

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

Roman Tremmel<sup>1#</sup>, Daniel Hübschmann<sup>2,3,4,5#</sup>, Elke Schaeffeler<sup>1,6</sup>, Sebastian Pirmann<sup>2</sup>, Stefan Fröhling<sup>3,7,8,9#</sup>, Matthias Schwab<sup>1,6,10,11,12\*#</sup>

<sup>1</sup> Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany and University of Tuebingen, Germany

<sup>2</sup> Computational Oncology Group, Molecular Precision Oncology Program, National Center for Tumor Diseases (NCT), NCT Heidelberg, a partnership between the German Cancer Research Center (DKFZ) and Heidelberg University Hospital, Heidelberg, Germany.

<sup>3</sup> German Cancer Consortium (DKTK), DKFZ, Core Center Heidelberg, Heidelberg, Germany.

<sup>4</sup> Innovation and Service Unit for Bioinformatics and Precision Medicine, DKFZ, Heidelberg, Germany.

<sup>5</sup> Pattern Recognition and Digital Medicine Group, Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), Heidelberg, Germany.

<sup>6</sup> Cluster of Excellence iFIT (EXC2180) "Image-Guided and Functionally Instructed Tumor Therapies," University of Tuebingen, Tuebingen, Germany.

<sup>7</sup> Division of Translational Medical Oncology, DKFZ, Heidelberg, Germany.

<sup>8</sup> NCT Heidelberg, a partnership between DKFZ and Heidelberg University Hospital, Heidelberg, Germany.

<sup>9</sup> Institute of Human Genetics, Heidelberg University, Heidelberg, Germany.

<sup>10</sup> Departments of Clinical Pharmacology, and Pharmacy and Biochemistry, University of Tuebingen, Tuebingen, Germany.

<sup>11</sup> DKTK, DKFZ, Partner Site Tuebingen, Tuebingen, Germany.

<sup>12</sup> NCT SouthWest, a partnership between DKFZ and University Hospital Tuebingen, Tuebingen, Germany

#Contributed equally

## Running title: Pharmacogenomics in cancer pharmacotherapy

### \*Corresponding author

Matthias Schwab

Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany

matthias.schwab@ikp-stuttgart.de

Auerbachstrasse 112,

70376 Stuttgart, Germany

Phone: +49 711 8101-3700

Fax: +49-711-85 92 95

### Manuscript statistics

Number of text pages:	91
Number of tables:	3
Number of figures:	6
Number of references:	359
Word count abstract:	216

### 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.

## **Table of Contents**

### **I. Introduction**

- A. Precision medicine and pharmacotherapy
- B. Prediction of drug response and adverse drug reactions
- C. Relevance of ADME processes
- D. The Human Genome and Pharmacogenomics

### **II. Germline Genome and cancer therapy**

- A. Genetic Variation in ADME genes
- B. Genotype-Phenotype correlation in selected ADME genes
- C. In silico prediction of functional consequences of genetic variation
- D. Validation of in silico functional predictions
- E. Implementation of pharmacogenomics
- F. Pharmacogenomics-guided supportive care
- G. Polygenic risk scores and prediction of drug response

### **III. Somatic variation and cancer therapy**

- A. Somatic mutations in cancer
- B. Targetability of somatic alterations
- C. Somatic alterations in pharmacogenes
- D. Risk prediction and drug treatment of cancer

### **IV. Integration of germline and somatic variation for drug therapy**

- A. Precision oncology
- B. Additional data layers and multi-omics integration
- C. Tumor heterogeneity and combination therapies
- D. Implementation of PGx in Precision Oncology

### **V. Concluding remarks**

### **VII. Acknowledgements**

### **VIII. Data availability**

### **IX. Author contribution**

### **X. References**

### **XI. Footnotes**

### **XII. Tables**

### **XIII. Figure legends**

## 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](http://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](http://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](http://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'](http://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](http://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 $\alpha$  (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](http://pharmvar.org/gene/CYP3A4), [pharmvar.org/gene/CYP3A5](http://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- $\alpha$  (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](http://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](http://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](http://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](https://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](https://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](https://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](http://cpicpgx.org); Dutch Pharmacogenetics Working Group, DPWG, [knmp.nl/dossiers/farmacogenetica/pharmacogenetics](http://knmp.nl/dossiers/farmacogenetica/pharmacogenetics); Canadian Pharmacogenomics Network for Drug Safety, CPNDS, [cpnds.ubc.ca](http://cpnds.ubc.ca); the French National Network of Pharmacogenetics, RNPGx, [pharmgkb.org/page/rnpgx](http://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](http://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](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](http://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](http://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](http://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](http://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](http://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  $\leq 10$  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/](https://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) (Agostinetto *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](https://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](https://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.

## VII. Acknowledgements

We thank Malgorzata Oles, Heidelberg for supporting the preparation of figures.

## 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.

## X. References

- Abdel-Kahaar E, Winter S, Tremmel R, Schaeffeler E, Olbricht CJ, Wieland E, Schwab M, Shipkova M, and Jaeger SU (2019) The Impact of CYP3A4\*22 on Tacrolimus Pharmacokinetics and Outcome in Clinical Practice at a Single Kidney Transplant Center. *Front Genet* **10**:871.
- Abdullah-Koolmees H, van Keulen AM, Nijenhuis M, and Deneer VHM (2020) Pharmacogenetics Guidelines: Overview and Comparison of the DPWG, CPIC, CPNDS, and RNPgX Guidelines. *Front Pharmacol* **11**:595219.
- Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, Smith-McCune K, Broaddus R, Lu KH, Chen J, Tran TV, Williams D, Iliev D, Jammulapati S, FitzGerald LM, Krivak T, DeLoia JA, Gutin A, Mills GB, and Lanchbury JS (2012) Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* **107**:1776–1782.

- Adiwidjaja J, Gross AS, Boddy AV, and McLachlan AJ (2022) Physiologically-based pharmacokinetic model predictions of inter-ethnic differences in imatinib pharmacokinetics and dosing regimens. *Br J Clin Pharmacol* **88**:1735–1750.
- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, and Sunyaev SR (2010) A method and server for predicting damaging missense mutations. *Nat Methods* **7**:248–249.
- Agarwal N, Azad AA, Carles J, Fay AP, Matsubara N, Heinrich D, Szczylik C, De Giorgi U, Young Joung J, Fong PCC, Voog E, Jones RJ, Shore ND, Dunshee C, Zschäbitz S, Oldenburg J, Lin X, Healy CG, Di Santo N, Zohren F, and Fizazi K (2023) Talazoparib plus enzalutamide in men with first-line metastatic castration-resistant prostate cancer (TALAPRO-2): a randomised, placebo-controlled, phase 3 trial. *The Lancet* **402**:291–303.
- Agostinetto E, Gligorov J, and Piccart M (2022) Systemic therapy for early-stage breast cancer: learning from the past to build the future. *Nat Rev Clin Oncol* **19**:763–774.
- Akhoundova D, and Rubin MA (2022) Clinical application of advanced multi-omics tumor profiling: Shaping precision oncology of the future. *Cancer Cell* **40**:920–938.
- Alam S, Doherty E, Ortega-Prieto P, Arizanova J, and Fets L (2023) Membrane transporters in cell physiology, cancer metabolism and drug response. *Dis Model Mech* **16**:dmm050404.
- Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, Boot A, Covington KR, Gordenin DA, Bergstrom EN, Islam SMA, Lopez-Bigas N, Klimczak LJ, McPherson JR, Morganella S, Sabarinathan R, Wheeler DA, Mustonen V, PCAWG Mutational Signatures Working Group, Alexandrov LB, Bergstrom EN, Boot A, Boutros P, Chan K, Covington KR, Fujimoto A, Getz G, Gordenin DA, Haradhvala NJ, Huang MN, Islam SMA, Kazanov M, Kim J, Klimczak LJ, Lopez-Bigas N, Lawrence M, Martincorena I, McPherson JR, Morganella S, Mustonen V, Nakagawa H, Tian Ng AW, Polak P, Prokopec S, Roberts SA, Rozen SG, Sabarinathan R, Saini N, Shibata T, Shiraishi Y, Stratton MR, Teh BT, Vázquez-García I, Wheeler DA, Wu Y, Yousif F, Yu W, Getz G, Rozen SG, Stratton MR, PCAWG Consortium, Aaltonen LA, Abascal F, Abeshouse A, Aburatani H, Adams DJ, Agrawal N, Ahn KS, Ahn S-M, Aikata H, Akbani R, Akdemir KC, Al-Ahmadie H, Al-Sedairy ST, Al-Shahrour F, Alawi M, Albert M, Aldape K, Alexandrov LB, Ally A, Alsop K, Alvarez EG, Amary F, Amin SB, Aminou B, Ammerpohl O, Anderson MJ, Ang Y, Antonello D, Anur P, Aparicio S, Appelbaum EL, Arai Y, Aretz A, Arihiro K, Ariizumi S, Armenia J, Arnould L, et al. (2020) The repertoire of mutational signatures in human cancer. *Nature* **578**:94–101.
- Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY, and Stratton MR (2015) A mutational signature in gastric cancer suggests therapeutic strategies. *Nat Commun* **6**:8683.
- Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale A-L, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörd JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Illicic T, Imbeaud S, Imielinski M, Jäger N, Jones DTW, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otín C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt ANJ, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Australian Pancreatic Cancer Genome Initiative, ICGC Breast Cancer Consortium, ICGC MMML-Seq Consortium, ICGC PedBrain, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M,

- Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, and Stratton MR (2013) Signatures of mutational processes in human cancer. *Nature* **500**:415–421.
- Alix-Panabières C, and Pantel K (2021) Liquid Biopsy. *Cancer Discov* **11**:858–873.
- Alving AS, Carson PE, Flanagan CL, and Ickes CE (1956) Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* **124**:484–485.
- Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, Klein TE, McLeod HL, Caudle KE, Diasio RB, and Schwab M (2018) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther* **103**:210–216.
- Ancien F, Pucci F, Godfroid M, and Rooman M (2018) Prediction and interpretation of deleterious coding variants in terms of protein structural stability. *Sci Rep* **8**:4480.
- Ando Y, Saka H, Asai G, Sugiura S, Shimokata K, and Kamataki T (1998) UGT1A1 genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. *Ann Oncol* **9**:845–847.
- Argelaguet R, Velten B, Arnol D, Dietrich S, Zenz T, Marioni JC, Buettner F, Huber W, and Stegle O (2018) Multi-Omics Factor Analysis—a framework for unsupervised integration of multi-omics data sets. *Mol Syst Biol* **14**:e8124.
- Bagger FO, Borgwardt L, Jespersen AS, Hansen AR, Bertelsen B, Kodama M, and Nielsen FC (2024) Whole genome sequencing in clinical practice. *BMC Med Genomics* **17**:39.
- Barker CIS, Groeneweg G, Maitland-van der Zee AH, Rieder MJ, Hawcutt DB, Hubbard TJ, Swen JJ, and Carleton BC (2022) Pharmacogenomic testing in paediatrics: Clinical implementation strategies. *Br J Clin Pharmacol* **88**:4297–4310.
- Beaubier N, Bontrager M, Huether R, Igartua C, Lau D, Tell R, Bobe AM, Bush S, Chang AL, Hoskinson DC, Khan AA, Kudalkar E, Leibowitz BD, Lozachmeur A, Michuda J, Parsons J, Perera JF, Salahudeen A, Shah KP, Taxter T, Zhu W, and White KP (2019) Integrated genomic profiling expands clinical options for patients with cancer. *Nat Biotechnol* **37**:1351–1360.
- Begicevic R-R, and Falasca M (2017) ABC Transporters in Cancer Stem Cells: Beyond Chemoresistance. *Int J Mol Sci* **18**:2362.
- Békés M, Langley DR, and Crews CM (2022) PROTAC targeted protein degraders: the past is prologue. *Nat Rev Drug Discov* **21**:181–200.
- Bell GC, Caudle KE, Whirl-Carrillo M, Gordon RJ, Hikino K, Prows CA, Gaedigk A, Agundez J, Sadhasivam S, Klein TE, and Schwab M (2017) Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 genotype and use of ondansetron and tropisetron. *Clin Pharmacol Ther* **102**:213–218.
- Belloni S, Caruso R, Cattani D, Mandelli G, Donizetti D, Mazzoleni B, and Tedeschi M (2022) Occurrence rate and risk factors for long-term central line-associated bloodstream infections in patients with cancer: A systematic review. *Worldviews Evid Based Nurs* **19**:100–111.

- Berger MF, and Mardis ER (2018) The emerging clinical relevance of genomics in cancer medicine. *Nat Rev Clin Oncol* **15**:353–365.
- Birdwell K, Decker B, Barbarino J, Peterson J, Stein C, Sadee W, Wang D, Vinks A, He Y, Swen J, Leeder J, Van Schaik R, Thummel K, Klein T, Caudle K, and MacPhee I (2015) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. *Clin Pharmacol Ther* **98**:19–24.
- Bosma PJ, Chowdhury JR, Bakker C, Gantla S, De Boer A, Oostra BA, Lindhout D, Tytgat GNJ, Jansen PLM, Elferink RPJO, and Chowdhury NR (1995) The Genetic Basis of the Reduced Expression of Bilirubin UDP-Glucuronosyltransferase 1 in Gilbert's Syndrome. *N Engl J Med* **333**:1171–1175.
- Brauch H, Schroth W, Goetz MP, Mürdter TE, Winter S, Ingle JN, Schwab M, and Eichelbaum M (2013) Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. *J Clin Oncol Off J Am Soc Clin Oncol* **31**:176–180.
- Bruckmueller H, and Cascorbi I (2021) Drug-Drug-Gene Interactions: A Call for Clinical Consideration. *Clin Pharmacol Ther* **110**:549–551.
- Brufsky A, Lenz L, Pudusseri A, Wright D, Patel KB, Clifford BT, O'Connor TL, Strain S, Kayali F, Ratzel S, Cummings S, Kronenwett R, and Slavin TP (2022) Adherence to EndoPredict test scores for extended endocrine therapy management in the prospective EndoPredict Extended Endocrine Trial (EXET). *J Clin Oncol* **40**:537–537.
- Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. *Nature* **490**:61–70.
- Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, and Stuart JM (2013) The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet* **45**:1113–1120.
- Cardoso F, Van'T Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, Pierga J-Y, Brain E, Causeret S, DeLorenzi M, Glas AM, Goulinopoulos V, Goulioti T, Knox S, Matos E, Meulemans B, Neijenhuis PA, Nitz U, Passalacqua R, Ravdin P, Rubio IT, Saghatchian M, Smilde TJ, Sotiriou C, Stork L, Straehle C, Thomas G, Thompson AM, Van Der Hoeven JM, Vuylsteke P, Bernardis R, Tryfonidis K, Rutgers E, and Piccart M (2016) 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* **375**:717–729.
- Carter H, Douville C, Stenson PD, Cooper DN, and Karchin R (2013) Identifying Mendelian disease genes with the Variant Effect Scoring Tool. *BMC Genomics* **14**:S3.
- Caspar SM, Schneider T, Meienberg J, and Matyas G (2020) Added Value of Clinical Sequencing: WGS-Based Profiling of Pharmacogenes. *Int J Mol Sci* **21**:2308.
- Caudle KE, Klein TE, Hoffman JM, Muller DJ, Whirl-Carrillo M, Gong L, McDonagh EM, Sangkuhl K, Thorn CF, Schwab M, Agundez JAG, Freimuth RR, Huser V, Lee MTM, Iwuchukwu OF, Crews KR, Scott SA, Wadelius M, Swen JJ, Tyndale RF, Stein CM, Roden D, Relling MV, Williams MS, and Johnson SG (2014) Incorporation of pharmacogenomics into routine clinical practice: the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline development process. *Curr Drug Metab* **15**:209–217.
- Caudle KE, Sangkuhl K, Whirl-Carrillo M, Swen JJ, Haidar CE, Klein TE, Gammal RS, Relling MV, Scott SA, Hertz DL, Guchelaar H, and Gaedigk A (2020) Standardizing

CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. *Clin Transl Sci* **13**:116–124.

Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, Seligmann J, De Baere T, Osterlund P, Yoshino T, and Martinelli E (2023) Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* **34**:10–32.

Chai X, Zeng S, and Xie W (2013) Nuclear receptors PXR and CAR: implications for drug metabolism regulation, pharmacogenomics and beyond. *Expert Opin Drug Metab Toxicol* **9**:253–266.

Chang MT, Asthana S, Gao SP, Lee BH, Chapman JS, Kandath C, Gao J, Socci ND, Solit DB, Olshen AB, Schultz N, and Taylor BS (2016) Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol* **34**:155–163.

Chen M, Neul C, Schaeffeler E, Frisch F, Winter S, Schwab M, Koepsell H, Hu S, Laufer S, Baker SD, Sparreboom A, and Nies AT (2020) Sorafenib Activity and Disposition in Liver Cancer Does Not Depend on Organic Cation Transporter 1. *Clin Pharmacol Ther* **107**:227–237.

Chen T (2010) Overcoming drug resistance by regulating nuclear receptors. *Adv Drug Deliv Rev* **62**:1257–1264.

Chen Y, Tang Y, Guo C, Wang J, Boral D, and Nie D (2012) Nuclear receptors in the multidrug resistance through the regulation of drug-metabolizing enzymes and drug transporters. *Biochem Pharmacol* **83**:1112–1126.

Cheng J, Novati G, Pan J, Bycroft C, Žemgulytė A, Applebaum T, Pritzel A, Wong LH, Zielinski M, Sargeant T, Schneider RG, Senior AW, Jumper J, Hassabis D, Kohli P, and Avsec Ž (2023) Accurate proteome-wide missense variant effect prediction with AlphaMissense. *Science* **381**:eadg7492.

Chiasson M, Dunham MJ, Rettie AE, and Fowler DM (2019) Applying Multiplex Assays to Understand Variation in Pharmacogenes. *Clin Pharmacol Ther* **106**:290–294.

Choi Y, Sims GE, Murphy S, Miller JR, and Chan AP (2012) Predicting the Functional Effect of Amino Acid Substitutions and Indels. *PLoS ONE* **7**:e46688.

Chun S, and Fay JC (2009) Identification of deleterious mutations within three human genomes. *Genome Res* **19**:1553–1561.

Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M, Okamoto A, Moore KN, Efrat Ben-Baruch N, Werner TL, Cloven NG, Oaknin A, DiSilvestro PA, Morgan MA, Nam J-H, Leath CA, Nicum S, Hagemann AR, Littell RD, Cella D, Baron-Hay S, Garcia-Donas J, Mizuno M, Bell-McGuinn K, Sullivan DM, Bach BA, Bhattacharya S, Ratajczak CK, Ansell PJ, Dinh MH, Aghajanian C, and Bookman MA (2019) Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer. *N Engl J Med* **381**:2403–2415.

Colucci G, Gebbia V, Paoletti G, Giuliani F, Caruso M, Gebbia N, Carteni G, Agostara B, Pezzella G, Manzione L, Borsellino N, Misino A, Romito S, Durini E, Cordio S, Di Seri M, Lopez M, and Maiello E (2005) Phase III Randomized Trial of FOLFIRI Versus

FOLFOX4 in the Treatment of Advanced Colorectal Cancer: A Multicenter Study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol* **23**:4866–4875.

Cooper-DeHoff RM, Niemi M, Ramsey LB, Luzum JA, Tarkiainen EK, Straka RJ, Gong L, Tuteja S, Wilke RA, Wadelius M, Larson EA, Roden DM, Klein TE, Yee SW, Krauss RM, Turner RM, Palaniappan L, Gaedigk A, Giacomini KM, Caudle KE, and Voora D (2022) The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1, ABCG2, and CYP2C9 genotypes and Statin-Associated Musculoskeletal Symptoms. *Clin Pharmacol Ther* **111**:1007–1021.

Couch FJ, Nathanson KL, and Offit K (2014) Two Decades After BRCA: Setting Paradigms in Personalized Cancer Care and Prevention. *Science* **343**:1466–1470.

Crews KR, Monte AA, Huddart R, Caudle KE, Kharasch ED, Gaedigk A, Dunnenberger HM, Leeder JS, Callaghan JT, Samer CF, Klein TE, Haidar CE, van Driest SL, Ruano G, Sangkuhl K, Cavallari LH, Müller DJ, Prows CA, Nagy M, Somogyi AA, and Skaar TC (2021) Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6, OPRM1, and COMT Genotypes and Select Opioid Therapy. *Clin Pharmacol Ther* **110**:888–896.

Cui J, Li G, Yin J, Li L, Tan Y, Wei H, Liu B, Deng L, Tang J, Chen Y, and Yi L (2020) GSTP1 and cancer: Expression, methylation, polymorphisms and signaling (Review). *Int J Oncol*, doi: 10.3892/ijo.2020.4979.

Danciu I, Cowan JD, Basford M, Wang X, Saip A, Osgood S, Shirey-Rice J, Kirby J, and Harris PA (2014) Secondary use of clinical data: The Vanderbilt approach. *J Biomed Inform* **52**:28–35.

Darmofal M, Suman S, Atwal G, Toomey M, Chen J-F, Chang JC, Vakiani E, Varghese AM, Balakrishnan Rema A, Syed A, Schultz N, Berger MF, and Morris Q (2024) Deep-Learning Model for Tumor-Type Prediction Using Targeted Clinical Genomic Sequencing Data. *Cancer Discov* OF1–OF18.

Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, and Batzoglou S (2010) Identifying a High Fraction of the Human Genome to be under Selective Constraint Using GERP++. *PLoS Comput Biol* **6**:e1001025.

De Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, Chi KN, Sartor O, Agarwal N, Olmos D, Thiery-Vuillemin A, Twardowski P, Mehra N, Goessl C, Kang J, Burgents J, Wu W, Kohlmann A, Adelman CA, and Hussain M (2020) Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N Engl J Med* **382**:2091–2102.

Dewachter L, Brooks AN, Noon K, Cialek C, Clark-ElSayed A, Schalck T, Krishnamurthy N, Versées W, Vranken W, and Michiels J (2023) Deep mutational scanning of essential bacterial proteins can guide antibiotic development. *Nat Commun* **14**:241.

Dilli Batcha J, Raju A, Matcha S, Raj S. E, Udupa K, Gota V, and Mallayasamy S (2022) Factors Influencing Pharmacokinetics of Tamoxifen in Breast Cancer Patients: A Systematic Review of Population Pharmacokinetic Models. *Biology* **12**:51.

DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, Konopleva M, Döhner H, Letai A, Fenaux P, Koller E, Havelange V, Leber B, Esteve J, Wang J, Pejsa V, Hájek R, Porkka K, Illés Á, Lavie D, Lemoli RM, Yamamoto K, Yoon S-S, Jang J-H, Yeh S-P, Turgut M, Hong W-J, Zhou Y, Potluri J, and Pratz KW (2020) Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. *N Engl J Med* **383**:617–629.



- Dong C, Wei P, Jian X, Gibbs R, Boerwinkle E, Wang K, and Liu X (2015) Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. *Hum Mol Genet* **24**:2125–2137.
- Duarte JD, and Cavallari LH (2021) Pharmacogenetics to guide cardiovascular drug therapy. *Nat Rev Cardiol* **18**:649–665.
- Eichelbaum M, Spannbrucker N, Steincke B, and Dengler HJ (1979) Defective N-oxidation of sparteine in man. *Eur J Clin Pharmacol* **16**, *Eur J Clin Pharmacol*.
- Emami Riedmaier A, Fisel P, Nies AT, Schaeffeler E, and Schwab M (2013) Metformin and cancer: from the old medicine cabinet to pharmacological pitfalls and prospects. *Trends Pharmacol Sci* **34**:126–135.
- Esserman LJ, Yau C, Thompson CK, Van 'T Veer LJ, Borowsky AD, Hoadley KA, Tobin NP, Nordenskjöld B, Fornander T, Stål O, Benz CC, and Lindström LS (2017) Use of Molecular Tools to Identify Patients With Indolent Breast Cancers With Ultralow Risk Over 2 Decades. *JAMA Oncol* **3**:1503.
- Evans DA, Manley KA, and McKUSICK VA (1960) Genetic control of isoniazid metabolism in man. *Br Med J* **2**:485–491.
- Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, Dietze O, Greil R, Jelen A, Sevela P, Freibauer C, Müller V, Jänicke F, Schmidt M, Kölbl H, Rody A, Kaufmann M, Schroth W, Brauch H, Schwab M, Fritz P, Weber KE, Feder IS, Hennig G, Kronenwett R, Gehrman M, and Gnant M (2011) A New Molecular Predictor of Distant Recurrence in ER-Positive, HER2-Negative Breast Cancer Adds Independent Information to Conventional Clinical Risk Factors. *Clin Cancer Res* **17**:6012–6020.
- Fisel P, Nies AT, Schaeffeler E, and Schwab M (2017) The importance of drug transporter characterization to precision medicine. *Expert Opin Drug Metab Toxicol* **13**:361–365.
- Flaherty KT, Gray R, Chen A, Li S, Patton D, Hamilton SR, Williams PM, Mitchell EP, Iafrate AJ, Sklar J, Harris LN, McShane LM, Rubinstein LV, Sims DJ, Routbort M, Coffey B, Fu T, Zwiebel JA, Little RF, Marinucci D, Catalano R, Magnan R, Kibbe W, Weil C, Tricoli JV, Alexander B, Kumar S, Schwartz GK, Meric-Bernstam F, Lih C-J, McCaskill-Stevens W, Caimi P, Takebe N, Datta V, Arteaga CL, Abrams JS, Comis R, O'Dwyer PJ, Conley BA, and for the NCI-MATCH Team (2020) The Molecular Analysis for Therapy Choice (NCI-MATCH) Trial: Lessons for Genomic Trial Design. *JNCI J Natl Cancer Inst* **112**:1021–1029.
- Frankell AM, Dietzen M, Al Bakir M, Lim EL, Karasaki T, Ward S, Veeriah S, Colliver E, Huebner A, Bunkum A, Hill MS, Grigoriadis K, Moore DA, Black JRM, Liu WK, Thol K, Pich O, Watkins TBK, Naceur-Lombardelli C, Cook DE, Salgado R, Wilson GA, Bailey C, Angelova M, Bentham R, Martínez-Ruiz C, Abbosh C, Nicholson AG, Le Quesne J, Biswas D, Rosenthal R, Puttick C, Hessey S, Lee C, Prymas P, Toncheva A, Smith J, Xing W, Nicod J, Price G, Kerr KM, Naidu B, Middleton G, Blyth KG, Fennell DA, Forster MD, Lee SM, Falzon M, Hewish M, Shackcloth MJ, Lim E, Benafif S, Russell P, Boleti E, Krebs MG, Lester JF, Papadatos-Pastos D, Ahmad T, Thakrar RM, Lawrence D, Navani N, Janes SM, Dive C, Blackhall FH, Summers Y, Cave J, Marafioti T, Herrero J, Quezada SA, Peggs KS, Schwarz RF, Van Loo P, Miedema DM, Birbak NJ, Hiley CT, Hackshaw A, Zaccaria S, TRACERx Consortium, Jamal-Hanjani M, McGranahan N, and Swanton C (2023) The evolution of lung cancer and impact of subclonal selection in TRACERx. *Nature* **616**:525–533.

- Frederiksen T, Areberg J, Schmidt E, Stage TB, and Brøsen K (2023) Does ethnicity impact CYP2D6 genotype–phenotype relationships? *Clin Transl Sci* **16**:1012–1020.
- Frigo DE, Bondesson M, and Williams C (2021) Nuclear receptors: from molecular mechanisms to therapeutics. *Essays Biochem* **65**:847–856.
- Froehlich TK, Amstutz U, Aebi S, Joerger M, and Largiadèr CR (2015) Clinical importance of risk variants in the dihydropyrimidine dehydrogenase gene for the prediction of early-onset fluoropyrimidine toxicity. *Int J Cancer* **136**:730–739.
- Fukami T, Yokoi T, and Nakajima M (2022) Non-P450 Drug-Metabolizing Enzymes: Contribution to Drug Disposition, Toxicity, and Development. *Annu Rev Pharmacol Toxicol* **62**:405–425.
- Fuselli S (2019) Beyond drugs: the evolution of genes involved in human response to medications. *Proc Biol Sci* **286**:20191716.
- Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, and Leeder JS (2008) The CYP2D6 activity score. *Clin Pharmacol Ther* **83**:234–242.
- Galetin A, Brouwer KLR, Tweedie D, Yoshida K, Sjöstedt N, Aleksunes L, Chu X, Evers R, Hafey MJ, Lai Y, Matsson P, Riselli A, Shen H, Sparreboom A, Varma MVS, Yang J, Yang X, Yee SW, Zamek-Gliszczynski MJ, Zhang L, and Giacomini KM (2024) Membrane transporters in drug development and as determinants of precision medicine. *Nat Rev Drug Discov* **23**:255–280.
- Gammal RS, Pirmohamed M, Somogyi AA, Morris SA, Formea CM, Elchynski AL, Oshikoya KA, McLeod HL, Haidar CE, Whirl-Carrillo M, Klein TE, Caudle KE, and Relling MV (2023) Expanded Clinical Pharmacogenetics Implementation Consortium Guideline for Medication Use in the Context of *G6PD* Genotype. *Clin Pharmacol Ther* **113**:973–985.
- Garber M, Guttman M, Clamp M, Zody MC, Friedman N, and Xie X (2009) Identifying novel constrained elements by exploiting biased substitution patterns. *Bioinformatics* **25**:i54–i62.
- Geck RC, Boyle G, Amorosi CJ, Fowler DM, and Dunham MJ (2022) Measuring Pharmacogene Variant Function at Scale Using Multiplexed Assays. *Annu Rev Pharmacol Toxicol* **62**:531–550.
- Gerstung M, Jolly C, Leshchiner I, Dentro SC, Gonzalez S, Rosebrock D, Mitchell TJ, Rubanova Y, Anur P, Yu K, Tarabichi M, Deshwar A, Wintersinger J, Kleinheinz K, Vázquez-García I, Haase K, Jerman L, Sengupta S, Macintyre G, Malikic S, Donmez N, Livitz DG, Cmero M, Demeulemeester J, Schumacher S, Fan Y, Yao X, Lee J, Schlesner M, Boutros PC, Bowtell DD, Zhu H, Getz G, Imielinski M, Beroukhim R, Sahinalp SC, Ji Y, Peifer M, Markowitz F, Mustonen V, Yuan K, Wang W, Morris QD, PCAWG Evolution & Heterogeneity Working Group, Dentro SC, Leshchiner I, Gerstung M, Jolly C, Haase K, Tarabichi M, Wintersinger J, Deshwar AG, Yu K, Gonzalez S, Rubanova Y, Macintyre G, Adams DJ, Anur P, Beroukhim R, Boutros PC, Bowtell DD, Campbell PJ, Cao S, Christie EL, Cmero M, Cun Y, Dawson KJ, Demeulemeester J, Donmez N, Drews RM, Eils R, Fan Y, Fittall M, Garsed DW, Getz G, Ha G, Imielinski M, Jerman L, Ji Y, Kleinheinz K, Lee J, Lee-Six H, Livitz DG, Malikic S, Markowitz F, Martincorena I, Mitchell TJ, Mustonen V, Oesper L, Peifer M, Peto M, Raphael BJ, Rosebrock D, Sahinalp SC, Salcedo A, Schlesner M, Schumacher S, Sengupta S, et al. (2020) The evolutionary history of 2,658 cancers. *Nature* **578**:122–128.

- Giessler KM, Kleinheinz K, Huebschmann D, Balasubramanian GP, Dubash TD, Dieter SM, Siegl C, Herbst F, Weber S, Hoffmann CM, Fronza R, Buchhalter I, Paramasivam N, Eils R, Schmidt M, Von Kalle C, Schneider M, Ulrich A, Scholl C, Fröhling S, Weichert W, Brors B, Schlesner M, Ball CR, and Glimm H (2017) Genetic subclone architecture of tumor clone-initiating cells in colorectal cancer. *J Exp Med* **214**:2073–2088.
- Gligorijević V, and Pržulj N (2015) Methods for biological data integration: perspectives and challenges. *J R Soc Interface* **12**:20150571.
- Goetz MP, Sangkuhl K, Guchelaar H-J, Schwab M, Province M, Whirl-Carrillo M, Symmans WF, McLeod HL, Ratain MJ, Zembutsu H, Gaedigk A, van Schaik RH, Ingle JN, Caudle KE, and Klein TE (2018) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. *Clin Pharmacol Ther* **103**:770–777.
- Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park J-O, Hochhauser D, Arnold D, Oh D-Y, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, McGuinness D, Cui KY, Schlienger K, Locker GY, and Kindler HL (2019) Maintenance Olaparib for Germline *BRCA* -Mutated Metastatic Pancreatic Cancer. *N Engl J Med* **381**:317–327.
- González-Martín A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, McCormick C, Lorusso D, Hoskins P, Freyer G, Baumann K, Jardon K, Redondo A, Moore RG, Vulsteke C, O'Cearbhaill RE, Lund B, Backes F, Barretina-Ginesta P, Haggerty AF, Rubio-Pérez MJ, Shahin MS, Mangili G, Bradley WH, Bruchim I, Sun K, Malinowska IA, Li Y, Gupta D, and Monk BJ (2019) Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* **381**:2391–2402.
- Griese E-U, Ilett KF, Kitteringham NR, Eichelbaum M, Powell H, Spargo RM, LeSouef PN, Musk AW, and Minchin RF (2001) Allele and genotype frequencies of polymorphic cytochromes P4502D6, 2C19 and 2E1 in Aborigines from Western Australia: *Pharmacogenetics* **11**:69–76.
- Griese EU, Zanger UM, Brudermanns U, Gaedigk A, Mikus G, Mörike K, Stüven T, and Eichelbaum M (1998) Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics* **8**:15–26.
- Grigoriadis K, Huebner A, Bunkum A, Colliver E, Frankell AM, Hill MS, Thol K, Birkbak NJ, Swanton C, Zaccaria S, and McGranahan N (2024) CONIPHER: a computational framework for scalable phylogenetic reconstruction with error correction. *Nat Protoc* **19**:159–183.
- Gröschel S, Hübschmann D, Raimondi F, Horak P, Warsow G, Fröhlich M, Klink B, Geldon L, Hutter B, Kleinheinz K, Bonekamp D, Marschal O, Chudasama P, Mika J, Groth M, Uhrig S, Krämer S, Heining C, Heilig CE, Richter D, Reisinger E, Pfützte K, Eils R, Wolf S, Von Kalle C, Brandts C, Scholl C, Weichert W, Richter S, Bauer S, Penzel R, Schröck E, Stenzinger A, Schlenk RF, Brors B, Russell RB, Glimm H, Schlesner M, and Fröhling S (2019) Defective homologous recombination DNA repair as therapeutic target in advanced chordoma. *Nat Commun* **10**:1635.
- Grussy K, Łaska M, Moczurad W, Król-Kulikowska M, and Ścisłowska M (2023) The importance of polymorphisms in the genes encoding glutathione S-transferase isoenzymes in development of selected cancers and cardiovascular diseases. *Mol Biol Rep* **50**:9649–9661.
- Hall PS, Smith A, Hulme C, Vargas-Palacios A, Makris A, Hughes-Davies L, Dunn JA, Bartlett JMS, Cameron DA, Marshall A, Campbell A, Macpherson IR, Dan Rea,

- Francis A, Earl H, Morgan A, Stein RC, and McCabe C (2017) Value of Information Analysis of Multiparameter Tests for Chemotherapy in Early Breast Cancer: The OPTIMA Prelim Trial. *Value Health* **20**:1311–1318.
- Hanahan D, and Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**:646–674.
- Hanahan D, and Weinberg RA (2000) The hallmarks of cancer. *Cell* **100**:57–70.
- Hanrahan AJ, Chen Z, Rosen N, and Solit DB (2024) BRAF - a tumour-agnostic drug target with lineage-specific dependencies. *Nat Rev Clin Oncol* **21**:224–247.
- Harada G, Yang S-R, Cocco E, and Drilon A (2023) Rare molecular subtypes of lung cancer. *Nat Rev Clin Oncol* **20**:229–249.
- Hawes EM, Claxton DP, Oeser JK, and O'Brien RM (2024) Identification of structural motifs critical for human G6PC2 function informed by sequence analysis and an AlphaFold2-predicted model. *Biosci Rep* **44**:BSR20231851.
- Hecht M, Bromberg Y, and Rost B (2015) Better prediction of functional effects for sequence variants. *BMC Genomics* **16**:S1.
- Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, Creemers G-J, Baars A, Dezentjé VO, Imholz ALT, Jeurissen FJF, Portielje JEA, Jansen RLH, Hamberg P, Ten Tije AJ, Droogendijk HJ, Koopman M, Nieboer P, van de Poel MHW, Mandigers CMPW, Rosing H, Beijnen JH, Werkhoven E van, van Kuilenburg ABP, van Schaik RHN, Mathijssen RHJ, Swen JJ, Gelderblom H, Cats A, Guchelaar H-J, and Schellens JHM (2018) DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* **19**:1459–1467.
- Henry NL, Somerfield MR, Dayao Z, Elias A, Kalinsky K, McShane LM, Moy B, Park BH, Shanahan KM, Sharma P, Shatsky R, Stringer-Reasor E, Telli M, Turner NC, and DeMichele A (2022) Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update. *J Clin Oncol* **40**:3205–3221.
- Heo YJ, Hwa C, Lee G-H, Park J-M, and An J-Y (2021) Integrative Multi-Omics Approaches in Cancer Research: From Biological Networks to Clinical Subtypes. *Mol Cells* **44**:433–443.
- Hertz DL, and McLeod HL (2016) Integrated patient and tumor genetic testing for individualized cancer therapy. *Clin Pharmacol Ther* **99**:143–146.
- Hertz DL, and McLeod HL (2013) Use of pharmacogenetics for predicting cancer prognosis and treatment exposure, response and toxicity. *J Hum Genet* **58**:346–352.
- Hertz DL, and Rae J (2015) Pharmacogenetics of cancer drugs. *Annu Rev Med* **66**:65–81.
- Hlaváč V, Holý P, and Souček P (2020) Pharmacogenomics to Predict Tumor Therapy Response: A Focus on ATP-Binding Cassette Transporters and Cytochromes P450. *J Pers Med* **10**:108.
- Hoffman J, Tan H, Sandoval-Cooper C, De Villiers K, and Reed SM (2024) GTExome: Modeling commonly expressed missense mutations in the human genome. *PLoS ONE* **19**:e0303604.

- Hofman J, Vagiannis D, Chen S, and Guo L (2021) Roles of CYP3A4, CYP3A5 and CYP2C8 drug-metabolizing enzymes in cellular cytostatic resistance. *Chem Biol Interact* **340**:109448.
- Horak P, and Fröhling S (2024) Measuring Progress in Precision Oncology. *Cancer Discov* **14**:18–19.
- Horak P, Fröhling S, and Glimm H (2016) Integrating next-generation sequencing into clinical oncology: strategies, promises and pitfalls. *ESMO Open* **1**:e000094.
- Horak P, Heining C, Kreutzfeldt S, Hutter B, Mock A, Hülle J, Fröhlich M, Uhrig S, Jahn A, Rump A, Geldon L, Möhrmann L, Hanf D, Teleanu V, Heilig CE, Lipka DB, Allgäuer M, Ruhnke L, Laßmann A, Endris V, Neumann O, Penzel R, Beck K, Richter D, Winter U, Wolf S, Pfütze K, Georg C, Meißburger B, Buchhalter I, Augustin M, Aulitzky WE, Hohenberger P, Kroiss M, Schirmacher P, Schlenk RF, Keilholz U, Klauschen F, Folprecht G, Bauer S, Siveke JT, Brandts CH, Kindler T, Boerries M, Illert AL, von Bubnoff N, Jost PJ, Spiekermann K, Bitzer M, Schulze-Osthoff K, von Kalle C, Klink B, Brors B, Stenzinger A, Schröck E, Hübschmann D, Weichert W, Glimm H, and Fröhling S (2021) Comprehensive Genomic and Transcriptomic Analysis for Guiding Therapeutic Decisions in Patients with Rare Cancers. *Cancer Discov* **11**:2780–2795.
- Hu DG, Mackenzie PI, Nair PC, McKinnon RA, and Meech R (2020) The Expression Profiles of ADME Genes in Human Cancers and Their Associations with Clinical Outcomes. *Cancers* **12**:3369.
- Hu J, Jarusiewicz J, Du G, Nishiguchi G, Yoshimura S, Panetta JC, Li Z, Min J, Yang L, Chepyala D, Actis M, Reyes N, Smart B, Pui C-H, Teachey DT, Rankovic Z, and Yang JJ (2022) Preclinical evaluation of proteolytic targeting of LCK as a therapeutic approach in T cell acute lymphoblastic leukemia. *Sci Transl Med* **14**:eabo5228.
- Huebner T, Steffens M, and Scholl C (2023) Current status of the analytical validation of next generation sequencing applications for pharmacogenetic profiling. *Mol Biol Rep* **50**:9587–9599.
- Hulshof EC, Deenen MJ, Guchelaar H-J, and Gelderblom H (2020) Pre-therapeutic UGT1A1 genotyping to reduce the risk of irinotecan-induced severe toxicity: Ready for prime time. *Eur J Cancer* **141**:9–20.
- Hustert E, Haberl M, Burk O, Wolbold R, He Y-Q, Klein K, Nuessler AC, Neuhaus P, Klattig J, Eiselt R, Koch I, Zibat A, Brockmüller J, Halpert JR, Zanger UM, and Wojnowski L (2001) The genetic determinants of the CYP3A5 polymorphism: *Pharmacogenetics* **11**:773–779.
- Hutchcraft ML, Lin N, Zhang S, Sears C, Zacholski K, Belcher EA, Durbin EB, Villano JL, Cavnar MJ, Arnold SM, Ueland FR, and Kolesar JM (2021) Real-World Evaluation of Universal Germline Screening for Cancer Treatment-Relevant Pharmacogenes. *Cancers* **13**:4524.
- Hwang S, Lee Y, Jang Y, Cho J, Yoon S, and Chung J (2024) Comprehensive Evaluation of OATP - and BCRP -Mediated Drug–Drug Interactions of Methotrexate Using Physiologically-Based Pharmacokinetic Modeling. *Clin Pharmacol Ther* **cpt.3329**.
- Hyman DM, Puzanov I, Subbiah V, Faris JE, Chau I, Blay J-Y, Wolf J, Rajc NS, Diamond EL, Hollebecque A, Gervais R, Elez-Fernandez ME, Italiano A, Hofheinz R-D, Hidalgo M, Chan E, Schuler M, Lasserre SF, Makrutzki M, Sirzen F, Veronese ML, Taberero J,

- and Baselga J (2015) Vemurafenib in Multiple Nonmelanoma Cancers with *BRAF* V600 Mutations. *N Engl J Med* **373**:726–736.
- Incze E, Mangó K, Fekete F, Kiss ÁF, Póti Á, Harkó T, Moldvay J, Szüts D, and Monostory K (2023) Potential Association of Cytochrome P450 Copy Number Alteration in Tumour with Chemotherapy Resistance in Lung Adenocarcinoma Patients. *Int J Mol Sci* **24**:13380.
- Ingelman-Sundberg M, and Lauschke VM (2020) Can CYP Inhibition Overcome Chemotherapy Resistance? *Trends Pharmacol Sci* **41**:503–506.
- Ingelman-Sundberg M, Mkrtchian S, Zhou Y, and Lauschke VM (2018) Integrating rare genetic variants into pharmacogenetic drug response predictions. *Hum Genomics* **12**:26.
- Innocenti F, Mills SC, Sanoff H, Ciccolini J, Lenz H-J, and Milano G (2020) All You Need to Know About DPYD Genetic Testing for Patients Treated With Fluorouracil and Capecitabine: A Practitioner-Friendly Guide. *JCO Oncol Pract* **16**:793–798.
- International Human Genome Sequencing Consortium (2004) Finishing the euchromatic sequence of the human genome. *Nature* **431**:931–945.
- Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, Cannon-Albright LA, Teerlink CC, Stanford JL, Isaacs WB, Xu J, Cooney KA, Lange EM, Schleutker J, Carpten JD, Powell IJ, Cussenot O, Cancel-Tassin G, Giles GG, MacInnis RJ, Maier C, Hsieh C-L, Wiklund F, Catalona WJ, Foulkes WD, Mandal D, Eeles RA, Kote-Jarai Z, Bustamante CD, Schaid DJ, Hastie T, Ostrander EA, Bailey-Wilson JE, Radivojac P, Thibodeau SN, Whittemore AS, and Sieh W (2016) REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet* **99**:877–885.
- Isvoran A, Peng Y, Ceauranu S, Schmidt L, Nicot AB, and Miteva MA (2022) Pharmacogenetics of human sulfotransferases and impact of amino acid exchange on Phase II drug metabolism. *Drug Discov Today* **27**:103349.
- Ittisoponpisan S, Islam SA, Khanna T, Alhuzimi E, David A, and Sternberg MJE (2019) Can Predicted Protein 3D Structures Provide Reliable Insights into whether Missense Variants Are Disease Associated? *J Mol Biol* **431**:2197–2212.
- Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, Coffman BL, and Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* **101**:847–854.
- Jaaks P, Coker EA, Vis DJ, Edwards O, Carpenter EF, Leto SM, Dwane L, Sassi F, Lightfoot H, Barthorpe S, Van Der Meer D, Yang W, Beck A, Mironenko T, Hall C, Hall J, Mali I, Richardson L, Tolley C, Morris J, Thomas F, Lleshi E, Aben N, Benes CH, Bertotti A, Trusolino L, Wessels L, and Garnett MJ (2022) Effective drug combinations in breast, colon and pancreatic cancer cells. *Nature* **603**:166–173.
- Janaszkiwicz A, Tóth Á, Faucher Q, Martin M, Chantemargue B, Barin-Le Guellec C, Marquet P, and Di Meo F (2022) Insights into the structure and function of the human organic anion transporter 1 in lipid bilayer membranes. *Sci Rep* **12**:7057.

- Jarrar Y, and Lee S-J (2021) The Functionality of UDP-Glucuronosyltransferase Genetic Variants and their Association with Drug Responses and Human Diseases. *J Pers Med* **11**:554.
- Jayaraman S, Reid JM, Hawse JR, and Goetz MP (2021) Endoxifen, an Estrogen Receptor Targeted Therapy: From Bench to Bedside. *Endocrinology* **162**:bqab191.
- Jena A, Birda CL, Choudhury A, and Sharma V (2023) Safety and efficacy of personalized versus standard initial dosing of thiopurines: Systematic review and meta-analysis of randomized trials. *Expert Opin Drug Saf* **22**:1253–1263.
- Johnson D, Wilke MAP, Lyle SM, Kowalec K, Jorgensen A, Wright GEB, and Drögemöller BI (2022) A Systematic Review and Analysis of the Use of Polygenic Scores in Pharmacogenomics. *Clin Pharmacol Ther* **111**:919–930.
- Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, Lee MT, Gage BF, Kimmel SE, Perera MA, Anderson JL, Pirmohamed M, Klein TE, Limdi NA, Cavallari LH, and Wadelius M (2017) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. *Clin Pharmacol Ther* **102**:397–404.
- Junk SV, Schaeffeler E, Zimmermann M, Mörcke A, Beier R, Schütte P, Fedders B, Alten J, Hinze L, Klein N, Kulozik A, Muckenthaler MU, Koehler R, Borkhardt A, Vijayakrishnan J, Ellinghaus D, Forster M, Franke A, Wintering A, Kratz CP, Schrappe M, Schwab M, Houlston RS, Cario G, and Stanulla M (2023) Chemotherapy-related hyperbilirubinemia in pediatric acute lymphoblastic leukemia: a genome-wide association study from the AIEOP-BFM ALL study group. *J Exp Clin Cancer Res* **42**:21.
- Kalow W, and Genest K (1957) A METHOD FOR THE DETECTION OF ATYPICAL FORMS OF HUMAN SERUM CHOLINESTERASE. DETERMINATION OF DIBUCAINE NUMBERS. *Can J Biochem Physiol* **35**:339–346.
- Karas S, and Innocenti F (2022) All You Need to Know About UGT1A1 Genetic Testing for Patients Treated With Irinotecan: A Practitioner-Friendly Guide. *JCO Oncol Pract* **18**:270–277.
- Katsonis P, Wilhelm K, Williams A, and Lichtarge O (2022) Genome interpretation using in silico predictors of variant impact. *Hum Genet* **141**:1549–1577.
- Khor CC, Winter S, Sutiman N, Mürdter TE, Chen S, Lim JSL, Li Z, Li J, Sim KS, Ganchev B, Eccles D, Eccles B, Tapper W, Zgheib NK, Tfayli A, Ng RCH, Yap YS, Lim E, Wong M, Wong NS, Ang PCS, Dent R, Tremmel R, Klein K, Schaeffeler E, Zhou Y, Lauschke VM, Eichelbaum M, Schwab M, Brauch HB, Chowbay B, and Schroth W (2023) Cross-Ancestry Genome-Wide Association Study Defines the Extended CYP2D6 Locus as the Principal Genetic Determinant of Endoxifen Plasma Concentrations. *Clin Pharmacol Ther* **113**:712–723.
- King M-C (2014) “The Race” to Clone BRCA1. *Science* **343**:1462–1465.
- Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, and Shendure J (2014) A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* **46**:310–315.

- Klanderma BJ, Koch C, Machini K, Parpattedar SS, Bandyadka S, Lin C-F, Hynes E, Lebo MS, and Amr SS (2022) Automated Pharmacogenomic Reports for Clinical Genome Sequencing. *J Mol Diagn* **24**:205–218.
- Klein K, and Zanger UM (2013) Pharmacogenomics of Cytochrome P450 3A4: Recent Progress Toward the “Missing Heritability” Problem. *Front Genet* **4**.
- Klug LR, Khosroyani HM, Kent JD, and Heinrich MC (2022) New treatment strategies for advanced-stage gastrointestinal stromal tumours. *Nat Rev Clin Oncol* **19**:328–341.
- Klyushova LS, Perepechaeva ML, and Grishanova AY (2022) The Role of CYP3A in Health and Disease. *Biomedicines* **10**:2686.
- Knikman JE, Wilting TA, Lopez-Yurda M, Henricks LM, Lunenburg CATC, De Man FM, Meulendijks D, Nieboer P, Droogendijk HJ, Creemers G-J, Mandigers CMPW, Imholz ALT, Mathijssen RHJ, Portielje JEA, Valkenburg-van Iersel L, Vulink A, Van Der Poel MHW, Baars A, Swen JJ, Gelderblom H, Schellens JHM, Beijnen JH, Guchelaar H-J, and Cats A (2023) Survival of Patients With Cancer With *DPYD* Variant Alleles and Dose-Individualized Fluoropyrimidine Therapy—A Matched-Pair Analysis. *J Clin Oncol* **41**:5411–5421.
- Koeck K, Grube M, Jedlitschky G, Oevermann L, Siegmund W, Ritter CA, and Kroemer HK (2007) Expression of Adenosine Triphosphate-Binding Cassette (ABC)??Drug Transporters in Peripheral??Blood Cells: Relevance for Physiology and Pharmacotherapy. *Clin Pharmacokinet* **46**:449–470.
- Kölz C, Schaeffeler E, Schwab M, and Nies AT (2021) Genetic and Epigenetic Regulation of Organic Cation Transporters, in *Organic Cation Transporters in the Central Nervous System* (Daws LC ed) pp 81–100, Springer International Publishing, Cham.
- Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, Wasan H, Ciardiello F, Loupakis F, Hong YS, Steeghs N, Guren TK, Arkenau H-T, Garcia-Alfonso P, Pfeiffer P, Orlov S, Lonardi S, Elez E, Kim T-W, Schellens JHM, Guo C, Krishnan A, Dekervel J, Morris V, Calvo Ferrandiz A, Tarpgaard LS, Braun M, Gollerkeri A, Keir C, Maharry K, Pickard M, Christy-Bittel J, Anderson L, Sandor V, and Tabernero J (2019) Encorafenib, Binimetinib, and Cetuximab in *BRAF* V600E–Mutated Colorectal Cancer. *N Engl J Med* **381**:1632–1643.
- Kovac M, Blattmann C, Ribi S, Smida J, Mueller NS, Engert F, Castro-Giner F, Weischenfeldt J, Kovacova M, Krieg A, Andreou D, Tunn P-U, Dürr HR, Rechl H, Schaser K-D, Melcher I, Burdach S, Kulozik A, Specht K, Heinemann K, Fulda S, Bielack S, Jundt G, Tomlinson I, Korbelt JO, Nathrath M, and Baumhoer D (2015) Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat Commun* **6**:8940.
- Kovar C, Loer HLH, Rüdeshcim S, Fuhr LM, Marok FZ, Selzer D, Schwab M, and Lehr T (2024) A physiologically-based pharmacokinetic precision dosing approach to manage dasatinib drug–drug interactions. *CPT Pharmacomet Syst Pharmacol* **13**:1144–1159.
- Krynetski EY, Tai H-L, Yates CR, Fessing MY, Loennechen T, Schuetz JD, Relling MV, and Evans WE (1996) Genetic polymorphism of thiopurine S-methyltransferase: clinical importance and molecular mechanisms: *Pharmacogenetics* **6**:279–290.
- Kuebler JP, Wieand HS, O’Connell MJ, Smith RE, Colangelo LH, Yothers G, Petrelli NJ, Findlay MP, Seay TE, Atkins JN, Zapas JL, Goodwin JW, Fehrenbacher L,



- Ramanathan RK, Conley BA, Flynn PJ, Soori G, Colman LK, Levine EA, Lanier KS, and Wolmark N (2007) Oxaliplatin Combined With Weekly Bolus Fluorouracil and Leucovorin As Surgical Adjuvant Chemotherapy for Stage II and III Colon Cancer: Results From NSABP C-07. *J Clin Oncol* **25**:2198–2204.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, and Schuetz E (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27**:383–391.
- Lanillos J, Carcajona M, Maietta P, Alvarez S, and Rodriguez-Antona C (2022) Clinical pharmacogenetic analysis in 5,001 individuals with diagnostic Exome Sequencing data. *NPJ Genomic Med* **7**:12.
- Lauschke VM, Zhou Y, and Ingelman-Sundberg M (2024) Pharmacogenomics Beyond Single Common Genetic Variants: The Way Forward. *Annu Rev Pharmacol Toxicol* **64**:33–51.
- Lee AM, Shi Q, Pavey E, Alberts SR, Sargent DJ, Sinicrope FA, Berenberg JL, Goldberg RM, and Diasio RB (2014) DPYD Variants as Predictors of 5-fluorouracil Toxicity in Adjuvant Colon Cancer Treatment (NCCTG N0147). *JNCI J Natl Cancer Inst* **106**.
- Lee CAA, Banerjee P, Wilson BJ, Wu S, Guo Q, Berg G, Karpova S, Mishra A, Lian JW, Tran J, Emmerich M, Murphy GF, Frank MH, and Frank NY (2020) Targeting the ABC transporter ABCB5 sensitizes glioblastoma to temozolomide-induced apoptosis through a cell-cycle checkpoint regulation mechanism. *J Biol Chem* **295**:7774–7788.
- Lee JS, Nair NU, Dinstag G, Chapman L, Chung Y, Wang K, Sinha S, Cha H, Kim D, Schperberg AV, Srinivasan A, Lazar V, Rubin E, Hwang S, Berger R, Beker T, Ronai Z, Hannenhalli S, Gilbert MR, Kurzrock R, Lee S-H, Aldape K, and Ruppin E (2021) Synthetic lethality-mediated precision oncology via the tumor transcriptome. *Cell* **184**:2487-2502.e13.
- Lemke LK, Alam B, Williams R, Starostik P, Cavallari LH, Cicali EJ, and Wiisanen K (2023) Reimbursement of pharmacogenetic tests at a tertiary academic medical center in the United States. *Front Pharmacol* **14**:1179364.
- Lenk HÇ, Klöditz K, Johansson I, Smith RL, Jukić MM, Molden E, and Ingelman-Sundberg M (2022) The Polymorphic Nuclear Factor NFIB Regulates Hepatic CYP2D6 Expression and Influences Risperidone Metabolism in Psychiatric Patients. *Clin Pharmacol Ther* **111**:1165–1174.
- Li J, Chen T, Jie F, Xiang H, Huang L, Jiang H, Lu F, Zhu S, Wu L, and Tang Y (2022) Impact of VKORC1, CYP2C9, CYP1A2, UGT1A1, and GGCX polymorphisms on warfarin maintenance dose: Exploring a new algorithm in South Chinese patients accept mechanical heart valve replacement. *Medicine (Baltimore)* **101**:e29626.
- Lin BC, Katneni U, Jankowska KI, Meyer D, and Kimchi-Sarfaty C (2023) In silico methods for predicting functional synonymous variants. *Genome Biol* **24**:126.
- Lin T, Islam O, and Heese K (2006) ABC transporters, neural stem cells and neurogenesis – a different perspective. *Cell Res* **16**:857–871.

- Liu S, Wu I, Yu Y-P, Balamotis M, Ren B, Ben Yehezkel T, and Luo J-H (2021) Targeted transcriptome analysis using synthetic long read sequencing uncovers isoform reprogramming in the progression of colon cancer. *Commun Biol* **4**:506.
- Liu Z, Tian Y, Zhang X, Wang J, and Yang J (2023) Identification of a novel prognostic ADME-related signature associated with tumor immunity for aiding therapy in head and neck squamous cell carcinoma. *Cancer Gene Ther* **30**:659–670.
- Loibl S, André F, Bachelot T, Barrios CH, Bergh J, Burstein HJ, Cardoso MJ, Carey LA, Dawood S, Del Mastro L, Denkert C, Fallenberg EM, Francis PA, Gamal-Eldin H, Gelmon K, Geyer CE, Gnani M, Guarneri V, Gupta S, Kim SB, Krug D, Martin M, Meattini I, Morrow M, Janni W, Paluch-Shimon S, Partridge A, Poortmans P, Pusztai L, Regan MM, Sparano J, Spanic T, Swain S, Tjulandin S, Toi M, Trapani D, Tutt A, Xu B, Curigliano G, and Harbeck N (2024) Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* **35**:159–182.
- Lord CJ, and Ashworth A (2016) BRCAness revisited. *Nat Rev Cancer* **16**:110–120.
- Lord CJ, and Ashworth A (2012) The DNA damage response and cancer therapy. *Nature* **481**:287–294.
- Ly RC, Shugg T, Ratcliff R, Osei W, Lynnes TC, Pratt VM, Schneider BP, Radovich M, Bray SM, Salisbury BA, Parikh B, Sahinalp SC, Numanagić I, and Skaar TC (2022) Analytical Validation of a Computational Method for Pharmacogenetic Genotyping from Clinical Whole Exome Sequencing. *J Mol Diagn JMD* **24**:576–585.
- MacPhail A, Dendle C, Slavin M, and McQuilten Z (2024) Hospital-acquired bloodstream infections in patients with cancer: current knowledge and future directions. *J Hosp Infect* **148**:39–50.
- Maeda K, Takano J, Ikeda Y, Fujita T, Oyama Y, Nozawa K, Kumagai Y, and Sugiyama Y (2011) Nonlinear pharmacokinetics of oral quinidine and verapamil in healthy subjects. *Clin Pharmacol Ther* **90**:263–270.
- Magliocco G, Desmeules J, Matthey A, Quirós-Guerrero LM, Bararpour N, Joye T, Marcourt L, Queiroz EF, Wolfender J-L, Gloor Y, Thomas A, and Daali Y (2021) Metabolomics reveals biomarkers in human urine and plasma to predict cytochrome P450 2D6 (CYP2D6) activity. *Br J Pharmacol* **178**:4708–4725.
- Mahgoub A, Idle JR, Dring LG, Lancaster R, and Smith RL (1977) Polymorphic hydroxylation of Debrisoquine in man. *Lancet Lond Engl* **2**:584–586.
- Maillard M, Nishii R, Yang W, Hoshitsuki K, Chepyala D, Lee SHR, Nguyen JQ, Relling MV, Crews KR, Leggas M, Singh M, Suang JLY, Yeoh AEJ, Jeha S, Inaba H, Pui C-H, Karol SE, Trehan A, Bhatia P, Antillon Klussmann FG, Bhojwani D, Haidar CE, and Yang JJ (2024) Additive effects of TPMT and NUDT15 on thiopurine toxicity in children with acute lymphoblastic leukemia across multiethnic populations. *JNCI J Natl Cancer Inst* djae004.
- Mannheimer B, Haslemo T, Lindh JD, Eliasson E, and Molden E (2016) Risperidone and Venlafaxine Metabolic Ratios Strongly Predict a CYP2D6 Poor Metabolizing Genotype. *Ther Drug Monit* **38**:127–134.
- Manson LEN, Swen JJ, and Guchelaar H-J (2020) Diagnostic Test Criteria for HLA Genotyping to Prevent Drug Hypersensitivity Reactions: A Systematic Review of

Actionable HLA Recommendations in CPIC and DPWG Guidelines. *Front Pharmacol* **11**:567048.

Mateo J, Steuten L, Aftimos P, André F, Davies M, Garralda E, Geissler J, Husereau D, Martinez-Lopez I, Normanno N, Reis-Filho JS, Stefani S, Thomas DM, Westphalen CB, and Voest E (2022) Delivering precision oncology to patients with cancer. *Nat Med* **28**:658–665.

Matthaei J, Bonat WH, Kerb R, Tzvetkov MV, Strube J, Brunke S, Sachse-Seeboth C, Sehr D, Hofmann U, Bornemann Hjelmberg J, Schwab M, and Brockmüller J (2020) Inherited and Acquired Determinants of Hepatic CYP3A Activity in Humans. *Front Genet* **11**:944.

Maulana Y, Toro Jimenez R, Twesigomwe D, Sani L, Irwanto A, Bertin N, and Gonzalez-Porta M (2024) The variation landscape of CYP2D6 in a multi-ethnic Asian population. *Sci Rep* **14**:16725.

Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, Tyrer JP, Chen T-H, Wang Q, Bolla MK, Yang X, Adank MA, Ahearn T, Aittomäki K, Allen J, Andrulis IL, Anton-Culver H, Antonenkova NN, Arndt V, Aronson KJ, Auer PL, Auvinen P, Barrdahl M, Beane Freeman LE, Beckmann MW, Behrens S, Benitez J, Bermisheva M, Bernstein L, Blomqvist C, Bogdanova NV, Bojesen SE, Bonanni B, Børresen-Dale A-L, Brauch H, Bremer M, Brenner H, Brentnall A, Brock IW, Brooks-Wilson A, Brucker SY, Brüning T, Burwinkel B, Campa D, Carter BD, Castelao JE, Chanock SJ, Chlebowski R, Christiansen H, Clarke CL, Collée JM, Cordina-Duverger E, Cornelissen S, Couch FJ, Cox A, Cross SS, Czene K, Daly MB, Devilee P, Dörk T, dos-Santos-Silva I, Dumont M, Durcan L, Dwek M, Eccles DM, Ekici AB, Eliassen AH, Ellberg C, Engel C, Eriksson M, Evans DG, Fasching PA, Figueroa J, Fletcher O, Flyger H, Försti A, Fritschi L, Gabrielson M, Gago-Dominguez M, Gapstur SM, García-Sáenz JA, Gaudet MM, Georgoulas V, Giles GG, Gilyazova IR, Glendon G, Goldberg MS, Goldgar DE, González-Neira A, Grenaker Alnæs GI, Grip M, Gronwald J, Grundy A, Guénel P, Haeberle L, Hahnen E, Haiman CA, Håkansson N, et al. (2019) Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am J Hum Genet* **104**:21–34.

McCarty CA, Chisholm RL, Chute CG, Kullo IJ, Jarvik GP, Larson EB, Li R, Masys DR, Ritchie MD, Roden DM, Struewing JP, Wolf WA, and eMERGE Team (2011) The eMERGE Network: a consortium of biorepositories linked to electronic medical records data for conducting genomic studies. *BMC Med Genomics* **4**:13.

McDermott JH, Wolf J, Hoshitsuki K, Huddart R, Caudle KE, Whirl-Carrillo M, Steyger PS, Smith RJH, Cody N, Rodriguez-Antona C, Klein TE, and Newman WG (2022) Clinical Pharmacogenetics Implementation Consortium Guideline for the Use of Aminoglycosides Based on *MT-RNR1* Genotype. *Clin Pharmacol Ther* **111**:366–372.

McInnes G, Dalton R, Sangkuhl K, Whirl-Carrillo M, Lee S, Tsao PS, Gaedigk A, Altman RB, and Woodahl EL (2020) Transfer learning enables prediction of CYP2D6 haplotype function. *PLOS Comput Biol* **16**:e1008399.

McLeod HL, Sargent DJ, Marsh S, Green EM, King CR, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Thibodeau SN, Grothey A, Morton RF, and Goldberg RM (2010) Pharmacogenetic Predictors of Adverse Events and Response to Chemotherapy in Metastatic Colorectal Cancer: Results From North American Gastrointestinal Intergroup Trial N9741. *J Clin Oncol* **28**:3227–3233.

- Meyer MJ, Neumann VE, Friesacher HR, Zdrzil B, Brockmöller J, and Tzvetkov MV (2019) Opioids as Substrates and Inhibitors of the Genetically Highly Variable Organic Cation Transporter OCT1. *J Med Chem* **62**:9890–9905.
- Michael M, and Doherty MM (2005) Tumoral Drug Metabolism: Overview and Its Implications for Cancer Therapy. *J Clin Oncol* **23**:205–229.
- Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, and Saijo N (2007) Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of UGT1A1\*6 and \*28. *Pharmacogenet Genomics* **17**:497–504.
- Miners JO, Polasek TM, Hulin J-A, Rowland A, and Meech R (2023) Drug-drug interactions that alter the exposure of glucuronidated drugs: Scope, UDP-glucuronosyltransferase (UGT) enzyme selectivity, mechanisms (inhibition and induction), and clinical significance. *Pharmacol Ther* **248**:108459.
- Mock A, Teleanu M-V, Kreutzfeldt S, Heilig CE, Hüllelein J, Möhrmann L, Jahn A, Hanf D, Kerle IA, Singh HM, Hutter B, Uhrig S, Fröhlich M, Neumann O, Hartig A, Brückmann S, Hirsch S, Grund K, Dikow N, Lipka DB, Renner M, Bhatti IA, Apostolidis L, Schlenk RF, Schaaf CP, Stenzinger A, Schröck E, Hübschmann D, Heining C, Horak P, Glimm H, and Fröhling S (2023) NCT/DKFZ MASTER handbook of interpreting whole-genome, transcriptome, and methylome data for precision oncology. *NPJ Precis Oncol* **7**:109.
- Moore JM, Bell EL, Hughes RO, and Garfield AS (2023) ABC transporters: human disease and pharmacotherapeutic potential. *Trends Mol Med* **29**:152–172.
- Moriyama B, Obeng AO, Barbarino J, Penzak S, Henning S, Scott S, Agúndez J, Wingard J, McLeod H, Klein T, Cross S, Caudle K, and Walsh T (2017) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C19 and Voriconazole Therapy. *Clin Pharmacol Ther* **102**:45–51.
- Moriyama T, Nishii R, Perez-Andreu V, Yang W, Klussmann FA, Zhao X, Lin T-N, Hoshitsuki K, Nersting J, Kihira K, Hofmann U, Komada Y, Kato M, McCorkle R, Li L, Koh K, Najera CR, Kham SK-Y, Isobe T, Chen Z, Chiew EK-H, Bhojwani D, Jeffries C, Lu Y, Schwab M, Inaba H, Pui C-H, Relling MV, Manabe A, Hori H, Schmiegelow K, Yeoh AEJ, Evans WE, and Yang JJ (2016) NUDT15 Polymorphisms Alter Thiopurine Metabolism and Hematopoietic Toxicity. *Nat Genet* **48**:367–373.
- Morris SA, Alsaidi AT, Verbyla A, Cruz A, Macfarlane C, Bauer J, and Patel JN (2022) Cost Effectiveness of Pharmacogenetic Testing for Drugs with Clinical Pharmacogenetics Implementation Consortium ( CPIC ) Guidelines: A Systematic Review. *Clin Pharmacol Ther* **112**:1318–1328.
- Mueller-Schoell A, Klopp-Schulze L, Schroth W, Mürdter T, Michelet R, Brauch H, Huisinga W, Joerger M, Neven P, Koolen SLW, Mathijssen RHJ, Copson E, Eccles D, Chen S, Chowbay B, Tfayli A, Zgheib NK, Schwab M, and Kloft C (2020) Obesity Alters Endoxifen Plasma Levels in Young Breast Cancer Patients: A Pharmacometric Simulation Approach. *Clin Pharmacol Ther* **108**:661–670.
- Mulder TAM, Van Eerden RAG, De With M, Elens L, Hesselink DA, Matic M, Bins S, Mathijssen RHJ, and Van Schaik RHN (2021) CYP3A4\*22 Genotyping in Clinical Practice: Ready for Implementation? *Front Genet* **12**:711943.

- Müller JP, Sarömba J, Ziegler P, Tremmel R, Rengelshausen J, Schaeffeler E, Just KS, Schwab M, Kraus T, and Stingl JC (2023) Nutrimeric Validation of Solanidine as Dietary-Derived CYP2D6 Activity Marker In Vivo. *Clin Pharmacol Ther* **cpt.3106**.
- Mürdter TE, Schroth W, Bacchus-Gerybadze L, Winter S, Heinkele G, Simon W, Fasching PA, Fehm T, Eichelbaum M, Schwab M, and Brauch H (2011) Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. *Clin Pharmacol Ther* **89:708–717**.
- Muriithi W, Wanjiku Macharia L, Pilotto Heming C, Lima Echevarria J, Nyachieo A, Niemeyer Filho P, and Moura Neto V (2020) ABC transporters and the hallmarks of cancer: roles in cancer aggressiveness beyond multidrug resistance. *Cancer Biol Med* **17:253–269**.
- Murray GI, Taylor MC, McFadyen MC, McKay JA, Greenlee WF, Burke MD, and Melvin WT (1997) Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res* **57:3026–3031**.
- Nelson RS, Seligson ND, Bottiglieri S, Carballido E, Cueto AD, Imanirad I, Levine R, Parker AS, Swain SM, Tillman EM, and Hicks JK (2021) UGT1A1 Guided Cancer Therapy: Review of the Evidence and Considerations for Clinical Implementation. *Cancers* **13:1566**.
- Neul C, Schaeffeler E, Sparreboom A, Laufer S, Schwab M, and Nies AT (2016) Impact of Membrane Drug Transporters on Resistance to Small-Molecule Tyrosine Kinase Inhibitors. *Trends Pharmacol Sci* **37:904–932**.
- Ng PC, and Henikoff S (2001) Predicting Deleterious Amino Acid Substitutions. *Genome Res* **11:863–874**.
- Nguyen DG, Morris SA, Hamilton A, Kwange SO, Steuerwald N, Symanowski J, Moore DC, Hanson S, Mroz K, Lopes KE, Larck C, Musselwhite L, Kadakia KC, Koya B, Chai S, Osei-Boateng K, Kalapurakal S, Swift K, Hwang J, and Patel JN (2024) Real-World Impact of an In-House Dihydropyrimidine Dehydrogenase ( *DPYD* ) Genotype Test on Fluoropyrimidine Dosing, Toxicities, and Hospitalizations at a Multisite Cancer Center. *JCO Precis Oncol* **e2300623**.
- Nies AT, Koepsell H, Damme K, and Schwab M (2011) Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. *Handb Exp Pharmacol* **105–167**.
- Nies AT, Schaeffeler E, and Schwab M (2022) Hepatic solute carrier transporters and drug therapy: Regulation of expression and impact of genetic variation. *Pharmacol Ther* **238:108268**.
- Nies AT, Schaeffeler E, van der Kuip H, Cascorbi I, Bruhn O, Kneba M, Pott C, Hofmann U, Volk C, Hu S, Baker SD, Sparreboom A, Ruth P, Koepsell H, and Schwab M (2014) Cellular uptake of imatinib into leukemic cells is independent of human organic cation transporter 1 (OCT1). *Clin Cancer Res Off J Am Assoc Cancer Res* **20:985–994**.
- Niu J, Straubinger RM, and Mager DE (2019) Pharmacodynamic Drug–Drug Interactions. *Clin Pharmacol Ther* **105:1395–1406**.
- Noll EM, Eisen C, Stenzinger A, Espinet E, Muckenhuber A, Klein C, Vogel V, Klaus B, Nadler W, Rösli C, Lutz C, Kulke M, Engelhardt J, Zickgraf FM, Espinosa O,

- Schlesner M, Jiang X, Kopp-Schneider A, Neuhaus P, Bahra M, Sinn BV, Eils R, Giese NA, Hackert T, Strobel O, Werner J, Büchler MW, Weichert W, Trumpp A, and Sprick MR (2016) CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. *Nat Med* **22**:278–287.
- Osanlou O, Pirmohamed M, and Daly AK (2018) Pharmacogenetics of Adverse Drug Reactions, in *Advances in Pharmacology* pp 155–190, Elsevier.
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, and Wolmark N (2004) A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer. *N Engl J Med* **351**:2817–2826.
- Pak MA, Markhieva KA, Novikova MS, Petrov DS, Vorobyev IS, Maksimova ES, Kondrashov FA, and Ivankov DN (2023) Using AlphaFold to predict the impact of single mutations on protein stability and function. *PLOS ONE* **18**:e0282689, Public Library of Science.
- Palumbo S, Mariotti V, and Pellegrini S (2024) A Narrative Review on Pharmacogenomics in Psychiatry: Scientific Definitions, Principles, and Practical Resources. *J Clin Psychopharmacol* **44**:49–56.
- Pan X, Ning M, and Jeong H (2017) Transcriptional Regulation of CYP2D6 Expression. *Drug Metab Dispos Biol Fate Chem* **45**:42–48.
- Pandi M-T, Koromina M, Tsafaridis I, Patsilnakos S, Christoforou E, Van Der Spek PJ, and Patrinos GP (2021) A novel machine learning-based approach for the computational functional assessment of pharmacogenomic variants. *Hum Genomics* **15**:51.
- Park JJH, Hsu G, Siden EG, Thorlund K, and Mills EJ (2020) An overview of precision oncology basket and umbrella trials for clinicians. *CA Cancer J Clin* **70**:125–137.
- Patel JN, Arnall J, Jandrisevits E, Morse AL, Steuerwald N, Copelan E, and Walsh D (2021) Pharmacogenomics-guided supportive oncology: A tale of two trials. *Contemp Clin Trials* **105**:106391.
- Pejaver V, Babbi G, Casadio R, Folkman L, Katsonis P, Kundu K, Lichtarge O, Martelli PL, Miller M, Moulton J, Pal LR, Savojardo C, Yin Y, Zhou Y, Radivojac P, and Bromberg Y (2019) Assessment of methods for predicting the effects of PTEN and TPMT protein variants. *Hum Mutat* **40**:1495–1506.
- Petkov VI, Miller DP, Howlader N, Gliner N, Howe W, Schussler N, Cronin K, Baehner FL, Cress R, Deapen D, Glaser SL, Hernandez BY, Lynch CF, Mueller L, Schwartz AG, Schwartz SM, Stroup A, Sweeney C, Tucker TC, Ward KC, Wiggins C, Wu X-C, Penberthy L, and Shak S (2016) Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *Npj Breast Cancer* **2**:16017.
- Pirmohamed M (2023) Pharmacogenomics. *Nat Rev Genet* **24**:350–362.
- Pollard KS, Hubisz MJ, Rosenbloom KR, and Siepel A (2010) Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res* **20**:110–121.
- Popova T, Manié E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, Delattre O, Sigal-Zafrani B, Bollet M, Longy M, Houdayer C, Sastre-Garau X, Vincent-Salomon A, Stoppa-Lyonnet D, and Stern M-H (2012) Ploidy and Large-Scale Genomic Instability

- Consistently Identify Basal-like Breast Carcinomas with *BRCA1/2* Inactivation. *Cancer Res* **72**:5454–5462.
- Porubsky D, and Eichler EE (2024) A 25-year odyssey of genomic technology advances and structural variant discovery. *Cell* **187**:1024–1037.
- Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, and Bernards R (2012) Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **483**:100–103.
- Pratt VM, Wang WY, Boone EC, Broeckel U, Cody N, Edelmann L, Gaedigk A, Lynnes TC, Medeiros EB, Moyer AM, Mitchell MW, Scott SA, Starostik P, Turner A, and Kalman LV (2022) Characterization of Reference Materials for TPMT and NUDT15. *J Mol Diagn* **24**:1079–1088.
- Pretz JL, Barysaukas CM, George S, Hornick JL, Raut ChandrajitP, Chen Y-LE, Marcus KJ, Choy E, Hornicek F, Ready JE, DeLaney TF, and Baldini EH (2017) Localized Adult Ewing Sarcoma: Favorable Outcomes with Alternating Vincristine, Doxorubicin, Cyclophosphamide, and Ifosfamide, Etoposide (VDC/IE)-Based Multimodality Therapy. *The Oncologist* **22**:1265–1270.
- Pristup J, Schaeffeler E, Arjune S, Hofmann U, Angel Santamaria-Araujo J, Leuthold P, Friedrich N, Nauck M, Mayr S, Haag M, Muerdter T, Marner F-J, Relling MV, Evans WE, Schwarz G, and Schwab M (2022) Molybdenum Cofactor Catabolism Unravels the Physiological Role of the Drug Metabolizing Enzyme Thiopurine S-Methyltransferase. *Clin Pharmacol Ther* **112**:808–816.
- Pui C-H, Yang JJ, Hunger SP, Pieters R, Schrappe M, Biondi A, Vora A, Baruchel A, Silverman LB, Schmiegelow K, Escherich G, Horibe K, Benoit YCM, Izraeli S, Yeoh AEJ, Liang D-C, Downing JR, Evans WE, Relling MV, and Mullighan CG (2015) Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. *J Clin Oncol* **33**:2938–2948.
- Puszkiel A, Arellano C, Vachoux C, Evrard A, Le Morvan V, Boyer J, Robert J, Delmas C, Dalenc F, Debled M, Venat-Bouvet L, Jacot W, Suc E, Sillet-Bach I, Filleron T, Roché H, Chatelut E, White-Koning M, and Thomas F (2019) Factors Affecting Tamoxifen Metabolism in Patients With Breast Cancer: Preliminary Results of the French PHACS Study. *Clin Pharmacol Ther* **106**:585–595.
- Puszkiel A, Arellano C, Vachoux C, Evrard A, Le Morvan V, Boyer J-C, Robert J, Delmas C, Dalenc F, Debled M, Venat-Bouvet L, Jacot W, Dohollou N, Bernard-Marty C, Laharie-Mineur H, Filleron T, Roché H, Chatelut E, Thomas F, and White-Koning M (2021) Model-Based Quantification of Impact of Genetic Polymorphisms and Co-Medications on Pharmacokinetics of Tamoxifen and Six Metabolites in Breast Cancer. *Clin Pharmacol Ther* **109**:1244–1255.
- Quang D, Chen Y, and Xie X (2015) DANN: a deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics* **31**:761–763.
- Rahmioglu N, Heaton J, Clement G, Gill R, Surdulescu G, Zlobecka K, Hodgkiss D, Smith NW, and Ahmadi KR (2013) Genome-wide association study reveals a complex genetic architecture underpinning-induced CYP3A4 enzyme activity. *Eur J Drug Metab Pharmacokinet* **38**:63–67.

- Raimondi D, Tanyalcin I, Ferté J, Gazzo A, Orlando G, Lenaerts T, Rooman M, and Vranken W (2017) DEOGEN2: prediction and interactive visualization of single amino acid variant deleteriousness in human proteins. *Nucleic Acids Res* **45**:W201–W206.
- Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, Fujiwara K, Vergote I, Colombo N, Mäenpää J, Selle F, Sehouli J, Lorusso D, Guerra Alía EM, Reinthaller A, Nagao S, Lefevre-Plesse C, Canzler U, Scambia G, Lortholary A, Marmé F, Combe P, De Gregorio N, Rodrigues M, Buderath P, Dubot C, Burges A, You B, Pujade-Lauraine E, and Harter P (2019) Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. *N Engl J Med* **381**:2416–2428.
- Ray-Coquard I, Pujade Lauraine E, Le Cesne A, Pautier P, Vacher Lavenue MC, Trama A, Casali P, Coindre JM, and Blay JY (2017) Improving treatment results with reference centres for rare cancers: where do we stand? *Eur J Cancer* **77**:90–98.
- Recondo G, Facchinetti F, Olaussen KA, Besse B, and Friboulet L (2018) Making the first move in EGFR-driven or ALK-driven NSCLC: first-generation or next-generation TKI? *Nat Rev Clin Oncol* **15**:694–708.
- Reizine NM, and O'Donnell PH (2022) Modern developments in germline pharmacogenomics for oncology prescribing. *CA Cancer J Clin* **72**:315–332.
- Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui C-H, Yee SW, Stein CM, Carrillo M, Evans WE, Hicks JK, Schwab M, and Klein TE (2013) Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clin Pharmacol Ther* **93**:324–325.
- Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, Pui C-H, and Evans WE (1999) Mercaptopurine Therapy Intolerance and Heterozygosity at the Thiopurine S-Methyltransferase Gene Locus. *JNCI J Natl Cancer Inst* **91**:2001–2008.
- Relling MV, and Klein TE (2011) CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin Pharmacol Ther* **89**:464–467.
- Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui C-H, Stein CM, Moyer AM, Evans WE, Klein TE, Antillon-Klussmann FG, Caudle KE, Kato M, Yeoh AEJ, Schmiegelow K, and Yang JJ (2019) Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clin Pharmacol Ther* **105**:1095–1105.
- Reva B, Antipin Y, and Sander C (2011) Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res* **39**:e118–e118.
- Riedmaier S, Klein K, Hofmann U, Keskitalo JE, Neuvonen PJ, Schwab M, Niemi M, and Zanger UM (2010) UDP-Glucuronosyltransferase (UGT) Polymorphisms Affect Atorvastatin Lactonization In Vitro and In Vivo. *Clin Pharmacol Ther* **87**:65–73.
- Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, and Gottesman MM (2018) Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* **18**:452–464.
- Roden DM, McLeod HL, Relling MV, Williams MS, Mensah GA, Peterson JF, and Van Driest SL (2019) Pharmacogenomics. *The Lancet* **394**:521–532.



- Rosmarin D, Palles C, Church D, Domingo E, Jones A, Johnstone E, Wang H, Love S, Julier P, Scudder C, Nicholson G, Gonzalez-Neira A, Martin M, Sargent D, Green E, McLeod H, Zanger UM, Schwab M, Braun M, Seymour M, Thompson L, Lacas B, Boige V, Ribelles N, Afzal S, Enghusen H, Jensen SA, Etienne-Grimaldi M-C, Milano G, Wadelius M, Glimelius B, Garmo H, Gusella M, Lecomte T, Laurent-Puig P, Martinez-Balibrea E, Sharma R, Garcia-Foncillas J, Kleibl Z, Morel A, Pignon J-P, Midgley R, Kerr D, and Tomlinson I (2014) Genetic Markers of Toxicity From Capecitabine and Other Fluorouracil-Based Regimens: Investigation in the QUASAR2 Study, Systematic Review, and Meta-Analysis. *J Clin Oncol* **32**:1031–1039.
- Rosti G, Castagnetti F, Gugliotta G, and Baccarani M (2017) Tyrosine kinase inhibitors in chronic myeloid leukaemia: which, when, for whom? *Nat Rev Clin Oncol* **14**:141–154.
- Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu Y-M, Cao X, Kalyana-Sundaram S, Sam L, Balbin OA, Quist MJ, Barrette T, Everett J, Siddiqui J, Kunju LP, Navone N, Araujo JC, Troncoso P, Logothetis CJ, Innis JW, Smith DC, Lao CD, Kim SY, Roberts JS, Gruber SB, Pienta KJ, Talpaz M, and Chinnaiyan AM (2011) Personalized Oncology Through Integrative High-Throughput Sequencing: A Pilot Study. *Sci Transl Med* **3**.
- Saad F, Clarke NW, Oya M, Shore N, Procopio G, Guedes JD, Arslan C, Mehra N, Parnis F, Brown E, Schlürmann F, Joung JY, Sugimoto M, Sartor O, Liu Y-Z, Poehlein C, Barker L, Del Rosario PM, and Armstrong AJ (2023) Olaparib plus abiraterone versus placebo plus abiraterone in metastatic castration-resistant prostate cancer (PROpel): final prespecified overall survival results of a randomised, double-blind, phase 3 trial. *Lancet Oncol* **24**:1094–1108.
- Sadee W, Wang D, Hartmann K, and Toland AE (2023) Pharmacogenomics: Driving Personalized Medicine. *Pharmacol Rev* **75**:789–814.
- Saito Y, Stamp L, Caudle K, Hershfield M, McDonagh E, Callaghan J, Tassaneeyakul W, Mushiroda T, Kamatani N, Goldspiel B, Phillips E, Klein T, and Lee M (2016) Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for human leukocyte antigen B (HLA-B) genotype and allopurinol dosing: 2015 update. *Clin Pharmacol Ther* **99**:36–37.
- Saladores P, Mürdter T, Eccles D, Chowbay B, Zgheib NK, Winter S, Ganchev B, Eccles B, Gerty S, Tfayli A, Lim JSL, Yap YS, Ng RCH, Wong NS, Dent R, Habbal MZ, Schaeffeler E, Eichelbaum M, Schroth W, Schwab M, and Brauch H (2015) Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. *Pharmacogenomics J* **15**:84–94.
- Sanchez-Spitman AB, Böhringer S, Dezentjé VO, Gelderblom H, Swen JJ, and Guchelaar H (2024) A Genome-Wide Association Study of Endoxifen Serum Concentrations and Adjuvant Tamoxifen Efficacy in Early-Stage Breast Cancer Patients. *Clin Pharmacol Ther* **cpt.3255**.
- Santos M, Niemi M, Hiratsuka M, Kumondai M, Ingelman-Sundberg M, Lauschke VM, and Rodríguez-Antona C (2018) Novel copy-number variations in pharmacogenes contribute to interindividual differences in drug pharmacokinetics. *Genet Med Off J Am Coll Med Genet* **20**:622–629.
- Sawers L, Ferguson MJ, Ihrig BR, Young HC, Chakravarty P, Wolf CR, and Smith G (2014) Glutathione S-transferase P1 (GSTP1) directly influences platinum drug chemosensitivity in ovarian tumour cell lines. *Br J Cancer* **111**:1150–1158.

- Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, Zanger UM, and Schwab M (2004) Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics* **14**:407–417.
- Schaeffeler E, Jaeger SU, Klumpp V, Yang JJ, Igel S, Hinze L, Stanulla M, and Schwab M (2019) Impact of NUDT15 genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. *Genet Med Off J Am Coll Med Genet* **21**:2145–2150.
- Schärfe CPI, Tremmel R, Schwab M, Kohlbacher O, and Marks DS (2017) Genetic variation in human drug-related genes. *Genome Med* **9**:117.
- Scheinfeldt LB, Brangan A, Kusic DM, Kumar S, and Gharani N (2021) Common Treatment, Common Variant: Evolutionary Prediction of Functional Pharmacogenomic Variants. *J Pers Med* **11**:131.
- Schickhardt C, Fleischer H, and Winkler EC (2020) Do patients and research subjects have a right to receive their genomic raw data? An ethical and legal analysis. *BMC Med Ethics* **21**:7.
- Schlessinger A, Zatorski N, Hutchinson K, and Colas C (2023) Targeting SLC transporters: small molecules as modulators and therapeutic opportunities. *Trends Biochem Sci* **48**:801–814.
- Scholl C, Gilliland DG, and Fröhling S (2008) Deregulation of Signaling Pathways in Acute Myeloid Leukemia. *Semin Oncol* **35**:336–345.
- Schroth W (2009) Association Between CYP2D6 Polymorphisms and Outcomes Among Women With Early Stage Breast Cancer Treated With Tamoxifen. *JAMA* **302**:1429.
- Schuetz JD, Molowa DT, and Guzelian PS (1989) Characterization of a cDNA encoding a new member of the glucocorticoid-responsive cytochromes P450 in human liver. *Arch Biochem Biophys* **274**:355–365.
- Schwab M, Eichelbaum M, and Fromm MF (2003) Genetic Polymorphisms of the Human MDR1 Drug Transporter. *Annu Rev Pharmacol Toxicol* **43**:285–307.
- Schwab M, and Schaeffeler E (2012) Pharmacogenomics: a key component of personalized therapy. *Genome Med* **4**:93.
- Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, Kerb R, Bliedernicht J, Fischer J, Hofmann U, Bokemeyer C, and Eichelbaum M (2008) Role of Genetic and Nongenetic Factors for Fluorouracil Treatment-Related Severe Toxicity: A Prospective Clinical Trial by the German 5-FU Toxicity Study Group. *J Clin Oncol* **26**:2131–2138.
- Schwaederle M, Parker BA, Schwab RB, Fanta PT, Boles SG, Daniels GA, Bazhenova LA, Subramanian R, Coutinho AC, Ojeda-Fournier H, Datnow B, Webster NJ, Lippman SM, and Kurzrock R (2014) Molecular Tumor Board: The University of California San Diego Moores Cancer Center Experience. *The Oncologist* **19**:631–636.
- Shihab HA, Gough J, Cooper DN, Stenson PD, Barker GLA, Edwards KJ, Day INM, and Gaunt TR (2013) Predicting the Functional, Molecular, and Phenotypic Consequences of Amino Acid Substitutions using Hidden Markov Models. *Hum Mutat* **34**:57–65.

- Shihab HA, Rogers MF, Gough J, Mort M, Cooper DN, Day INM, Gaunt TR, and Campbell C (2015) An integrative approach to predicting the functional effects of non-coding and coding sequence variation. *Bioinformatics* **31**:1536–1543.
- Shriver SP, Adams D, McKelvey BA, McCune JS, Miles D, Pratt VM, Ashcraft K, McLeod HL, Williams H, and Fleury ME (2024) Overcoming Barriers to Discovery and Implementation of Equitable Pharmacogenomic Testing in Oncology. *J Clin Oncol* JCO.23.01748.
- Shugg T, Ly RC, Rowe EJ, Philips S, Hyder MA, Radovich M, Rosenman MB, Pratt VM, Callaghan JT, Desta Z, Schneider BP, and Skaar TC (2022) Clinical Opportunities for Germline Pharmacogenetics and Management of Drug-Drug Interactions in Patients With Advanced Solid Cancers. *JCO Precis Oncol* e2100312.
- Siamoglou S, Koromina M, Hishinuma E, Yamazaki S, Tsermpini E-E, Kordou Z, Fukunaga K, Chantratita W, Zhou Y, Lauschke VM, Mushiroda T, Hiratsuka M, and Patrinos GP (2022) Identification and functional validation of novel pharmacogenomic variants using a next-generation sequencing-based approach for clinical pharmacogenomics. *Pharmacol Res* **176**:106087.
- Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, Clawson H, Spieth J, Hillier LW, Richards S, Weinstock GM, Wilson RK, Gibbs RA, Kent WJ, Miller W, and Haussler D (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res* **15**:1034–1050.
- Simmons SK, Lithwick-Yanai G, Adiconis X, Oberstrass F, Iremadze N, Geiger-Schuller K, Thakore PI, Frangieh CJ, Barad O, Almogy G, Rozenblatt-Rosen O, Regev A, Lipson D, and Levin JZ (2023) Mostly natural sequencing-by-synthesis for scRNA-seq using Ultima sequencing. *Nat Biotechnol* **41**:204–211.
- Simona A, Song W, Bates DW, and Samer CF (2023) Polygenic risk scores in pharmacogenomics: opportunities and challenges—a mini review. *Front Genet* **14**:1217049.
- Singh S, Stocco G, Theken KN, Dickson A, Feng Q, Karnes JH, Mosley JD, and El Rouby N (2024) Pharmacogenomics polygenic risk score: Ready or not for prime time? *Clin Transl Sci* **17**:e13893.
- Smith D, He B, Shi J, Zhu H-J, and Wang X (2024) Novel Independent Trans- and Cis-Genetic Variants Associated with CYP2D6 Expression and Activity in Human Livers. *Drug Metab Dispos* **52**:143–152.
- Sneha S, Baker SC, Green A, Storr S, Aiyappa R, Martin S, and Pors K (2021) Intratumoural Cytochrome P450 Expression in Breast Cancer: Impact on Standard of Care Treatment and New Efforts to Develop Tumour-Selective Therapies. *Biomedicines* **9**:290.
- Sosinsky A, Ambrose J, Cross W, Turnbull C, Henderson S, Jones L, Hamblin A, Arumugam P, Chan G, Chubb D, Noyvert B, Mitchell J, Walker S, Bowman K, Pasko D, Buongiorno Pereira M, Volkova N, Rueda-Martin A, Perez-Gil D, Lopez J, Pullinger J, Siddiq A, Zainy T, Choudhury T, Yavorska O, Fowler T, Bentley D, Kingsley C, Hing S, Deans Z, Rendon A, Hill S, Caulfield M, and Murugaesu N (2024) Insights for precision oncology from the integration of genomic and clinical data of 13,880 tumors from the 100,000 Genomes Cancer Programme. *Nat Med* **30**:279–289.

- Stein D, Kars ME, Wu Y, Bayrak ÇS, Stenson PD, Cooper DN, Schlessinger A, and Itan Y (2023) Genome-wide prediction of pathogenic gain- and loss-of-function variants from ensemble learning of a diverse feature set. *Genome Med* **15**:103.
- Stein RA, and Mchaourab HS (2023) *Rosetta Energy Analysis of AlphaFold2 models: Point Mutations and Conformational Ensembles*, Biochemistry.
- Stratton MR, Campbell PJ, and Futreal PA (2009) The cancer genome. *Nature* **458**:719–724.
- Subbiah V, Puzanov I, Blay J-Y, Chau I, Lockhart AC, Raje NS, Wolf J, Baselga J, Meric-Bernstam F, Roszik J, Diamond EL, Riely GJ, Sherman EJ, Riehl T, Pitcher B, and Hyman DM (2020) Pan-Cancer Efficacy of Vemurafenib in *BRAF* V600-Mutant Non-Melanoma Cancers. *Cancer Discov* **10**:657–663.
- Swen J, Wilting I, Goede AD, Grandia L, Mulder H, Touw D, Boer AD, Conemans J, Egberts T, Klungel O, Koopmans R, Weide JVD, Wilffert B, Guchelaar H-J, and Deneer V (2008) Pharmacogenetics: From Bench to Byte. *Clin Pharmacol Ther* **83**:781–787.
- Swen JJ, Nijenhuis M, De Boer A, Grandia L, Maitland-van Der Zee AH, Mulder H, Rongen GAPJM, Van Schaik RHN, Schalekamp T, Touw DJ, Van Der Weide J, Wilffert B, Deneer VHM, and Guchelaar H-J (2011) Pharmacogenetics: From Bench to Byte—An Update of Guidelines. *Clin Pharmacol Ther* **89**:662–673.
- Swen JJ, van der Wouden CH, Manson LE, Abdullah-Koolmees H, Blagec K, Blagus T, Böhringer S, Cambon-Thomsen A, Cecchin E, Cheung K-C, Deneer VH, Dupui M, Ingelman-Sundberg M, Jonsson S, Joefield-Roka C, Just KS, Karlsson MO, Konta L, Koopmann R, Kriek M, Lehr T, Mitropoulou C, Rial-Sebbag E, Rollinson V, Roncato R, Samwald M, Schaeffeler E, Skokou M, Schwab M, Steinberger D, Stingl JC, Tremmel R, Turner RM, van Rhenen MH, Dávila Fajardo CL, Dolžan V, Patrinos GP, Pirmohamed M, Sunder-Plassmann G, Toffoli G, and Guchelaar H-J (2023) A 12-gene pharmacogenetic panel to prevent adverse drug reactions. *Lancet Lond Engl* **401**:347–356.
- Syed YY (2020) Oncotype DX Breast Recurrence Score®: A Review of its Use in Early-Stage Breast Cancer. *Mol Diagn Ther* **24**:621–632.
- Tafazoli A, Guchelaar H-J, Milyk W, Kretowski AJ, and Swen JJ (2021) Applying Next-Generation Sequencing Platforms for Pharmacogenomic Testing in Clinical Practice. *Front Pharmacol* **12**:693453.
- Tamm R, Magi R, Tremmel R, Winter S, Mihailov E, Smid A, Moricke A, Klein K, Schrappe M, Stanulla M, Houlston R, Weinshilboum R, Mlinaric Rascan I, Metspalu A, Milani L, Schwab M, and Schaeffeler E (2016) Polymorphic variation in *TPMT* is the principal determinant of *TPMT* phenotype: a meta-analysis of three genome-wide association studies. *Clin Pharmacol Ther*, doi: 10.1002/cpt.540.
- Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, Szallasi Z, Barry WT, Winer EP, Tung NM, Isakoff SJ, Ryan PD, Greene-Colozzi A, Gutin A, Sangale Z, Iliev D, Neff C, Abkevich V, Jones JT, Lanchbury JS, Hartman A-R, Garber JE, Ford JM, Silver DP, and Richardson AL (2016) Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. *Clin Cancer Res* **22**:3764–3773.
- The ENCODE Project Consortium, Abascal F, Acosta R, Addleman NJ, Adrian J, Afzal V, Ai R, Aken B, Akiyama JA, Jammal OA, Amrhein H, Anderson SM, Andrews GR, Antoshechkin I, Ardlie KG, Armstrong J, Astley M, Banerjee B, Barkal AA, Barnes

IHA, Barozzi I, Barrell D, Barson G, Bates D, Baymuradov UK, Bazile C, Beer MA, Beik S, Bender MA, Bennett R, Bouvrette LPB, Bernstein BE, Berry A, Bhaskar A, Bignell A, Blue SM, Bodine DM, Boix C, Boley N, Borrman T, Borsari B, Boyle AP, Brandsmeier LA, Breschi A, Bresnick EH, Brooks JA, Buckley M, Burge CB, Byron R, Cahill E, Cai L, Cao L, Carty M, Castanon RG, Castillo A, Chaib H, Chan ET, Chee DR, Chee S, Chen Hao, Chen Huaming, Chen J-Y, Chen S, Cherry JM, Chhetri SB, Choudhary JS, Chrast J, Chung D, Clarke D, Cody NAL, Coppola CJ, Coursen J, D'Ippolito AM, Dalton S, Danyko C, Davidson C, Davila-Velderrain J, Davis CA, Dekker J, Deran A, DeSalvo G, Despacio-Reyes G, Dewey CN, Dickel DE, Diegel M, Diekhans M, Dileep V, Ding B, Djebali S, Dobin A, Dominguez D, Donaldson S, Drenkow J, Dreszer TR, Drier Y, Duff MO, Dunn D, Eastman C, et al. (2020) Expanded encyclopaedias of DNA elements in the human and mouse genomes. *Nature* **583**:699–710.

The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium, Aaltonen LA, Abascal F, Abeshouse A, Aburatani H, Adams DJ, Agrawal N, Ahn KS, Ahn S-M, Aikata H, Akbani R, Akdemir KC, Al-Ahmadie H, Al-Sedairy ST, Al-Shahrour F, Alawi M, Albert M, Aldape K, Alexandrov LB, Ally A, Alsop K, Alvarez EG, Amary F, Amin SB, Aminou B, Ammerpohl O, Anderson MJ, Ang Y, Antonello D, Anur P, Aparicio S, Appelbaum EL, Arai Y, Aretz A, Arihiro K, Ariizumi S, Armenia J, Arnould L, Asa S, Assenov Y, Atwal G, Aukema S, Auman JT, Aure MRR, Awadalla P, Aymerich M, Bader GD, Baez-Ortega A, Bailey MH, Bailey PJ, Balasundaram M, Balu S, Bandopadhyay P, Banks RE, Barbi S, Barbour AP, Barenboim J, Barnholtz-Sloan J, Barr H, Barrera E, Bartlett J, Bartolome J, Bassi C, Bathe OF, Baumhoer D, Bavi P, Baylin SB, Bazant W, Beardsmore D, Beck TA, Behjati S, Behren A, Niu B, Bell C, Beltran S, Benz C, Berchuck A, Bergmann AK, Bergstrom EN, Berman BP, Berney DM, Bernhart SH, Beroukhim R, Berrios M, Bersani S, Bertl J, Betancourt M, Bhandari V, Bhosle SG, Biankin AV, Bieg M, Bigner D, Binder H, Birney E, Birrer M, Biswas NK, Bjerkehagen B, Bodenheimer T, et al. (2020) Pan-cancer analysis of whole genomes. *Nature* **578**:82–93.

The International Cancer Genome Consortium (2010) International network of cancer genome projects. *Nature* **464**:993–998.

Theken KN, Lee CR, Gong L, Caudle KE, Formea CM, Gaedigk A, Klein TE, Agúndez JAG, and Grosser T (2020) Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for *CYP2C9* and Nonsteroidal Anti-Inflammatory Drugs. *Clin Pharmacol Ther* **108**:191–200.

Thomas CD, Mosley SA, Kim S, Lingineni K, El Roubi N, Langae TY, Gong Y, Wang D, Schmidt SO, Binkley PF, Estores DS, Feng K, Kim H, Kinjo M, Li Z, Fang L, Chapman AB, Cooper-DeHoff RM, Gums JG, Hamadeh IS, Zhao L, Schmidt S, Frye RF, Johnson JA, and Cavallari LH (2020) Examination of Metoprolol Pharmacokinetics and Pharmacodynamics Across *CYP2D6* Genotype-Derived Activity Scores. *CPT Pharmacomet Syst Pharmacol* **9**:678–685.

Tirona RG, Lee W, Leake BF, Lan L-B, Cline CB, Lamba V, Parviz F, Duncan SA, Inoue Y, Gonzalez FJ, Schuetz EG, and Kim RB (2003) The orphan nuclear receptor HNF4 $\alpha$  determines PXR- and CAR-mediated xenobiotic induction of *CYP3A4*. *Nat Med* **9**:220–224.

Toksvang LN, Lee SHR, Yang JJ, and Schmiegelow K (2022) Maintenance therapy for acute lymphoblastic leukemia: basic science and clinical translations. *Leukemia* **36**:1749–1758.

- Tremmel R, Hofmann U, Haag M, Schaeffeler E, and Schwab M (2024) Circulating Biomarkers Instead of Genotyping to Establish Metabolizer Phenotypes. *Annu Rev Pharmacol Toxicol* **64**:annurev-pharmtox-032023-121106.
- Tremmel R, Klein K, Battke F, Fehr S, Winter S, Scheurenbrand T, Schaeffeler E, Biskup S, Schwab M, and Zanger UM (2020) Copy number variation profiling in pharmacogenes using panel-based exome resequencing and correlation to human liver expression. *Hum Genet* **139**:137–149.
- Tremmel R, Nies AT, Van Eijck BAC, Handin N, Haag M, Winter S, Büttner FA, Kölz C, Klein F, Mazzola P, Hofmann U, Klein K, Hoffmann P, Nöthen MM, Gaugaz FZ, Artursson P, Schwab M, and Schaeffeler E (2022) Hepatic Expression of the Na<sup>+</sup>-Taurocholate Cotransporting Polypeptide Is Independent from Genetic Variation. *Int J Mol Sci* **23**:7468.
- Tremmel R, Pirmann S, Zhou Y, and Lauschke VM (2023) Translating pharmacogenomic sequencing data into drug response predictions—How to interpret variants of unknown significance. *Br J Clin Pharmacol* bcp.15915.
- Tremmel R, Zhou Y, Schwab M, and Lauschke VM (2023) Structural variation of the coding and non-coding human pharmacogenome. *NPJ Genomic Med* **8**:24.
- Tseng E, Walsky RL, Luzietti RA, Harris JJ, Kosa RE, Goosen TC, Zientek MA, and Obach RS (2014) Relative Contributions of Cytochrome CYP3A4 Versus CYP3A5 for CYP3A-Cleared Drugs Assessed In Vitro Using a CYP3A4-Selective Inactivator (CYP3cide). *Drug Metab Dispos* **42**:1163–1173.
- Turner AJ, Haidar CE, Yang W, Boone EC, Offer SM, Empey PE, Haddad A, Tahir S, Scharer G, Broeckel U, and Gaedigk A (2024) Updated DPYD HapB3 haplotype structure and implications for pharmacogenomic testing. *Clin Transl Sci* **17**:e13699.
- Turner N, Tutt A, and Ashworth A (2004) Hallmarks of “BRCAness” in sporadic cancers. *Nat Rev Cancer* **4**:814–819.
- Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, Gelber RD, De Azambuja E, Fielding A, Balmaña J, Domchek SM, Gelmon KA, Hollingsworth SJ, Korde LA, Linderholm B, Bando H, Senkus E, Suga JM, Shao Z, Pippas AW, Nowecki Z, Huzarski T, Ganz PA, Lucas PC, Baker N, Loibl S, McConnell R, Piccart M, Schmutzler R, Steger GG, Costantino JP, Arahmani A, Wolmark N, McFadden E, Karantza V, Lakhani SR, Yothers G, Campbell C, and Geyer CE (2021) Adjuvant Olaparib for Patients with *BRCA1* - or *BRCA2* -Mutated Breast Cancer. *N Engl J Med* **384**:2394–2405.
- Twesigomwe D, Drögemöller BI, Wright GEB, Adebamowo C, Agongo G, Boua PR, Matshaba M, Paximadis M, Ramsay M, Simo G, Simuunza MC, Tiemessen CT, Lombard Z, and Hazelhurst S (2023) Characterization of CYP2D6 Pharmacogenetic Variation in Sub-Saharan African Populations. *Clin Pharmacol Ther* **113**:643–659.
- Twesigomwe D, Drögemöller BI, Wright GEB, Siddiqui A, da Rocha J, Lombard Z, and Hazelhurst S (2021) StellarPGx: A Nextflow Pipeline for Calling Star Alleles in Cytochrome P450 Genes. *Clin Pharmacol Ther* **110**:741–749.
- Van De Geer WS, Mathijssen RHJ, Van Riet J, Steeghs N, Labots M, Van Herpen C, Devriese LA, Tjan-Heijnen VCG, Voest EE, Sleijfer S, Martens JWM, Cuppen E, Van De Werken HJG, and Bins S (2023) Identifying somatic changes in drug transporters

- using whole genome and transcriptome sequencing data of advanced tumors. *Biomed Pharmacother* **159**:114210.
- Van Der Graaf WTA, Tesselaar MET, McVeigh TP, Oyen WJG, and Fröhling S (2022) Biology-guided precision medicine in rare cancers: Lessons from sarcomas and neuroendocrine tumours. *Semin Cancer Biol* **84**:228–241.
- van der Lee M, Allard WG, Bollen S, Santen GWE, Ruivenkamp CAL, Hoffer MJV, Kriek M, Guchelaar H-J, Anvar SY, and Swen JJ (2020) Repurposing of Diagnostic Whole Exome Sequencing Data of 1,583 Individuals for Clinical Pharmacogenetics. *Clin Pharmacol Ther* **107**:617–627.
- Van Der Lee M, Allard WG, Vossen RHAM, Baak-Pablo RF, Menafrá R, Deiman BALM, Deenen MJ, Neven P, Johansson I, Gastaldello S, Ingelman-Sundberg M, Guchelaar H-J, Swen JJ, and Anvar SY (2021) Toward predicting CYP2D6-mediated variable drug response from CYP2D6 gene sequencing data. *Sci Transl Med* **13**:eabf3637.
- Van Der Lee M, Guchelaar H-J, and Swen JJ (2021) Substrate specificity of CYP2D6 genetic variants. *Pharmacogenomics* **22**:1081–1089.
- van der Lee M, Rowell WJ, Menafrá R, Guchelaar H-J, Swen JJ, and Anvar SY (2022) Application of long-read sequencing to elucidate complex pharmacogenomic regions. *Pharmacogenomics J* **22**:75–81.
- van der Pol KH, Nijenhuis M, Soree B, de Boer-Veger NJ, Buunk AM, Guchelaar H-J, Risselada A, van Schaik RHN, Swen JJ, Touw D, van der Weide J, van Westrhenen R, Deneer VHM, Houwink EJJ, and Rongen GA (2024) Dutch pharmacogenetics working group guideline for the gene-drug interaction of ABCG2, HLA-B and Allopurinol, and MTHFR, folic acid and methotrexate. *Eur J Hum Genet EJHG* **32**:155–162.
- Van Der Velden DL, Hoes LR, Van Der Wijngaart H, Van Berge Henegouwen JM, Van Werkhoven E, Roepman P, Schilsky RL, De Leng WWJ, Huitema ADR, Nuijen B, Nederlof PM, Van Herpen CML, De Groot DJA, Devriese LA, Hoeben A, De Jonge MJA, Chalabi M, Smit EF, De Langen AJ, Mehra N, Labots M, Kapiteijn E, Sleijfer S, Cuppen E, Verheul HMW, Gelderblom H, and Voest EE (2019) The Drug Rediscovery protocol facilitates the expanded use of existing anticancer drugs. *Nature* **574**:127–131.
- Van Der Weide K, and Van Der Weide J (2014) The Influence of the CYP3A4\*22 Polymorphism on Serum Concentration of Quetiapine in Psychiatric Patients. *J Clin Psychopharmacol* **34**:256–260.
- van Eijk M, Boosman RJ, Schinkel AH, Huitema ADR, and Beijnen JH (2019) Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother Pharmacol* **84**:487–499.
- Van Leeuwen RWF, Jansman FGA, Van Den Bemt PMLA, De Man F, Piran F, Vincenten I, Jager A, Rijnveld AW, Brugma JD, Mathijssen RHJ, and Van Gelder T (2015) Drug–drug interactions in patients treated for cancer: a prospective study on clinical interventions. *Ann Oncol* **26**:992–997.
- Van 'T Veer LJ, Dai H, Van De Vijver MJ, He YD, Hart AAM, Mao M, Peterse HL, Van Der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley

- PS, Bernards R, and Friend SH (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature* **415**:530–536.
- Vasilogianni A, Al-Majdoub ZM, Achour B, Annie Peters S, Barber J, and Rostami-Hodjegan A (2022) Quantitative Proteomics of Hepatic Drug-Metabolizing Enzymes and Transporters in Patients With Colorectal Cancer Metastasis. *Clin Pharmacol Ther* **112**:699–710.
- Venook AP, Niedzwiecki D, Lenz H-J, Innocenti F, Fruth B, Meyerhardt JA, Schrag D, Greene C, O’Neil BH, Atkins JN, Berry S, Polite BN, O’Reilly EM, Goldberg RM, Hochster HS, Schilsky RL, Bertagnolli MM, El-Khoueiry AB, Watson P, Benson AB, Mulkerin DL, Mayer RJ, and Blanke C (2017) Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With *KRAS* Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. *JAMA* **317**:2392.
- Wallden B, Storhoff J, Nielsen T, Dowidar N, Schaper C, Ferree S, Liu S, Leung S, Geiss G, Snider J, Vickery T, Davies SR, Mardis ER, Gnant M, Sestak I, Ellis MJ, Perou CM, Bernard PS, and Parker JS (2015) Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. *BMC Med Genomics* **8**:54.
- Wang D, Papp AC, and Sun X (2015) Functional characterization of CYP2D6 enhancer polymorphisms. *Hum Mol Genet* **24**:1556–1562.
- Wang F, Zhang X, Wang Y, Chen Y, Lu H, Meng X, Ye X, and Chen W (2023) Activation/Inactivation of Anticancer Drugs by CYP3A4: Influencing Factors for Personalized Cancer Therapy. *Drug Metab Dispos* **51**:543–559.
- Wang S-M, Lin W-C, Lin H-Y, Chen Y-L, Ko C-Y, and Wang J-M (2021) CCAAT/Enhancer-binding protein delta mediates glioma stem-like cell enrichment and ATP-binding cassette transporter ABCA1 activation for temozolomide resistance in glioblastoma. *Cell Death Discov* **7**:8.
- Wang X, Wu J, Ye H, Zhao X, and Zhu S (2024) Research Landscape of Physiologically Based Pharmacokinetic Model Utilization in Different Fields: A Bibliometric Analysis (1999–2023). *Pharm Res*, doi: 10.1007/s11095-024-03676-4.
- Weinshilboum RM, and Sladek SL (1980) Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* **32**:651–662.
- Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, and Wrighton SA (2002) Comparative Metabolic Capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* **30**:883–891.
- Winkler EC, and Knoppers BM (2022) Ethical challenges of precision cancer medicine. *Semin Cancer Biol* **84**:263–270.
- Wojtyniak J-G, Britz H, Selzer D, Schwab M, and Lehr T (2020) Data Digitizing: Accurate and Precise Data Extraction for Quantitative Systems Pharmacology and Physiologically-Based Pharmacokinetic Modeling. *CPT Pharmacomet Syst Pharmacol* **9**:322–331.
- Wolbold R, Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, Schwab M, and Zanger UM (2003) Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* **38**:978–988.



- Wolking S, Schaeffeler E, Lerche H, Schwab M, and Nies AT (2015) Impact of Genetic Polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on Drug Disposition and Potential Clinical Implications: Update of the Literature. *Clin Pharmacokinet* **54**:709–735.
- Wong AK, Somogyi AA, Rubio J, and Philip J (2022) The Role of Pharmacogenomics in Opioid Prescribing. *Curr Treat Options Oncol* **23**:1353–1369.
- Wong M, Mayoh C, Lau LMS, Khuong-Quang D-A, Pinese M, Kumar A, Barahona P, Wilkie EE, Sullivan P, Bowen-James R, Syed M, Martincorena I, Abascal F, Sherstyuk A, Bolanos NA, Baber J, Priestley P, Dolman MEM, Fleuren EDG, Gauthier M-E, Mould EVA, Gayevskiy V, Gifford AJ, Grebert-Wade D, Strong PA, Manouvrier E, Warby M, Thomas DM, Kirk J, Tucker K, O'Brien T, Alvaro F, McCowage GB, Dalla-Pozza L, Gottardo NG, Tapp H, Wood P, Khaw S-L, Hansford JR, Moore AS, Norris MD, Trahair TN, Lock RB, Tyrrell V, Haber M, Marshall GM, Ziegler DS, Ekert PG, and Cowley MJ (2020) Whole genome, transcriptome and methylome profiling enhances actionable target discovery in high-risk pediatric cancer. *Nat Med* **26**:1742–1753.
- Worst BC, van Tilburg CM, Balasubramanian GP, Fiesel P, Witt R, Freitag A, Boudalil M, Previti C, Wolf S, Schmidt S, Chotewutmontri S, Bewerunge-Hudler M, Schick M, Schlesner M, Hutter B, Taylor L, Borst T, Sutter C, Bartram CR, Milde T, Pfaff E, Kulozik AE, Stackelberg A, Meisel R, Borkhardt A, Reinhardt D, Klusmann J-H, Fleischhack G, Tippelt S, Dirksen U, Jurgens H, Kramm CM, Bueren AO, Westermann F, Fischer M, Burkhardt B, Wossmann W, Nathrath M, Bielack SS, Fruhwald MC, Fulda S, Klingebiel T, Koscielniak E, Schwab M, Tremmel R, Driever PH, Schulte JH, Brors B, Deimling A, Lichter P, Eggert A, Capper D, Pfister SM, Jones DTW, and Witt O (2016) Next-generation personalised medicine for high-risk paediatric cancer patients - The INFORM pilot study. *Eur J Cancer Oxf Engl* **1990** **65**:91–101.
- Wray NR, Goddard ME, and Visscher PM (2007) Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Res* **17**:1520–1528.
- Xiang R, Kelemen M, Xu Y, Harris LW, Parkinson H, Inouye M, and Lambert SA (2024) Recent advances in polygenic scores: translation, equitability, methods and FAIR tools. *Genome Med* **16**:33.
- Xu Y, Liu D, and Gong H (2023) *Improving the prediction of protein stability changes upon mutations by geometric learning and a pre-training strategy*, Bioinformatics.
- Yang S-K, Hong M, Baek J, Choi H, Zhao W, Jung Y, Haritunians T, Ye BD, Kim K-J, Park SH, Park S-K, Yang D-H, Dubinsky M, Lee I, McGovern DPB, Liu J, and Song K (2014) A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. *Nat Genet* **46**:1017–1020.
- Yang W, Karol SE, Hoshitsuki K, Lee S, Larsen EC, Winick N, Carroll WL, Loh ML, Raetz EA, Hunger SP, Winter SS, Dunsmore KP, Devidas M, Relling MV, and Yang JJ (2022) Association of Inherited Genetic Factors With Drug-Induced Hepatic Damage Among Children With Acute Lymphoblastic Leukemia. *JAMA Netw Open* **5**:e2248803.
- Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, Gaedigk A, Suver C, Zhong H, Leeder JS, Guengerich FP, Strom SC, Schuetz E, Rushmore TH, Ulrich RG, Slatter JG, Schadt EE, Kasarskis A, and Lum PY (2010) Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. *Genome Res* **20**:1020–1036.

- Ye Z, Mayer J, Leary EJ, Kitchner T, Dart RA, Brilliant MH, and Hebring SJ (2023) Estimating the efficacy of pharmacogenomics over a lifetime. *Front Med* **10**:1006743.
- Yee SW, and Giacomini KM (2021) Emerging Roles of the Human Solute Carrier 22 Family. *Drug Metab Dispos Biol Fate Chem* **50**:1193–1210.
- Yu DMT, Huynh T, Truong AM, Haber M, and Norris MD (2015) ABC Transporters and Neuroblastoma, in *Advances in Cancer Research* pp 139–170, Elsevier.
- Zack TI, Schumacher SE, Carter SL, Cherniack AD, Saksena G, Tabak B, Lawrence MS, Zhang C-Z, Wala J, Mermel CH, Sougnez C, Gabriel SB, Hernandez B, Shen H, Laird PW, Getz G, Meyerson M, and Beroukhim R (2013) Pan-cancer patterns of somatic copy number alteration. *Nat Genet* **45**:1134–1140.
- Zanger UM, Fischer J, Raimundo S, Stüven T, Evert BO, Schwab M, and Eichelbaum M (2001) Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* **11**:573–585.
- Zanger UM, Momoi K, Hofmann U, Schwab M, and Klein K (2021) Tri-Allelic Haplotypes Determine and Differentiate Functionally Normal Allele CYP2D6\*2 and Impaired Allele CYP2D6\*41. *Clin Pharmacol Ther* **109**:1256–1264.
- Zanger UM, and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* **138**:103–141.
- Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF, DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ, Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen H-W, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, et al. (2017) Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* **23**:703–713.
- Zhang L, Sarangi V, Ho M-F, Moon I, Kalari KR, Wang L, and Weinshilboum RM (2021) SLCO1B1: Application and Limitations of Deep Mutational Scanning for Genomic Missense Variant Function. *Drug Metab Dispos* **49**:395–404.
- Zhang L, Sarangi V, Moon I, Yu J, Liu D, Devarajan S, Reid JM, Kalari KR, Wang L, and Weinshilboum R (2020) CYP2C9 and CYP2C19: Deep Mutational Scanning and Functional Characterization of Genomic Missense Variants. *Clin Transl Sci* **13**:727–742.
- Zhao L, Zhou S, and Gustafsson J-Å (2019) Nuclear receptors: recent drug discovery for cancer therapies. *Endocr Rev*, doi: 10.1210/er.2018-00222.

- Zhou Y, and Lauschke VM (2024) Next-generation sequencing in pharmacogenomics - fit for clinical decision support? *Expert Rev Clin Pharmacol* **17**:213–223.
- Zhou Y, and Lauschke VM (2022) Population pharmacogenomics: an update on ethnogeographic differences and opportunities for precision public health. *Hum Genet* **141**:1113–1136.
- Zhou Y, Mkrтчian S, Kumondai M, Hiratsuka M, and Lauschke VM (2019) An optimized prediction framework to assess the functional impact of pharmacogenetic variants. *Pharmacogenomics J* **19**:115–126.
- Zhu H, Wang J, Zhang Q, Pan X, and Zhang J (2023) Novel strategies and promising opportunities for targeted protein degradation: An innovative therapeutic approach to overcome cancer resistance. *Pharmacol Ther* **244**:108371.
- Zhu Z, Mu Y, Qi C, Wang J, Xi G, Guo J, Mi R, and Zhao F (2015) CYP1B1 enhances the resistance of epithelial ovarian cancer cells to paclitaxel in vivo and in vitro. *Int J Mol Med* **35**:340–348.

## XI. Footnotes

This work was supported by the NCT Molecular Precision Oncology Program, DKFZ, and DKTK; and the Robert-Bosch Foundation, Stuttgart. A patent application regarding a pharmacogenetic diagnostic pipeline for next-generation sequencing data has been filed by the authors.

## XII. Tables

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

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

<sup>#</sup> according reference GRCh37 (NC\_000022.10) and including reference (\*1) allele  
<sup>##</sup> 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

<b>In silico tools and criteria for prediction scores</b>	<b>Year of publication</b>	<b>Estimated proportional use in PGx studies over the last five years<sup>#</sup></b>	<b>Reference</b>
<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% <sup>o</sup>	(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)

<sup>#</sup>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 <sup>#</sup>	Class	Somatic target	Clinically associated germline or somatic variants <sup>##</sup>		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	<b>EGFR (Resistance mutation T790M, rs121434569)</b> , 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	<b>EGFR (Somatic testing)</b> , 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

Downloaded from pharmgkb.com at ASPET Journals on December 20, 2024

			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, <b>FCGR3A (Reduced response, rs396991)</b> , 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 ( <b>risk of toxicity, rs7557402</b> ), 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	

Downloaded from pharmrev.aspenjournals.com at ASPET Journals on December 20, 2024



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

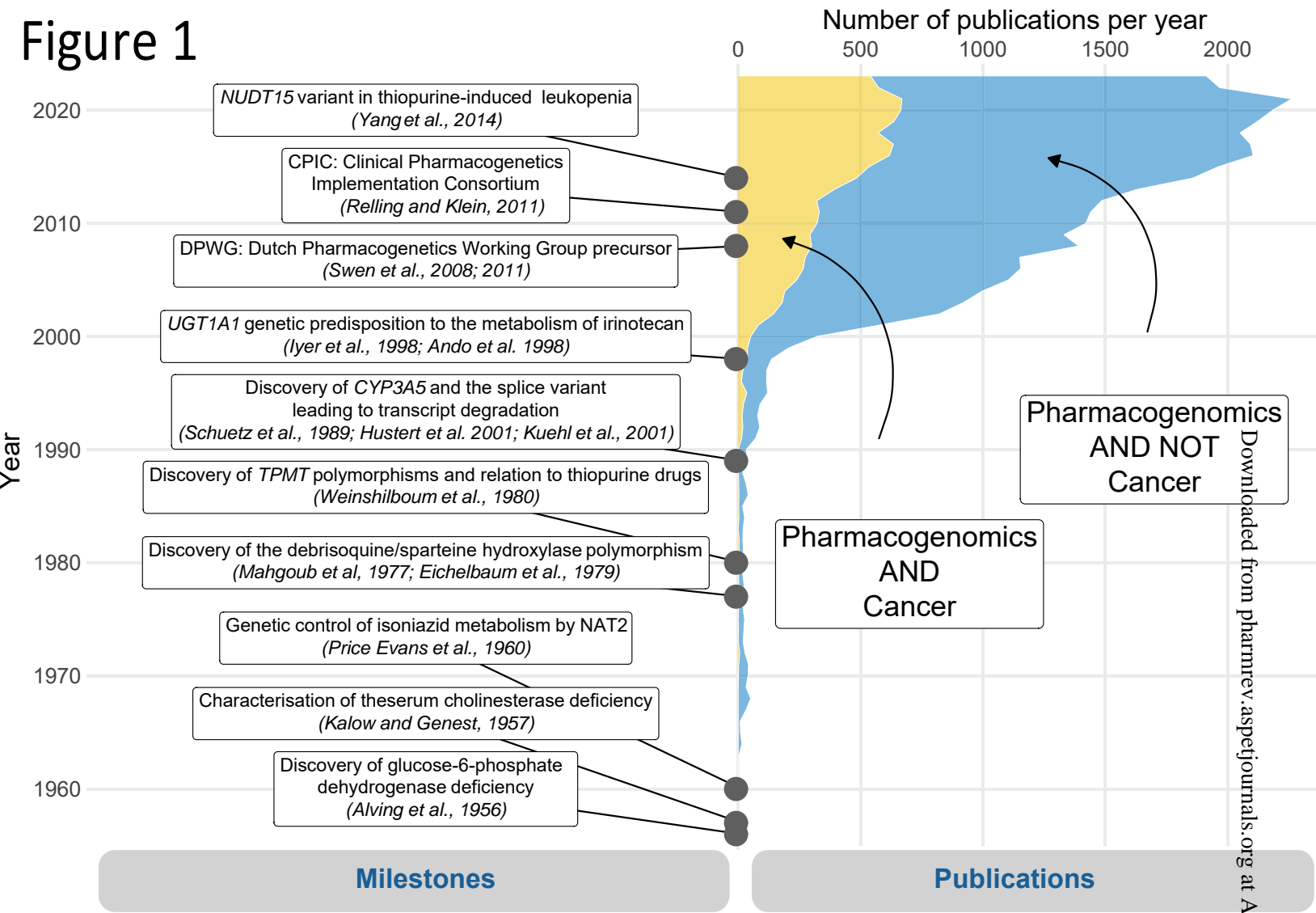
identified, allowing predictions regarding treatment response and risk of toxicities. **B.** The progression from molecular testing via multidisciplinary tumor boards involving experts from various fields, resulting in a drug treatment recommendation. Abbreviations: SNV: single nucleotide variant, CNV: 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

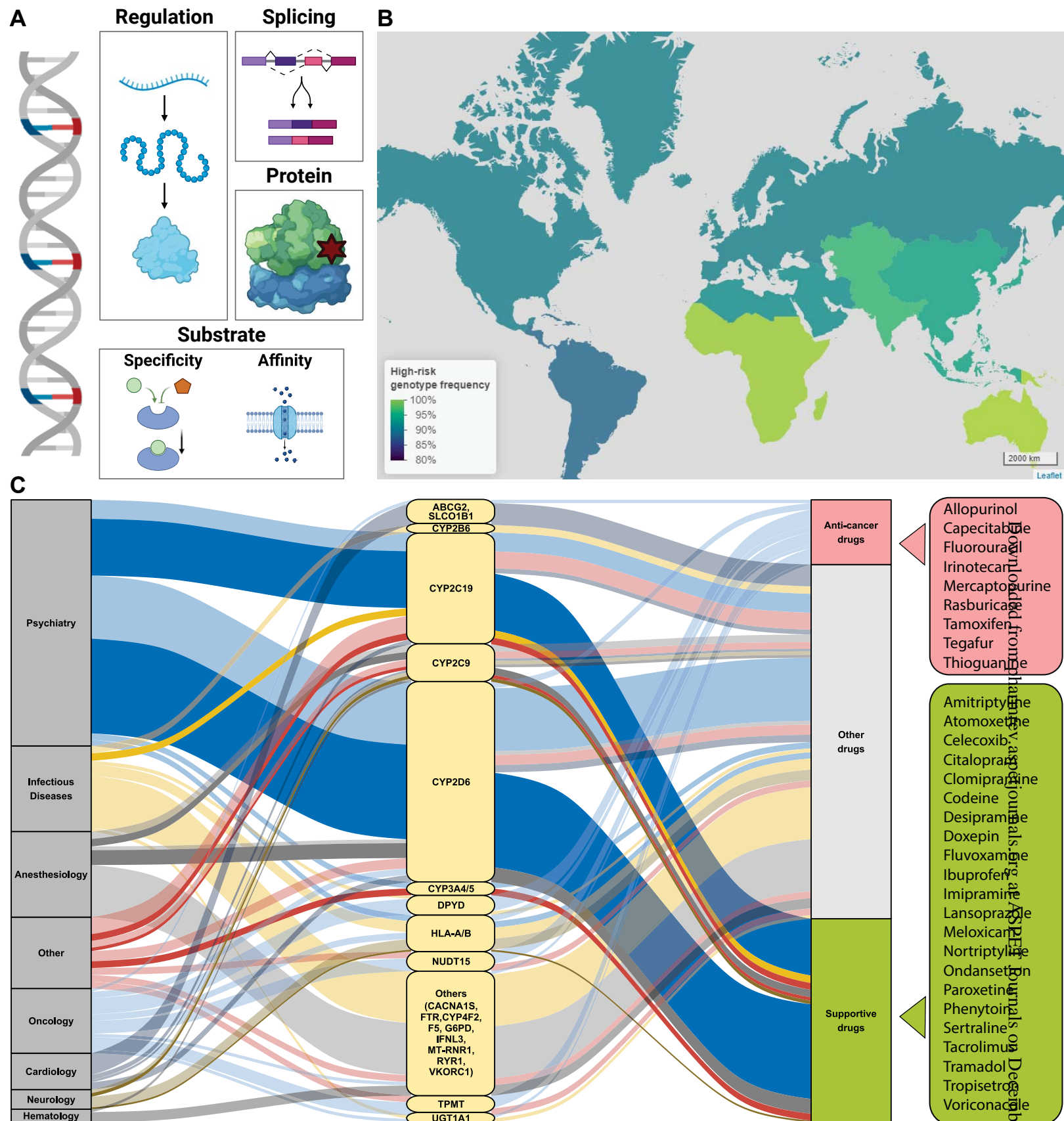
toxicities was analyzed in 442 patients genotyped for the *DPYD* variants (\*2A, \*13, c.2846A>T (p.D949V), c.1236G>A (rs56038477, proxy for *HapB3* (c.1129-5923C>G)). The incidence was estimated as 1-Kaplan-Meier survival estimate, and log-rank test was used to compare groups. Grade 3+ toxicities occurred 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/m<sup>2</sup> weekly or 150 mg/m<sup>2</sup> 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/m<sup>2</sup> + FU, FOLFOX: oxaliplatin + FU, IROX: oxaliplatin + IRN 200 mg/m<sup>2</sup>) (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



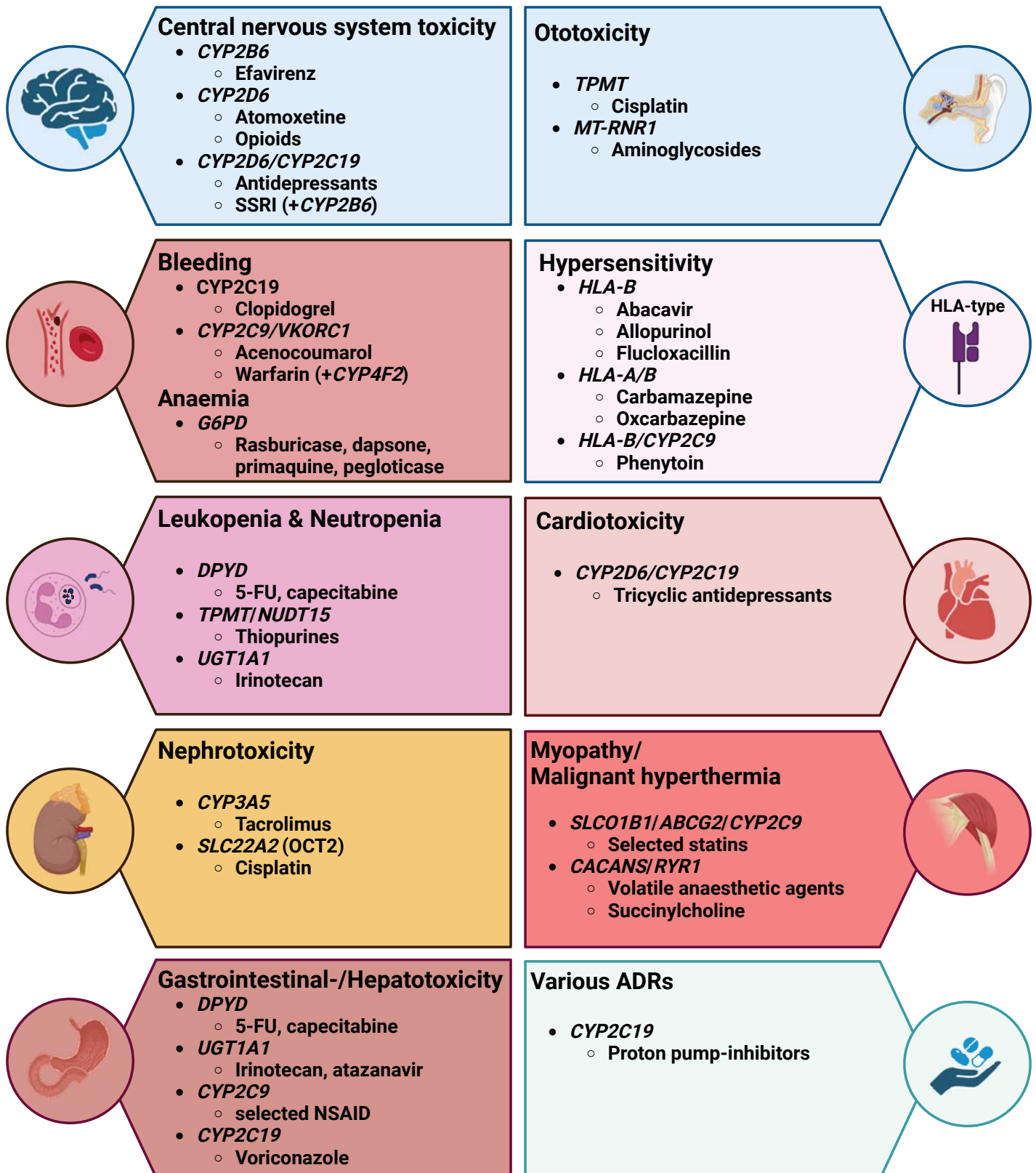
# Figure 2



Downloaded from https://academic.oup.com/ajph/article/114/12/2024/7248787 by guest on December 20, 2024

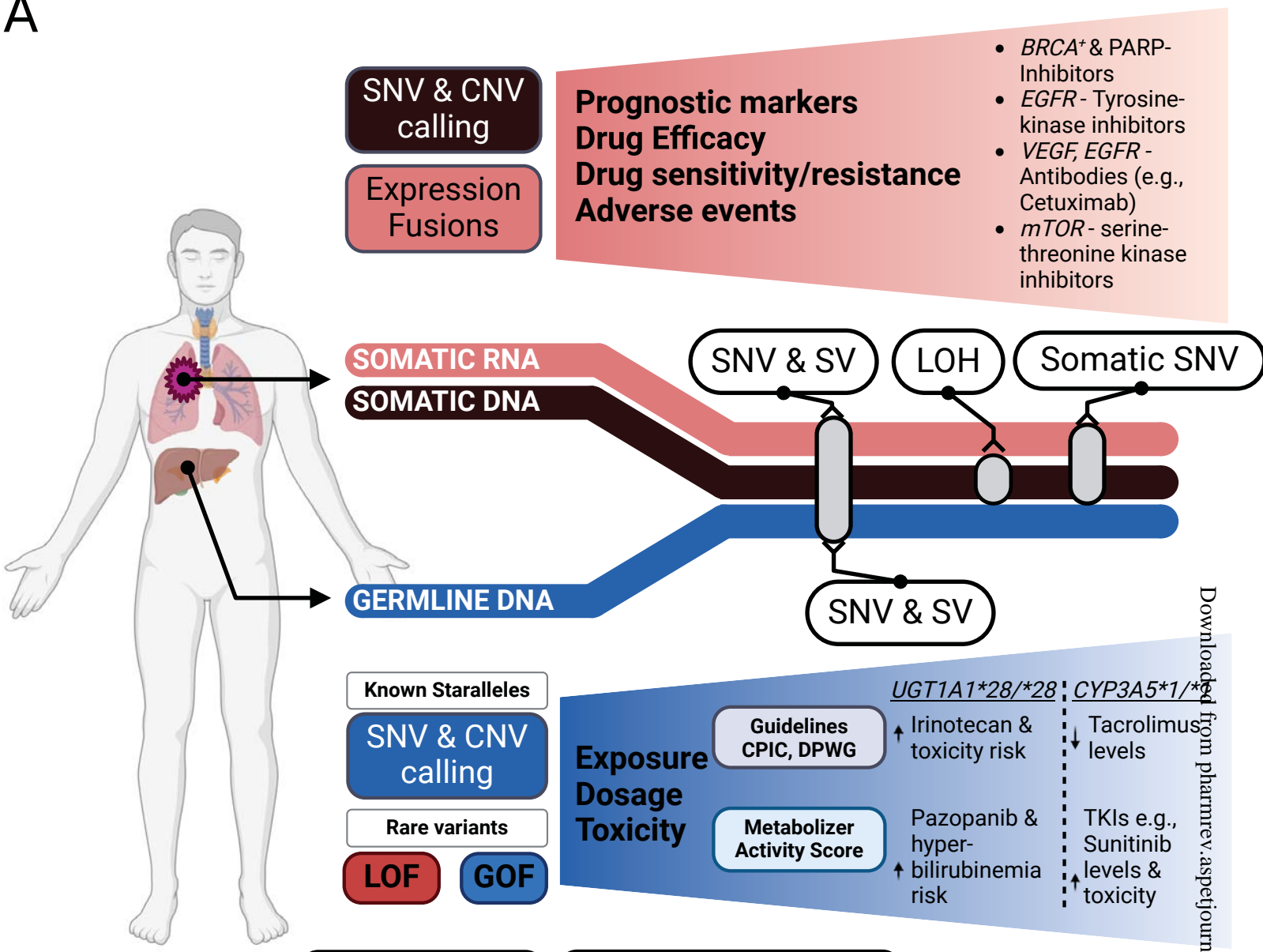
# Figure 3

## Documented PGx biomarker for organ toxicities



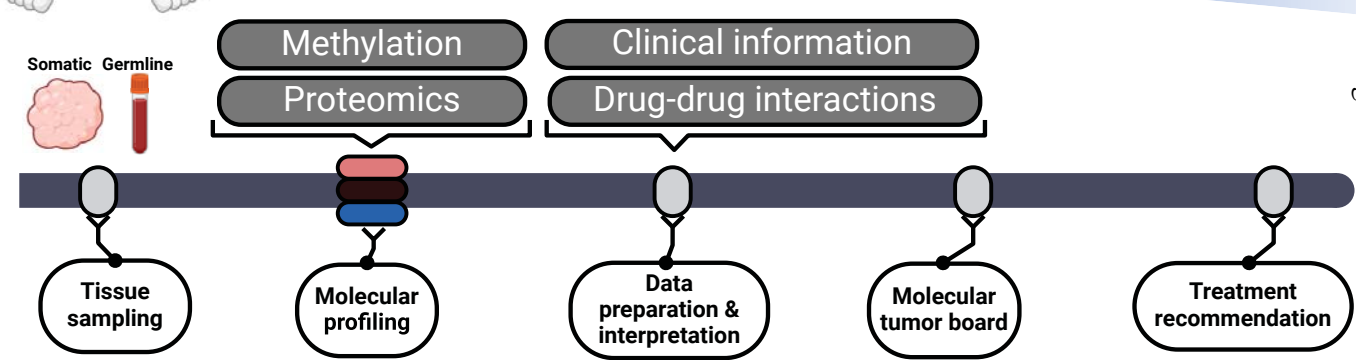
# Figure 4

A



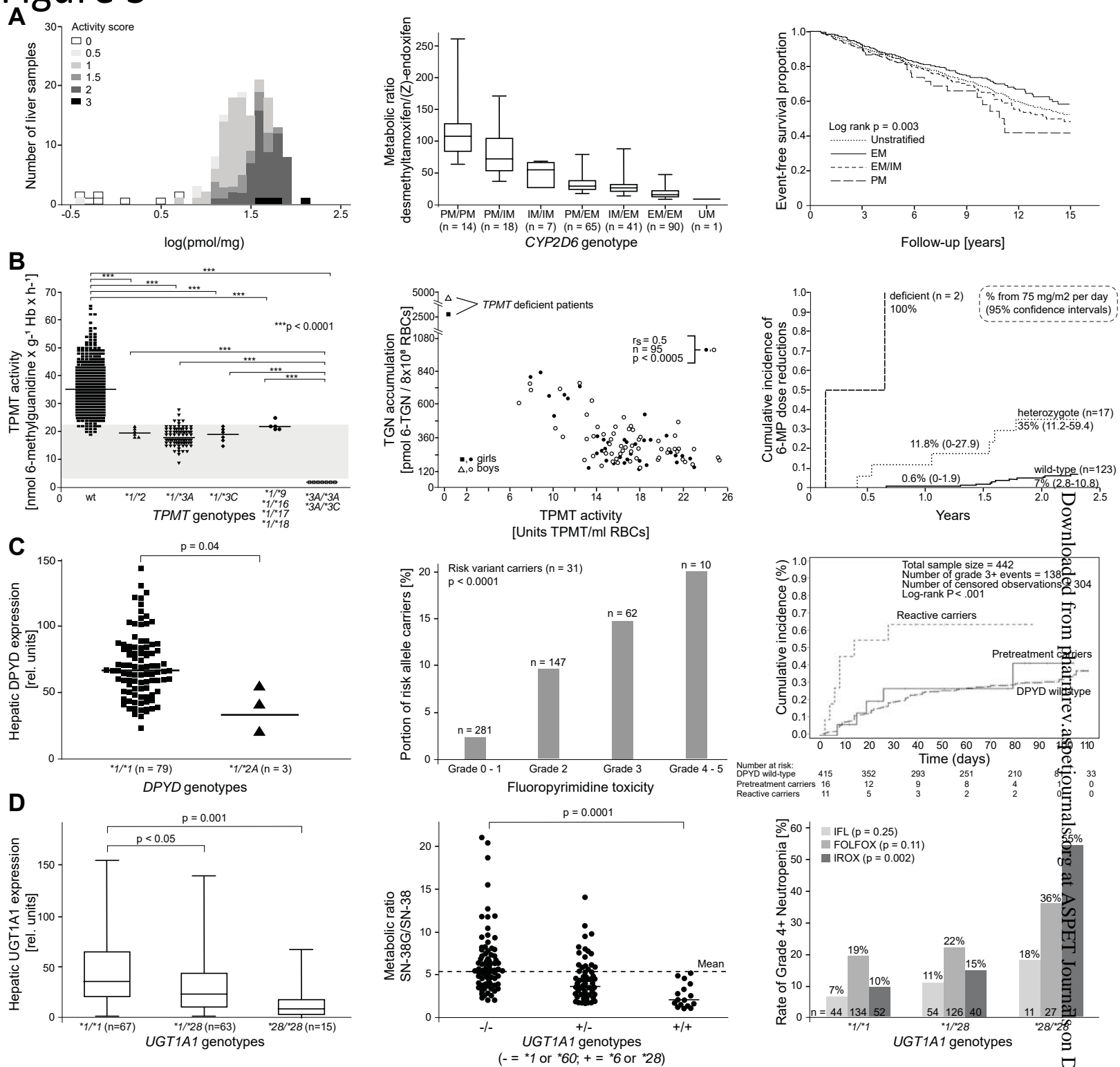
Downloaded from pharmrev.aspetjournals.org at ASPET Journals on December 20, 2024

B





# Figure 5



# Figure 6

