

Innovation in cancer pharmacotherapy through integrative consideration of germline and tumor genomes

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Abbreviations

5-FU	5-Fluorouracil
6-MP	6-mercaptopurine
ABC	ATP-binding cassette
ADME	Absorption, Distribution, Metabolism, and Excretion
ADR	Adverse drug reactions
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AS	Activity score
AUC	Area under the curve
CRC	Colorectal cancer
CYP	CytochromCytochrome P450
DDI	Drug-drug interactions
DDGI	Drug-drug-gene interactions
DGI	Drug-gene-interactions
DF	Decreased function
DME	Drug metabolizing enzyme
DSBs	DNA double-strand breaks
EGFR	Epidermal growth factor receptor
FFPE	Formalin-fixed paraffin-embedded
GOF	Gain of function
GWAS	Genome-wide association studies
HRD	Homologous recombination deficiency
IF	Increased function
IM	Intermediate metabolizer
Indel	Insertion-deletion
IRN-	Irinotecan
LOF	Loss of function

LOH	Loss of heterozygosity
LST	Large-scale state transitions
mAbs	Monoclonal antibodies
MASTER	Molecularly Aided Stratification for Tumor Eradication Research
mCRPC	Metastatic castration-resistant prostate cancer
MMR	Mismatch repair
MOFA	Multi-Omics factor analysis
MR	Metabolic ratio
MTB	Molecular tumor boards
NF	Normal function
NGS	Next generation sequencing
NHEJ	Non-homologous end joining
NM	Normal metabolizer
NR	Nuclear receptor
NSCLC	Non-small cell lung carcinoma
PARP	Poly ADP-ribose polymerase
PARPi	Poly ADP-ribose polymerase (PARP) inhibition
PDAC	Pancreatic ductal adenocarcinoma
PF	Poor function
PGx	Pharmacogenomics
PM	Poor metabolizer
PROTAC	Proteolysis-targeting chimera
PRS	Polygenic risk scores
qPCR	Quantitative PCR
RBC	Red blood cell
RCT	Randomized controlled trials
sCNA	Somatic copy number aberration
SLC	Solute carrier
SNV	Single nucleotide variant
TPD	Targeted protein degradation
UM	Ultrarapid metabolizers
VKORC	Vitamin K epoxide reductase complex
WES	Whole exome sequencing
WGS	Whole genome sequencing

Keywords: Pharmacogenomics, pharmacogenetics, cancer, drug therapy, cancer, treatment recommendation, personalized medicine, precision oncology, next generation sequencing, molecular profiling, molecular tumor board

Abstract

Precision cancer medicine is widely established, and numerous molecularly targeted drugs for various tumor entities are approved or in development. Personalized pharmacotherapy in oncology has so far been based primarily on tumor characteristics, e.g., somatic mutations. However, the response to drug treatment also depends on pharmacological processes summarized under the term ADME (absorption, distribution, metabolism, and excretion). Variations in ADME genes have been the subject of intensive research for more than five decades, considering individual patients' genetic makeup, referred to as pharmacogenomics (PGx). The combined impact of a patient's tumor and germline genome is only partially understood and often not adequately considered in cancer therapy. This may be attributed, in part, to the lack of methods for combined analysis of both data layers. Optimized personalized cancer therapies should, therefore, aim to integrate molecular information which derives from both the tumor and the germline genome, and taking into account existing PGx guidelines for drug therapy. Moreover, such strategies should provide the opportunity to consider genetic variants of previously unknown functional significance. Bioinformatic analysis methods and corresponding algorithms for data interpretation need to be developed to integrate PGx data in cancer therapy with a special meaning for interdisciplinary molecular tumor boards, where cancer patients are discussed to provide evidence-based recommendations for clinical management based on individual tumor profiles.

Significance Statement

The era of personalized oncology has seen the emergence of drugs tailored to genetic variants associated with cancer biology. However, the full potential of targeted therapy remains untapped due to the predominant focus on acquired tumor-specific alterations. Optimized cancer care must integrate tumor and patient genomes, guided by pharmacogenomic principles. An essential prerequisite for realizing truly personalized drug treatment of cancer patients is the development of bioinformatic tools for comprehensive analysis of all data layers generated in modern precision oncology programs.

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I. Introduction

A. Precision medicine and pharmacotherapy

The concept of "one drug fits all" has been outdated in recent years by the approach of personalized medicine. Significant progress has been made in the therapy of tumor diseases, with various targets identified through innovative drug development (Mateo *et al.*, 2022). An impressive example is the cancer therapy of non-small cell lung carcinoma (NSCLC) with > 20 different molecular subtypes for which targets have been identified (Harada *et al.*, 2023). In consequence drugs have been developed to compensate for gain-of-function mutations for instance in the epidermal growth factor receptor (EGFR) resulting in innovative approved drugs such as afatinib, and erlotinib (<https://www.thelancet.com/pb-assets/Lancet/infographics/nsclc/image-1709218498257.pdf>). This approach is supported by a recent article that highlights the benefits of pan-genomic markers. Specifically, it emphasizes the linkage between the discovery of cancer driver genes, mutational signatures, and the use of real-world clinical data to improve the stratification of treatment outcomes and prognosis (Sosinsky *et al.*, 2024). Moreover, precision pharmacotherapy in oncology requires consideration of a broader portfolio of factors compared to non-oncological therapies. Recent efforts are spent to consider technologies, such as RNA sequencing, DNA methylation, gene expression profiling, and proteomics to close the gap of so far unexplained interindividual variability of response to cancer therapy by the use of tumor or metastasis biomaterial and/or liquid biopsies to promote precision cancer therapy (Alix-Panabières and Pantel, 2021; Akhoundova and Rubin, 2022) . However, precision medicine in cancer needs to take into consideration not only the tumor and related molecular targets but also genetic variability in the germline (Schwab and Schaeffeler, 2012). For instance adverse drug reactions (ADR) that affect organ systems of the human body, such as the liver or blood cells, are influenced by the germline and not the tumor genome (Hertz and Rae, 2015; Osanlou *et al.*, 2018). Given that cancer therapy often necessitates the use of combination therapies, including innovative immunotherapies, the demand for complex analyses and prediction tools is required. Those tools that comprehensively cover a remarkable array of pharmacologically

relevant data will enable treatment strategies that address also cancer heterogeneity and allow for more effective and personalized approaches in oncological care.

Another important area in precision cancer therapy is the concept of targeted protein degradation (TPD) to modulate proteins which are unable to be targeted with small molecules (Békés *et al.*, 2022). In this context, proteolysis-targeting chimera (PROTAC) protein degraders, which are heterobifunctional molecules, chemically induce selective, proteasome-dependent degradation of target proteins that are crucial in cancer e.g. lymphocyte-specific protein tyrosine kinase (LCK) (Hu *et al.*, 2022). These degraders are highly promising for novel therapeutic options, as recently reviewed (Zhu *et al.*, 2023).

The goal of this review is to summarize up-to-date information on the clinical relevance of germline and somatic alterations to emphasize how important both aspects are in cancer therapy including supportive care and to develop approaches for implementing this knowledge into clinical practice.

B. Prediction of drug response and adverse drug reactions

Prediction of drug therapy response depends on multiple factors, including age, gender, weight, ethnic background, and interactions between prescribed medications, all of which play significant roles (Sadee *et al.*, 2023). This is particularly relevant in patients undergoing cancer therapy, where supportive care is often necessary alongside causal oncological treatments. The consideration of genetic variability in drug therapy is covered by the term pharmacogenomics (PGx). This indicates the elucidation of individual genetic variation in genes related to pharmacokinetics of drugs represented by absorption, distribution, metabolism, and excretion (ADME) processes which may affect drug efficacy and safety. Moreover, the use of multiomics approaches will further enable optimization of drug therapy and contribute to the discovery of novel targeted therapies (Pirmohamed, 2023).

PGx is a relatively young scientific discipline, in light of the fact that the decoding of the human genome in 2000 significantly promoted research activities. However, notable examples were already discovered in the 60s and 70s (Figure 1A) including the glucose-6-

phosphate dehydrogenase deficiency (Alving *et al.*, 1956), the N-acetyltransferase polymorphism (Evans *et al.*, 1960), and the sparteine/debrisoquine cytochrome P450 (CYP2D6) polymorphism (Mahgoub *et al.*, 1977; Eichelbaum *et al.*, 1979). The number of scientific articles on PGx has steadily increased over the past 15 years, with over 2000 publications in 2023 (Figure 1B). The growing importance of PGx in the context of cancer therapy is the subject of scientific investigations, but is still significantly underrepresented compared to non-cancer research as demonstrated by our literature review as well as the limited number of cancer drugs taken into account in PGx guideline articles in comparison to non-cancer drugs (<https://www.pharmgkb.org/guidelineAnnotations>). The significant achievement of PGx in recent years is that data from randomized controlled trials (RCT) provide evidence for preemptive PGx testing for selected drugs and underpin the functional relevance of variation in ADME genes by experimental studies (Roden *et al.*, 2019). However outside the frame of dedicated trials the use of population-scale and hospital-based biobanks linked to electronic health records (EHR) provide evidence to validate PGx associations particularly regarding rare variants as demonstrated by the US eMERGE (Electronic Medical Records and Genetics) network (McCarty *et al.*, 2011) and the Biobank at Vanderbilt University (BioVU) concept (Danciu *et al.*, 2014).

In addition to genetic alterations in drug targets, factors such as impaired organ function - particularly relevant for drugs primarily excreted through the kidneys (e.g. platinum-based drugs)- should also be considered for dose adjustments. Moreover, drug-drug interactions (DDI) are widely accepted in drug therapy as well as drug-gene-interactions (DGI) (see below) whereas potentially synergistically or antagonistically acting drug-drug-gene interactions (DDGI), i.e. the cumulative effect of DDIs and DGIs (Bruckmueller and Cascorbi, 2021) are so far underestimated. These interactions are particularly relevant in cancer therapy since multiple PGx drugs, including supportive care, are concomitantly administered to cancer patients (see II.F).

C. Relevance of ADME processes

Pharmacological processes related to efficacy or occurrence of ADR are associated with the absorption, distribution, metabolism, and excretion of the active substance and/or related metabolites. In addition to age-related changes genetic variations in drug-metabolizing enzymes (DME) (Lauschke *et al.*, 2024) and membrane transporters (Nies *et al.*, 2022) substantially influence these ADME processes. Generally, DMEs are categorized into phase I and phase II enzymes, with major consequence for the elimination, but in the case of phase I enzymes, also for the bioactivation of so-called prodrugs (Zanger and Schwab, 2013; Fukami *et al.*, 2022). More specific information related to DME and PGx is given in section II. Drug transporters are membrane-bound proteins that facilitate the movement of drugs into or out of the cell. They are for instance expressed in the apical membrane of enterocytes, the biliary canalicular membrane of hepatocytes, the luminal membrane of the kidney's proximal tubules, and the epithelial cells of the blood-brain barrier, but much more locations are well described (Galetin *et al.*, 2024). The ATP-binding cassette (ABC) efflux transporter family comprises 48 proteins (seven subfamilies, labeled ABC-A to ABC-G) that play a critical role in actively transporting various molecules, including ions, lipids, and a wide array of xenobiotic compounds, including chemotherapeutic agents (Moore *et al.*, 2023). The solute carrier (SLC) uptake transporter superfamily represents the largest group of membrane transporters in the human genome, encompassing over 400 proteins grouped into 65 subfamilies (Schlessinger *et al.*, 2023). There is a significant diversity in substrate specificity among the subfamilies, reflecting the complexity of these transporters in cellular and physiological processes. While subfamilies like SLC2 and SLC27 are specialized for a narrow range of substrates with similar physicochemical properties like carbohydrates or long-chain fatty acids, the SLC22 subfamily for instance exhibits a broader specificity, facilitating the transport of a diverse portfolio of ions like organic cations, anions, and zwitterions (Yee and Giacomini, 2021). Specific drug transport profiles make transporters (ABC and SLC) crucial in modulating drug failure or drug resistance, especially in cancer therapy (see III.C) (Alam *et al.*, 2023). Nuclear receptors (NR) are a family of 48 ligand-

activated transcription factors, that directly regulate the expression of genes in many physiological and pathophysiological processes, including organogenesis, cell differentiation, and metabolism (Frigo *et al.*, 2021). While most NRs are activated by binding endogenous substances such as thyroid hormones, steroids, and vitamins, some, such as PXR and CAR, are activated by xenobiotics, including drugs. These xenosensing NRs regulate drug metabolism and transport and thus have particular importance for cancer drug therapy (Zhao *et al.*, 2019).

Because drugs must be actively taken up by tumor cells, and their effectiveness is significantly influenced by intracellular metabolism, tumor-specific data on ADME-relevant processes are crucial, but currently limited. The assumption that liver-specific data on DME and transporters related for cancer drugs also apply to tumor cells is misleading, since cancer cells, depending on the tumor entity, exhibit in most cases a different ADME expression profile (see III.C) (Hu *et al.*, 2020; Liu *et al.*, 2023). Thus, it becomes apparent that an integrative understanding of ADME processes across the whole human body and tumor tissue requires methods that allow an estimation of drug concentrations in specific cell fractions including cancer cells (Hertz and McLeod, 2013). Mathematical modeling, covering various pharmacological processes, is the basis for a variety of physiologically-based pharmacokinetic models (PBPK) (Wojtyniak *et al.*, 2020; Wang *et al.*, 2024). Cancer drugs, such as tyrosine kinase inhibitors, have benefited from these models (Adiwidjaja *et al.*, 2022; Hwang *et al.*, 2024; Kovar *et al.*, 2024). So far, PBPK models considered only germline information, but neglected somatic variants and their significance for tumor-associated ADME processes. Thus, there is a strong need to incorporate relevant cancer specific information as well as laboratory data (e.g., intracellular plasma concentration in tumor cells), together with information about the body's own organ systems (e.g., liver).

D. The Human Genome and Pharmacogenomics

Even before the structure of DNA was fully understood, researchers observed distinct inheritance patterns in drug response (Figure 1A) and were able to identify hereditary causes for variation in enzyme activities among patients and their family members including

cholinesterase deficiency (Kalow and Genest, 1957), debrisoquine/sparteine CYP2D6 polymorphism (Mahgoub *et al.*, 1977; Eichelbaum *et al.*, 1979), and thiopurine S-methyltransferase polymorphism (TPMT) (Weinshilboum and Sladek, 1980). Since the 70s, novel molecular technologies allowed correlation analysis of phenotypes and variation in DNA sequences, e.g. using restriction fragment length polymorphism (RFLP) analysis. Consequently, these techniques enabled population studies that firstly revealed the prevalence of frequent but also rare genetic variation in the context of PGx (e.g. *TPMT* polymorphism, see II. A), and offered the possibility to consider inter-ethnic differences of frequency distributions. Generating the first sequence of the human genome, declared completed in April 2003 (<https://www.genome.gov/human-genome-project>), with a comprehensive map of about 25,000 genes (International Human Genome Sequencing Consortium, 2004) marked a turning point in PGx research. High-throughput screening methods facilitated a comprehensive view of the genome. Short-read next-generation sequencing (NGS) like whole exome sequencing (WES) or whole genome sequencing (WGS) enabled the systematic detection of novel and rare variants including single nucleotide variants (SNVs), structural variants, and copy number variations (CNV) in population-scale cohorts (e.g. 1000Genome Project, ExAC, and gnomAD) (Porubsky and Eichler, 2024). In general, loss and gain of function mechanisms have been described, including effects on mRNA translation, splicing, protein expression, and substrate specificity (Figure 2A). The NIH-funded Pharmacogenomics Knowledgebase (PharmGKB; pharmgkb.org) has collected clinical data on *in vitro* or *in vivo* functional consequences on drug metabolism and transport for over 1000 pharmacogenes, encompassing more than 160 genes listed in the FDA's table of PGx-biomarkers (www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations); Figure 2C). Moreover, very recently the Pharmacogene Variation (PharmVar) Consortium has been established as a central repository for pharmacogene variation (pharmvar.org). PharmVar aims to focus on the haplotype structure and allelic variation of ADME genes to facilitate research activities and particularly the interpretation of PGx results.

While the analysis of more than 60,000 exomes suggested that approximately 80% of individuals carry at least one genetic variant in a pharmacogene (Schärfe *et al.*, 2017; Pirmohamed, 2023), a similar study showed that each individual carries around 40 functional SNVs in 208 pharmacogenes, and 10% of those were rare (Ingelman-Sundberg *et al.*, 2018). Furthermore, in a recent study which comprehensively assessed the structural variability across pharmacogenes (344 ADME genes and 564 drug targets) in 10,847 WGS samples, each individual carried on average 11.8 structural variants with potential functional impact on the coding regions of pharmacogenes (Tremmel, *et al.*, 2023). Another study demonstrated, that across individuals 97% of 201 analyzed pharmacogenes are affected by rare deletions and/or duplications (Santos *et al.*, 2018). When comparing longitudinal and nearly comprehensive electronic health records with PGx data, 80% of patients are prescribed at least three medications in their lifetime that could be affected by actionable genetic variants (Ye *et al.*, 2023).

II. Germline Genome and cancer therapy

A. Genetic Variation in ADME genes

As outlined before, ADME comprises various pharmacologically relevant processes including DME, drug transporters, and nuclear receptors. The elucidation of heritable genetic variation in DME spans almost six decades and comprehensive overviews regarding the occurrence, the frequency and the functionality of SNVs in CYP450 enzymes (Zanger and Schwab, 2013), UGTs (Miners *et al.*, 2023), SULTs (Isvoran *et al.*, 2022) and other DME like TPMT and NUDT15 (Pratt *et al.*, 2022) are publicly available.

Genetic variation is related to various molecular mechanisms resulting in different functional consequences and subsequently in diverse phenotypes. Loss-of-function (LOF) variants in CYP450 genes often influence RNA splicing and thereby alter gene expression, as well as have an impact on transcription or structural configuration of proteins. Alternative splicing is observed for several DMEs, particularly for CYP450 enzymes due to intronic variants (e.g. CYP2B6*4/*6/*9, CYP2C19*2, CYP2D6*4/*41, CYP3A4*22, CYP3A5*3) but also for the

dihydropyrimidine dehydrogenase (DPD) enzyme (*DPYD hapB3 c.1129-5923C>G*). Gain-of-function variants include promoter variants (e.g. *CYP2B6*22*, *CYP2C19*17*), coding variants that result in decreased substrate turnover (e.g. *CYP2D6*10*), and CNV characterized by an increased number of functional gene copies (e.g., *CYP2D6*, *CYP2A6*, *SULT1A1*) (Table 1; pharmvar.org/gene/'gene_name'). In contrast, deletions of the whole gene or a partial gene region (e.g. *CYP2D6*5*) result in missing protein expression and activity. *GSTT1*, along with *GSTM1* or *UGT2B17*, are notable for highly frequent null genotypes due to homozygous deletions, which have been studied extensively related to drug metabolism, but also disease susceptibility, including cancer (Tremmel *et al.*, 2020; Isvoran *et al.*, 2022; Grussy *et al.*, 2023).

Variation in transporter genes is also frequent and various molecular mechanisms have been reported with functional consequences on pharmacokinetic properties of transporter substrates (Fisel *et al.*, 2017; Tremmel *et al.*, 2022; Galetin *et al.*, 2024). Prominent examples of genetic variation in drug transporters are *ABCB1* (encoding P-glycoprotein or Multidrug Resistance 1), *ABCC1* (encoding MRP1), and *ABCG2* (encoding BCRP) although their clinical relevance for drug therapy remains limited. For instance, while the pharmacokinetics and the response of selected drugs has been extensively investigated in association with the frequent *ABCB1* haplotype, consisting of three variants (rs1128503, p.G412G; rs2032582, p.A893S/T; rs1045642, p.I1145I), even across different ethnic populations, the findings have been largely inconsistent (Schwab *et al.*, 2003; Wolking *et al.*, 2015). In contrast, drug dosing guidelines for allopurinol (van der Pol *et al.*, 2024) and rosuvastatin (Cooper-DeHoff *et al.*, 2022) consider genotyping of *ABCG2* at onset of therapy to be potentially beneficial for drug effectiveness.

Genetic variation in SLC transporters has also been extensively studied. For instance, statin (e.g. simvastatin) related myopathy is linked to *SLCO1B1* (encoding OATP1) variants (Duarte and Cavallari, 2021; Cooper-DeHoff *et al.*, 2022), and *SLC22A1* (encoding OCT1) genetic variation is associated with metformin response (Emami Riedmaier *et al.*, 2013; Kölz *et al.*, 2021).

Genetic variation in NRs and the aryl hydrocarbon receptor (AhR) have been described, but the clinical significance is currently very limited. One reason is that in the case of functional impairment of a certain NR, other NRs mostly compensate for the defect (Chai *et al.*, 2013). In addition, the frequency of genetic variation in ADME genes (DME, transporter, NR) in different ethnic populations varies and has been the subject of extensive research in recent decades. Generally, large ethnic population groups are distinct, such as Europeans, Americans, Asians, and Africans. Genetic drift, i.e. random fluctuation in the frequency of an allele in a population due to evolutionary reasons, admixture, i.e. the consequence of interbreeding between previously isolated populations, and other factors contribute to differences in allele frequencies and/or haplotypes. A prime example for inter-ethnic variability of genetic variation is the *CYP2D6* gene (Figure 2B), for which gene amplifications (e.g. *CYP2D6*2xN*) occur in up to 3% in Europeans (Griese *et al.*, 1998) and Africans, e.g. Sub-Saharan African populations (Twesigomwe *et al.*, 2023), while in the Middle East the gene amplification occurs with a frequency of up to 30% (Zhou and Lauschke, 2022). Moreover, using again the example of *CYP2D6*, aborigines in Australia only exhibit a frequency of about <1% for *CYP2D6* LOF gene variants resulting in missing enzyme expression and function (Griese *et al.*, 2001), in line with data from other Asian populations (e.g. Chinese, Malay, and Indian descent) (Maulana *et al.*, 2024), whereas in Europe about 10% of the population carry *CYP2D6* LOF variants. Ethnicity and its impact on genetic variation in different populations have implications for drug therapy and the implementation of genetic diagnostics into clinical practice (Frederiksen *et al.*, 2023). Again, taking the example of *CYP2D6* and the use of antidepressants, a significantly higher drug failure rate occurs in the Middle Eastern population compared to the Europeans due to higher frequency of the *CYP2D6* gene amplification (Palumbo *et al.*, 2024). Another example is the prevalence of nudix hydrolase 15 (*NUDT15*) variants (see II.B). In particular, individuals of East Asian descent, including Chinese, Japanese, and Korean populations, exhibit a higher prevalence of *NUDT15* variants compared to other ethnicities (Yang *et al.*, 2014). Research indicates that up to 10% of East Asians, 7% of South Asians and in contrast less than 1% of

Europeans (Schaeffeler *et al.*, 2019) carry at least one copy of a *NUDT15* variant associated with increased sensitivity to thiopurine drugs (Relling *et al.*, 2019). Thus, in the context of PGx diagnostics in clinical routine, broad coverage of genetic variation must be ensured, e.g. using molecular techniques such as NGS, to avoid misinterpretation of the patients' correct phenotype.

B. Genotype-Phenotype correlation in selected ADME genes

Genetic variability in ADME genes results in phenotypic consequences. There are numerous examples of DMEs (e.g. CYP450 enzymes) and drug transporters (e.g. OCT1) for which a well-established genotype-phenotype correlation has been reported based on extensive *in vitro*, animal (knockout) and *in vivo* studies. In the following, we will describe in more detail some clinically relevant examples.

Cytochrome P450 2D6

The gene *CYP2D6*, located on chromosome 22q13.2, consists of nine exons and is recognized as the most polymorphic gene among the CYP450 DME. It harbors over 402 SNVs, which cover approximately 26-30% of all coding base pair positions, along with structural variations which affect the gene copy number and include whole gene deletions, duplications as well as hybrid alleles formed with its neighboring homologue pseudogene *CYP2D7*. These variants result in more than 160 core alleles (Table 1, pharmvar.org/gene/CYP2D6). The most common functional variants which are recommended to be clinically tested are null function alleles (*3: 2550delA, frameshift; *4: 1847G>A, splicing; *5: gene deletion; *6: 1708delT, frameshift), decreased function alleles (*9: 2616delAAG, deletion; *10: 100C>T, P34S; *14: 1758G>A, G169R; *17: 1022C>T, T107I; *41: 2989G>A, splicing) and increased function alleles (*xN: duplication allele). Up to 50% of subjects carry one *CYP2D6* variant that potentially alter the metabolism of approximately 25% of clinically used medications including several drugs with PGx guideline as illustrated in Figure 2C, such as opioids (e.g. codeine, tramadol), antiemetics (e.g. ondansetron, tropisetron), antidepressants (e.g. amitriptyline, fluoxetine) and antiarrhythmics (e.g. propafenone). The patient's genotype or the related star allele diplotype can be

translated either into four phenotypic groups, i.e. poor metabolizers (PM), intermediate metabolizers (IM), normal metabolizers (NM), and ultrarapid metabolizers (UM) or, as recently suggested, in a continuous activity score (AS) (Gaedigk *et al.*, 2008). The AS indicates the hepatic metabolic capacity of the CYP2D6 enzyme, commonly described as metabolic ratio (MR), i.e. the calculated ratio of the parent drug and the metabolite concentration. Specific probe drugs (e.g. sparteine (Griese *et al.*, 1998) or labeled medications (e.g. metoprolol (Thomas *et al.*, 2020) or risperidone (Mannheimer *et al.*, 2016) are used to determine the MR through measurement of concentrations in the blood or the urine. Very recently, solanidine, a steroidal alkaloid found in potatoes (Magliocco *et al.*, 2021) has been proven as dietary-derived activity marker for CYP2D6 activity (Müller *et al.*, 2023). A consensus method translating the AS to one of the four CYP2D6 phenotypes has recently been recommended, indicating that the AS 0 corresponds to the PM phenotype, the AS $0 < x < 1.25$ to the IM phenotype, the AS $1.25 \leq x \leq 2.25$ to the NM phenotype and AS >2.25 to the UM phenotype (Caudle *et al.*, 2020).

Several genotype-phenotype correlation studies have contributed to the robust validation of the CYP2D6 phenotypic classification which includes human liver samples (Zanger *et al.*, 2021), studies on healthy volunteers and patient cohorts (Zanger and Schwab, 2013) (Figure 5A). Notably, the PM phenotype or the AS 0 can be predicted in almost 100% by LOF *CYP2D6* variants in a homozygous or compound heterozygous manner (Zanger *et al.*, 2001, 2021), while *CYP2D6* gene amplifications explain the UM phenotype only in approximately 30% (Griese *et al.*, 1998). Moreover, for selected *CYP2D6* alleles (*CYP2D6**2, *10 and *17) the AS may depend on the substrate specificity since discrepancies have recently been reported for some CYP2D6 substrates (e.g. dextromethorphan, venlafaxine) demonstrating the complexity of a correct genotype -phenotype assignment (Van Der Lee, Guchelaar, *et al.*, 2021). Finally, multiple putative regulatory noncoding variants in the extended *CYP2D6* region, located either in up or downstream enhancer elements, have been described (Yang *et al.*, 2010; Khor *et al.*, 2023; Sanchez- Spitman *et al.*, 2024), that may interact with the *CYP2D6* promoter (Wang *et al.*, 2015; Smith *et al.*, 2024), or may affect the binding motifs of

transcription factors e.g. HNF4 α (Pan *et al.*, 2017) or NFIB (Lenk *et al.*, 2022). However, additional studies are needed to explore the potential functional consequences.

The role of CYP2D6 in cancer therapy is significant due to its role in the metabolism of tamoxifen, the mainstay in endocrine therapy of breast cancer. The bioactivation of tamoxifen to its hundred-fold more potent metabolite, endoxifen, significantly relies on CYP2D6 (Brauch *et al.*, 2013). Numerous studies have demonstrated that plasma concentrations of endoxifen are significantly reduced in pre- and postmenopausal breast cancer women who were treated with tamoxifen standard dosage (20 mg) and classified as CYP2D6 PMs (Mürdter *et al.*, 2011; Saladores *et al.*, 2015; Puszkiel *et al.*, 2019) (Figure 5A). Lower endoxifen plasma levels were associated with poor response and increased relapse of breast cancer (Goetz *et al.*, 2018). The application of model-based pharmacokinetic analyses including physiologically-based pharmacokinetic modeling (PBPK) provided further insight in the tamoxifen metabolism confirming the primary role of CYP2D6, but indicate substantial impact of age, anthropometric characteristics (e.g. obesity), menopausal status (Mueller- Schoell *et al.*, 2020), and co-medication with CYP2D6 inhibitors on Z-endoxifen pharmacokinetics (Maeda *et al.*, 2011; Puszkiel *et al.*, 2021; Dilli Batcha *et al.*, 2022). For implementation of PGx in clinical practice tamoxifen is an excellent example emphasizing the complexity of PGx testing since tumor DNA is limited for accurate *CYP2D6* genotyping due to loss of heterozygosity (LOH) at chromosome 22q13.2 in breast cancer DNA (Brauch *et al.*, 2013) corroborating the use of germline DNA. Finally, to overcome worse outcome in tamoxifen-treated CYP2D6 PM breast cancer patients, independently from the switch of those patients to an aromatase inhibitor therapy, concepts are under review to use endoxifen monotherapy (Jayaraman *et al.*, 2021), or supplementation of tamoxifen standard therapy with low-dose endoxifen (<https://tamendox.de>).

Cytochrome P450 3A4/5

The enzymes CYP3A4 and CYP3A5 are the most important isoforms of the CYP3A subfamily, which also includes CYP3A7 and CYP3A43. The gene cluster spans approximately 200 kb on chromosome 7q21-22.1. *CYP3A4* and *CYP3A5* consist of 13 exons

and show high structural similarity (>70%). Known variants are summarized in 45 core alleles for *CYP3A4* and 6 core alleles for *CYP3A5* (Table 1, pharmvar.org/gene/CYP3A4, pharmvar.org/gene/CYP3A5). *CYP3A4* is crucial in drug metabolism since more than 30% of clinically used drugs across various therapeutic indications are *CYP3A4* substrates which may be explained by its large and flexible active site capable of accommodating and metabolizing numerous lipophilic compounds (Zanger and Schwab, 2013). The high sequence similarity between *CYP3A4* and *CYP3A5* (>85%) results in comparable substrate selectivity (Williams *et al.*, 2002), making it challenging to discriminate their activities. Some specific probe drugs have been identified targeting more selectively *CYP3A4* (e.g. erythromycin, everolimus, quetiapine) or *CYP3A5* (e.g. tacrolimus, vincristine), whereas midazolam metabolism depends on both *CYP3A4* and *CYP3A5* (Tseng *et al.*, 2014). Several studies assessed the impact of *CYP3A* on cancer drugs, however, with conflicting results hampering the clinical relevance (Wang *et al.*, 2023). A substantial contribution of *CYP3A4* and *CYP3A5* to the metabolism of the anti-cancer agents everolimus, sirolimus, etoposide, exemestane, imatinib, sorafenib, sunitinib, and paclitaxel has been claimed, with potential consequences on drug response (Table 3).

CYP3A4 is highly expressed in human liver, while the protein expression of *CYP3A5*, and of other *CYP3A* gene family members (*CYP3A7*, *CYP3A43*) is much lower. Hepatic *CYP3A* expression is highly interindividually variable (>100-fold) and several underlying mechanisms have been proposed, such as the promiscuity in substrate and inhibitor binding (Klyushova *et al.*, 2022), sex-dependent differences (Wolbold *et al.*, 2003), as well as its inducibility by NRs such as the pregnane X-receptor (PXR), the constitutive androstane receptor (CAR), the vitamin D receptor, as well as the peroxisome proliferator-activated receptor- α (PPARA) (Tirona *et al.*, 2003). Interestingly, the hepatic *CYP3A4* and *CYP3A5* protein expression in histologically normal livers derived from cancer patients was significantly lower compared to liver tissue from healthy subjects, and even much lower and more variable in tumor tissue of various cancer types (Vasilogianni *et al.*, 2022).

Significant contribution of genetic variation on interindividual variability of CYP3A expression was only found for CYP3A5, and data from several studies including genome-wide association studies (GWAS) (Rahmioglu *et al.*, 2013) as well as the large-scale gnomAD cohort failed to show a major impact of genetics on CYP3A4 variability (Klein and Zanger, 2013). Notably, CYP3A5 expression varies globally, and only up to 20% of Europeans are expressors of this isozyme. Thus, approximately 80% are so called CYP3A5 non-expressors carrying the *CYP3A5*3* allele, an intronic variant (intron 3, c.219-237A>G) that leads to a cryptic splice acceptor site which, in turn, leads to a truncated nonfunctional protein (Hustert *et al.*, 2001). Of note, Africans are carrying the *CYP3A5*3* allele only in 30%. Since CYP3A5 significantly influences the tacrolimus metabolism with consequences on drug efficacy, dose adjustment is required in transplant patients, who are CYP3A5 expressors (*CYP3A5*1*) (Birdwell *et al.*, 2015).

Regarding *CYP3A4* almost all variants are rare to super rare (<0.01-0.01) (frameshift variants *6, *20 and missense variants *8, *11, *13, *16). Only the splicing variant in intron 6 (c.522-191C>T; *CYP3A4*22*) for instance with a frequency of 5% in Europeans is suggested to be clinically relevant since it is associated with reduced CYP3A4 enzyme activity (Abdel-Kahaar *et al.*, 2019). Whether dose adjustment for selected drugs such as tacrolimus (Mulder *et al.*, 2021), or the antipsychotic agent quetiapine (Van Der Weide and Van Der Weide, 2014) should be performed particularly in homozygous carriers of the *CYP3A4*22* allele is still a matter of discussion. In contrast to the data on *CYP3A* genetic variation, twin studies suggest an important genetic contribution (>60%) on the interindividual variability of CYP3A metabolic capacity that could be explained only in part by known variants in *CYP3A4* and *CYP3A5* (Matthaei *et al.*, 2020). Thus, further investigations are needed to fully understand the genetic variability of *CYP3A*, particularly in the context of CYP3A5 expressors.

Thiopurine S-methyltransferase and nudix hydrolase 15

The chemotherapeutic agent 6-mercaptopurine (6-MP) is the mainstay in treatment of childhood acute lymphoblastic leukemia (ALL) and the cytosolic thiopurine S-

methyltransferase (TPMT) catalyzes the inactivation by methylation of 6-MP (Relling *et al.*, 2019). Very recently the endogenous substrate of TPMT has been identified indicating a link between molybdenum cofactor catabolism and drug metabolism (Pristup *et al.*, 2022). First described in 1980 (Weinshilboum and Sladek, 1980), the TPMT polymorphism has been studied intensively.

The *TPMT* gene, located on chromosome 6p22.3, consists of 11 exons and several pseudogenes on chromosomes 3, 18 and X are described. Currently 45 core alleles (*TPMT*2-TPMT*46*) of the *TPMT* gene, have been identified (liu.se/en/research/tpmt-nomenclature-committee, Table 1). Based on *in vitro*, TPMT knock-out mouse and extensive *in vivo* studies *TPMT* variation correctly predicts the IM and PM TPMT phenotype (i.e. enzyme activity determined in red blood cells or hepatic cytosol) with concordance rates > 95% (Figure 5B) (Schaeffeler *et al.*, 2004; Tamm *et al.*, 2016).

In addition, the nudix hydrolase 15 (*NUDT15*) has been identified as additional polymorphic pharmacogene, located at chromosome 13q14.2 (Yang *et al.*, 2014), and consisting of five exons. Currently 20 *NUDT15* core alleles are known, in part associated with significant alteration of *NUDT15* enzyme activity based on *in vitro* and *in vivo* data, as well as *NUDT15* knock-out mouse (Table 1, pharmvar.org/gene/NUDT15). The enzyme dephosphorylates the 6-MP active metabolite 6-thio-GTP to 6-thio-GDP, thereby limiting the incorporation into DNA (Moriyama *et al.*, 2016). Several retro- but also prospective studies provide evidence that severe hematotoxicity (e.g. leukopenia, pancytopenia) in patients treated with standard dosage of 6-MP is the consequence of genetically-driven reduced or absent TPMT and/or *NUDT15* enzyme activity (Figure 3), leading to increased blood levels of active metabolites (Figure 5B) (Relling *et al.*, 2019; Jena *et al.*, 2023). Thus, prospective genetic testing of *TPMT* and *NUDT15* offers personalized dose adjustment of 6-MP. Consequently CPIC PGx guidelines (see II.E) have been developed, first in 2011 for *TPMT*, updated in 2013, (Relling *et al.*, 2013) and in 2018 extended by *NUDT15* as second relevant PGx marker for thiopurine therapy (Relling *et al.*, 2019). Interestingly, very recently first data indicates that the *TPMT/NUDT15* IM/IM phenotype shows additive effects on 6-MP-related hematotoxicity in

children with ALL and stronger dose reduction of 6-MP is required compared to patients with a TPMT IM or NUDT15 IM phenotype alone (Maillard *et al.*, 2024). Of note, *TPMT* and *NUDT15* variation allows only risk prediction for the development of hematotoxicity whereas thiopurine-related liver toxicity or the flu-like syndrome are not associated (Toksvang *et al.*, 2022).

Dihydropyrimidine dehydrogenase

Fluoropyrimidines such as 5-fluorouracil (5-FU) and capecitabine are metabolized by dihydropyrimidine dehydrogenase (DPD), encoded by the *DPYD* gene, and with over 80% of the administered dose catalyzed through this pathway. The gene *DPYD*, located on chromosome 1p21.3, consists of 26 exons, and currently more than 400 genetic variants have been identified (Table 1, pharmvar.org/gene/DPYD), for which *in vitro* and *in vivo* evidence confirms a most deleterious or moderately reduced impact on DPD expression/function (Figure 5C). Of note, *DPYD* genetic variations are classified using star (*) allele nomenclature, but dbSNP rs-identifiers, nucleotide or amino acid changes according HGVS nomenclature (hgvs-nomenclature.org) are also used. As an example, the *DPYD**2A allele is characterized by an intronic variant (c.1905+1G>A) of the *DPYD* gene, which functionally leads to the skipping of an entire exon and a non-functional-protein. 5 FU-related grade 3/4 toxicities are observed in about 20% up to 40% in patients treated with adjuvant 5-FU with or without oxaliplatin as well as in the metastatic setting (Kuebler *et al.*, 2007; Venook *et al.*, 2017) and are commonly characterized by gastrointestinal ADR (e.g. diarrhea, nausea/vomiting, mucositis) and neutropenia or myelosuppression associated with infections, while cases of neurotoxicity or cardiotoxicity are rare. In cases of genetically determined deleterious *DPYD* variants, the risk of severe, sometimes life-threatening side effects under standard dosages of 5-FU is increased (Schwab *et al.*, 2008; Rosmarin *et al.*, 2014), and most of these ADR have an early onset, i.e. after two to three cycles of respective treatment regimens. Furthermore, RCTs and meta-analyses showed the need for action concerning the *DPYD* variants *2A (rs3918290, c.1905+1G>A), *13 (rs55886062, c.1679T>G, p.I560S), c.2846A>T (rs67376798, p.D949V) and *HapB3* (rs75017182, c.1129-

5923C>G) to prevent severe 5-FU-associated neutropenia or mucositis, how to handle specific *DPYD* genotypes (see II.E) and to adjust the dosage accordingly (Amstutz *et al.*, 2018; Henricks *et al.*, 2018). The four *DPYD* variants (*2A, *13, c.2846A>T, and *HapB3*) considered in the 5-FU CPIC guideline show a global frequency distribution of 0.02% to 0.96% (based on data from the 1,000 Genomes Project) and an estimation under the Hardy-Weinberg equilibrium law indicates that at least 2% of 1,000 patients treated with FU may be carriers of at least 1 of these 4 variants (Innocenti *et al.*, 2020). Data on the sensitivity and specificity and negative and positive predictive values of *DPYD* genetic testing for three variants (*2A, *13, c.2846A>T) in predicting grade 3+ toxicities showed 5.3%, 99.4%, 68.0% and 81.8%, respectively (Lee *et al.*, 2014). The high specificity and positive predictive value illustrate that patients carrying *DPYD* variants are at high risk to develop severe toxicity, justifying preemptive *DPYD* diagnostics. Nevertheless, there are independent factors which so far insufficiently identified to explain 5-FU toxicity demonstrated by the low sensitivity. Prospective data from 500 patients treated with fluoropyrimidine-based chemotherapy corroborates this finding that the *DPYD* variants *2A, *13, c.2846A>T and *HapB3* could explain 20 to 30% of early-onset 5-FU toxicities (Froehlich *et al.*, 2015). With regard to the *HapB3* haplotype, very recently a new study indicated that the so far assumed complete linkage disequilibrium between the functionally relevant intronic splice site variant c.1129-5923C>G and the synonymous variant c.1236G>A (rs56038477, p.Glu412=) does not exist in all cases (Turner *et al.*, 2024). Moreover *DPYD* illustrates the complexity of PGx diagnostics related to cancer drugs, i.e. avoiding ADR through dosage adjustments in the presence of genetic variation, but potentially resulting in poorer treatment response. A retrospective analysis indicates that the recommended dose reduction by 25% of standard dose in the presence of the *HapB3* genotype may be associated with a worse treatment outcome which requires further systematic investigations (Knikman *et al.*, 2023). Furthermore, it can be hypothesized that different penetrance of the genotype-phenotype relationship between tumor and the rest of the body may be at least partially due to the modulating effect of somatic mutations (see IV). Notably, in 2020 the European Medicines

Agency (EMA) issued a recommendation that genetic testing for *DPYD* is mandatory before onset of 5-FU therapy which led to the implementation of *DPYD* testing at national levels in Europe (e.g. Federal Institute for Drugs and Medicinal Products, BfArM, Germany; ema.europa.eu/en/news/ema-recommendations-dpd-testing-prior-treatment-fluorouracil-capecitabine-tegafur-and-flucytosine). In Germany subsequently reimbursement of costs for *DPYD* diagnostics by health insurance companies has been introduced, specified by an official guidance of the Federal Joint Committee (GBA) in Germany that health insurance companies must cover the costs.

Uridine-diphosphate-glucuronosyltransferase 1A1

UDP-glucuronosyltransferase 1A1 (*UGT1A1*), a phase II DME, mainly catabolizes the active metabolite SN38 of the topoisomerase I inhibitor irinotecan to its glucuronide, thus playing a crucial role in irinotecan elimination in patients with solid tumors, such as colorectal and pancreatic cancer. The *UGT1A* gene locus, located at chromosome 2q37.1, is transcribed into nine individual enzymes, namely *UGT1A1* and *UGT1A3* to *UGT1A10*, each consisting of five exons. Through exon sharing, one of nine unique exon 1 sequences at the 5' end is combined with four common exons at the 3' end (Jarrar and Lee, 2021). More than 113 distinct functional *UGT1A1* variants have been documented (Table 1, pharmacogenomics.pha.ulaval.ca/wp-content/uploads/2015/04/UGT1A1-allele-nomenclature.html), among them the clinically significant and prevalent variants *6 and *28. The *UGT1A1**28 allele corresponds to a TA-Indel (rs3064744) located in the TAA-box within the promoter region and contains seven TA repeats, resulting in decreased hepatic *UGT1A1* transcription (Figure 5D). Other repeat numbers are associated with normal expression (*UGT1A1**1, 6 repeats), increased expression (*UGT1A1**36, 5 repeats), or decreased expression (*UGT1A1**37, 8 repeats). Interestingly, there is another promoter variant (*UGT1A1**80, -364C>T) which is in very high linkage disequilibrium with *28 and *37, but its own effect on enzyme expression and activity has not been fully elucidated (Nelson *et al.*, 2021).

Overall, UGT1A enzymes show considerable overlap of substrate specificity whereas only UGT1A1 is accountable for bilirubin glucuronidation, a factor implicated in hyperbilirubinemia upon inherited enzyme deficiency. Consequently, two syndromes of hyperbilirubinemia are recognized and correlated to the degree of enzyme deficiency: Crigler–Najjar type I (complete enzyme deficiency), type II (residual enzyme activity) and Gilbert's (Meulengracht) syndrome (enzyme activity decreased by app. 70% (Bosma *et al.*, 1995)).

Severe neutropenia and diarrhea are the predominant irinotecan-related ADRs. Additional risk factors are age, sex, performance status, impaired liver function and concurrent use of CYP3A4 and/or UGT1A1 inhibitors. Carriers of *UGT1A1**28 accumulate toxic levels of SN-38 (Karas and Innocenti, 2022). Retrospective and prospective clinical trials provide evidence that dose adjustment of irinotecan in patients carrying *UGT1A1**28 or *6 reduces significantly the risk of neutropenia (Figure 3) which holds also true for other cancer drugs such as etoposide (Hulshof *et al.*, 2020). In consequence drug regulatory authorities, such as the FDA and the EMA as well as recently in 2021 also the German Regulatory Agency for Drugs and Medicinal Products (BfArM; [bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/EN/RHB/2021/rhb-irinotecan.html?nn=966164](https://www.bfarm.de/SharedDocs/Risikoinformationen/Pharmakovigilanz/EN/RHB/2021/rhb-irinotecan.html?nn=966164)) have issued instructions for dose adjustment in carriers of *UGT1A1**28, initiating irinotecan treatment with 70% of the standard dose followed by neutrophil count-guided uptitration of the dose when irinotecan is well tolerated. Very recently, an association between the frequently occurring chemotherapy-related high hyperbilirubinemia during all intensive treatment phases of pediatric ALL (e.g. AIEOP-BFM ALL 2000 protocol) linked to the anticancer agents asparaginase, mercaptopurine, and methotrexate and variation in the *UGT1A* gene cluster was proposed as an independent prognostic factor of treatment outcome (Yang *et al.*, 2022; Junk *et al.*, 2023). Therefore, prediction for hepatotoxicity and risk-adapted treatment strategies for childhood ALL may be complemented by both the assessment of hyperbilirubinemia and UGT1A genotyping.

C. *In silico* prediction of functional consequences of genetic variation

As NGS technologies lead to the discovery of hundreds of new variants with unknown function, strategies are needed for reliable prediction of their functional consequences since classical *in vitro* assessment of functional consequences is not feasible due to the volume of variants, the time and resource-intensive nature of *in vitro* experiments, and the complexity of biological systems. Generally, genetic variants can occur in coding or non-coding regions of the genome. Although coding regions cover only 1.2% of the human genome (The ENCODE Project Consortium *et al.*, 2020), they are typically used for functional interpretations as they can directly affect protein expression or function for instance due to frameshift, nonsense, missense, or insertion variants. Nevertheless, also non-coding variants can be functional by influencing regulatory sequences and untranslated regions with consequences on mRNA translation and expression. The initial variant prioritization is, hence, conducted through (i) variant classification into non-coding regions, exons/introns, 3'UTR, CpG sites (i.e. DNA sequences comprising cytosine followed by guanine from 5' to 3' direction), or histone marks, taking into consideration publicly available catalogs of genomic and epigenomic features, (ii) population frequency distributions, and (iii) clinical evidence using for instance ClinVar, a freely accessible archive of information on the relationship of human variations and phenotypes (clinicalgenome.org/data-sharing/clinvar).

Synonymous variants are commonly considered benign, although there is compelling evidence that they can affect protein expression by influencing RNA structure, stability, and miRNA binding and *in silico* prediction methods are increasingly available (Lin *et al.*, 2023). In contrast, a plethora of prediction tools exists for non-synonymous SNVs and have been used in PGx studies (Table 2). All tools employ diverse approaches, including comparisons on interspecies homology and protein structure data (e.g., AlphaFold), application of machine learning techniques trained on extensive variant annotations (e.g., from high-throughput *in vitro* or *in vivo* experiments or large-scale study cohorts), and utilization of ensemble models, that combine multiple individual models to improve accuracy and robustness (Katsonis *et al.*, 2022). Each tool is trained and optimized for specific gene categories e.g., disease causing

variants. This has also been recognized for pharmacogenes as recently demonstrated for *TPMT* and *PTEN* based on the initiative Critical Assessment of Genome Interpretation (CAGI; genomeinterpretation.org). Here, different predictions were compared against experimentally characterized phenotypes and major differences in accuracy have been observed (Pejaver *et al.*, 2019).

Several pharmacogenes exhibit co-evolution signatures with different lifestyles and diets, and signs of evolutionary positive selection, particularly enzymes with primarily exogenous substrate profile (Fuselli, 2019). As a consequence, these genes can harbor common functional variants with population allele frequencies surpassing 10-20% in contrast to much rarer disease-causing gene variants under high evolutionary pressure. Prediction tools optimized for the latter often fall short in accurately predicting SNV functions in pharmacogenes (Tremmel, *et al.*, 2023). Therefore, several pharmacogene-optimized algorithms have been developed. One of the first ensemble classifiers, is an ADME-optimized Prediction Framework (APF), that combines 18 algorithms, and achieved 93% sensitivity and specificity in predicting LOF and functionally neutral pharmacogenomic variants for 44 pharmacogenes (Zhou *et al.*, 2019). An extreme gradient boost machine learning model (XGB-PGX) on evolutionary statistics for missense variants and functional annotations from UniProt that aimed to cover the population bias in PGx studies by including comprehensive global allele frequencies from the 1000 Genome Project (i.e. a comprehensive resource on genetic variant with frequencies of at least 1% in a large number of people who declared themselves to be healthy, <https://www.internationalgenome.org/1000-genomes-summary/>) outperformed classical predictors, such as SIFT, PolyPhen, and CADD (Scheinfeldt *et al.*, 2021). Furthermore, there are two machine learning approaches available incorporating several in silico prediction tools along with additional features, including conservation scores to create an ensemble variant classifications (Pandi *et al.*, 2021).

Two studies adopted a different approach for *CYP2D6* serving as an optimal starting point for machine learning proof-of-concepts. The first study, predicted the functional status of

CYP2D6 star alleles using a convolutional neural network. The algorithm was trained on 31 star allele sequences with known function and was able to predict haplotype phenotypes with 88% accuracy (McInnes *et al.*, 2020). Another study showed, that a neural network can predict

the interindividual variability of *CYP2D6* activity from phased NGS-derived variant data with higher accuracy (79%) compared with the conventional categorized star allele approach (54%), and additionally allowed functional prediction of uncharacterized combinations of variants (Van Der Lee, *et al.*, 2021). Moreover, the most exact prediction on protein structure from DeepMind's transformer neural network AlphaFold2 can be used to interrogate the functional effect of coding variants, although the accuracy of AlphaFold2 raw data is still under discussion (Pak *et al.*, 2023). State-of-the-art prediction of the protein structure may help to identify important structural motifs and critical positions in the amino acid sequence as shown for human *G6PC2*, encoding a glucose-6-phosphatase (G6Pase) catalytic subunit (Hawes *et al.*, 2024), as well as for the pharmacogene *SLC22A6*, encoding the organic anion transporter 1 (OAT1) (Janaszkiwicz *et al.*, 2022). A promising preprint study incorporated tissue-specific RNAseq data to categorize missense variants in commonly expressed human proteins (Hoffman *et al.*, 2024). Another approach used the combination of the previously developed *in silico* prediction method SPEACH_AF with other types of software (i.e. Rosetta Energy Analysis) (Stein and Mchaourab, 2023). These novel models yield successful predictions also for protein-protein and protein-drug interactions (Xu *et al.*, 2023).

D. Validation of *in silico* functional predictions

After the identification of putative functional variants through *in silico* prediction, *in vitro* or *in vivo* experiments are essential to validate their function before potential clinical application. So far *in vitro* laboratory experiments, including cell culture, gene expression, and biochemical assays are used to determine enzyme activity or drug transporter function. Animal and human *in vivo* studies (e.g. phase I trials) allow the elucidation of pharmacological consequences of genetic variation in a biological context using also innovative approaches like liquid biopsies (Tremmel *et al.*, 2024). An alternative approach

involves multiplex assays of variant effect (MAVE) which mostly are deep mutational scans to measure molecular phenotypes (Chiasson *et al.*, 2019). Such experiments combine NGS and high-throughput readouts in suitable cell systems to assess functional consequences of amino acid changes at every protein position on mRNA or protein abundance as well as on enzyme activity using covalent substrates. Those results can be used also as a training resource for machine learning methods. Major limitations of the application of MAVE are high workload and costs which could be decreased by reducing the number of variants e.g., only taking into account missense variants or variants, which were already identified in population-genome studies (e.g., GnomAD). Proof of concept for the successful application of MAVE has recently reported for the drug transporter *SLCO1B1* (Zhang *et al.*, 2021) and the DMEs *CYP2C9* and *CYP2C19* (Zhang *et al.*, 2020). Further optimization may consider high-throughput readouts with multiple substrates or substrate-inhibitor/activator combinations to more precisely assess enzyme specificity and phenoconversion. Moreover, the elucidation of pharmacogene pathways including drug targets, DME, and transporters at the same time in one analysis seems to be promising. The latter approach was already used to guide the development of novel antimicrobial drugs through a CRISPR-mutagenesis analysis of three essential *E. coli* proteins in their original genomic context. New insights into protein function, antimicrobial resistance, and drug target were found (Dewachter *et al.*, 2023). With respect to PGx, comprehensive deep mutational scanning including variant mapping and phenotyping through sequencing (Vamp-Seq) have been performed for selected ADME genes (*CYP2C9*, *CYP2C19*, *NUDT15*, *SLCO1B1*, *TPMT*, *VKORC1*) and pharmacodynamic targets (*ADRB2*, *LDLR*) (Geck *et al.*, 2022).

E. Implementation of pharmacogenomics

International and national consortia (Clinical Pharmacogenetics Implementation Consortium, CPIC, cpicpgx.org; Dutch Pharmacogenetics Working Group, DPWG, knmp.nl/dossiers/farmacogenetica/pharmacogenetics; Canadian Pharmacogenomics Network for Drug Safety, CPNDS, cpnds.ubc.ca; the French National Network of Pharmacogenetics, RNPGx, pharmgkb.org/page/rnpgx) have been established to compile

evidence-based PGx knowledge on clinically relevant genetic alterations in the germline genome in order to adapt drug therapy. These guidelines contain recommendations for adjusting the individual dose of the drug, which can result in both dose reduction or dose escalation (Caudle *et al.*, 2014). In extreme cases, for instance in the presence of homozygous variant genotypes with complete enzyme deficiency, an alternative therapy is recommended, assuming that the drug is mainly metabolized through this particular enzyme. CPIC and DPWG are the consortia that have developed the most comprehensive guidelines for the implementation of PGx so far and make them available on publicly accessible websites (pharmgkb.org/guidelineAnnotations). Defined procedures are available as a basis for these consortia, outlining how a corresponding guideline with recommendations should be developed, verified, and made publicly available. For CPIC, this means, for example, that for each guideline, a panel of experts is selected, whose central task is to review and evaluate all available literature, as well as to determine which study results can be used for guideline development for methodological reasons. In this context, it should be noted that studies with high levels of evidence, such as RCTs, hold significant importance, and case series or non-controlled study designs have less or no relevance in this evaluation procedure (Caudle *et al.*, 2014). In the case of CPIC, currently 191 guidelines are published and recommendations are available for 82 drug-gene combinations. The DPWG PGx guidelines can refer to 63 recommendations. To support worldwide implementation of the PGx guidelines, a very systematic analysis has been conducted, comparing the guidelines to specific target genes in order to identify any potential discrepancies. This analysis revealed that over 99% of the CPIC and Dutch Guidelines are identical, and there is also an extremely high overlap when compared to Canadian and French Guidelines (Abdullah-Koolmees *et al.*, 2020). Although the methodological approaches of the consortia are partially different, the validity of the findings and resulting recommendations is evident and demonstrates the congruence of existing consortia. The necessity of such guidelines not only for patient care but also for research activities is demonstrated by the recently successfully completed PREPARE study. This randomized trial comprising approximately 7000 patients across

Europe was conducted in a real-world setting as part of a PGx implementation strategy indicates that when following the DPWG PGx guidelines based on PGx testing ADRs can be reduced in up to 30% (Swen *et al.*, 2023). The available guidelines presuppose that in different healthcare systems, digital structures will be in place, empowering treating physicians, especially in outpatient settings, to access relevant information without having to rely on complicated scientific print materials.

F. Pharmacogenomics-guided supportive care

PGx-guided supportive therapy in the context of tumor diseases is of clinical relevance and includes both preemptive and side effect-related strategies (Patel *et al.*, 2021). Supportive drugs are used to manage cancer-related symptoms and to improve the quality of life. Antiemesis and pain management, and the use of 5HT3 antagonists (e.g. ondansetron (Bell *et al.*, 2017)), opioids/opioid analogues (Crews *et al.*, 2021) and nonsteroidal anti-inflammatory drugs (NSAID) (Theken *et al.*, 2020), respectively, are selected examples. In the case of opioid therapy, substances such as codeine, tramadol, oxycodone, and hydrocodone, which are included in the WHO stepwise approach for cancer pain management, undergo bioactivation via CYP2D6 to form active metabolites (e.g. O-desmethyltramadol, hydromorphone) which exhibit the analgesic effects through opioid receptors (Wong *et al.*, 2022). CPIC-guided recommendations for CYP2D6 genotype-related prescribing are so far provided for codeine and tramadol (Crews *et al.*, 2021). A decreased analgetic effect of codeine and tramadol at standard dosage can be expected for CYP2D6 PM (see II.B). Conversely, in the case of CYP2D6 gene amplification (UM phenotype) elevated plasma levels of the active metabolite morphine may occur with an increased risk for serious toxicities (Figure 3) (Crews *et al.*, 2021). So far, the evidence for CYP2D6 genotype-related prescribing in case of hydrocodone and oxycodone is limited since CYP2D6 contributes only to a smaller extent to the bioactivation of both agents.

The example of opioid use nicely shows the complexity of PGx-guided supportive care because other candidate genes such as the opioid receptor MU1 encoded by *OPRM1*, the catechol-O-methyltransferase (COMT) and the organic cation transporter 1 (OCT1) (Wong *et*

et al., 2022) that allows the uptake of opioids into cells (Meyer *et al.*, 2019) contribute to opioid pharmacology. The MU1 opioid receptor is part of the G-protein-coupled receptor (GPCR) family and binds opioids, primarily affecting nociception. *OPRM1* is highly polymorphic and genetic variants have been linked to reduced expression in vitro and in vivo with consequences for opioid response. Moreover, opioid response depends on pain perception and COMT is a key regulator of catecholamine levels in the pain perception pathway via methyl conjugation of catecholamines. Selected genetic variants in *COMT* have been associated with lower activity for methylation compared to the wild-type allele. The clinical relevance of *OPRM1* and *COMT* PGx for opioid agents including morphin or fentanyl is still a matter of debate, and data are limited to justify upfront genetic testing in clinical care (Crews *et al.*, 2021; Wong *et al.*, 2022).

Regarding OCT1, genetic variation of *SLC22A1* (encoding for OCT1) is known to significantly reduce the uptake function resulting in decreased intracellular drug concentrations (Kölz *et al.*, 2021). *SLC22A1* genetics was associated with several other cancer agents such as tyrosine kinase inhibitors (TKIs), and oxaliplatin (Table 3) (Nies *et al.*, 2011; Neul *et al.*, 2016), but inconsistent data is available regarding the clinical relevance of *SLC22A1* PGx (Nies *et al.*, 2014; Chen *et al.*, 2020).

Another important example with relevance for PGx is anti-infective supportive therapy for cancer patients. It is well accepted that cancer patients that are receiving chemotherapy, partly in combination with radiation, or immuno- or cell therapies have a significantly increased risk for severe systemic infections compared with non-cancer patients which not only includes outpatient-acquired but also healthcare-related infections (Belloni *et al.*, 2022; MacPhail *et al.*, 2024). PGx-guided use of antiinfective agents is well established for selected drugs and guideline recommendations (e.g. CPIC, DPWG) are available for the antibiotics flucloxacillin

(https://api.pharmgkb.org/v1/download/file/attachment/DPWG_HLA_flucloxacillin_4652.pdf), nitrofurantoin (Gammal *et al.*, 2023) and aminoglycosides (McDermott *et al.*, 2022) as well as

the antifungal agent voriconazole (Moriyama *et al.*, 2017) to improve efficacy or to avoid ADR (see Figure 3).

In addition to ADME PGx-guided supportive care there is substantial evidence that genetic variation in distinct human leukocyte antigen (HLA) genes is associated with severe in part life-threatening hypersensitivity reactions of several drugs (see Figure 3) (Manson *et al.*, 2020). For example, allopurinol which inhibits the xanthine oxidase is used during cancer chemotherapy to prevent acute uric acid nephropathy (tumor lysis syndrome) and a major cause of severe cutaneous adverse reactions (SCAR, e.g. Stevens-Johnson Syndrome) with up to 25% mortality. A high sensitivity (up to 100%) and specificity (up to 94%) for *HLA-B*58:01* testing in Asians have been shown regarding allopurinol induced SCAR (Manson *et al.*, 2020). Therefore, PGx recommendation guidelines strongly recommend not taking allopurinol in hetero- and homozygous carriers of *HLA-B*58:01* (Saito *et al.*, 2016).

Other supportive drug classes in cancer care are also influenced by PGx, and guidelines are available (Figure 2C) including antidepressants to support pain management, psychiatric symptoms and sleeping disorders (pharmgkb.org/guidelineAnnotations).

G. Polygenic risk scores and prediction of drug response

Several polygenic risk scores (PRS) have been established in the context of susceptibility to certain diseases and have been advanced through extensive genomic analyses, such as GWAS. One of the first approaches was already published in 2007 (Wray *et al.*, 2007), indicating the feasibility of a genetic risk prediction. PRS typically encompass a varying number of genetic variants, which usually have limited significance individually and/or occur rarely. However, within a complex network, combining various genetic factors significantly enhances their predictive value, allowing for a more valid prediction of a certain disease risk, disease progression or chronic clinical conditions. Numerous PRS have been described for cardiovascular (e.g. coronary artery disease) or neuropsychiatric (e.g. schizophrenia, depression) diseases as well as various cancers (e.g., breast cancer), but with limited relevance for clinical practice (Xiang *et al.*, 2024). PRS in the context of drug therapy have been largely overlooked so far, concerning both treatment response and ADRs. Initial

approaches in this direction involve combinations of candidate genes related to PGx data, as considered in some CPIC/DPWG guidelines (pharmgkb.org/guidelineAnnotations). One notable example of a PRS that also incorporates important clinical parameters (e.g. ethnicity) is the anticoagulant warfarin. The International Warfarin Pharmacogenetics Consortium (IWPC) proposed, as early as 2009, the inclusion of not only the CYP2C9 enzyme relevant for warfarin metabolism but also the Vitamin K epoxide Reductase Complex (VKORC) subunit 1, responsible for reducing vitamin K epoxide to its active form, for better instructing PGx-based warfarin dosing. Recently *CYP4F2* (Johnson *et al.*, 2017) and gamma-glutamyl carboxylase (*GGCX*) involved in the activation of vitamin K dependent clotting factors were further added (Li *et al.*, 2022). Other PGx examples related to cancer with combination of candidate genes are thiopurines (*TPMT*, *NUDT15*), antidepressants (*CYP2C19*, *CYP2D6*), potent volatile anesthetic agents/succinylcholine (*RYR1*, *CACNA1S*), and opioids (*CYP2D6*, *COMT*, *OPRM1*), where information has been incorporated into the CPIC guidelines in recent years (pharmgkb.org/guidelineAnnotations) (Johnson *et al.*, 2022). Thus, the perception has shifted, recognizing the importance and relevance of PRS due to the increasing number of independently identified genomic factors explaining drug response (Simona *et al.*, 2023; Singh *et al.*, 2024). The challenges regarding PRS comprise, among others, the varying frequencies of relevant genetic variants in different ethnic populations (see II.A), consideration of other clinical parameters as well as missing implementation strategies and bioinformatic support. Other aspects, like the fact that the application of PRS entails additional costs and requires specific medical expertise for the interpretation of results are significant barriers to implementation.

III. Somatic variation and cancer therapy

A. Somatic mutations in cancer

In a multicellular organism, every cell acquires mutations over its lifetime (Stratton *et al.*, 2009) which are called somatic mutations. Most of them are innocuous, and many are

cleared by DNA repair mechanisms quickly. Every cell thus acquires its own set of somatic mutations, and they differ between cells of the same individual. Upon malignant transformation, one cell clonally expands, and all daughter cells inherit all mutations, both germline and somatic, from the initially transformed cell (Hanahan and Weinberg, 2000, 2011). This renders somatic mutations in a tumor clonal. Over the past years, the field of cancer genomics has characterized the mutational landscapes of various tumor entities by WES and/or WGS (The International Cancer Genome Consortium, 2010; Cancer Genome Atlas Research Network *et al.*, 2013; The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium *et al.*, 2020). The overall amount of somatic mutations varies over a wide range between cancer samples, roughly between 0.001 per megabase (Mb) and 400 per Mb, and between tumor entities, on average ranging from less than 0.05 per Mb in pilocytic astrocytoma or 0.5 in pediatric acute leukemia to more than 10 per Mb in melanoma (Alexandrov *et al.*, 2013). Figure 6 exemplifies the density of somatic mutations across cohorts of rare adult cancers aggregated from the German Cancer Research Center (DKFZ), National Center for Tumor Diseases (NCT) and German Cancer Consortium (DKTK) in the MASTER (Molecularly Aided Stratification for Tumor Eradication Research) program, a multicenter precision oncology program using broad multi-omics characterization including WGS/WES of tumor and matched normal control samples as well as RNA sequencing and DNA methylation profiling of tumor samples under accredited conditions, and clinical decision-making (Horak *et al.*, 2021; Mock *et al.*, 2023). Of note, as some of the investigated entities are extremely rare, a strict classification based on morphology and anatomic localization would lead to a plethora of subgroups, which partially would include very few samples. For the ease of display, cancer entities in MASTER were therefore grouped into so-called entity baskets following a pragmatic meta-classification system (Figure 6). Sample numbers across the entity baskets are displayed in Figure 6B, showing strong contributions from rare cancers and some contributions from rare subtypes of common entities. Similar cancer genomics landscapes have been published for more common cancers ((Cancer Genome Atlas Network, 2012), data available at portal.gdc.cancer.gov), at pan-cancer level

((The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium *et al.*, 2020), data available at dcc.icgc.org/projects), or for particular medically relevant questions, like metastatic cancer (Zehir *et al.*, 2017).

Genetic alterations in certain genes contribute particularly to malignant transformation, i.e., LOF mutations in tumor suppressor genes and gain-of-function mutations in oncogenes. These mutations confer a selective advantage and are therefore recurrent. The most recurrently mutated gene across all cancer entities is TP53 (Chang *et al.*, 2016), encoding a sequence-specific transcription factor with various tumor-suppressive functions. Figure 6C displays cumulative numbers of the different mutation types per gene across the MASTER cohort. Colors code for mutation type, and well established patterns can be retrieved, like frequent occurrence of amplifications in oncogenes, e.g., *MYC*, and deletions in tumor suppressor genes, e.g., *CDKN2A*. As opposed to *MYC* and *CDKN2A*, which are mainly affected by somatic copy number aberrations (sCNAs), other genes (e.g., *TP53*) are mainly affected by small variants (SNVs and insertion-deletion (indels)). It is furthermore worth noting that the genes analysed here are also affected by a varying fraction of germline variants, which often are small variants present in a heterozygous configuration, and the tumor loses the healthy allele by other mutational mechanisms (e.g., a deletion), thereby acquiring a hemizygous or homozygous configuration for the variant. However, the number of somatic mutations observed in a gene across large cancer genomics cohorts also depends on the size of the gene; and genes which neither harbor increased mutation density, nor are established tumor suppressors but are particularly large include *TTN* (Titin, encoding a large protein expressed in striated muscle, gene length: 304,814 bases) or *RYR2* (Ryanodine receptor 2, one of the components of a calcium channel found in cardiac muscle sarcoplasmic reticulum, gene length: 791,805 bases; Figure 6).

B. Targetability of somatic alterations

Somatic mutations are critical for the development and progression of cancer. In addition, some mutations in a tumor also are targetable, i.e., there are treatments specifically designed to target cellular processes or signaling pathways that are directly or indirectly

activated or deactivated by particular mutations. For example, a BRAF V600E mutation, which leads to constitutive activation of the RAF-MEK-ERK pathway, can be effectively targeted by an inhibitor such as vemurafenib. Tailoring therapy to a tumor's unique molecular profile, in particular, to somatic mutations, can significantly improve treatment outcomes. This is exemplified by the use of small-molecule inhibitors for entities characterized by abnormal kinase signaling due to activating mutations or gene arrangements. Examples include gastrointestinal stromal tumors, subsets of NSCLC, melanoma, various hematopoietic malignancies, and others (Scholl *et al.*, 2008; Rosti *et al.*, 2017; Recondo *et al.*, 2018; Klug *et al.*, 2022). However, challenges arise when dealing with molecular profiles where the clinical implications are less apparent. For instance, the question often arises about the "druggability" of a genetic variant in different tissue contexts. This complexity is exemplified by the use of mutation-specific BRAF V600 inhibitors, which yield objective responses in approximately half of the studied entities (Subbiah *et al.*, 2020; Hanrahan *et al.*, 2024), but in colorectal cancer (CRC) effective suppression of oncogenic RAF-MEK-ERK signaling requires consideration of the tissue of origin's physiological expression profile (Prahallad *et al.*, 2012; Kopetz *et al.*, 2019). PGx in CRC involves the study of genetic variation influencing individual responses to chemotherapy, targeted therapies, and other treatment modalities. Key oncogenes like *EGFR*, *MET*, *BRAF*, and others play a pivotal role in determining the efficacy and safety of treatments. Mutations in these genes are crucial for selecting targeted therapies, as they can predict the response to drugs such as gefitinib, erlotinib and osimertinib (EGFR inhibitors), or vemurafenib (BRAF inhibitor), as outlined, e.g., in a recent ESMO guideline on diagnosis, treatment and follow-up of metastatic colorectal cancer (Cervantes *et al.*, 2023): "Testing for mismatch repair (MMR) status and *KRAS*, *NRAS* exon 2, 3 and 4 as well as *BRAF* mutations is recommended in all patients at the time of mCRC diagnosis, due to its relevance in selecting first-line therapy. This can be carried out on either the primary tumor or any metastatic site, with a suggested turnaround of ≤ 10 days. As these mutations are negative predictive factors for the use of anti-epidermal growth factor receptor

(EGFR) monoclonal antibodies (mAbs), *RAS* testing is mandatory before this treatment is initiated.”

Targetability is not limited to single-gene alterations but can also apply to more complex biomarkers. A field in which biomarker design and identification have been intensively studied is DNA repair. Different DNA repair defects leave specific imprints on the genome of a cancer cell and, at the same time, result in synthetic lethal relationships that can be exploited therapeutically. Homologous recombination repair (HRR) plays a crucial role in repairing various mutations, including DNA double-strand breaks (DSBs) (Lord and Ashworth, 2016). LOF mutations in genes associated with HRR lead to homologous recombination deficiency (HRD), causing an accumulation of DSBs and increased reliance on alternative DNA repair pathways such as non-homologous end joining (NHEJ) (Lord and Ashworth, 2012). Cancers with HRD have more large structural variants and sCNA, referred to as “genomic scarring” (Abkevich *et al.*, 2012). Various techniques have been devised to measure the degree of genomic instability in such tumors, including the LOH-HRD score (Telli *et al.*, 2016) and the number of large-scale state transitions (LST) (Popova *et al.*, 2012). Mutational signatures are imprints that distinct mutational processes have left on the genomes of cancer cells (Alexandrov *et al.*, 2013, 2020). For their analysis, all mutations of a given type, e.g., SNVs, are categorized into more subtle features. This may be achieved by taking into consideration the motif context of the respective mutation. In an initial unsupervised pan-cancer analysis of 507 WGS and 6535 WES samples, 30 mutational signatures were identified (cancer.sanger.ac.uk/signatures), half of which were found to be associated with specific mutational mechanisms. Cancers with impaired HRR are characterized by mutational signatures 3 and 8 (Alexandrov *et al.*, 2015). Initially observed in tumors harboring mutations in the tumor suppressor genes *BRCA1* and *BRCA2*, similar genomic patterns were later identified in tumors with wildtype *BRCA1/2* but LOF mutations in other HRR genes (Couch *et al.*, 2014; King, 2014). This led to the concept of “BRCAness” (Turner *et al.*, 2004), which has since been broadened to include genes such as *PALB2* and others (Lord and Ashworth, 2016; Mateo *et al.*, 2022). Identifying HRD in tumor samples is

crucial for predicting their response to poly ADP-ribose polymerase (PARP) inhibition (PARPi) (Gröschel *et al.*, 2019). Complementing mutation-based predictions with the identification of HRD-specific genomic patterns can enhance prediction accuracy (Kovac *et al.*, 2015; Telli *et al.*, 2016). Prospective clinical trials in ovarian cancer have emphasized this approach, demonstrating that the presence of HRD beyond BRCA1/2 mutations significantly correlates with response to PARPi (Coleman *et al.*, 2019; González-Martín *et al.*, 2019; Ray-Coquard *et al.*, 2019). As of early 2024, PARP inhibitors are also approved for HER2-negative breast cancer (Tutt *et al.*, 2021), metastatic castration-resistant prostate cancer (mCRPC) (De Bono *et al.*, 2020; Agarwal *et al.*, 2023; Saad *et al.*, 2023), and metastatic pancreatic ductal adenocarcinoma (PDAC) (Golan *et al.*, 2019). In contrast to ovarian cancer, the indication for PARPi still mostly relies on germline and/or somatic mutations in BRCA1/2, but more complex biomarkers based on extended gene lists are finding their way into approval for mCRPC (De Bono *et al.*, 2020; Agarwal *et al.*, 2023).

C. Somatic alterations in pharmacogenes

Tailored cancer pharmacotherapy should consider not only tumor suppressor genes, and oncogenes, but also the expression and activity of ADME genes in tumor tissue, given their role in the metabolism or activation of many anticancer drugs (Table 3). Several transporters and CYPs are not only expressed in organs classically associated with pharmacokinetics like the liver, but also in tumor tissues (van Eijk *et al.*, 2019). Recent studies, including data from TCGA, reported non-hepatic expression levels of 157 of 300 ADME genes across cancer types, including breast, gastrointestinal, lung, and ovarian cancers. (Hu *et al.*, 2020; Sneha *et al.*, 2021). Furthermore, ADME expression may serve as prognostic biomarker for overall survival (Hu *et al.*, 2020). For most DMEs, an equal or reduced expression compared to the corresponding healthy tissue has been reported (Vasilogianni *et al.*, 2022), and only very few seem to be overexpressed depending on cancer entity (Zhu *et al.*, 2015, p. 20; Cui *et al.*, 2020). In contrast to the germline profile, somatic expression of *ABCB1* has been linked to drug resistance in acute myeloid leukemia (AML) (Robey *et al.*, 2018). The mechanism of overexpression is not completely understood, but somatic SNVs, CNVs, and aberrant DNA

methylation could be responsible. And indeed, analysis of TCGA mutation profiles identified ABC transporters and CYPs highly mutated in colorectal, lung and bronchus, breast, and prostate cancers (Hlaváč *et al.*, 2020; Van De Geer *et al.*, 2023). Following principles such as evolutionary dynamics under selection pressure, an almost ubiquitous mechanism of drug resistance in cancer is the increased expression of drug transporters mediating the efflux of xenobiotics.

Specific examples of somatic ABC expression concern ABCA1 and ABCB5, mediating temozolomide resistance in glioblastoma, or ABCC1, also known as MRP1, in various cancer types such as AML, ALL, glioma, and NSCLC (Lee *et al.*, 2020; Wang *et al.*, 2021). Notably, elevated ABCC1 levels have been associated with a diminished response to chemotherapy in neuroblastoma (Yu *et al.*, 2015). Another extensively studied family is the ABCG family, particularly ABCG2 (BCRP), initially discovered in multidrug-resistant breast cancer (Muriithi *et al.*, 2020). The relevance of ABC expression has been described in particular for specific slow-cycling subpopulations of tumor cells in certain entities, termed cancer stem cells (Begicevic and Falasca, 2017). Of note, for healthy hematopoietic stem cells, ABC transporters have even been proposed as phenotypic markers (Koeck *et al.*, 2007; Begicevic and Falasca, 2017), and also other types of healthy stem cells show characteristic expression patterns of ABC transporters, e.g., ABCA2, ABCA3, ABCB1, and ABCG2 in neural stem cells (Lin *et al.*, 2006).

The above examples illustrate that in addition to the identification of germline and somatic DNA mutations, obtaining other omics layers from cancer tissues adds substantial information. The above examples focus on gene expression, but informative layers also include DNA methylation, (phospho)proteomic, or metabolomic profiles. To fully understand the complexity of cancer, an integrative analysis of all these omics layers is warranted (see IVB). In a supervised setting, this may be achieved at a low level by, e.g., annotating RNA allele frequencies to variants identified by DNA sequencing (Horak *et al.*, 2021), whereas in an unsupervised discovery setting, tools like Multi-Omics Factor Analysis (MOFA) may be particularly useful (Argelaguet *et al.*, 2018).

Many studies have used a range of methods to quantify DME activity in tumors and cell lines; however, they lack comparability and common conclusions (Michael and Doherty, 2005). Therefore, comprehensive studies on protein and activity data to investigate the contribution on drug resistance via intratumoral deactivation of drugs are warranted. With a few exceptions, most of the research on such resistance mechanisms to date has been theoretical or at best providing indirect correlative evidence and is not yet supported by appropriate experiments, and exact underlying mechanisms have rarely been investigated. Systematic somatic proteomics data, e.g. such as provided by the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC, pdc.cancer.gov/pdc/) may reveal new insights in non-hepatic ADME expression and drug metabolism by tumors. Nevertheless, there are examples of intratumoral drug metabolism or its association to therapeutic response. A recent study on lung adenocarcinoma patients found that higher somatic copy numbers of *CYP2C8* and *CYP3A4*, which are involved in paclitaxel metabolism, were linked to non-responder patients with progressive disease during paclitaxel treatment (Incze *et al.*, 2023). In contrast, in the HepG2 cell line, *CYP3A4* and *CYP2C8* had no effect on paclitaxel efficacy, and only *CYP3A4* activity was found to contribute to docetaxel resistance (Hofman *et al.*, 2021). Another study assessed the activity of *CYP3A5* in patient-derived models of PDAC and showed an influence on resistance to TKIs (Noll *et al.*, 2016). The exocrine PDAC subtype showed intrinsic resistance to erlotinib and dasatinib. It was also shown that this effect can be effectively inhibited and induced. In other PDAC subtypes, acquired TKI and paclitaxel resistance was also observed and correlated with *CYP3A5* expression. However, these results are debated since expression is not a good measure of *CYP3A5* activity, as the common splicing variant (*3; see II.B) lead to an inactive protein (Ingelman-Sundberg and Lauschke, 2020). Various tumors exhibit increased levels of *CYP1B1*, leading to modifications in the biotransformation of taxanes such as paclitaxel and docetaxel (Murray *et al.*, 1997; Zhu *et al.*, 2015). Another gene whose complex role in cancer has already been more thoroughly described is *GSTP1*. *GSTP1* is overexpressed in many cancer types, and it is assumed that this leads to increased detoxification of

chemotherapeutic agents, especially platinum-based therapies (Sawers *et al.*, 2014; Cui *et al.*, 2020). As a potential regulation mechanism causing the altered activity of DMEs in tumors, drug-inducible NRs such as PXR are discussed (Chen, 2010; Chen *et al.*, 2012). In general, DMEs can only be seen as just one element in the complex interplay of tumor-specific mechanisms and pathways leading to the survival of tumor cells despite treatment.

D. Risk prediction and drug treatment of cancer

Combining different pieces of information has proven to be performant and beneficial also in risk prediction. In breast cancer, disease risk prediction started with *BRCA1* and *BRCA2* and was rapidly improved by polygenic scores. A comprehensive work on 313 germline variants showed that women in the top 1% of PRS were 3.6 times more likely to develop ER-positive breast cancer, and women in the lowest 1% of PRS were 6 times less likely to develop ER-positive breast cancer than women in the middle quintile (Mavaddat *et al.*, 2019).

The treatment options consist of surgical intervention, radiation, systemic therapy including anthracyclines such as doxorubicin or epirubicin, taxanes, e.g., docetaxel, and HER2-targeting drugs including trastuzumab, and finally commonly used hormonal therapy with estrogen receptor (ER) modulators (e.g. tamoxifen, raloxifene) or aromatase inhibitors (e.g. exemestane, letrozole) (Agostinetti *et al.*, 2022). More than one-third of women with early breast cancer receive adjuvant chemotherapy although the clinical benefit is not shown for more than half of the women. They would have survived without the additional treatment. The potential of risk stratification with respect to individualized treatment decisions is obvious i.e., which patient will most or least likely benefit from systemic chemotherapy. Given the complexity of tumor genomes and biology, multigenomic assays have been identified as suitable tools for predictive tests (Van 'T Veer *et al.*, 2002; Heo *et al.*, 2021). Therefore, exemplarily we review polygenic tests that are currently available for clinical use in the management of breast cancer.

In the latest clinical practice guidelines of the American Society of Clinical Oncology (ASCO) as well as the European Society of Medical Oncology (ESMO) (Henry *et al.*, 2022; Loibl *et al.*, 2024) there are four multigene tests considered to guide adjuvant treatment decisions in

breast cancer. All four tests provide information about an individual's recurrence risk and the benefit of chemotherapy in general, not about specific drugs. Across all tests, chemotherapy is indicated in patients with high-risk or high-score results. The clinical utility of the tests has been or is actually evaluated in large RCTs.

The *EndoPredict Test* (originally Sividon Diagnostics, Köln, Germany now Myriad International GmbH, Cologne, Germany) started as an 8-gene test and was expanded to a 12-gene quantitative PCR (qPCR) test on formalin-fixed paraffin-embedded (FFPE) tumor samples (Filipits *et al.*, 2011). The score summarizes the test result with tumor size and nodal status and is able to predict distant recurrence-free survival for up to 15 years post-diagnosis in ER-positive and HER2-negative tumor patients. The clinical utility of the test is evaluated in two large prospective RCTs: RESCUE (NCT03503799) and EndoPredict Extended Endocrine Trial (EXET; NCT04016935) (Brufsky *et al.*, 2022). *Oncotype DX* (Genomic Health, Redwood, CA) was developed 2004 and is a 21-gene qPCR test on FFPE samples. The score calculated upon the expression levels divides patients into the three groups of low, intermediate and high risk of recurrence in 10 years. Multiple studies showed that low score results predicted little to no benefit from chemotherapy, whereas patients with high scores showed significant benefit from additional chemotherapy on top of endocrine therapy (Paik *et al.*, 2004; Syed, 2020). Two separate reports for nodal status are available and clinical utility was demonstrated in prospective studies (West German Study Group (WSG) PLAN B trial, TAILORx and RxPONDER (SWOG 1007)). *Prosigna*, formerly known as PAM50, is a 50-gene signature test applied on Nanostring NCounter technology. The test has been analytically validated in patients under endocrine treatment (Wallden *et al.*, 2015). In addition to the prognostic value of recurrent disease, the Prosigna gene signature can assign tumor samples to the intrinsic subtypes Luminal A, Luminal B, HER2+, and basal-like tumors. In contrast to the other tests, *Mamma Print* uses a 70-gene microarray developed by Agendia (Irvine, CA) and can be used independently of ER status which was shown as clinically appropriate in the prospective MINDACT study (Cardoso *et al.*, 2016). Several studies have examined additional features of the tests available. For example,

MammaPrint and Oncotype DX are most cost-effective (Hall *et al.*, 2017) and can identify subgroups of breast cancer patients with an ultra-low risk of death over two decades (Petkov *et al.*, 2016; Esserman *et al.*, 2017). However, the challenge is now to choose the optimal test for the patient.

IV. Integration of germline and somatic variation for drug therapy

A. Precision oncology

Precision oncology is rapidly advancing based on increasingly comprehensive and high-fidelity molecular diagnostics, resulting in a deeper understanding of individual tumors' functional underpinnings. The primary aim of this personalized approach to cancer care is to predict which patients will likely respond to specific therapies (Mateo *et al.*, 2022). Sequencing of tumor DNA and RNA is an essential method for achieving molecular stratification of patients, as it can detect an increasing spectrum of genomic and transcriptomic alterations with direct clinical implications (Horak and Fröhling, 2024). In contrast to traditional cohort studies that rely on recurrence, precision oncology focuses on individual cancer patients and aims to enable more accurate and customized treatment approaches (Berger and Mardis, 2018). In oncology, an adequate and comprehensive view of a case, i.e., a patient and the tumor, is only possible when taking both somatic and germline genetics into account. For example, targetable lesions can be found in both the somatic and germline variant calls. Frequently, the combination of, e.g., a heterozygous germline variant and a somatic event, such as LOH leads to inactivation of a tumor suppressor gene and can drive malignant transformation. To distinguish between germline and somatic mutations, DNA from both the tumor sample and a matched normal tissue, often blood, needs to be sequenced, and the variant calling itself represents data integration. Ensuring reliable and interpretable results is crucial when clinical decisions rely on genomic analyses, and effective communication of these results holds paramount importance (Horak *et al.*, 2016). It has been recognized that organizing precision oncology efforts through interdisciplinary panels, including experts from various medical disciplines (e.g., (molecular)

oncologists & pathologists, medical geneticists), bioinformatics, and cancer biology, is an efficient approach. Initially termed “multidisciplinary sequencing tumor boards” (Roychowdhury *et al.*, 2011) and later known as molecular tumor boards (MTB) (Schwaederle *et al.*, 2014), these forums serve as platforms where multidisciplinary teams convene to discuss individual patient data including clinical, laboratory and other diagnostic information, analyze genomic data, and molecular profiling, quality, and devise personalized treatment strategies to assign evidence-based treatment recommendations (Mock *et al.*, 2023).

As precision oncology aims at the ideal drug-patient match for every individual case, it frequently performs drug repurposing. Many treatment recommendations of a MTB may be off-label, as a drug is often approved in a given set of entities, but targetable lesions and biomarkers may be found in other entities. Beyond evidence-based treatment recommendations based on targetable lesions (e.g. *EGFR* mutations, Figure 4A), other actionable observations may include refinement of diagnosis based on pathognomonic molecular alterations or the necessity of genetic counseling for the index patient and/or family members in the case of germline findings (Horak *et al.*, 2021; Darmofal *et al.*, 2024). With the dramatically increasing knowledge on the implications of PGx for cancer care, there is an urgent need to involve molecular pharmacologists in the MTB and to train the other specialists in PGx (Shriver *et al.*, 2024).

Finally, beyond classical RCT innovative trial designs are crucial for precision oncology and adaptive trial designs, such as basket or umbrella trials or even platform trials have been so far increasingly used to tailor drug therapy based on various risk factors (Park *et al.*, 2020). Rare cancers with an incidence of less than six per 100,000 persons per year in particular require innovative adaptive trial designs (Van Der Velden *et al.*, 2019). The molecular pathogenesis of many rare cancers is understudied, leading to a lack of prognostic and predictive factors, as well as a scarcity of rationally developed, molecular mechanism-aware therapies (Van Der Graaf *et al.*, 2022).

Moreover, trials on rare cancers often require the involvement of high-volume cancer centers and/or collaboration among multiple institutions (Flaherty *et al.*, 2020). Another obstacle to implementing precision oncology approaches in rare cancers is that negative evidence of drug efficacy in unstratified clinical trials likely underestimated the potential of therapies (Ray-Coquard *et al.*, 2017). The multicenter, prospective observational MASTER study (see III.A) was initiated to enhance the understanding of advanced rare cancers and early-onset common cancers, and to address the unmet clinical needs associated with these diseases. The aim is to inform the clinical management of patients and identify opportunities for the development of molecularly stratified clinical trials (Figure 6). So far the molecular profiles and clinical outcomes of the first 1310 patients highlight the practical diagnostic and therapeutic implications for a patient population with unfavorable prognosis (Horak *et al.*, 2021).

B. Additional data layers and multi-omics integration

Advancements in biomedical technologies continuously expand the availability of high-dimensional data layers, prompting the integration of multi-omics approaches in precision oncology to interrogate complex biomarkers aimed at identifying predictive, prognostic, or diagnostic information (Figure 4B). An important data integration strategy is to annotate read counts from gene expression data to the mutations identified by DNA sequencing, thereby providing information on whether a mutation is expressed or not (Beaubier *et al.*, 2019; Lee *et al.*, 2021). DNA methylation or proteomics can also be used to provide added value (Wong *et al.*, 2020). Whenever more than one layer is present, methods for data integration or multi-omics integration are necessary. Data integration methods can be grouped into type early (or full), for which the datasets of the different omics layers are combined into a single dataset on which the data model is built, which often requires transformations of the datasets into a common representation, and type late (or decision), for which models are built for each dataset separately. The models are then combined into a unified model, and by building isolate models from each dataset, the mutual relations of the different data layers are ignored (Gligorijević and Pržulj, 2015). In the research field of cancer drug treatments in which

various processes and influences are connected and modulate each other, PGx appears to be a promising hidden factor or hidden source of variation.

C. Tumor heterogeneity and combination therapies

After malignant transformation, tumors clonally expand. Often, additional somatic mutations and/or epigenetic or functional alterations are acquired, and subclones arise (Hanahan and Weinberg, 2011). The resulting diversification, usually referred to as tumor heterogeneity, has been known for decades based on histopathologic and radiologic examinations. When using bulk sequencing or other omics technologies for characterizing a tumor sample, an additional complication arises: tumor tissues are rarely pure; often, adjacent healthy tissue is admixed. However, based on algorithmic considerations in the detection and calling of sCNAs, an estimate for an optimal purity/ploidy combination can be given (Zack *et al.*, 2013). When combining sCNA information with allele frequency distributions of SNVs, subpopulations, subclones, and even the individual evolutionary history of a given tumor sample can be reconstructed using parsimony considerations (Giessler *et al.*, 2017), computational frameworks (Grigoriadis *et al.*, 2024) and by automated algorithms (Frankell *et al.*, 2023). When taking into consideration even more patterns identified in a tumor sample, like mutational signatures using e.g. single cell read outs (RNA, epigenetics, proteome) and spatial transcriptomics, detailed molecular clocks can be inferred, and even more time course information of the sample can be gained (Gerstung *et al.*, 2020).

Tumor heterogeneity is among the reasons why many cancer patients need combination treatment. This concept, first explored in childhood leukemia (Pui *et al.*, 2015), is the backbone of classical multi-agent chemotherapy for most hematologic and solid-organ malignancies such as FOLFIRI (5-FU, folinic acid, and irinotecan) and FOLFOX (5-FU, folinic acid, and oxaliplatin) (Colucci *et al.*, 2005), multimodality therapy of adult Ewing sarcoma (Pretz *et al.*, 2017), and prevails in newer molecular mechanism-aware treatment regimens such as venetoclax and azacitidine in AML (DiNardo *et al.*, 2020). The evaluation of pharmacodynamic DDI with respect to synergistic effects but also safety is warranted (Niu *et*

al., 2019). And indeed novel effective combinatorial drug treatments can be identified in distinct molecular cancer subpopulations (Jaaks *et al.*, 2022).

In contrast, combination therapies inevitably lead to DDI, whose number and potential detrimental effects increase with the number of co-prescriptions. This principle applies to cancer patients who frequently undergo concurrent treatments (Van Leeuwen *et al.*, 2015). In the precision oncology era, combination therapies are guided by co-occurring molecular biomarkers and associated evidence-based recommendations, whose proportion has increased steadily over time, e.g., in the MASTER program from 5% of cases in 2014 to 53.9% in 2018 (Horak *et al.*, 2021). Combination therapies are often prioritized if more than one recommendation was issued or evidence exists for the lack of efficacy of single agents in specific histologic contexts as in the case of BRAF inhibition in BRAFV600-mutated colorectal cancer (Hyman *et al.*, 2015). Another driving force are molecularly stratified clinical trials of combination treatments, such as the TOP-ART trial of the MASTER network (ClinicalTrials.gov Identifier NCT03127215), which tests olaparib, a PARP inhibitor, in conjunction with the chemotherapeutic agent trabectedin.

D. Implementation of PGx in Precision Oncology

As demonstrated, NGS technologies have been integral to PGx research. The increasing evolution of bioinformatics tools which provide functional prediction and specific algorithms to easily extract PGx information from NGS data, coupled with technological advancements, enables the implementation of PGx across diverse clinical environments (Tafazoli *et al.*, 2021; Reizine and O'Donnell, 2022). Cancer patients are particularly suited, as NGS of germline and somatic tissue is commonly employed, to inform targeted cancer drug treatments. Indeed, several studies have confirmed the potential clinical advantage for multidisciplinary PGx in cancer patients by repurposing germline NGS data (Hutchcraft *et al.*, 2021; Shugg *et al.*, 2022), but also in other clinical settings such as pediatric medicine (Barker *et al.*, 2022). However, WES data showed limitations, including missing coverage of intronic variants or limited CNV detection of key variants in several pharmacogenes (e.g. *CYP3A5*3*, *CYP2C19*17*, *VKORC1-rs9923231*, *CYP2D6*5*) (van der Lee *et al.*, 2020;

Lanillos *et al.*, 2022). In contrast, WGS data and long-range NGS have proven useful in this regard, providing comprehensive results for pharmacogenes (e.g., PGx guideline) (Twesigomwe *et al.*, 2021; van der Lee *et al.*, 2022) with sufficient accuracy, although standards of analytical validation (e.g. accuracy, precision, limit of detection, specificity, etc.) need to be addressed more intensively (Ly *et al.*, 2022; Huebner *et al.*, 2023). High-throughput WGS sequencing approaches identify an average of 4 to 5 million variants in a genome and the read (Fastq, BAM) and variant (vcf) files need up to hundreds of gigabytes disk space (Bagger *et al.*, 2024). Thus, extracting PGx-profiles, including known functional (and mainly common), but also rare PGx variants (see II.B) from that large amount of data, requires innovative and comprehensive bioinformatics tools. Several tools have been developed, which provide the key genotypes of SNVs and CNVs for a tool-dependent set of genes (e.g., Aldy, Pypgx, Stargazer, PharmCAT; up-to-date information is given in (Tremmel, *et al.*, 2023)). Consequently, the variants are combined into haplotypes/star alleles, and the final results are translated into the corresponding metabolizer phenotypes or activity scores. Based on these data the available PGx guidelines can be queried for all available or a selected set of gene-drug pairs. End-to-end solutions have been developed covering all aspects of genotyping to reporting in an automated pipeline (Klanderman *et al.*, 2022). But, there are still major limitations. Those tools mainly assess common variants and the large fraction of rare and/or undescribed variants is not captured, thus potentially resulting in inaccurate or even incorrect metabolizer or functional phenotypes. Even if rare variants are interrogated, a valid clinical functional classification and prediction is an yet unresolved issue (Siamoglou *et al.*, 2022). Furthermore, the resolution of short-read NGS is limited in repetitive and/or homologous genomic regions such as the *CYP2D6*, *HLA*, *SULT1A1*, or the *UGT1A* loci. Hence, the correct assignment of genotypes might be impossible (Caspar *et al.*, 2020), but long-read sequencing is able to overcome this limitation (Zhou and Lauschke, 2024). Therefore, some studies suggested to use a combination of two or more tools for the interrogation of a confident consensus genotype (Tafazoli *et al.*, 2021). However, the handling of different input files (vcf, bam), and accepted alignment of input NGS reads

(against GRCh37 or GRCh38), along with solutions how to resolve discrepancies between individual results complicate data evaluation, genotype accuracy and finally the clinical implementation process.

Reimbursement of PGx testing varies substantially between continents and countries, although PGx was favored in most studies assessing cost-effectiveness (Morris *et al.*, 2022). While, for instance, in Germany single gene-drug combinations (e.g. DPYD-5FU, UGT1A1-irinotecan, CYP2C9-siponimod, see II.B) are reimbursed by insurance companies, data from US shows that PGx panels have been more often reimbursed than single gene tests (Lemke *et al.*, 2023). Given the substantial costs required for the storage of NGS data, the use of newly generated NGS data in case of cancer patients offers an attractive alternative for PGx analysis. In light of the trend of decreasing NGS costs, competitors have promised significant price reductions (Liu *et al.*, 2021; Simmons *et al.*, 2023), probably reshaping the landscape of genomic testing services. For instance high storage costs may be circumvented by rather re-sequencing of samples than storing large amounts of NGS data long-term. Thus, NGS data analysis require a setup of efficient computational pipelines to extract and interpret known variants with respect to drug and dosage guidelines. The advantage of NGS is a comprehensive or full PGx profile including (rare) variants of unknown function in addition to known variants. Of note, expert-knowledge, in silico tools and datasets of large-scale functional annotation (e.g. VAMP-Seq) are required for the evaluation of variants with unknown or questionable function and subsequent clinical translation, taking into account the well-established PGx profiles. To the end, as illustrated in Figure 4, molecular profiling of both somatic and germline genomes enables prediction of individual drug response to specific cancer therapies (Hertz and McLeod, 2016). While the identification of specific somatic driver mutations offers the selection of targeted therapies designed to suppress the activated pathway, PGx enables the optimization of the drug dosage to reduce the risk for ADR. Moreover recommendations for accompanying therapeutic drug and/or ADR monitoring can be given.

When dealing with genomic data including PGx information, it is important to comply with ethical and legal requirements (Winkler and Knoppers, 2022). One source providing guidance is the “Ethical and Legal Aspects of Whole Human Genome Sequencing” project developed within the “Ethical and Legal Aspects of Translational Medicine” (EURAT) framework at Heidelberg University (uni-heidelberg.de/md/totalsequenzierung/informationen/mk_eurat_position_paper.pdf). It aims to analyze the ethical, legal, and economic implications of genome sequencing in clinical settings including the issue of incidental findings (Schickhardt *et al.*, 2020) and to develop practice-based recommendations. Concrete deliverables are the definition of milestones, a code of conduct, and patient consent models. Of relevance to precision oncology, EURAT states: “physicians who would like to make greater use of this diagnostic tool will have to navigate the attendant ethical, legal, and economic prospects and challenges; and patients who seek treatment in Heidelberg will have to consider these new genome-based diagnostic options and their associated opportunities and risks more extensively as part of the information and consent processes.” (uni-heidelberg.de/md/totalsequenzierung/informationen/mk_eurat_position_paper.pdf). The German Cancer Research Center (DKFZ) has adopted the code of conduct for non-physician scientists.

V. Concluding remarks

Technological advances in recent years have made it possible to describe the molecular landscapes of most cancers. Notably, a rapidly expanding spectrum of genomic alterations have prognostic and/or predictive value and/or represent targets for therapeutic intervention. The increasing availability and throughput and decreasing cost of screening technologies, particularly NGS, have led to the introduction of systematic molecular profiling into modern cancer medicine. However, the paradigm of individualized precision oncology is mainly limited to the consideration and clinical use of somatically acquired genetic alterations. In contrast, information on the germline genome is only gradually being acquired more systematically, primarily to detect hereditary cancer predisposition. The highly dynamic field

of PGx, although also based on genetic analyses, has developed mainly in parallel to molecularly guided precision oncology. The increasing clinical application of truly comprehensive molecular profiling, including WGS of tumor and matched normal control (germline) tissue, offers a unique opportunity to merge somatic and germline genetics in oncology and improve patient outcomes by taking a holistic view of a tumor and its host organism. Here particularly safety aspects and the avoidance of ADR of innovative cancer agents as well supportive medication need to be considered. Moreover, PGx information should be part of the interpretation of somatic tumor genomes to capture as many determinants of response and resistance to cancer therapies as possible and to tailor clinical management.

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VIII. Data availability

This review article contains no datasets generated or analyzed during the current study.

IX. Author contribution

All authors contributed to the conception, drafting the article, creation of figures and table, and critical review of the manuscript. All authors approved the final version of the manuscript.

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XI. Footnotes

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XII. Tables

Table 1. Selected pharmacogenes and their known variant portfolio and corresponding phenotype consequences

Gene	Number of core (star)alleles[#]	Structural variants	Clinical important phenotypes^{##}	Link
CYP2B6	46	Deletions, Hybrids with CYP2B7	PM, IM, NM, RM, UM	pharmvar.org/gene/CYP2B6
CYP2C19	34	Rare partial deletions & deletions	PM, IM, NM, RM	pharmvar.org/gene/CYP2C19
CYP2C9	85	No	PM, IM low, IM high, NM	pharmvar.org/gene/CYP2C9
CYP2D6	163	Deletions, Duplications, Hybrids with CYP2D7	PM, IM, NM, UM	pharmvar.org/gene/CYP2D6
CYP3A4	45	No	PM, IM, NM	pharmvar.org/gene/CYP3A4
CYP3A5	6	No	PM, IM, NM	pharmvar.org/gene/CYP3A5
DPYD	433	No	PM, IM, NM	pharmvar.org/gene/DPYD
NUDT15	20	No	PM, IM, NM	pharmvar.org/gene/NUDT15
UGT1A1	113	No	PM, IM, NM	pharmacogenomics.pha.ulaval.ca/wp-content/uploads/2015/04/UGT1A1-allele-nomenclature.html
SLCO1B1	42	Rare partial deletions & deletions	PF, DF, NF, IF	pharmvar.org/gene/SLCO1B1
TPMT	45	No	PM, IM, NM	liu.se/en/research/tpmt-nomenclature-6m

[#] according reference GRCh37 (NC_000022.10) and including reference (*1) allele

^{##} according to CPIC/PharmGKB. PM: poor metabolizer; IM: intermediate metabolizer; NM: normal metabolizer; RM: rapid metabolizer; UM: ultra metabolizer; PF: poor function; DF: decreased function; NF: normal function; IF: increased function

Table 2. Functional prediction tools for exonic variants

In silico tools and criteria for prediction scores	Year of publication	Estimated proportional use in PGx studies over the last five years[#]	Reference
<i>SIFT</i>	2001	10%	(Ng and Henikoff, 2001)
<i>Polyphen/ Polyphen2</i>	2002/ 2010	33.2%	(Adzhubei <i>et al.</i> , 2010)
<i>PhastCons</i>	2005	<1%	(Siepel <i>et al.</i> , 2005)
<i>Likelihood ratio tests</i>	2009	1.8%	(Chun and Fay, 2009)
<i>SiPhy</i>	2009	<1% ^o	(Garber <i>et al.</i> , 2009)
<i>GERP++</i>	2010	2.5%	(Davydov <i>et al.</i> , 2010)
<i>PhyloP</i>	2010	2.2%	(Pollard <i>et al.</i> , 2010)
<i>MutationAssessor</i>	2011	4.9%	(Reva <i>et al.</i> , 2011)
<i>PROVEAN</i>	2012	7.8%	(Choi <i>et al.</i> , 2012)
<i>FATHMM</i>	2013	2.4%	(Shihab <i>et al.</i> , 2013)
<i>VEST3</i>	2013	2.0%	(Carter <i>et al.</i> , 2013)
<i>CADD</i>	2014	13.9%	(Kircher <i>et al.</i> , 2014)
<i>DANN</i>	2014	1.5%	(Quang <i>et al.</i> , 2015)
<i>FATHMM-MKL</i>	2013	1.8%	(Shihab <i>et al.</i> , 2015)
<i>MetaSVM,MetaLR</i>	2015	2.5%	(Dong <i>et al.</i> , 2015)
<i>SNAP2</i>	2015	2.2%	(Hecht <i>et al.</i> , 2015)
<i>REVEL</i>	2016	6.4%	(Ioannidis <i>et al.</i> , 2016)
<i>DEOGEN2</i>	2017	<1%	(Raimondi <i>et al.</i> , 2017)
<i>SNPMuSiC</i>	2018	<1%	(Ancien <i>et al.</i> , 2018)
<i>Missense3D</i>	2019	1.3%	(Ittisoponpisan <i>et al.</i> , 2019)
<i>LoGoFunc</i>	2023	<1%	(Stein <i>et al.</i> , 2023)
<i>AlphaMissense</i>	2023	<1%	(Cheng <i>et al.</i> , 2023)

[#]The proportional use of functional variant prediction tools in pharmacogenomics studies from 2019 to 2024 was evaluated through PubMed queries. Searches were conducted using terms 'Pharmacogenetics', 'Pharmacogenomics', 'ADME Gene', 'drug transporter', or 'drug metabolizing enzyme'. Then, 184,974 citations of extracted pubmed entries were screened for the prediction tools, using unique parts of their publication titles e.g., for the tool CADD following part of the manuscript title was used: 'general framework for estimating the relative pathogenicity of human'. There are several limitations of this analysis. We have not distinguished between original research papers and reviews, and we neglected annotation workflows such as ANNOVAR, SnpSift, or Ensembl VEP. These workflows encompass multiple tools as annotation layers and are often cited as the only resource.

Table 3. Clinical association between variants of pharmacogenes & drug targets and selected anti-cancer drugs extracted from PharmGKB database (last accessed March 2024)

Drug [#]	Class	Somatic target	Clinically associated germline or somatic variants ^{##}		Associated phenotype category	Cancer entity in which the association was reported
			Gene family	Gene		
Alemtuzumab	Antibody	CD52	Drug target	CXCL12	Efficacy	B-Cell, Chronic, Leukemia
Axitinib	TKI	VEGFR1-3, PDGFRA/B, KIT	Drug target	HIF1A	Efficacy	NA
Bevacizumab	Antibody	VEGF	Drug target	ARMS2, CFH, CXCL8, CXCR4, EDN1, GGH, HSP90AB1, HTRA1, MGAT4A, MTHFR, PRKCA, RGS5, SHMT1, VEGFA	Dosage Efficacy, Toxicity	Breast Neoplasms, Colorectal Neoplasms, Non-Small-Cell Lung
Cetuximab	Antibody	EGFR	Drug target	AREG, CCND1, EGF, EGFR, FCGR2A, FCGR3A, KRAS, MGAT4A, RASSF1	Efficacy Toxicity	Colorectal Neoplasms, Head And Neck Neoplasms
Dasatinib	TKI	ABL1, KIT, SRC	Transporter	ABCG2	Other	NA
Erlotinib	TKI	EGFR	Drug target	EGFR (Resistance mutation T790M, rs121434569) , MAP3K1	Efficacy Toxicity	Adenocarcinoma, Drug Resistance, Lung Neoplasms
			Phase I	CYP1A2	Metabolism/PK	
Everolimus	STKI*	mTOR	Drug target	FGFR4, MTOR, PIK3R1, RPTOR	Efficacy Toxicity	Breast Neoplasms, Kidney Neoplasms, Leukopenia, Neuroendocrine Tumors
			Phase I	CYP3A4, CYP3A5	Metabolism/PK	
			Transporter	ABCB1	Toxicity	
Gefitinib	TKI	EGFR	Drug target	EGFR (Somatic testing) , IKBKB, IKBKE, MAP3K1, NFKBIA, NFKBIB, NR1H2, RELA, SIRT2, TAB2	Efficacy Toxicity	Adenocarcinoma, Lung Neoplasms
			Phase I	CYP2D6	Toxicity	
			Transporter	ABCB1, ABCG2	Toxicity	
Gemtuzumab Ozogamicin	Antibody- drug conjugate	CD33	Drug target	CD33	Efficacy	Acute Myeloid Leukemia

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			Phase I	CYP2E1	Toxicity	
			Phase II	SULT2B1	Toxicity	
			Transporter	SLC22A12, SLCO1B1	Toxicity	
Imatinib	TKI	ABL1, PDGFRA/B	Drug target	BCL2L11, CHST1, EGFR, NQO1, RUNDC3B, ULK3	Dosage, Toxicity, Efficacy	Gastrointestinal Stromal Tumors, Leukemia
			Phase I	CYP1A2, CYP2B6, CYP2F1, CYP3A4, CYP3A5	Dosage, Efficacy, Metabolism/PK, Toxicity	
			Phase II	GSTT1, UGT2A1	Efficacy, Toxicity	
			Transporter	ABCB1, ABCB4, ABCC2, ABCC4, ABCG2, SLC19A1, SLC22A1, SLC22A4, SLC22A5, SLCO1A2	Dosage, Efficacy, Metabolism/PK, Toxicity	
Lapatinib	TKI	EGFR, ERBB2-4	Immune system	HLA-DQA1, HLA-DRB1	Toxicity	NA
Nilotinib	TKI	ABL1	Phase II	UGT1A1	Toxicity	NA
			Transporter	ABCG2	Other	
Panitumumab	Antibody	EGFR	Drug target	AREG, EGFR, KRAS	Efficacy	Colorectal Neoplasms
Pazopanib	TKI	FGFR1-4, KDR, KIT	Drug target	KDR	Efficacy, Metabolism/PK	Carcinoma, Kidney Neoplasms
			Phase II	UGT1A1	Toxicity	
Regorafenib	TKI	VEGFR, TIE2, KIT, RET, RAF1, BRAF, PDGFR, FGFR	Drug target	KDR	Toxicity	NA
Rituximab	Antibody	CD20	Drug target	CXCL12, FCGR2A, FCGR3A (Reduced response, rs396991) , IL2, TGFB1	Efficacy	Diffuse Large B-Cell Leukemia, Non-Hodgkin Lymphoma
			Phase II	GSTA1	Efficacy	
			Transporter	ABCB1	Toxicity	
Sirolimus	STKI	mTOR	Drug target	IL10, TCF7L2	Toxicity	Urinary Bladder Neoplasms
			Other	NR1I2, POR	Metabolism/PK, Toxicity	
			Phase I	CYP3A4, CYP3A5	Dosage, Metabolism/PK	

			Phase II Transporter	UGT1A8 ABCB1	Toxicity Metabolism/PK, Toxicity	
Sorafenib	TKI, STKI	FLT3, KIT, RAF	Drug target	ADAMTS18, CDH13, EGFR, EPAS1 (risk of toxicity, rs7557402), GALNT14, HIF1A, KDR, MAP2K6, NOS3, PIK3R5, PRKCE, TNF, VEGFA, VEGFB, WVVOX	Efficacy, Toxicity	Carcinoma, Liver Neoplasms, Kidney Neoplasms
			Phase II Transporter	UGT1A1, UGT1A9 ABCB1, ABCC2, SLC15A2, SLCO1B1	Toxicity Efficacy, Toxicity	
Sunitinib	TKI	FGFR1-3, KIT	Drug target	CXCL8, FLT3, FLT4, IL13, VEGFA	Efficacy, Toxicity	Carcinoma, Gastrointestinal Stromal Tumors, Kidney Neoplasms
	TKI	FGFR1-3, KIT	Other	NR1I2, POR	Efficacy, Toxicity	Carcinoma, Gastrointestinal Stromal Tumors, Kidney Neoplasms
			Phase I Transporter	CYP3A5 ABCB1, ABCG2, SLCO1B3	Dosage, Toxicity Efficacy, Toxicity	
Temsirolimus	STKI	mTOR	Other	NR1I2	Metabolism/PK, Toxicity	Urinary Bladder Neoplasms
			Transporter	ABCB1	Metabolism/PK	
Tocilizumab	Antibody	IL6R	Drug target	CD69, FCGR3A, GALNT18, IL6R	Efficacy	NA
Trastuzumab	Antibody	ERBB2	Drug target	BARD1, ERBB2, ERBB3, FCGR2A, FCGR3A, PPCDC, RNF8	Efficacy, Toxicity	Breast Neoplasms
Valproic Acid**	HDAC inhibitor	HDAC	Drug target	ANKK1, COL1A1, GABRA1, GRIN2B, LEPR, POLG, RABEP1, SCN1A, SCN2A, SH2B1, SOD2	Dosage, Efficacy, Toxicity, Other	NA
			Phase I	CYP1A1, CYP2C19, CYP2C9	Dosage, Efficacy, Metabolism/PK, Toxicity	

Phase II	UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT2B7	Dosage, Metabolism/PK
Transporter	ABCB1	Efficacy

#73 drugs were retrieved from (Worst *et al.*, 2016). For 48 drugs no clinical association could be extracted from PharmGKB

Clinical association with PharmGKB level of evidence 1 or 2 are highlighted in bold type and the effective variant is reported.

*Serine/threonine kinase inhibitor

**Only targets relevant for oncology are listed here

XIII. Figure legends

Figure 1. Selected pivotal findings in the field of pharmacogenomics (PGx) and temporal trend of scientific publications related to PGx without (blue) and with (yellow) consideration of cancer therapy. The following key words has been used for the search of PGx related publications: (Pharmacogenetics OR Pharmacogenomics) NOT cancer, (Pharmacogenetics OR Pharmacogenomics) AND cancer.

Figure 2.A. Schematic representation of the most common functional effects of variants in pharmacogenes. Variants may influence translation and transcription, splicing, protein structure and stability with consequences on substrate specificity and transporter affinity. Created with BioRender.com.

B. Proportion of individuals of the main geographical subgroups expected to carry a high-risk diplotype for pharmacogenes with PGx guideline recommendations (Clinical Pharmacogenetics Implementation Consortia, CPIC and The Dutch Pharmacogenetics Working Group, DPWG). Data was retrieved from PharmGKB.org. The frequencies of following functional alleles were summarized: *CYP2B6* (*4, *6, *18), *CYP2C19* (*2, *3, *17), *CYP2C9* (*2, *3), *CYP2D6* *3, *4, *5, *6, *7, *8, *9, *10, *14, *41, *1x2, *2x2), *CYP3A5* (*3), *DPYD* (*2A, *13, c.2846A>T, *HabB3*), *HLA-A**31:01, *HLA-B* (*15:02, *57:01, *58:01), *SLCO1B1* (*5, *15), *TPMT* (*2, *3A/B/C), *UGT1A1* (*28) for the populations of Central/South Asia, East Asia, Europe, Middle East & North Africa, North America, Oceania, South America, and Sub-Saharan Africa. **C.** Overview of various therapeutic indications and PGx drugs in relationship to pharmacogenes.

Figure 3. Clinical relevant organ toxicities and damages as result of PGx-related adverse drug reactions of anticancer and other drugs. Created with BioRender.com.

Figure 4. Integrative clinical workflow in precision oncology including PGx. **A.** NGS analysis of somatic and germline tissue. Selective tumor markers, often active that can be targeted therapeutically are identified within the tumor. In the germline, genomic variants are

identified, allowing predictions regarding treatment response and risk of toxicities. **B.** The progression from molecular testing via multidisciplinary tumor boards involving experts from various fields, resulting in a drug treatment recommendation. Abbreviations: SNV: single nucleotide variant, CNV: copy number variation, LOF: loss of function variant, GOF: gain of function variant. Created with BioRender.com.

Figure 5. Genotype-phenotype correlation for selected pharmacogenes and their association with gene expression and/or function, pharmacokinetic and outcome data of selected cancer drugs. **A.** CYP2D6 and tamoxifen. Left, hepatic CYP2D6 protein expression (n=150) vs. CYP2D6 activity score (AS 0 to 3.0) (Zanger *et al.*, 2021); middle, steady-state metabolic ratio (MR) of desmethyltamoxifen/(Z)-endoxifen plasma levels (n=236, 20 mg tamoxifen) vs CYP2D6 genotypes (homozygous for PM alleles, homo-/heterozygous for IM or one PM alleles, NM or UMs). The data presented as median, 25%/75% percentiles, and range (Mürdter *et al.*, 2011); right, event-free survival indicating NM (patients with NM alleles), heterozygous NM/IM (patients with IM or one PM allele), and PM (patients with two PM alleles). Follow-up of 15 years after diagnosis (median 6.3 years) was considered (Schroth, 2009). **B.** TPMT and 6-mercaptopurine (6-MP). Left, TPMT activity in red blood cells (RBC) among 1214 individuals in relation to *TPMT* genotypes. The grey area depicts the range of intermediate TPMT activity (Schaeffeler *et al.*, 2004); middle, relationship between TPMT activity and thioguanine nucleotide (TGN, active 6-MP metabolites) levels in RBC in children with ALL (standard 6-MP therapy) (Krynetski *et al.*, 1996); right, cumulative incidence of the end of 6-MP therapy for PM and 1 year for IM and NM requiring a decrease in 6-MP dose to prevent hematotoxicity in ALL children ($P < .001$) (Relling *et al.*, 1999). **C.** DPYD and 5-fluorouracil. Left, hepatic dihydropyrimidine dehydrogenase (DPD) protein content (n=82) and *DPYD*2A* (Schwab *et al.*, 2008); middle, proportion of patients carrying combined *DPYD* risk variants (*c.1129-5923C>G/hapB3*, *c.1679T>G*, *c.1905+1G>A*, *c.2846A>T*) in association with fluoropyrimidine-related severity of toxicity in a cohort of 500 patients (Froehlich *et al.*, 2015); right, the cumulative incidence of grade 3+ fluoropyrimidine-related

toxicities was analyzed in 442 patients genotyped for the *DPYD* variants (*2A, *13, c.2846A>T (p.D949V), c.1236G>A (rs56038477, proxy for *HapB3* (c.1129-5923C>G)). The incidence was estimated as 1-Kaplan-Meier survival estimate, and log-rank test was used to compare groups. Grade 3+ toxicities occurred earlier and more frequent in reactive *DPYD* carriers in comparison to pretreatment carriers and wild-type patients ($P < .001$) (Nguyen *et al.*, 2024). **D.** *UGT1A1* and irinotecan. Left, hepatic UDP-glucuronosyl-transferase 1A1 protein content (n=145) and *UGT1A1**28 polymorphism (Riedmaier *et al.*, 2010); middle, haplotypes harboring either *6 or *28 (*6/*1, *6/*60, *28/*1, *28/*60) alleles were associated with lower SN-38G/SN-38 area under the curve (AUC) ratios compared to patients without *6 or *28 (*1/*1, *60/*1, *60/*60) alleles. The two haplotypes *6 or *28 (*6/*6, *28/*28, *28/*6) had the lowest AUC ratio ($P < 0.0001$). An irinotecan dose of 100 mg/m² weekly or 150 mg/m² biweekly was used in 177 cancer patients (Minami *et al.*, 2007); right, *UGT1A1**28 genotype and association with high dose irinotecan (IRN)-related severe neutropenia in patients with colorectal cancer and various regimens (IFL: IRN 25 mg/m² + FU, FOLFOX: oxaliplatin + FU, IROX: oxaliplatin + IRN 200 mg/m²) (McLeod *et al.*, 2010). All figures are reproduced with publisher permission.

Figure 6. Cancer genomics. **A.** Number of mutations per megabase (Mb) per entity basket. The latter are groups of entities following a pragmatic metaclassification system. **B.** Sample numbers across entity baskets, showing strong contributions from rare cancers and some contributions from rare subtypes of common entities. **C.** Cumulative counts of different mutation types per gene across the DKFZ/NCT/DKTK MASTER cohort. Abbreviations: GIST, gastrointestinal stromal tumor; PNET, primitive neuroectodermal tumor; STS, soft-tissue sarcoma; NSCLC, non-small cell lung cancer; CUP, cancer of unknown primary. The entity basket “STS: other” contains various uncommon STS subtypes. SNV, single-nucleotide variant; indel, short (< 50 bp) insertion and deletion; amp, amplification; hdel: homozygous deletion.

Figure 1

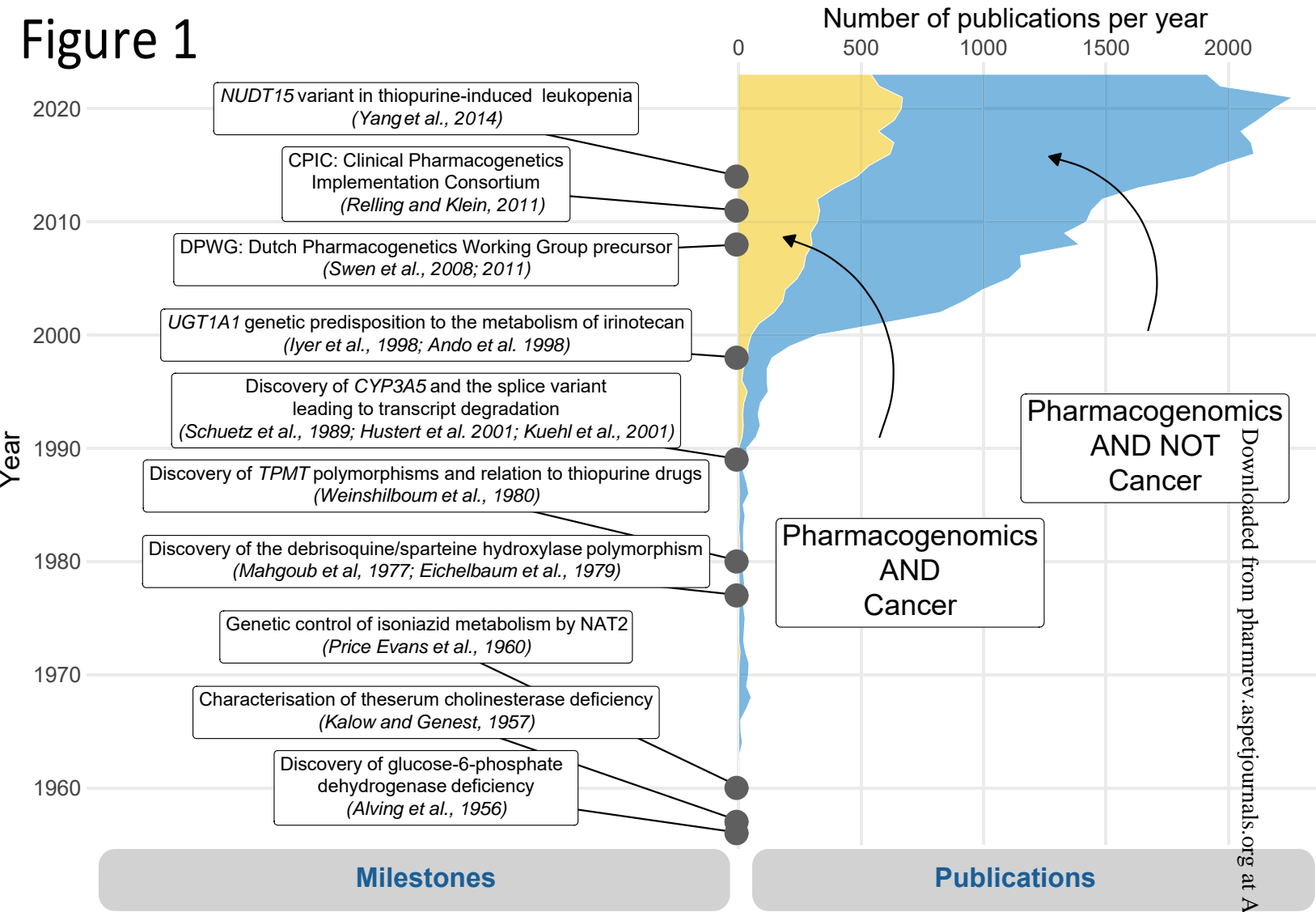
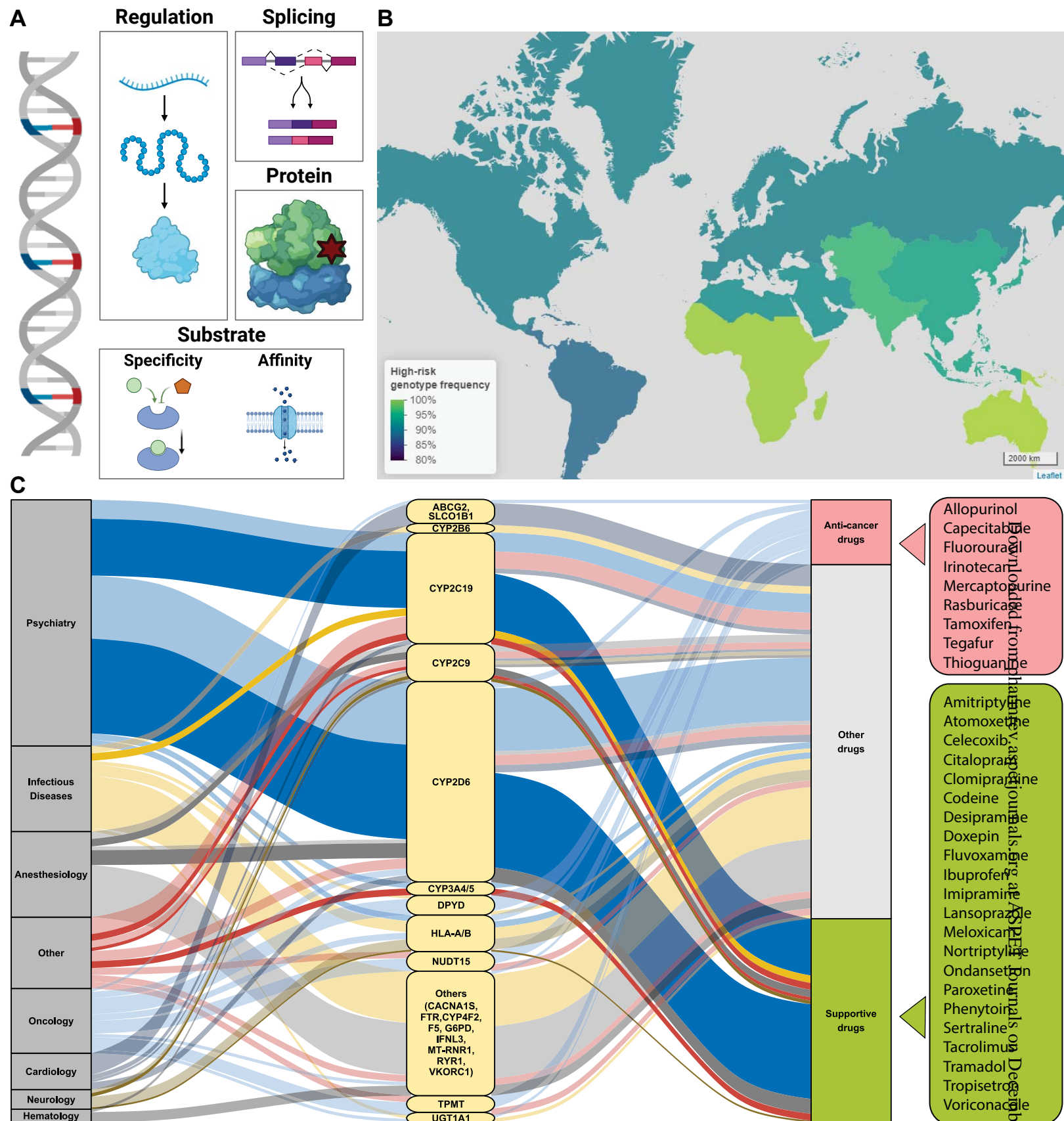


Figure 2



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Figure 3

Documented PGx biomarker for organ toxicities

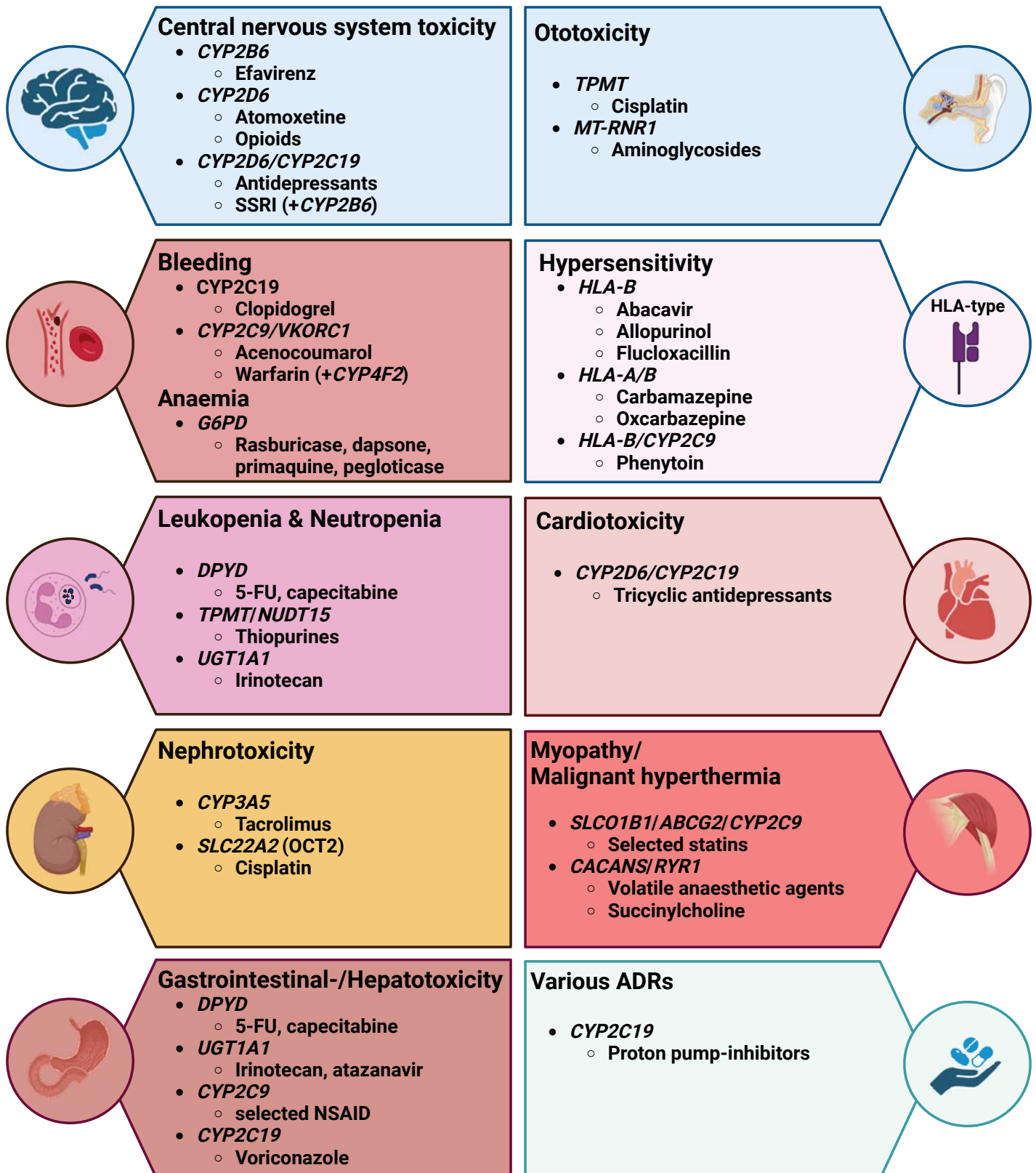
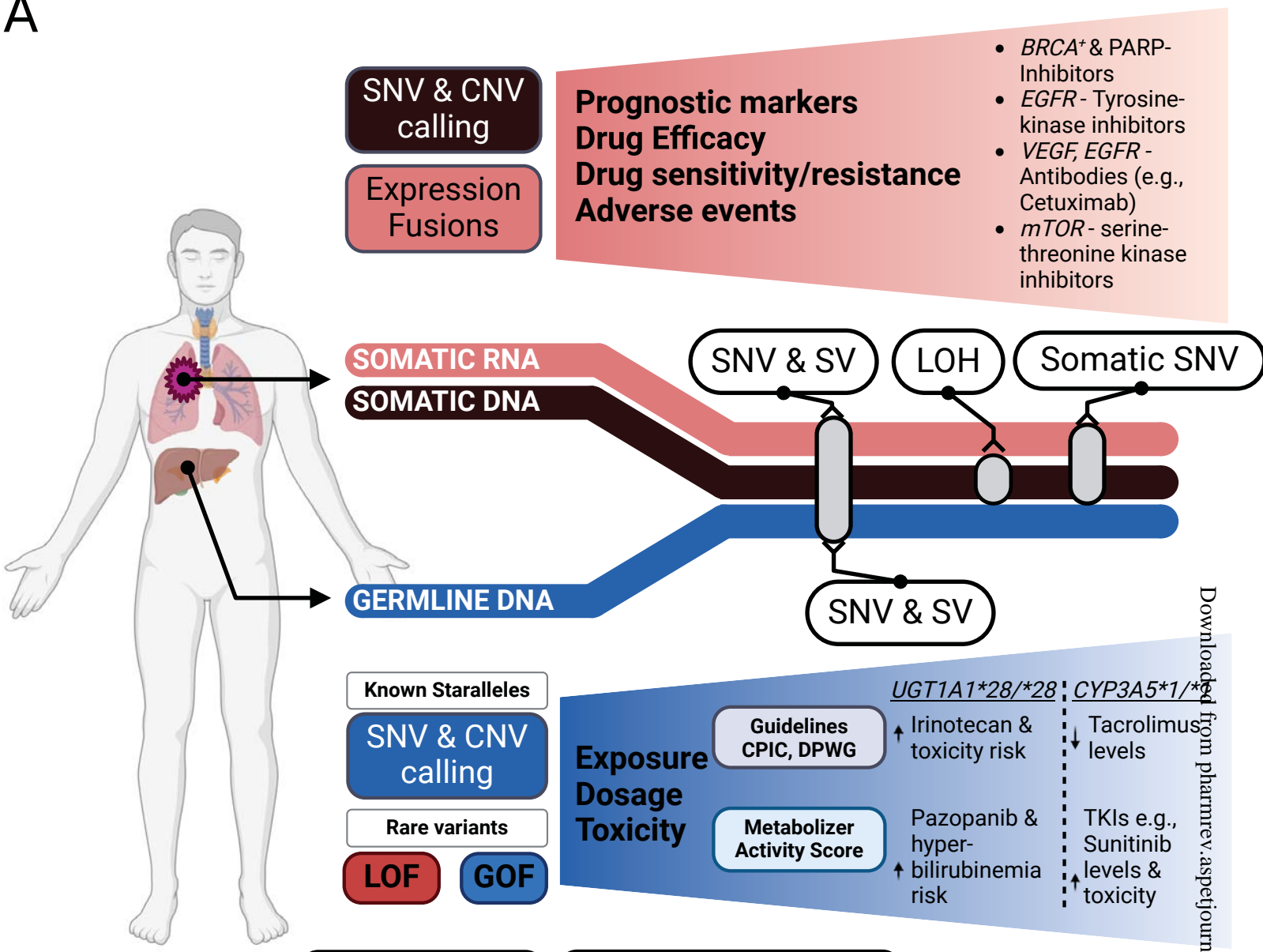


Figure 4

A



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B

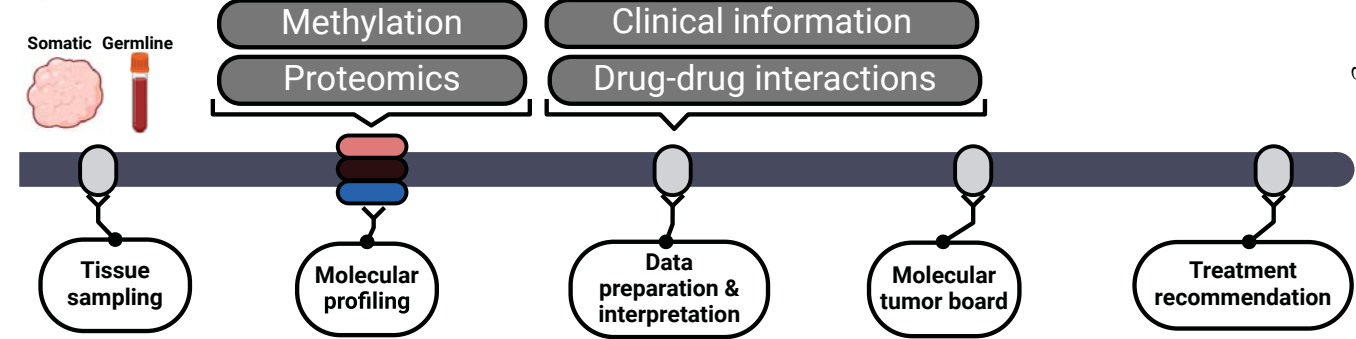


Figure 5

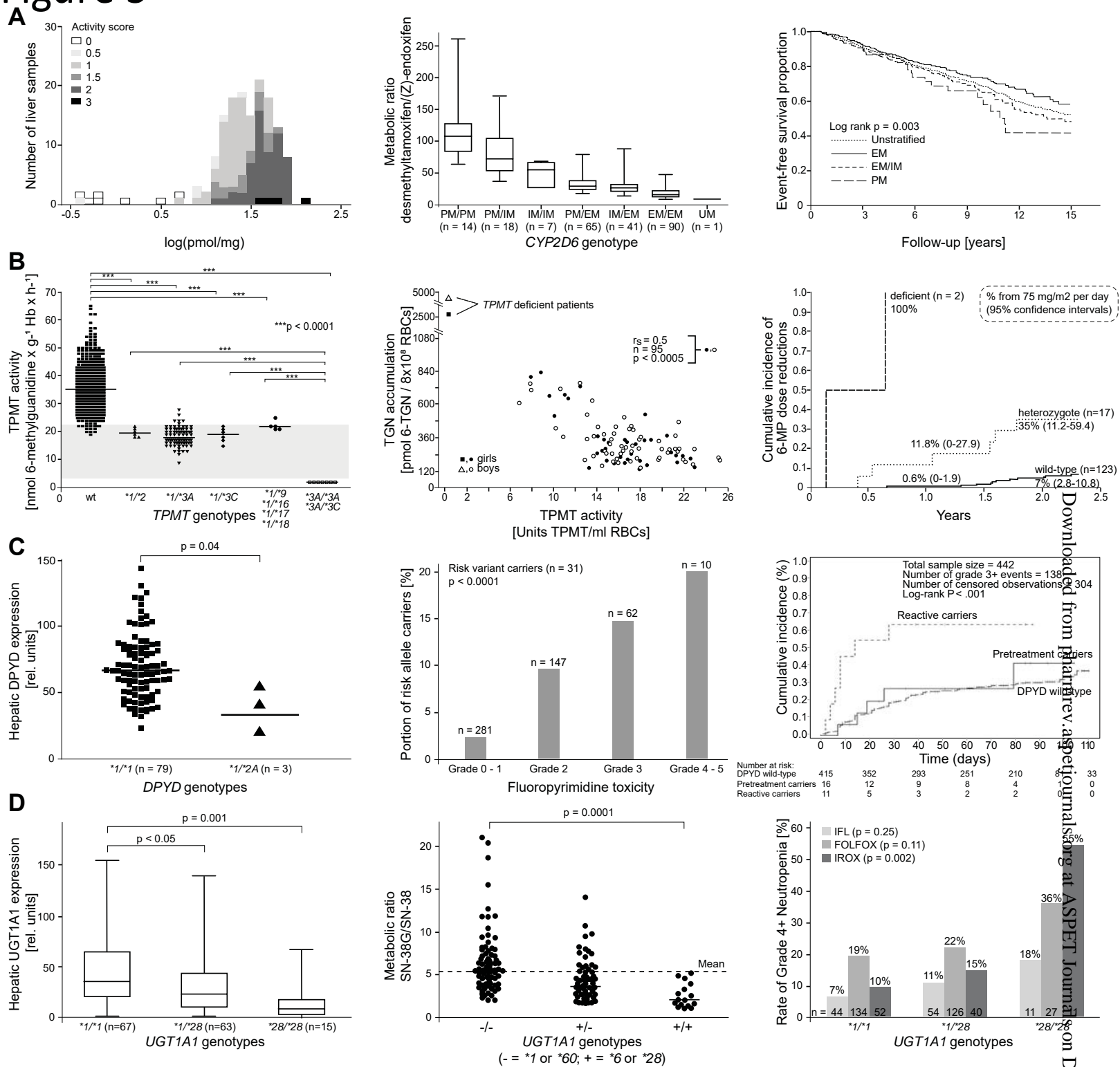


Figure 6

