optimal individual regimens could benefit the entire public health system.

Given the importance of inter-individual genetic variability in predicting drug response by exploiting a TDM approach, it is obvious that PGx testing and the formulation of standardized guidelines to associate drug dosage with validated onco-PGx markers are urgently required.

Nevertheless, the complications of PGx studies described herein, which are related to the aforementioned difficulties of TDM, can explain existing discrepancies in drugs-use guidelines, in relation to both polymorphisms to be evaluated and treatment dosages in the presence of specific variants. For example, the CPIC and DPWG provide dose-adjustment guidance for the pro-drug capecitabine, based on the presence of *DPYD* alleles that decrease enzyme activity [40, 10], whereas the EMA and US FDA only recommend the use of alternative drugs in case of DPD deficiency.

In conclusion, when the clinical relevance and utility of germline onco-PGx markers remain unclear, the introduction of germline marker genotyping into clinical practice is not considered.

3 Validated and potential onco-PGx markers for use in cancer treatment

3.1 Fluoropyrimidine drugs: 5-FU, capecitabine, and tegafur

5-FU has been widely used since 1957 as a chemotherapeutic drug against several solid tumors, including gastrointestinal tract, breast, and head and neck cancers [41]. This anti-metabolite drug is an analogue of uracil, with the hydrogen at the C-5 position substituted by a fluorine atom. 5-FU exerts its cytotoxic effects by repressing RNA transcription and DNA synthesis via direct incorporation of toxic metabolites, and repression of pyrimidine synthesis via inhibition of thymidylate synthase (TS) [41–43]. To this day, 5-FU still represents a cornerstone of many chemotherapy regimens, and its benefits are increased by combination therapy with molecular modulators, such as folinic acid/leucovorin (LV), and synergistic drugs, such as oxaliplatin (FOLFOX) [44] and irinotecan (FOLFIRI) [45], or targeted monoclonal antibodies against *VEGF* or *EGFR* [46, 47].

The toxicity of 5-FU is the major factor limiting its use, as 10–40 % of patients exhibit severe adverse events that are responsible for suspension treatment: myelosuppression, cardiac toxicity, neurotoxicity, neutropenia, diarrhea, mucositis, and hand–foot syndrome [48, 49]. The activity and toxicity of 5-FU can be modulated by changing the schedule between bolus and infusion: in bolus, the main mechanism of cytotoxicity appears to be inhibition of RNA synthesis seems, whereas in infusion, the main effect of the drug metabolites action is mediated by inhibition of TS and DNA synthesis [50, 51]. Finally, the incidence of neutropenia appears to be higher on a bolus schedule [48], whereas the incidences of cardiotoxicity and hand–foot syndrome are prominent when the drug is administered by infusion [52].

Capecitabine and tegafur are two orally administrated 5-FU prodrugs that can modulate 5-FU–related toxicity. Direct oral administration of 5-FU is not possible, due to the high concentration of DPD, the enzyme primarily responsible for 5-FU catabolism, in the gut wall. Capecitabine is absorbed as an intact molecule through the gastrointestinal tract, and is then converted in the 5-FU in the liver by the sequential catalytic activity of three enzymes: carboxylesterase, cytidine deaminase (CDA), and thymidine or uridine phosphorylase [53].

By contrast, after intestinal absorption tegafur is converted by the hepatic enzyme CYP2A6 into 5-hydroxytegafur, which is then converted into 5-FU. Tegafur is often administered in combination with uracil (tegafur–uracil: UFT), the natural substrate of DPD, to slow the degradation of 5-FU and increase the response rate [54].

The toxicity and efficacy of 5-FU are influenced by multiple genetic factors and patient characteristics, such as age and sex (e.g., females are more likely to exhibit severe toxic effects). However, constitutional variability in proteins involved in pharmacodynamics and pharmacokinetics is the main factor that determines the outcome of 5-FU treatment outcome [51, 55, 56].

5-FU enters cells via transporters such as SLC22A7 and SLC29A1, and is then converted into three cytotoxic active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP). FdUMP competes with uracil for TS, a key enzyme in the synthesis of pyrimidines incorporated into DNA during *de novo* replication. TS inhibition by FdUMP is achieved through the formation of a ternary complex of FdUMP, TS, and 5,10-methylenetetrahydrofolate (5,10-MTHF). The latter compound is a component of the folate pathway, which is responsible for the recycling of methyl

groups and methionine synthesis, and which is also blocked by fluoropyrimidines. The conversion of 5-FU in FdUMP is performed by thymidine phosphorylase TYMP and thymidine kinase. A recent study showed that 5-FU can be also directly incorporated into RNA or DNA during their synthesis after it is converted into the cytotoxic molecules FUTP and FdUTP, respectively [57, 58].

Only 1–3 % of administered 5-FU is activated into cytotoxic metabolites: approximately 80 % is rapidly catabolized and degraded by the sequential catalytic activity of DPD, DPYS, and UPB1; and up to 20 % is eliminated in the urine [53]. DPD is the rate-limiting enzyme in the degradative pathway, and it is predominantly expressed in liver. The activities of all enzymes involved in the complex 5-FU network vary within the population due to genetic differences that could lead to reduction or elevation of their activities; thus, inter-individual germline variability influences the response to 5-FU in terms of resistance and toxicity to treatment.

3.1.1 DPYD

The pharmacokinetics of 5-FU varies significantly among patients, with a half-life ranging between 4 and 25 min [11].

As already mentioned, DPD is the key enzyme in the catabolism of 5-FU, and variations in its activity are strongly related to drug response. A decrease in DPD activity is the cause of severe side effects in roughly 60 % of 5-FU–treated patients [59], whereas resistance to 5-FU-based therapy can be attributed to elevated DPD expression and activity, especially in tumor cells [60]. DPD activity differs greatly within populations, with an estimated 3–5 % of Caucasians exhibiting partial or complete deficiency of DPD function [61]; this proportion is higher (~12 %) in African-Americans, and it is higher in females than in males [17, 51, 62].

The complete absence of DPD protein is responsible for an autosomal recessive disorder characterized by accumulation of thymine and uracil in the urine and neurological symptoms such as epilepsy and mental retardation [63].

The DPD enzyme is encoded by the *DPYD* gene (1p22), which consists of 23 exons. *DPYD* harbors several thousand known variations, most of which do not affect the enzyme activity of DPD [64]. Table 3 lists *DPYD* polymorphisms associated with altered DPD enzymatic activity and approved by CPIC and DPWG for drug dosage guidelines [10, 40].

About 30 % of patients deficient for DPD activity and exhibiting severe toxicity to 5-FU have the intronic variant *DPYD**2A (or c.1905 +1G>A, IVS 14+1G>A or rs3918290), due to a SNP in the splice site of intron 14 that results in skipping of exon 14 and a non-functional enzyme [65]. *DPYD**2A is considered moderately rare, with an estimated frequency in the general population of 0.5–1 % [65]; it is absent in Asians and African-Americans, although both of these populations exhibit a high prevalence of DPD deficiency [64]. Recently, new *DPYD*-deficient variants, such as T760I, P92A, and Y304H, have been shown to be more frequent among African-Americans than in Europeans [62].

Two other SNPs are associated with low enzyme activity and 5-FU toxicity, *DPYD**13 (c.1679T>G, p.Ile560Ser, or rs55886062) and rs67376798 (c.2846A>T or p.Asp949Val) [49, 51, 59, 66]. Genotyping for *2A, *13, and rs67376798 is strongly suggested by the CPIC and DPGW guidelines, according to which individuals homozygous for one or more of these three alleles are considered deficient in DPD [10, 40]. In such cases, the US FDA recommends the administration of alternative drugs (e.g., raltitrexed) that are metabolized by pathways that do not involve DPD [52]. Heterozygous individuals are considered partially defective in DPD, with a reduction in 5-FU clearance of approximately 50 %, relative to wild-type individuals [46, 57]. Based on these findings,

Table 3 Selection of the most relevant PGx variants associated to fluoropyrimidines response

Gene	Gene variant	rs ID	Annotations	References
DPYD	c.1905 + 1G>A (DPYD*2A)[a]	rs3918290	exon 14 skipping, associated with significantly decreased DPD activity and toxicity to fluoropyrimidines	[18, 51, 66]
	c.1679T>G (DPYD*13A) [a]	rs55886062	maybe destabilize FMN binding domain associated with decreased DPD activity and toxicity to fluoropyrimidines	[59, 66, 114].
	c.2846A>T	rs67376798	may affect electron transport associated with decreased DPD activity and toxicity to fluoropyrimidines	[51, 66, 114].
TYMS	TYMS 28bp-VNTR (2R/3R)	rs34743033	the number of repeats affect mRNA translational efficiency and TS expression	
	TYMS SNP C>G (on 3R allele)	rs2853542	C allele decreases mRNA translational and TS expression	[80, 83, 84, 85, 94]
	c.1494del6	rs34489327	Deletion of 6bp reduces mRNA stability and TS expression	
MTHFR	c.677C>T	rs1801133	aminoacid substitution in co-factor binding site that causes decreased activity	[90], [97, 102]
	c.1298A>C	rs1801131	aminoacid substitution in SAM regulatory domain that causes decreased activity	

[a] DPYD followed by an asterisk and an arabic number indicate the international standardized nomenclature [58]

CPIC and DPWG suggest a reduction of the starting dose of at least 50 %, followed by sequential monitoring of drug response to establish the proper dosage [10, 40].

Other variants have been associated with alterations in DPD activity, although the data are often conflicting and weak (the complete list with respective references is available online [58]). Among these variants, Y186C is associated with reduced DPD activity, and it has recently been identified in approximately 26 % of African-American patients. The substitution of tyrosine 186 with a cysteine could affect enzyme dimerization and thereby influence enzymatic activity; however, these results are ambiguous, with the reduction in catalytic activity ranging among studies from 15 to 46 % [18, 64]. Another intronic mutation (c.1129-5923C>G or rs75017182) probably correlates with reduced DPD activity; this variant creates a cryptic splice donor site that causes the inclusion of 44 nucleotides of intron 10 into the mature mRNA [67]. This mutation is in linkage disequilibrium with the synonymous exon variant c.1236G>A [68–70].

Polymorphisms can also be related to an increased enzyme activity: variants such as C29R, S534R, and P1023T exhibit a hyperactive phenotype. More data are necessary to confirm these findings, as C29R was previously linked to reduced DPD functionality [18, 64].

Compound heterozygosity for multiple polymorphisms results in heterogeneous phenotypes, because such variation can lead to unexpected impacts on phenotype. This phenomenon could explain the inconsistencies in results from different studies. For example, in contrast to previously reports [71], Tsunoda et al. [72] did not observe correlations between *DPYD* *5, *6, or *9, and low DPD activity. Consequently, several researchers have proposed that DPD activity should be determined on the basis of classes of haplotypes that comprise more variants, to provide complete information about DPD functionality for each individual patient [69]. Combinatorial genetic approaches could help more precisely determine DPD activity level, which would in turn be useful for establishing proper dosage guidelines.

Other explanations for the conflicting data include the presence of co-medications that could modify the influence of a polymorphism on DPD activity [51]; inappropriate sampling, e.g., harvesting of DNA from tumor tissue instead of constitutional tissue such as blood; or patient characteristics such as ethnicity and gender.

Given the importance of *DPYD* in 5-FU toxicity, inter-individual epigenetic differences in regulation of DPD expression have been proposed as a possible explanation for DPD deficiency. High levels of somatic methylation on the *DPYD* promoter, resulting in low levels of DPD enzyme, are associated with 5-FU resistance in colorectal cancer cells. More recent studies carried out on constitutional tissues have not revealed a contribution of promoter methylation to 5-FU side effects [73, 74]. Furthermore, large genome rearrangements do not seem to contribute to the development of 5-FU toxicity [73, 75]. Finally, DPD expression can be post-transcriptionally regulated in cancer, as well as in normal cells [76]. For example, miRNA can modulate DPD expression in lung cancer cells [77] and in normal liver [78].

Despite the importance of *DPYD* germline variants in predicting 5-FU treatment outcome, polymorphisms cannot explain all cases of 5-FU toxicity; other genes involved in drug responses should be evaluated before 5-FU–based therapy is initiated, although to date no specific recommendations have been made.

3.1.2 TYMS

TYMS (18p11.3), which consists of 13 exons, encodes thymidylate synthase (TS), the principal target of fluoropyrimidines; this enzyme is responsible for the conversion of dUMP in dTMP. TS is a folate-dependent protein that also uses the oxidation of 5,10-MTHF in its reaction, thus taking part in folate metabolism [79]. This enzyme plays a fundamental role in DNA synthesis and DNA repair, and thus in cellular proliferation [80]. Given the function of TS in determining cell viability, its pharmacologic inhibition is a useful way to slow cancer-cell proliferation; its activity can be repressed by various agents, including fluoropyrimidines and folate analogues [81].

Germline polymorphisms that affect TS expression or the binding sites of inhibitors may be associated with low clinical response to treatment [82]. To date, three variants have been deeply analyzed: rs34743033, rs2853542, and rs34489327 (Table 3). The rs34743033 polymorphism comprises a variable number of 28-bp tandem repeats (TYMS 28 bp-VNTR) in the 5′-UTR/enhancer region of *TYMS* promoter (TSER). Most of the population has two or three repeats on each allele, but higher numbers of repeats have been detected in African and Asian individuals [83]. The presence of at least one repeat is necessary to allow *TYMS* transcription, because this repeat contains a binding site for the upstream stimulating factors (USFs). The number of repeats may be associated with protein expression level and, subsequently, to cancer susceptibility and treatment outcome [84, 85].

rs2853542 is a SNP (C>G) that modifies the USF recognition site, and consequently leads to a reduction in the mRNA level [86, 87]. Several studies reported that the number of repeats of VNTR alleles and SNP rs2853542 are associated not only with drug resistance and poor prognosis [86–89], but also with drug toxicity. These phenotypes can also be present when *TYMS* variants are combined with polymorphisms in other genes involved in fluoropyrimidine response [51, 86, 90, 91]. However, other studies did not detect the same associations [68, 92, 93]; thus, the involvement of these *TYMS* variants in onco-PGx remains to be elucidated.

rs34489327 is an insertion/deletion of 6 bp at the 3′-UTR of *TYMS* (1494del6) that may regulate translation and mRNA stability. The 3′UTR–deleted pre-mRNA may be preferentially bound by an RNA-binding protein (AUF1) in its adenylate/uridylate-rich elements, leading to a less stable and easily-degraded mRNA [93]. This variant has been linked to an elevated risk of cancer development [86, 94, 95], but the data reported to date regarding the effect of this polymorphism on treatment outcome are ambiguous: in one study, individuals homozygous for the deletion responded better to treatment, even if they experienced increased toxicity [96]; however, other studies failed to confirm this observation [68, 97–99].

In conclusion, the effect of *TYMS* variants on therapy outcomes remains controversial. Possible explanations for the discrepancies among studies include the absence of solid statistical analyses, the presence of co-treatments and co-variants in other genes, and the use of tumor samples to investigate the clinical relevance of *TYMS* polymorphisms.

Currently, genetic analysis of these variants remains unreliable, and further studies are necessary to assess their utility in clinical practice. Moreover, a recent study [100] identified a new candidate gene involved in fluoropyrimidine-related toxicity: *ENSOF1*, which is adjacent to *TYMS* and could therefore explain phenotypes related to *TYMS* polymorphisms.

3.1.3 MTHFR

The 5,10-methlenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate (5,10-MTH), a key step in folate metabolism, which is essential for purine synthesis [101]. The *MTHFR* gene (1p36.3) consists of 11 exons and contains two SNPs that have been extensively investigated in the context of PGx: c.677C>T and c.1298A>C (Table 3).

C677T (rs1801133) is localized in exon 4 and causes a conservative substitution (Ala222Val) in the co-factor binding site associated with reduced enzyme activity (at least 30 % in in vitro analysis). A1298C (rs1801131) is located in exon 7 and results in a non-synonymous substitution (Glu429Ala) in the S-adenosyl methionine (SAM) regulatory domain, causing a conformational change and altered enzymatic activity. Both of these polymorphisms are associated with elevated cancer risk [102].

Data about the relationship between these variants and drug response are inconsistent. Some studies found a significant correlation between these SNPs and clinical outcome [90, 97, 103] or risk of toxicity [102], whereas others failed to confirm these associations [104–108]. Recently, Loganayagam and co-workers [68] have shown that the *MTHFR* 1298CC genotype is strongly related to the onset of hand–foot syndrome in patients treated with capecitabine.

Thus, it is probably premature to introduce genotyping of *MTHFR* into clinical practice, because more consistent results are needed to confirm its value as a potential onco-PGx marker.

3.1.4 Other genes

The complex metabolism of fluoropyrimidines involves many proteins, and many studies have reported that variants in other members of this metabolic pathway could play important roles in fluoropyrimidine activity and toxicity.

CDA is the enzyme responsible for activation of the pro-drug capecitabine (i.e., its conversion into 5-FU), and the *CDA* promoter variants c-451C>T and c-92A>C have been associated with elevated toxicity; by contrast, *CDA* rs315400insC seems to protect against the adverse effects of capecitabine [68, 109]. Similarly, tegafur is converted into 5-FU by CYP2A6, and many polymorphisms in *CYP2A6* have been linked to alterations in enzyme activity and drug response [110, 111]. DPYS is the second enzyme involved in 5-FU catabolism, and several polymorphisms within its sequence have been studied, but the results of these studies are not concordant [68, 112–114]. Recently, we showed that expression levels of *OPRT* correlate with the efficacy and toxicity of 5-FU treatment. OPRT is one of the enzymes implicated in 5-FU activation, and this preliminary finding suggests that it might be valuable as an onco-PGx marker [115].

Many other markers have been described. Therefore, once again, it is clear that a great deal of work is still necessary to identify all of the effective onco-PGx variants before treating patients with fluoropyrimidines.

3.2 Thiopurine drugs: 6-mercaptopurine and 6-thioguanine

The thiopurine drugs, 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) are purine analogues that exert their cytotoxic activity by competing with endogenous purines in fundamental pathways involved in DNA/RNA synthesis and stability, as well as in coenzyme formation [116]. These compounds are widely used for remission induction and maintenance therapy of myeloid leukemias and lymphoid malignancies [117]. Another thiopurine drug is azathioprine (AZA), but it is not a anticancer agent, indeed is mainly indicated for rheumatoid arthritis, Crohn's disease, and colitis, as well as to prevent renal transplant rejection. Since it is not prescribed in cancer treatments, polymorphisms related to its response are not discussed here.

Although 6-MP and 6-TG are extensively exploited in many chemotherapeutic regimens, they exhibit heterogeneous efficacy and toxicity among patients, potentially resulting in suspension of treatment. Hematologic and hepatic toxicities are the most common adverse effects related to accumulation of toxic metabolites in plasma [118, 119], but in some cases, myelosuppression and secondary neoplasia have also been reported [120, 121].

The wide variability observed in response to thiopurine treatments can be attributed to differences in the genetic constitution, age, and gender of patients. Heritable deficiency in enzymes of the thiopurine pathway can increase the concentration of toxic metabolites, resulting in adverse events; on the other hand, hyperactivation of these enzymes can reduce drug efficacy.

Following oral administration, the inactive prodrugs 6-MP and 6-TG must be activated within cells to be functional. Cell uptake involves several transporters, including SLC28A2, SLC28A3, SLC29A1, and SLC29A2. Once being taken up by cells, 6-MP and 6-TG are converted by hypoxanthine guanine phosphoribosyl transferase (HPRT1) into thioinosine monophosphate (TIMP) and thioguanosine monophosphate (TGMP), respectively. TIMP is then transformed into TGMP by a two-step process, and then into several cytotoxic thioguanine nucleotides (TGN), including thiodeoxyguanosine triphosphate TdGTP (incorporated into DNA) and thioguanosine triphosphate TGTP (incorporated into RNA); thus, the metabolites of this drug affect both DNA and RNA synthesis and stability. The cytotoxic effects of thiopurines are also achieved by inhibition of *de novo* purine synthesis via secondary metabolites, such as methylmercaptopurine nucleotides (MeMPR) for 6-MP, and by induction of apoptosis in activated T-cells via inhibition of Rac1 [12].

The pathways leading to the synthesis of active metabolites compete with those responsible for drug inactivation, comprising the activities of xanthine oxidase (XO) and thiopurine methyltransferase (TPMT) enzymes. In addition, the accumulation of potentially toxic molecules, such as inosine triphosphate (ITP) and deoxyinosine triphosphate (de-ITP) formed from TIMP, is prevented by the catalytic activity of another enzyme, inosine triphosphate pyrophosphohydrolase (ITPA) [58, 122, 123].

PGx has provided essential tools for studying the relationship between heritable variability and the outcome of thiopurine therapy. The identification of genetic variants that determine response to this class of drugs represents one of the most successful demonstrations of the power of onco-PGx in clinical practice.

3.2.1 TPMT

TPMT catalyzes the S-methylation of thiopurines. Because this reaction reduces the pool of drug available and the formation of TGNs, its activity is inversely proportional to the concentration of TGNs, the principal active metabolites of thiopurine drugs [118]. High TPMT activity (and low levels of TGNs) has been associated with poor prognosis and increased risk of tumor relapse, whereas reduced TPMT activity has been observed in patients that exhibited severe adverse effects following treatment with thiopurines, especially myelotoxicity [124, 125]. Response to thiopurine therapy is thus mainly dependent on the activity of TMPT, which is in turn mainly determined by heritable genetic polymorphisms in the *TPMT* gene (Table 2) [126]. In addition, non-genetic factors such as sex, age, and the presence of co-medications influence TPMT activity [122]. For example, TPMT activity is higher in males than in females [127–129].

The *TPMT* gene (6p22.33) contains 10 exons; to date, more than 20 polymorphisms have been described for this gene. Among these numerous variants, *TPMT*2* (rs1800462), *TPMT*3B* (rs1800460), and *TPMT*3C* (rs1142345) have been identified as the responsible SNPs in 90–95 % of individuals with reduced enzyme activity, with a genotype–phenotype concordance of about 90 % [127, 129]. *TPMT*2* is a SNP (c.238G>C) that leads to the Ala80Pro substitution. *TPMT*3B* corresponds to the SNP c.460G>A, causing the substitution Ala154Thr, which is very rare and usually in linkage disequilibrium with *TPMT*3C*, resulting in a more common haplotype associated with reduced activity, *TPMT*3A*. Finally, *TPMT*3C*, a SNP (c.719A>G) that leads to the Tyr240Cys substitution, is the most common variant in Americans and Asians (roughly 2 %); this variant is associated with a smaller reduction in activity than the other two alleles [130].

As with the genes discussed above, discordance between the *TPMT* genotype and phenotype attributed to experimental factors, such as the subject population, presence of co-treatments, and genetic factors, such as the presence of other unknown *TPMT* variants or other related genes [131]. Based on the evidence assembled to date, *TPMT* genotyping should be considered prior to thiopurine-based treatment; indeed, it is recommended by the US FDA and mentioned by EMA. Individuals who are homozygous for one or more of these polymorphisms exhibit null TPMT activity, whereas heterozygotes exhibit partial activity. On the basis of these findings, the CPIC and DPWG formulated drug dose guidelines for the purpose of decreasing toxicity without affecting chemotherapeutic efficacy. A dose reduction of at least 10-fold, as well as a reduction in the frequency of administration, is recommended for completely TPMT-deficient patients, and starting with 30–70 % of the full dose is recommended for partially TPMT-deficient patients [10, 117].

Several other variants found in patients have been correlated with a decreased enzyme activity, including *TPMT*4A*, which results in a G-to-A transition that disrupts the intron 9–exon 10 acceptor splice site, causing the transcription of two abnormal transcripts [128]; and *TPMT*8* (c.644G>A), frequently identified in African and Asian individuals with intermediate enzyme activity [129]. In addition, several polymorphisms have also been observed in the *TPMT* promoter, although more studies are necessary to confirm their possible role in the response to thiopurine treatment [132].

The relevance of *TPMT* in therapy outcome and the increasing number of known variants prompted the creation of a website in which new variants are described and classified [133, 134] (Table 2).

Despite the importance of *TPMT* as a germline PGx marker for thiopurine treatment, other genes should also be evaluated for determination of drug response.

3.2.2 ITPA and other genes

ITPA encodes an enzyme involved in the detoxification of compounds formed from TIMP. To date, several polymorphisms have been found in this locus. Some of them (such as c.94C>A) are associated with reduced activity, but results obtained from phenotypic analyses of this variant have not been consistent [122, 123, 135].

Germline polymorphisms in other genes related to thiopurine pharmacokinetics or pharmacodynamics, such as *ABCC4* and *ABCC5 (*ATP-binding cassette transporter C4 and C5), have been described as potential PGx variants [131]; however, inconsistencies in the available data prevent their inclusion in clinical practice.

3.3 Cisplatin

Cisplatin and its analogues (such as carboplatin and oxaliplatin) are widely administered to treat several types of cancers; including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas and germ cell tumors. They work through three different mechanisms: direct alkylation of DNA bases, formation of cross-links in the DNA double-helix, and alterations in DNA repair pathways. These pharmacological actions result in compromised DNA stability and cell death [136]. The high cytotoxic activity of cisplatin entails severe side effects that strictly limit its use [137]. Numerous studies have shown that the variability in response to this type of treatment may be caused by inter-individual genetic variations in several genes, including the gene *GSTP1* [138, 139]. However, the currently available data are insufficient to confirm the clinical validity of *GSTP1* testing in regard to the onco-PGx of cisplatin.

The most relevant complication that prevents its use is ototoxicity; in particular, since cisplatin is extensively used in childhood solid tumors, including hepatoblastoma and brain tumors [140], it can cause serious hearing loss. The inter-individual variability in hearing loss risk suggests that ototoxicity could be a result of genetic, in conjunction with other factors, such as age, cranial irradiation, and concomitant vincristine therapy and that it is increased in pediatric patients [141, 142].

Recently, Ross and co-workers [143] used a candidate gene approach to identify candidate genetic variants for ototoxicity. These polymorphisms are mainly located at *TPMT* (thiopurine S-methyltransferase; rs12201199, rs1800460 or TMPT*3B, and rs1142345 or TMPT*3C) (Table 2) and *COMT* (catechol O-methyltransferase; rs9332377 and rs4646316), and seem to cause reduced activity in the encoded enzymes. The connection between these genes and the effects of cisplatin are not completely clear, but two hypotheses have been formulated. First, cisplatin also binds thiol-containing molecules and purines that are synthetized by a pathway involving TPMT and COMT; thus, reduced activity in these enzymes could increase the cytotoxic effect of cisplatin via formation of increased DNA cross-links. Second, TPMT and COMT are methyltransferases dependent on S-adenosyl methionine (SAM), and a reduction in their activities could increase the level of SAM, thereby increasing the cytotoxic effect of cisplatin [143].

Although Pussegoda et al. [140] confirmed the aforementioned results, Yang et al. [144] maintained that no correlation exists between *TPMT* and *COMT* polymorphisms and cisplatin-dependent ototoxicity. Despite the inconsistencies among studies, the US FDA included a recommendation for *TPMT* genotyping on the cisplatin label; in particular genetic variants in TMPT, such as TMPT*3B and TMPT*3C, are associated with increased risk of hearing loss in children administered with standard doses of cisplatin. Nevertheless, also children without these polymorphisms remain at risk of ototoxicity, thus all pediatric patients receiving cisplatin should be subjected to audiometric testing also for several years after therapy [5]. Subsequently, critics to this amendment were raised up [145]. Therefore, a deep review of all available data may be necessary to understand if *TPMT* genotyping is useful to predict cisplatin-related ototoxicity and if it is possible to edit proper dosing guidelines.

3.4 Irinotecan

Irinotecan (IRI) is an antineoplastic agent originally used in colorectal cancer therapy (in a first-line treatment, it is often administered together with 5-FU and leucovorin, a regimen known as FOLFIRI); currently, it is also used in lung cancer (usually in combination with cisplatin) and other solid tumors, including gastric and gynecological cancers [146, 147].

IRI is a synthetic pro-drug analogue of camptothecin, an inhibitor of topoisomerase I; its active metabolite, SN-38, destabilizes topoisomerase I–DNA complexes generated during DNA replication or DNA repair. By this mechanism of action, the ligation step of single- and double-stranded breaks is blocked. Persistent DNA breaks lead to apoptosis and cell death.

Although the combination of IRI and fluoropyrimidines has greatly improved the survival rate in colorectal cancer patients [148], the response to IRI is highly variable. Moreover, side effects such as myelotoxicity, neutropenia, gastrointestinal complications, and infections represent major limitations for the clinical use of IRI [149]. Because IRI is

often administered in combination therapies, the variability of the IRI response could be explained by drug–drug interactions [150], but also by genetic variation in enzymes involved in irinotecan metabolism.

Once administered, IRI is transported into the liver by proteins such as ABCB1 and ABCG2. In the liver, the carboxylesterases (CES) hydrolyze IRI to SN-38, which is then transported into cancer cells. However, only a small percentage of active SN-38 is taken up by tumor cells; the majority of this compound undergoes glucuronidation to SN-38G by the uridine diphosphate glucoronosyltransferase family member UGT1A, and SN-38G is then released into the intestine for its elimination. The bacterial microflora are able to reactivate SN-38G into SN-38, which is reabsorbed by enterohepatic circulation; this step is specifically associated with the gastrointestinal toxicity of IRI [151]. In addition, a small amount of IRI is directly excreted into the bile. Furthermore, IRI is oxidized by the P450 CYP3A into the inactive metabolites APC (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin) and NPC (7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecin), which is once more metabolized into SN-38. CYP3A and UGTA1A are key enzymes involved in the pharmacokinetics of IRI, and their activity is correlated with drug toxicity. In fact, impairment of either enzyme's activity modifies the concentration of available IRI and its metabolite SN-38, which is responsible for the development of myelosuppression, neutropenia, and diarrhea [58].

The genotyping of genes involved in IRI metabolism revealed the genetic variations associated with adverse drug effects. Furthermore, recent studies in several tumor cell lines have shown that resistance to IRI may be due to somatic modification of proteins involved in IRI pharmacodynamics, such as proteins involved in signaling pathways (e.g., the EGFR and MAP kinase cascades) [152, 153].

3.4.1 UGT1A

The uridine diphosphate glucoronosyltransferase (UGT) superfamily comprises numerous enzymes responsible for the glucuronidation of different target substrates, a fundamental step in biliary or renal elimination.

The *UGT1A* locus (2q37) encodes nine enzymes through a combination of nine alternative first exons with the remaining common exons. UGT1A1 (Table 2) is a UGT1A isoform expressed in the liver and gastrointestinal tract. In the liver, UGT1A1 is the sole enzyme responsible for bilirubin metabolism, and alterations in its activity cause abnormal serum levels of bilirubin associated with pathological conditions, such as Crigler-Najjar type I disease and Gilbert's syndrome [154, 155].

The variability of UGT1A1 activity depends on genetic variants within the encoding gene: to date, about a hundred of alleles conferring increased or reduced catalytic activity have been described. Individuals homozygous or heterozygous for inactive alleles suffer from the aforementioned diseases; in addition, some of these alleles have been associated with an increased risk of developing various cancers, such as colorectal or breast cancers [156].

UGT1A1 is the principal isoform responsible for the glucuronidation of SN-38, although some studies have also demonstrated the involvement of UGT1A7 and UGT1A9 [13]. Several alleles that confer reduced enzymatic activity have been correlated with toxicity to IRI; these include *UGT1A1*28* and *UGT1A1*6*, which occur at different frequencies among populations: *UGT1A1*28* is mainly present in Caucasians and African-Americans (with a frequencies of roughly 0.30), whereas *UGT1A1*6* is mainly distributed in Asia [157, 158].

The *UGT1A1*28* allele (rs8175347) is characterized by an additional thymine–adenine repeat in the promoter (seven rather than six repeats of the wild-type allele *1). This extra repeat severely reduces transcription. When patients with this allele are treated with IRI, excess SN-38 is responsible for severe neutropenia and diarrhea [159]. *UGT1A1*28* genotyping for IRI-based therapies has been recommended by the US FDA since 2005, and the label suggests a reduced initial dosage for *UGT1A1*28* homozygous individuals [5]. Several meta-analyses have subsequently shown that *28/*28 homozygous patients have an elevated risk of developing IRI toxicity only when treated with high doses; therefore, the DPWG recommends a dose reduction of 30 % when the initial dose of treatment would have been more than 250 mg/m^2 [10].

Recently, Innocenti and co-workers [149] proposed new genotype-guided dosages for patients undergoing IRI therapy, based on their identification of the maximum tolerated dose (MTD) and the dose-limiting toxicity (DLT) in patients with advanced solid tumors bearing the *1/*1, *1/*28, and *28/*28 genotypes. Tolerable doses can be further adjusted in the case of FOLFIRI regimens in patients with metastatic colorectal cancers. Therefore, the impact of co-medications are relevant to the response to IRI. For example, administration of cyclosporin, a bio-modulator of IRI pharmacokinetics, should be avoided because it mimics the phenotype of the *UGT1A1*28* variant [150]. Although the role of *UGT1A1*28* variant in the prediction of IRI response is quite strengthened, meta-analyses did not demonstrate its association with IRI toxicity, probably because other variants can also influence toxicity and should also be taken into account [160].

*UGT1A1*6* (rs4148323) results from the substitution of glycine 71 with arginine due to SNP c.211G>A, which causes a decrease in both expression and activity of UGT1A1. Individuals carrying this allele show the same phenotypes as individuals carrying the *UGT1A1*28* allele. Because this allele is associated with reduced UGT1A1 functionality, patients homozygous or heterozygous for the *UGT1A1*6* allele

exhibit side effects when treated with IRI. Many studies, including different meta-analyses, have demonstrated that genotyping of this variant, in combination with *UGT1A1*28*, is essential for prediction of enzyme activity and drug response, especially within Asian populations [157, 158]. However, further information is needed before recommendations are formulated for this variant: in particular, the influence of concomitant treatments or the existence of other non-considered variants, as well as technical limitations, such as sample size, that could invalidate the preliminary evidence described above.

In addition, variants in other UGT1A isoforms such as UGT1A7, UGT1A9, and UGT1A6 were included in analyses of IRI metabolism in several other studies [161]. Of these, UGT1A7 is the predominant isoform in the intestine, where it is responsible for the detoxification of the reactivated SN-38. Variants such as *UGT1A7*3* or *UGT1A7**4 were correlated with a low glucuronidation activity and, consequently, with adverse effects due to higher concentrations of SN-38 [162, 163]. *UGTA19* is expressed in the liver, and the allele *UGT1A9*-688A/C results in elevated gastrointestinal toxicity in patients treated with IRI. Moreover, UGT1A6 is also able to glucuronidate SN-38; however, further data are necessary to define its relevance in IRI treatment [164, 165].

In conclusion, recent studies suggested that evaluation of several *UGT1A* haplotypes could aid in accurately predicting IRI response and formulating personalized drug dosage. Finally, because numerous chemicals are metabolized by UGT1A isoforms, their influence on UGT1A activity must be taken into account in predictions of IRI treatment outcome.

3.4.2 Other genes

CYP3A4 and CYP3A5 are essential for the oxidative metabolism of IRI. Preliminary in vitro and in vivo results related to several variants, such as *CYP3A4*16*, *CYP3A4*18*, and *CYP3A5*3*, have correlated these isoforms with a reduction in enzymatic activity and consequent impairment of IRI metabolite levels. These findings suggested that these variants influence the response to IRI treatment.

In addition, genetic variability in ABC transporters such as ABCC2 or ABCG2, which are involved in the uptake and transport of IRI and its metabolites, could play roles in the development of IRI-related side effects [166].

3.5 Rasburicase

Rasburicase is a recombinant urate oxidase that catalyzes the oxidation of uric acid into the soluble metabolites allantoin and hydrogen peroxide. Accumulation of toxic amounts of uric acid in plasma (hyperuricemia) is a direct consequence of chemotherapy; therefore, this drug is often necessary as a co-treatment to decrease the concentration of uric acid [167].

The toxicity of rasburicase is related to the hydrogen peroxide it produces. This compound causes oxidative stresses that are not tolerated well in G6PD-deficient patients; such stresses lead to hemolytic anemia (AHA) and, in some extreme cases, methemoglobinemia.

G6PD (Table 2) encodes a ubiquitous essential enzyme of the pentose phosphate pathway that catalyzes the conversion of glucose 6-phosphate into 6-phosphogluconolactone and simultaneous reduction of nicotinamide adenine dinucleotide phosphate (NADPH). In erythrocytes, G6PD is the only enzyme that produces NADPH, a reducing agent fundamental to protecting cells from oxidative stress. Some G6PD variants cause defects in recovery from oxidative stress due to the resultant low levels of NADPH in erythrocytes [168]. Currently, more than 100 alleles of *G6PD* have been reported, and a complete list of their respective haplotype is available in the PharmGKB database [58]. Large rearrangements within the G6PD gene are lethal in males, and are therefore negatively selected. The variants present in populations are missense mutations causing amino-acid substitutions or small in-frame deletions or insertions that primarily affect the protein's stability and affinity for substrates. These variations in G6PD usually result in a reduction of catalytic activity that becomes evident in the presence of certain drugs or consumption of fava beans, which stimulate the oxidative stress response, resulting in a condition known as favism [169, 170].

G6PD polymorphisms are present in approximately 5 % of the world's population, especially in Africans and Europeans [171]. Variants have been divided into five classes by the World Health Organization [172], on the basis of both G6PD enzyme activity in erythrocytes and the clinical manifestations of each variant. Subsequently, the CPIC provided guidelines for rasburicase-based therapy related to G6PD activity [173], and stated that rasburicase is absolutely contraindicated in patients with CNSHA (chronic non-spherocytic hemolytic anemia) associated with a drastic reduction in G6PD (less than 10 % compared to wild-type protein). However, patients with only partially compromised G6PD activity should be monitored frequently; in cases of large decreases in G6PD activity, alternative treatments, such as allopurinol, should be considered. The EMA and FDA recommend simply avoiding rasburicase treatments in patients with a known G6PD deficiency.

G6PD deficiency is an X-linked trait. The assessment of genotype–phenotype correlation is relatively easy in males, whereas in females it can be tricky for patients heterozygous for *G6PD* variants due to X-chromosome inactivation [173]. Therefore, especially in females, a quantitative screening of enzyme activity is often carried out. Because the interpretation of genetic tests and G6PD activity status can be difficult, CPIC guidelines have provided a workflow summarizing all the steps for diagnosis of G6PD deficiency. However, more

data are still required to set the dosage on the basis of *G6PD* variants.

In summary, individuals with germline reduced activity variants of G6PD are more susceptible to oxidative stress, often generated by drugs, which can lead to hemolytic anemia.

3.6 Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKIs) are small molecules responsible for the inhibition of several constitutively activated tyrosine kinases whose corresponding pathways, if altered in cancer cells, can promote cellular proliferation. TKIs represent an important targeted therapeutic alternative to classical cytotoxic chemotherapy, and they are usually administered in combination with other treatments [174]. Because they are directed against specific (onco-)proteins to modulate several signaling pathways that are altered in tumors, TKIs cause more limited side effects; nevertheless, the response to these drugs is variable, due not only to genetic variability in their respective cellular targets, but also to inter-individual constitutional variability in pharmacokinetics. Furthermore, TKIs are able to influence the activity of proteins not directly involved in their metabolism; this is relevant because they are usually combined with other drugs. These drug–drug interactions may become more serious when the patients treated with TKI have polymorphisms in genes that affect drug metabolism [175]. Therefore, it is clear that patients' pharmacogenetic backgrounds could help define optimal TKI therapy; moreover, the introduction of a TDM approach to precisely assess the therapeutic range of each TKI, as already mentioned in the Introduction, could further improve the efficacy of these drugs by reducing their toxicity.

Among the possible side effects of TKI therapy, drug-induced liver injury (DILI) remains the principal cause of treatment suspension. Because biomarkers to assess hepatotoxicity are not always available, metabolite concentration is usually determined to predict DILI. The US FDA has provided guidelines for monitoring liver functions and identifying liver injury: the concomitant increase of ALT (alanine aminotransferase) and TBL (total bilirubin) is the most informative sign of hepatocellular damage; this observation is classified as "Hy's law" by the US FDA, based on Hyman Zimmerman's original observations in cases of hepatotoxicity [176].

TKIs cause hepatotoxicity by different mechanisms that frequently involve oxidative stress, immunological response, mitochondrial dysfunctions, and disruption of hepatic bile transport. Currently, several drug labels contain indications and warnings related to potential DILI; below we provide a brief summary.

Pazopanib, erlotinib, nilotinib and regorafenib are TKIs. In addition to their various molecular targets, these drugs also inhibit UGT1A1. Consequently, they cause severe side effects when administered to patients with constitutionally low UGT1A1 activity. Subsequently, such patients exhibit clinical manifestations of pathological conditions related to high bilirubin levels. We have already illustrated the importance of UGT1A1 germline variants as PGx indicators for IRI-based treatments; however, UGT1A1 enzymes are not essential for the metabolism of TKIs (Table 2).

Pazopanib is an angiogenesis inhibitor that acts on vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR). It is used to treat metastatic renal cancer and soft-tissue sarcoma [177]. Elevation of TBL in patients treated with pazopanib often correlates with the presence of several UGT1A1 variants, such as *UGT1A1*28*, *UGT1A1*6*, and *UGTA1A1*36*, and therefore with a predisposition to Gilbert's syndrome [178, 179]. Gilbert's syndrome has a benign clinical course; however, it is possible that drugs or other factors could trigger a transition from this mild condition to a more severe disease.

Similarly, nilotinib and erlotinib are able to inhibit the glucuronidation activity of UGT1A1, so that their administration leads to a significant increase in bilirubin levels in patients that are poor metabolizers (for example, those carrying *UGT1A1*28* and similar variants) [180, 181]. Nilotinib is a competitive inhibitor of the breakpoint cluster region-Abelson1 (BCR-ABL1) kinase, and it was originally developed to treat chronic myeloid leukemia. On the other hand, erlotinib, an inhibitor of epidermal growth factor receptor (EGFR), is prescribed for a number of solid tumors, including colorectal and lung cancer, often in combination with IRI and fluoropyrimidines (the FOLFIRI regimen). The US FDA and EMA recommend genetic testing of *UGT1A1* before initiation of treatments with these TKIs, and they suggest monitoring the onset of hepatotoxicity in patients with the *UGT1A1*28* variants (the only variant mentioned by these agencies) and suspension of treatment in cases with severe side effects [6].

Regorafenib is an oral anticancer agent that blocks several protein kinases, including kinases involved in tumor angiogenesis (VEGFR1, −2, −3, TIE2), oncogenesis (KIT, RET, RAF-1, BRAF), and in the tumor microenvironment (PDGFR, FGFR). In preclinical studies regorafenib has demonstrated antitumor activity in various tumor models including colorectal tumor models. In addition, regorafenib has shown anti-metastatic effects in vivo. Furthermore, regorafenib is a UGT1A1 inhibitor and thus hyperbilirubinaemia may occur in patients with Gilbert's syndrome. For this reason, EMA label has recently reported information about possible consequences on bilirubin metabolism in patients treated with regorafenib; without mentioning any genetic variant that could be involved, because there are not enough evidences about variations in drug response [6].

Gefitinib is another EGFR inhibitor that is effective against NSCLC; as in the case of erlotinib, it causes hepatotoxicity more frequently in Japanese patients than in Europeans [182]. Gefitinib is predominantly metabolized by CYP3A4,

CYP3A5, and CYP1A1, but can also be converted in ortho-desmethyl-gefitinib by CYP2D6 (Table 2).

Polymorphisms in drug-metabolizing enzymes, such as the cytochrome P450 (CYP) family, are strongly associated with drug responses. In particular, *CYP2D6* genotyping allows the identification of four metabolizer groups: poor, intermediate, extensive, and ultra-rapid. Poor metabolizers are not able to metabolize gefitinib, and gefitinib itself can inhibit CYP2D6 [183].

Recently, several reports have demonstrated a correlation between gefitinib-induced hepatotoxicity and low-activity variants of CYP2D6, such as *CYP2D6*5* and *CYP2D6*10*, especially in patients treated with CYP3A4 inhibitors [184]. Based on these findings, the EMA decided to include a warning about gefitinib-based therapy for patients who are classified as poor metabolizers based in *CYP2D6* genotyping. Although drug dosage adjustment is not necessary for these patients, they must be frequently monitored for the onset of adverse effects, especially if they are concomitantly treated with CYP3A4 inhibitors [6].

Lapatinib, an inhibitor of human epidermal growth factor receptor 2 (HER2) and EGFR, is used for the treatment of HER2-overexpressing metastatic breast cancer, often in association with capecitabine and letrozole [185]. Lapatinib therapy can cause hepatotoxicity (ALT and TBL elevation). In particular, an increased risk of DILI, in patients treated with lapatinib, is correlated with the presence of HLA-DQA1*02:01/DRB1*07:01 alleles (Table 2). The relationship between lapatinib and HLA class II suggests an immune-mediated hepatocyte injury that involves CD4-positive T-cells [186]. The molecular mechanism is not completely understood, and it has been hypothesized that in the liver of individuals carrying HLA-DQA1*02:01 and DRB1*07:01 heterodimers, lapatinib metabolites bind cellular proteins recognized by antigen-presenting cells. This mechanism leads to downstream activation of CD4-positive T-cells and to development of an adaptive immune response in the liver [14, 187]. However, the majority of patients with these alleles do not develop DILI, probably because other factors are involved. Despite this, the EMA label for lapatinib reports the possibility of liver injury in patients with the DQA1*02:01/DRB1*07:01 alleles. No genetic test is recommended, however, because the association between this genotype and lapatinib treatment has not been sufficiently validated, but the surveillance of potential adverse effects in patients bearing these alleles is mandatory [6].

Another drug exhibiting G6PD-dependent toxicity is dabrafenib, a TKI that targets somatically mutated forms of BRAF, such as the V600E, that are often present in metastatic melanoma. Dabrafenib contains a sulfonamide moiety that increases the risk of hemolytic anemia in patients with G6PD deficiency; consequently, this drug causes a G6PD-dependent toxicity that must be monitored.

In addition to these TKIs, for which the EMA and US FDA provide official indications about relevant PGx markers, an increasing number of studies on onco-PGx variants is available for many other TKIs; however, these remain under evaluation. Among them, sunitinib and sorafenib have been studied extensively, and potential useful onco-PGx variants have been identified.

Orally administered sunitinib is a multi-targeted TKI that inhibits the VEGFRs types 1, 2, and 3; platelet-derived growth factor receptor-α and -β; Fms-related tyrosine kinase 3; colony stimulating factor-1 receptor; the cytokine receptor Kit; and the proto-oncogene tyrosine-protein kinase receptor Ret. Sunitinib is approved for the treatment of metastatic renal cell carcinoma (mRCC), imatinib-resistant metastatic gastrointestinal stromal tumors (GIST), and neuroendocrine tumors of the pancreas.

The most common side effects in patients treated with sunitinib are diarrhea, hand–foot syndrome, mucositis, vomiting, hypertension, leukopenia, neutropenia, and thrombocytopenia. Less common adverse events include cardiotoxicity, hypothyroidism, and hepatotoxicity. About 32 % of mRCC patients treated with sunitinib exhibit multiple adverse events and consequently require a significant dose reduction, or in extremis a suspension of treatment. The most likely reason for this varied response to sunitinib could be inter-individual variability in the pharmacokinetics and pharmacodynamics of this drug.

Van der Veldt et al. [187] hypothesized that the CYP3A5*1 allele could transform more rapidly sunitinib to its active metabolite SU12662, thus causing severe toxicity due to the increased exposure to toxic agents.

Van Erp and colleagues [188] performed an association study on 219 patients treated with sunitinib as a single agent. Their results suggested that development of sunitinib toxicity is related to several germline polymorphisms in genes involved in its pharmacokinetics, including the CYP450 CYP1A1, the drug transporters ABCB1 and ABCG2, and the CYP450 regulator NR1I3; and in its pharmacodynamics, as VEGFR2 and FLT3.

A recently published case report described a 76-year-old man with mRCC treated with the recommended dose of sunitinib who exhibited severe hepatotoxicity, probably due to the altered functionality of ABCB1 in the presence of the rs1045642, rs1128503, and rs2032582 polymorphisms [189]. This patient was successfully treated with another TKI, sorafenib, which differs slightly from sunitinib with respect to pharmacokinetics and pharmacodynamics.

Diekstra and colleagues [190] investigated whether polymorphisms in the candidate genes cited above truly affect sunitinib response. SNPs in CYP3A4, CYP3A5, and ABCB1 seem to be related to the clearance of sunitinib; however, the results were insufficient to directly modify the dosing regimens. Furthermore, like pazopanib, sunitinib

seems to cause hyperbilirubinemia in patients carrying UGT1A1 variants [178].

Another TKI that influences bilirubin levels in the presence of UGT1A1*28 allele is sorafenib [191], an antiangiogenic agent that inhibits VEGFR and other TKs and is currently used to treat hepatocellular carcinoma. Peer and colleagues [191] suggested that polymorphisms in UGT1A9, which directly metabolizes sorafenib, are involved in the development of toxicity, in particular the hand–foot–skin reaction (HFSR). Another study [192] hypothesized the involvement of polymorphisms in UGT1A9, in particular UGT1A9 IVS1-37431, in the onset of HFSR; furthermore, this study also proposed the involvement of genetic variants in an angiogenesis factor, such as VEGF or TNF-α, in the sorafenib-induced HFSR. However, all these reported preliminary findings require more supporting evidence before they are integrated in daily clinical practice.

In conclusion, further data are necessary to validate effective PGx markers for TKIs, especially considering the complications caused by drug–drug interactions, indeed the EMA European Public Assessment Report (EPAR) for sunitinib attests that potent CYP3A4 inhibitors or inducers should be avoided as they may affect sunitinib plasma levels [6].

3.7 Tamoxifen

Tamoxifen (TAM) is a selective estrogen receptor modulator. Starting in the 1970s, TAM was used in the treatment of hormone receptor-positive breast cancers in both the advanced and early stages. In patients diagnosed in the early stage, TAM is conventionally administered for 5 years to reduce the risk of cancer recurrence and mortality [193]. A recent study reported that the protective action of TAM is increased when treatment is prolonged [194]; accordingly, the updated American Society of Clinical Oncology (ASCO) clinical guidelines recommend that TAM should be offered for a total of 10 years to patients with early-onset breast cancer [195].

TAM is also useful for primary prevention in women with high-risk conditions, such as a positive familial history, *BRCA* gene mutations, breast in situ dysplasia, or atypical hyperplasia. Based on these findings, the National Institute for Health and Care Excellence in the UK updated its guidelines, recommending that high-risk women should undergo 5 years of chemoprevention with TAM [196]. It has been estimated that the number of subjects who will be treated with TAM, which is already high today, will increase in the near future. Because the use of TAM is accompanied by adverse events (principally, endometrial cancer, and thrombo-embolism), the risk/benefit ratio should be carefully evaluated on an individual basis, especially when TAM is used in chemoprevention [197].

TAM is a pro-drug that is converted into many different molecules, such as endoxifen (EN) and 4-OH TAM, some of which have an increased pharmacological activity relative to the parental drug. EN, which is generated by the addition of a hydroxyl group and the elimination of a methyl group, has 30–100-fold higher affinity for the estrogen receptor and a more pronounced anti-proliferative action than TAM. Besides, the plasma levels of EN are 5-fold higher than those of 4OH TAM; consequently, EN is considered primarily responsible for TAM's clinical activity. Importantly, EN plasma levels exhibit large inter-individual variability: between patients taking the same TAM dose, variations of ~20-fold can be detected in systemic concentrations of EN [198]. The cytochrome CYP2D6 exerts a primary role in the metabolic pathway involved in EN production, and its activity is associated with different treatment outcomes and systemic effects of EN. Some drugs frequently used in breast cancer patients, such as the anti-depressives paroxetine and fluoxetine (that are SSRIs), strongly inhibit CYP2D6 activity, thereby reducing EN plasma levels [23] and significantly increasing the risk of recurrence and death in breast cancer patients [199]. This drug-drug interaction prevents the concomitant prescription of TAM with SSRIs, especially in patients who have a constitutional decreased CYP2D6 activity due to the presence of specific allelic variants (described below).

3.7.1 CYP2D6

CYP2D6 is a member of the cytochrome P450 family, and it is one of the most important enzymes involved in the metabolism of xenobiotics: specifically, it is responsible for the metabolism and elimination of approximately 25 % of clinically used drugs [23].

CYP2D6 (22q13) has around 100 allelic variants associated with different protein activity levels. Table 4 lists the most frequent polymorphisms of *CYP2D6*, together with the phenotype of the protein. A complete catalogue of the known CYP2D6 variants is available on The Human Cytochrome

Table 4 Most frequent polymorphisms of CYP2D6

Allele	rs ID	Sequence variation	Enzyme activity
*1	-	Wild type	Normal
*1×2	-	*CYP2D6* duplication	High
*2	rs16947	2850C>T, 4180G>C	Normal
*2×2	rs16947	*CYP2D6* duplication	High
*3	rs4986774	2549delA	Null
*4	rs3892097	1846G>A	Null
*5	-	*CYP2D6* deletion	Null
*6	rs5030655	1707delT	Null
*9	rs5030656	2615_2617delAAG	Low
*10	rs1065852	100C>T	Low
*17	rs28371706	1023C>T, 2850C>T	Low
*41	rs28371725	2988G>A	Low

Table 5 Comprehensive report on available dosing guidelines and warnings for onco-PGx validated markers

Gene	Dosing guidelines		Warnings
	CPIC	DPWG	
DPYD	Alternative drug for patients who are homozygous for DPYD non-functional variants (*2A (rs3918290), *13 (rs55886062), and rs67376798) 50 % reduction in starting dose for heterozygous patients for DPYD non-functional variants; followed by titration of dose based on toxicity b or pharmacokinetic test (if available).	Alleles: -active=*1, *4, *5, *6, *9A -decreased activity=*9B, *10 -inactive=*2A, *3, *7, *8, *11, *12, *13, 496A>G, IVS10-15T>C, 1156G>T, 1845G>T For patients carrying two inactive or decreased activity alleles or one inactive and one decreased activity allele, alternative drug to 5-FU, capecitabine and tegafur are recommended. Reduce 5-FU, capecitabine or tegafur dose (by 50 %) or select an alternative drug for patients carrying one active and one inactive or decreased activity allele.	The US-FDA and EMA European Public Assessment Report (EPAR) state that 5-FU, capecitabine and tegafur are contraindicated in patients with DPD deficiency.
TMPT	Start with normal starting dose for patients who are homozygous for normal functional *1 allele. Reduce by 30–70 % the starting dose and adjust doses of thiopurines based on degree of myelosuppression and disease-specific guidelines for patients who are heterozygous for one non functional allele (*2, *3A, *3B, *3C, or *4). Reduce drastically doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of thiopurines based on degree of myelosuppression and disease-specific guidelines for patients who are homozygous for one non functional allele (*2, *3A, *3B, *3C, or *4). For non malignant conditions, consider alternative non thiopurine immunosuppressant therapy.	Select alternative drug or reduce dose by 50 % for patients who are heterozygous for one inactive allele (*2, *3, *4-*18). Select alternative drug or reduce dose by 90 % for patients who are homozygous for one inactive allele (*2, *3, *4–*18). Increase dose in response of hematologic monitoring and efficacy.	The US-FDA and EMA labels contain information about patients predisposed to thiopurines related toxicity based on TMPT genotype and phenotype. The FDA-approved drug label for cisplatin evidences warnings and precautions when treating children with certain TPMT gene variants (e.g., TPMT *3B and TPMT *3C) due to an increased risk of ototoxicity. The US FDA requires that all children administrated with cisplatin should undergo audiometric testing.
UGT1A1		Reduce the starting dose of irinotecan by 30 % for UGT1A1*28 homozygous patients receiving more than 250 mg/m^2.	The US-FDA requires a reduction in the starting dose of irinotecan in patients UGT1A1*28 homozygous. The US-FDA states that individuals with the UGT1A1*28 genotype are at an increased risk of hyperbilirubinemia when taking pazopanib and nilotinib. The EMA EPAR highlights that erlotinib, nilotinib, pazopanib and regorafenib should be used with caution in patients with low expression of UGT1A1 or Gilbert's disease (caused by genetic variants in UGT1A1 gene).
G6PD	G6PD variants have been shared into five classes by the World Health Organization [172], on the basis of G6PD enzyme activity and clinical manifestations. Rasburicase is contraindicated in G6PD deficient patients with or without CNSHA. In patients with a negative or inconclusive genetic test result an enzyme activity test is recommended prior to rasburicase treatment to determine whether a patient is G6PD deficient.		The Us-FDA and EMA state that rasburicase is contraindicated in patients with G6PD deficiency. The US-FDA establishes that patients treated with dabrafenib should be closely monitored if they are deficient in glucose-6-phosphate dehydrogenase (G6PD) activity.

Table 5 (continued)

Gene	Dosing guidelines		Warnings
	CPIC	DPWG	
CYP2D6		For CYP2D6 poor and intermediate metabolizers, who have null or low enzyme activity (Table 4), consider using aromatase inhibitors for postmenopausal women due to increased risk for relapse of breast cancer with tamoxifen. Avoid concomitant CYP2D6 inhibitor use for intermediate metabolizers.	The EMA EPAR assesses that CYP2D6 poor metabolisers should be closely monitored for adverse events.
HLA			The EMA EPAR reports pharmacogenetic information regarding an increased risk of lapatinib-induced hepatotoxicity in patients carrying the DQA1*02:01 or DRB1*07:01 HLA alleles.

All the information collected herein can be consulted on PharmGKB database [58], EMA [6] and FDA [5]

P450 (CYP) Allele Nomenclature Database (Table 1) [178]. The most common genotypes associated with normal activity (rapid metabolizers) are the *1 and *2 alleles; those associated with null activity (slow metabolizers) are *3, *4, *5, and *6; and the alleles leading to a reduced activity (intermediate metabolizers) of CYP2D6 are *9, *10, and *41.

CYP2D6 may also be duplicated, and subjects carrying *1×2 or *2×2 on one gene copy carrying the *1 or *2 alleles exhibit very high CYP2D6 activity (ultra-rapid metabolizers). *CYP2D6* polymorphisms exert a strong influence on EN plasma levels [200]. Recently, a meta-analysis study [201], analyzed the conflicting data regarding the association between *CYP2D6* polymorphisms and TAM adjuvant treatment outcome These discrepancies are possibly due to bias in retrospective approaches, incomplete profiles of the evaluated allele variants, and inadequate tissues for germline genotyping. In 2006, the US FDA evaluated the possibility of introducing *CY2D6* genotyping before initiation of TAM treatment, based on preliminary observations of the association between the *4 allele and worse outcomes in early breast cancer patients [202]. In the following years, these data were not confirmed, and the US FDA refused to go further. The results of two large collaborative trials, Big1-98 [203] and ATAC [204], concluded the debate: they did not confirm a correlation between CYP2D6 genotype and the risk of disease recurrence [205]. However, some months later, subsequent analysis showed that the allelic distributions in BIG 1–98 and ATAC studies did not correspond to Hardy–Weinberg equilibrium, suggesting a bias related to the choice of samples used for the genotyping. This finding prompted other authors [206] to review the previously published data, revealing that some haplotype analyses had been performed on DNAs from tumor specimens and not from normal tissues.

Although significant associations have been observed between EN plasma levels and TAM treatment outcomes, as well as between CYP2D6 genotype and risk of recurrence [207, 208], the data were not considered sufficient to definitively propose CYP2D6 analysis routinely as a predictive marker for adjuvant anti-estrogenic treatment [209]. At this time, the only known factor affecting TAM activation is the co-administration of strong CYP2D6 inhibitors (such as SSRIs), which as highlighted in the clinical guidelines should be carefully avoided.

4 Conclusions

In a number of cancers, the detection of somatic mutations is required for targeted therapy prescription; however, routine investigation of germline genetic variants associated with drug response is still confined to a few drugs, mainly because of the inconclusive nature of the underlying data. At this time, the validated constitutional onco-PGx variants are listed in Table 5, together with the dosing guidelines edited by CPIC and DPWG and the warnings included in US-FDA and EMA approved labels. US-FDA and EMA warnings generally refer to the genes involved in drug response, but not to the specific polymorphisms to test, that are instead included in dosing guidelines reviewed by CPIC and DPWG, on the basis of strong evidences reported in the literature and the calculated level of evidence [58].

These genetic variants should be analyzed in every patient before starting an anticancer therapy, to avoid severe side effects.

For the other genes and polymorphisms described in this review, the data reported in the literature do not reach a high level of evidence and therefore are not been included in Table 5.

In the most ideal situation, using onco-PGx tests that include all possible relevant variants, clinicians could prescribe the optimal cancer treatment with a drug dosage tailored to improve efficacy, reduce toxicity, and predict non-responders, thereby decreasing chemotherapy-associated morbidity and improving health benefits. This ideal condition could be achieved if onco-PGx is supported by a TDM approach. In fact, onco-PGx and TDM could be thought of as two players on the same team, working together to formulate the best anticancer therapy for each patient. The identification of new onco-PGx markers could be facilitated by TDM studies that accurately report any benefit or side effect of a treatment; on the other hand, the integration of information from onco-PGx tests in TDM could help to define proper drug dosages. The association of onco-PGx and TDM should be dynamic and continually updated. This is especially true today, as the rapid evolution of next-generation sequencing applications and the development of computational methods results in generation of massive amounts of data on genomic variability that must be understood and validated to promote the assessment of shared guidelines for PGx. Faster validation of onco-PGx markers requires not only TDM, but also prospective studies, which must be encouraged during pharmaceutical trials. Indeed, prevention of the administration of inappropriate drugs on the basis of inter-individual variability should reduce the cost of anticancer therapies. Furthermore, the identification of onco-PGx variants should be obtained through the application of certified genetic tests. Therefore, the public health community should be prepared to adopt a new way of dealing with anticancer treatments, in which the personalized medicine becomes a precision instrument finely tuned for each patient.

Mark G. Kris, a thoracic oncologist at Memorial Sloan Kettering Cancer Center in New York City, recently said, "if you have lung cancer in 2014, the first thing we do is a genetic test for potential drivers" [210] We feel that this should be done not only for somatic mutations, but also for germline variants that could influence therapeutic outcomes.

Conflict of interest The authors declare that they have no conflicts of interest.

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