Chapter 6
On the Verge of Impossibility: Accounting for Variability Arising from Permutations of Comorbidities that Affect the Fate of Drugs in the Human Body

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Abstract Contending with variability in drug exposure and effect in disease populations requires patient characterization for changes in drug metabolism and transport pathways and predictive modelling platforms within the framework of systems pharmacology. In this chapter, we explore current and emerging patient characterization approaches, the role of physiologically based pharmacokinetic modelling in stratified versus individualized predictions, the possibility of exploring the impact of permutations of comorbidities, and application of these elements in model-informed precision dosing.

Keywords Variability · Drug metabolism and disposition · In vitro–in vivo · Extrapolation (IVIVE) · Physiologically Based Pharmacokinetics (PBPK) · Quantitative proteomics · Disease perturbation

6.1 Introduction

> "*Variability is the law of life*, and as no two faces are the same, so no two bodies are alike, and no individuals react alike and behave alike under the abnormal conditions which we know as disease" – Sir William Osler (1849–1919), Professor of Medicine, Oxford, England

Current drug development mainly focuses on 'typical' representation of patients and many of the subtypes involving other comorbidities are studied at later stages after regulatory approval. The latter does not provide any evidence for potential

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requirements of dosage adjustment that might be necessary for effective and safe medication of patients with certain comorbidities or combinations of these. Hence, in recent years, drug regulatory agencies, as well as professional associations related to drug development and pharmacotherapy, advocated widening the recruitment criteria during clinical studies to provide information on the fate of drugs beyond what is known in a 'typical patient'. Two recent Guidance for the Industry documents issued by the US Food and Drug Administration (FDA) concerning "Enhancing the Diversity of Clinical Trial Populations — Eligibility Criteria, Enrollment Practices, and Trial Designs" [41] and "Diversity Plans to Improve Enrollment of Participants from Underrepresented Racial and Ethnic Populations in Clinical Trials" [42] are typical examples of such attempts. Although widening recruitment improves gathering of information, and subsequent data analysis can highlight some of the significant changes using sparse samples and non-linear mixed-effect models (so-called population pharmacokinetics or POP-PK), these are not a panacea for the huge lack of data in special populations that may suffer from more than one or two comorbidities. Requesting conduct of clinical studies for every given permutation of concurrent comorbidities places such an act on the verge of impossibility for any drug development entity. However, we cannot leave patients in such special groups without sound and scientific decisions on the best dosage regimen for a given drug and permit off-label use to become the norm for these patients.

The solution may lie within the so-called mechanistic models framing the fate of drugs. These are known as physiologically based pharmacokinetic (PBPK) models and they can accommodate and propagate the physiological and pathological changes in bodily systems to consequences for any given drug if the interplay of parameters with the drug is adequately characterized using in vitro systems. Even in the case of known comorbidities, such as organ impairment (mainly renal or hepatic), which are assessed much more often than other conditions regarding their impact on the fate of drugs, Jadhav et al. [49] reported in 2015 that over 50% of drugs released onto market did not have any information on the impact of severe impairment. These authors as well as others went on to say that PBPK models can fill the void in such conditions. The situation with the void of information on subpopulations has not improved since the report by Jadhav et al. in 2015 as evidenced by our internal unpublished data that demonstrate the case for renal impairment patients (Fig. 6.1). In this chapter, we explore the role of PBPK, in conjunction with existing and emerging patient characterization approaches, in addressing this lack of dosing information for special populations.

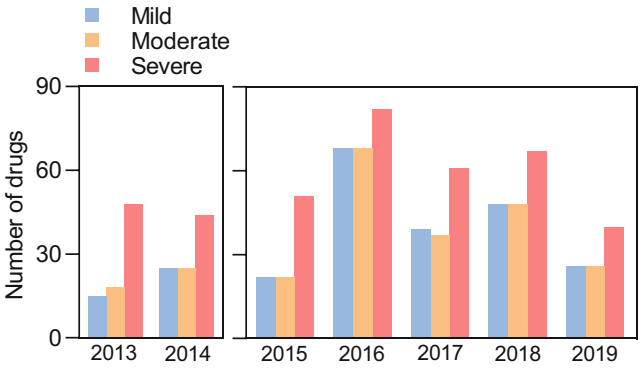

Fig. 6.1 The number of FDA-approved drugs without explicit dosing recommendation for patients with renal impairment at the point of entry to the market. The plot shows data (for 2013 and 2014) from Jadhav et al. [49] and unpublished in-house data (for 2015–2019)

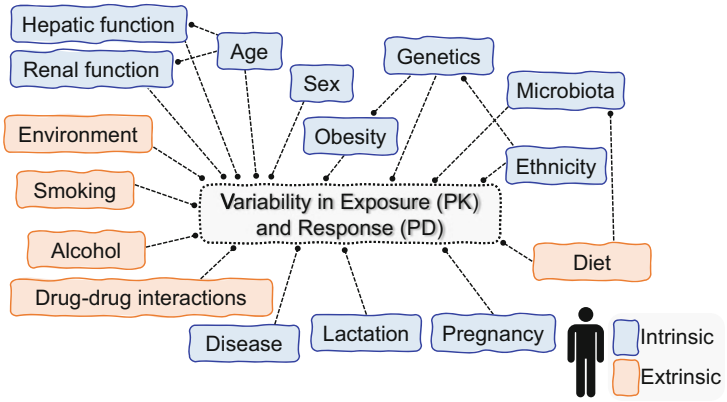

Fig. 6.2 Factors intrinsic and extrinsic to the patient which affect variability in drug exposure and response. Factors are inter-dependent making accounting for their effect challenging. The use of modelling allows prediction of the effect of different permutations of such factors. Abbreviations: *PD* pharmacodynamics, *PK* pharmacokinetics

6.2 Accounting for Sources of Interindividual Variability in Pharmacokinetics

Numerous internal and external factors, with complex interplay, affect between-patient variability in drug kinetics (Fig. 6.2); these have an impact on patient physiology, biology, and expression of proteins involved in drug disposition. Other factors unrelated to patient biology, such as compliance, can also add to the apparent PK variability [68]. Quantitative assessment of variability in the fate of drugs in the body due to these factors follows mathematical formalism of pharmacokinetics. This can be in the form of simple equations that describe temporal

changes of drug concentration after dosing. Finding the covariates that define interindividual differences in the various model parameters is an a posteriori activity within these models (e.g. using POP-PK methods for sparse samples that employ non-linear mixed-effect methods). However, predicting such individual variations in concentration–time profiles in advance of conducting clinical studies requires more mechanistic models in the form of physiologically based pharmacokinetics (PBPK) [19].

Adequate drug exposure, as defined by the area under the curve of the concentration-time profile (AUC) or either maximum or minimum exposure, respectively defined by the highest concentration (C_{max}) or trough concentration (C_{trough}), is an essential element of reaching therapeutic response. Together, absorption, distribution, metabolism, and excretion (ADME) of drugs determine the features of the concentration-time profile following drug administration. Data generated from in vitro studies are used to determine and understand ADME variations in different individuals by integration of data with PBPK models [80, 81]. However, the multi-scale nature of these mechanistic models [96] necessitates large efforts and wide expertise to create and verify the model elements, putting PBPK under the framework of systems pharmacology/biology [51] that requires drug-independent systems data, as enlisted below [52].

- Physiological, anatomical, biological, and biochemical data for each individual (some are defined based on demography, such as ethnicity, sex, age, and environment of the population that an individual belongs to when the actual individual values are not known).
- Trial design parameters, such as the conditions under which the drug is taken (e.g. fed versus fasted state) or any concomitant drugs interfering with the functions of the systems that handle the drug (e.g. perturbing enzyme expression or function).

The above are combined with drug data (physicochemical properties, e.g. LogP and pKa, drug intrinsic clearance by certain enzymes, affinity to certain transporters) to help not only understand but also predict the behaviour of the drug in certain individuals or a subgroup of patients using a realistic compartmental structure defined as a set of differential equations. Critical considerations are listed below.

- The factors affecting the variability of the absorption and bioavailability of orally administered drugs are described previously [53]. It is important to note that cytochrome P450 (CYP) 3A and multidrug resistance P-glycoprotein (P-gp), which have wide interindividual variability, are present at high levels in the villi tips of enterocytes in the small intestine [6] and they can cause variations in the bioavailability of drugs, as shown for tacrolimus controlled-release formulation in the case of Afro-Americans versus their Caucasian counterparts [60, 91]. Variations in these proteins as well as other CYP and non-CYP enzymes and transporters in the small intestine are demonstrated in disease states, such as Crohn's disease [9] and can play a significant role in altering the fate of drugs in such patients [10].

- Early screening tools can assess the relative importance of the routes of metabolism by various metabolic pathways. Hence, it is now possible to employ information on in vivo intrinsic clearance as well as transporter-mediated uptake to postulate about variability associated with hepatic clearance in human populations [80]. This is facilitated with knowledge of scaling factors [1, 2, 13, 67].
- Aspects defining variations in renal excretion are also formulated under systems pharmacology [83, 84] and capture the role of urine flow and pH alongside the physical chemistry, lipophilicity, and ionization of the compound that define plasma protein and erythrocyte binding and add knowledge of drug affinity to efflux transporters [85] and abundance of such transporters in human kidney [7].
- Variability in volume of distribution does not have an impact on overall exposure (as measured by AUC_0). However, it defines the shape of the temporal changes of the concentration-time profiles (C_{max} and C_{trough}); hence, defining/predicting its variability is important. The physical volume of tissues and their blood flows are components of PBPK models that capture population variability related to these parameters. Nonetheless, there are other aspects of the volume of distribution which are more relevant to protein binding in the systemic circulation as well as tissues. Many of these can be measured in vitro and used for in vitro–in vivo extrapolation (IVIVE) purposes through PBPK models [16, 72, 75].

6.3 The Growing Role of Physiologically Based Pharmacokinetics (PBPK)

In a recent survey, El-Khateeb et al. [37] demonstrated that the first two decades of the twenty-first century have witnessed a more than 40-fold increase in the applications of PBPK (based on the number of publications in the literature). This was in contrast to the general discipline of pharmacokinetics which had a relatively modest increase of around fourfold, in line with an increase in the bulk of scientific publications by threefold. The fastest growing area of PBPK applications according to the survey was focused on addressing alterations to kinetics (or lack thereof) in special populations. Indeed, this was one of the areas that regulatory scientists advocated for the use of PBPK over a decade ago [101] by harnessing the natural compatibility between PBPK and assessment of internal/external factors affecting the kinetics of drugs in various patients.

So, what are the attributes of PBPK that make it so popular with determining the impact of patient variability? The essence of PBPK modelling was described by Rostami-Hodjegan [77] in relation to separation of the system parameters from those of drugs and formulations (Fig. 6.3). Therapeutic effects of a minority of drugs can be monitored relatively easily using established biomarkers (e.g. international normalized ratio, INR, for anticoagulants, blood pressure for antihypertensive agents, and blood glucose for antidiabetic agents) for dose adjustment. However, for most

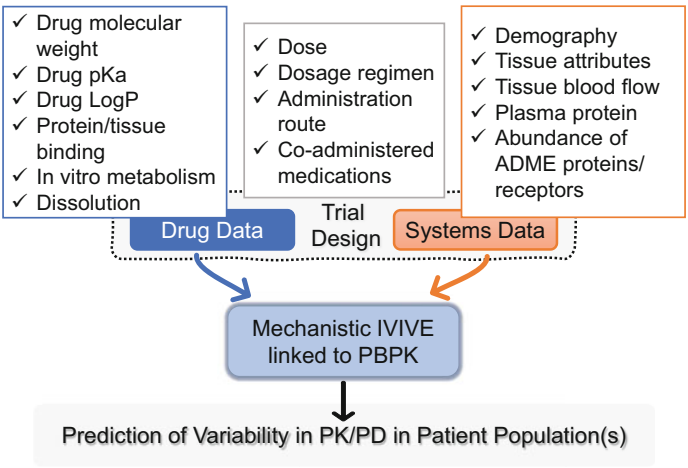

Fig. 6.3 Separation of parameters related to the drug from parameters related to the population in PBPK models. This approach enables testing different permutations of factors, allowing assessment of changes in PK (or PD) in the target population a priori to conducting clinical studies. Abbreviations: ADME, absorption, distribution, metabolism and excretion; IVIVE, in vitro–in vivo extrapolation; PBPK, physiologically based pharmacokinetics; PD, pharmacodynamics; PK, pharmacokinetics

drugs, such effects are not readily measurable or finding out the outcome takes a long time (e.g. patient survival). On the other hand, accounting for drug exposure differences can minimize a large part of the variation in patient outcomes that is related to kinetics. One of the major sources of variability in kinetics is related to interindividual differences in metabolic and transporter-mediated clearance. Clearance and first pass gut and liver metabolism together define internal exposure of the bioavailable dose after entering the gut wall. Many drugs have an optimal therapeutic window for exposure. Whereas for renal clearance, creatinine can be used as a general marker for glomerular filtration as well as active secretion of the drugs into the urine, hepatic clearance does not have a single universal marker that can be applied to all drugs. Characterization of metabolism becomes very important in various groups of patients when we consider that >70% of 698 orally administered marketed drugs have high levels of metabolism as part of their clearance [15]. As shown by Rostami-Hodjegan and Tucker [80], PBPK models can readily incorporate the known variations in drug metabolism and propagate them to projected clearance values using IVIVE techniques. Availability of liver [93, 99], intestinal [9, 33], brain [5, 17, 87], kidney [7, 57], skin [29], and lung [40] tissue for conducting quantitative analysis of proteins related to drug ADME has contributed to advancing a priori understanding of likely differences in kinetics in special populations before conducting any clinical studies. Table 6.1 summarizes prominent examples of such applications. With this approach, and if the baseline in healthy adults (or other control cohorts) is established, it is possible to simulate kinetics in special populations, such as foetal exposure to medications taken by pregnant mothers, or

Table 6.1 Prominent examples of recent applications of tissue proteomics in the investigation of disease impact on human drug metabolism and transport pathways

Disease/Condition	Tissue	Targets quantified	Drugs in PK simulations	Proteomic methods	Applications	References
Liver disease						
Cirrhosis (CP scores A-C)	Liver	14 CYPs, 9 UGTs, 8 non-CYP non-UGT enzymes, 19 transporters	Repaglinide, dabigatran etexilate, zidovudine	Targeted proteomics	Effect of disease; dose adjustment	El-Khateeb et al. [38]
Cirrhosis	Liver	7 CYPs, 4 UGTs, 6 non-CYP non-UGT enzymes	Zidovudine[a], morphine[a]; lamotrigine[b]	Targeted proteomics	Effect of disease; dose adjustment	Prasad et al. [74], Ladumor et al. [62]
Cirrhosis	Liver	12 transporters	Repaglinide[c]; rosuvastatin[d]	Targeted proteomics	Effect of disease; dose adjustment	Wang et al. [98], Kumar et al. [58]
Hepatitis C-induced, alcoholic, autoimmune, and cholestatic liver diseases	Liver	14 transporters	–	Targeted proteomics	Effect of disease	Drozdzik et al. [34]
Hepatitis C-induced, alcoholic, autoimmune, and cholestatic liver diseases	Liver	10 CYPs, 4 UGTs	–	Targeted proteomics	Effect of disease	Drozdzik et al. [35]
Liver disease (metastatic liver cancer, cirrhosis)	Liver (cancer set: tumorous and histologically normal tissue)	13 CYPs, 10 UGTs, 8 non-CYP non-UGT enzymes, 18 transporters	–	Targeted and global proteomics	Effect of disease	Vasilogianni et al. [93]
Metastatic liver cancer	Liver (tumorous and histologically normal tissue)	14 CYPs, 8 UGTs, 25 transporters	50 substrates (Simcyp library)	Targeted proteomics	Effect of disease; dose adjustment	Vasilogianni et al. [94]
		9 CYPs, 4 UGTs	–		Effect of disease	

(continued)

Table 6.1 (continued)

Disease/Condition	Tissue	Targets quantified	Drugs in PK simulations	Proteomic methods	Applications	References
Metastatic liver cancer	Liver (histologically normal tissue)			Targeted proteomics		Kurzawski et al. [59]
Primary liver cancer	Liver	10 transporters	–	Targeted proteomics	Effect of disease	Billington et al. [17]
Hepatitis C-induced liver disease	Liver	15 transporters	–	Targeted proteomics	Effect of disease	Drozdzik et al. [36]
NAFLD/NASH with type 2 diabetes mellitus	Liver	CYP3A4	Midazolam	Global proteomics	Effect of comorbidity on CYP3A substrates	Jamwal et al. [54]
Wilson's disease	Liver	10 CYPs, 4 UGTs, 16 transporters	–	Targeted proteomics	Effect of disease	Szeląg-Pieniek et al. [90]
Kidney disease						
Kidney cancer	Kidney cortex (histologically normal)	9 enzymes, 22 transporters	–	Targeted and global proteomics	Quantitative expression in disease; local kinetics in kidney	Al-Majdoub et al. [7]
Kidney cancer	Kidney cortex (histologically normal)	20 transporters	–	Targeted proteomics	Effect of disease on renal tissue expression	Oswald et al. [69]
Kidney cancer	Kidney cortex (histologically normal)	12 transporters	Metformin[e]	Targeted proteomics	Regional expression of transporters; IVIVE of renal secretory clearance	Prasad et al. [73], Kumar et al. [57]

Intestinal disease						
Crohn's disease	Ileum, colon	13 CYPs, 5 UGTs, 28 non-CYP non-UGT enzymes, 58 transports	Verapamil[f], digoxin[f], rosuvastatin[f]	Global proteomics	Effect of (inflamed and non-inflamed) disease; changes in oral bioavailability	Alrubia et al. [9], Alrubia et al. [10]
Ulcerative colitis	Colon	14 transporters	–	Targeted proteomics	Effect of disease and inflammation on enzymes[i] and transporters	Erdmann et al. [39]
Neuropathology						
Dementia (Alzheimer's disease, dementia with Lewy bodies)	Brain cortex (frontal lobe)	53 transporters	–	Targeted and global proteomics	Effect of disease on blood-brain barrier (BBB) transporters	Al-Majdoub et al. [5]
Alzheimer's disease	Hippocampus, parietal lobe, cerebellum	4 transporters and 1 receptor (LRP1)	–	Targeted proteomics	Effects of disease on BBB transporters in different regions of the brain	Storelli et al. [89]
Glioblastoma	Brain cortex	6 transporters	Fexofenadine	Targeted proteomics	Effect of disease on brain drug exposure	Bao et al. [14]
Epilepsy	Brain cortex	5 transporters	–	Targeted proteomics	Effects of epilepsy on transporter abundance and vasculature in the brain	Brukner et al. [22]
Epilepsy	Brain cortex	7 enzymes and 19 transporters	–	Targeted proteomics	Effect of disease on BBB transporters	Shawahna et al. [87]

(continued)

Table 6.1 (continued)

Disease/Condition	Tissue	Targets quantified	Drugs in PK simulations	Proteomic methods	Applications	References
Obesity						
	Liver	OATP1B1, OATP1B3, OATP2B1, NTCP	Rosuvastatin	Global proteomics	Prediction of individual drug exposure	Wegler et al. [99]
	Jejunum	CYP3A4, UGT2B7, P-gp, MRP2, MRP3	Morphine	Targeted proteomics	Oral kinetics in obesity	Lloret-Linares et al. [64]
	Jejunum	13 CYPs, 9 UGTs, 29 transporters	–	Targeted proteomics	Effect of disease on enzymes and transporters	Miyauchi et al. [66]
	Liver, jejunum	CYP3A4	Midazolam	Global proteomics	Dose individualization using 4-β-hydroxycholesterol in obesity	Kvitne et al. [61]
	Liver, jejunum	32 CYPs, 14 UGTs, 58 non-CYP non-UGT enzymes, 199 transporters	–	Global proteomics	Effect of obesity on expression profiles in liver and intestine	Wegler et al. [100]
Pregnancy						
Drug effects on foetus	Placenta	7 transporters	Nelfinavir[g], efavirenz[g], imatinib[g]; dexamethasone[h], betamethasone[h], darunavir[h], lopinavir[h]	Targeted proteomics	Prediction of foetal drug exposure	Anoshchenko et al. [11], Peng et al. [70], Anoshchenko et al. [12]

Paediatric disease						
Biliary atresia	Liver	21 ABC transporters	–	Global proteomics	Effect of biliary atresia on hepatobiliary transporter expression	Al-Majdoub et al. [8]

ABC ATP-binding cassette, *CP score* Child-Pugh score, *CYP* cytochrome P450, *UGT* UDP-glucuronosyltransferase, *P-gp* P-glycoprotein, *MRP* multidrug resistance protein, *OATP* organic anion transporting polypeptide, *NTCP* sodium-taurocholate co-transporting polypeptide, *LRP1* LDL receptor-related protein 1, *IVIVE* in vitro–in vivo extrapolation, *PK* pharmacokinetics, *NAFLD* non-alcoholic fatty liver disease, *NASH* non-alcoholic steatohepatitis

[a]Simulations reported in Prasad et al. [74]

[b]Simulations reported in Ladumor et al. [62]

[c]Simulations reported in Wang et al. [98]

[d]Simulations reported in Kumar et al. [58]

[e]Simulations reported in Kumar et al. [57]

[f]Simulations reported in Alrubia et al. [10]

[g]Simulations reported in Peng et al. [70]

[h]Simulations reported in Anoshchenko et al. [12]

[i]Quantification of enzymes (5 CYPs, 4 UGTs) and inflammation markers was done using transcriptomic methods

in disease groups, such as patients with hepatic impairment [67]. While availability of human samples for these applications is certainly increasing, access is still restricted by ethical and logistic obstacles, with samples largely limited to post-mortem or surgical surplus tissue.

The principles of PBPK can also be extended to propagation of interindividual variability to drug pharmacodynamics (PD) within the framework of quantitative systems pharmacology [25]. PBPK-PD models are mainly used to predict drug effects in special populations (e.g. predicting dental analgesic effect of ibuprofen in children [30]) and PD effects of drug-drug interactions (DDI) (e.g. the impact of coadministration of domperidone and ketoconazole on QT prolongation in the electrocardiogram of patients [65]). Application of quantitative proteomics to monitoring changes in drug receptors and other PD targets, such as the insulin receptor (INSR) in the human blood–brain barrier [92] and receptor tyrosine kinases in human metastatic liver cancer from colon [95], is expected to facilitate modelling of drug concentration–effect relationships in special/disease populations.

6.4 Predicting Pharmacokinetics in Subgroups of Patients Versus Predictions in an Individual

Despite advances made in the prediction of changes that occur in pharmacokinetics in subgroups of patients, predicting the fate of drugs in a specific individual who may not be the average patient in his or her subgroup requires characterization of changes that happen in ADME proteins in that particular individual as opposed to the average person in the relevant subgroup. Figure 6.4 summarizes current and emerging characterization methods.

Genotyping can identify the bracket of the pharmacogenetic subgroup for an individual patient, which is then linked to a specific activity score, such as the case of CYP2D6 genotype [44]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) [23] has published several guideline reports demonstrating the value of such tests in managing optimal dosing for many drugs, e.g. tacrolimus (CYP3A5 genotype), clopidogrel (CYP2C19 genotype), and efavirenz (CYP2B6 genotype) [18, 32, 86]. CPIC guidelines typically offer recommendation of dose adjustment, the use of therapeutic drug monitoring or consideration of alternative therapeutic agents for each genotype group. However, there are wide population variations in the activity of proteins encoded by the same gene, and indeed, some ADME proteins with large population variability in abundance and activity do not have known genotypes that correlate with changes in activity. Hence, endogenous biomarkers and exogenous probes have been used to characterize patients regarding sets of important ADME pathways (e.g. cocktails of drug substrates). Established probe cocktails include the Geneva cocktail (6 enzymes and 1 transporter [20]), the Cooperstown 5 + 1 cocktail (5 enzymes [24]), the Karolinska cocktail (5 enzymes [26]), and the Pittsburgh cocktail (5 enzymes [43]). The issue with these biomarkers

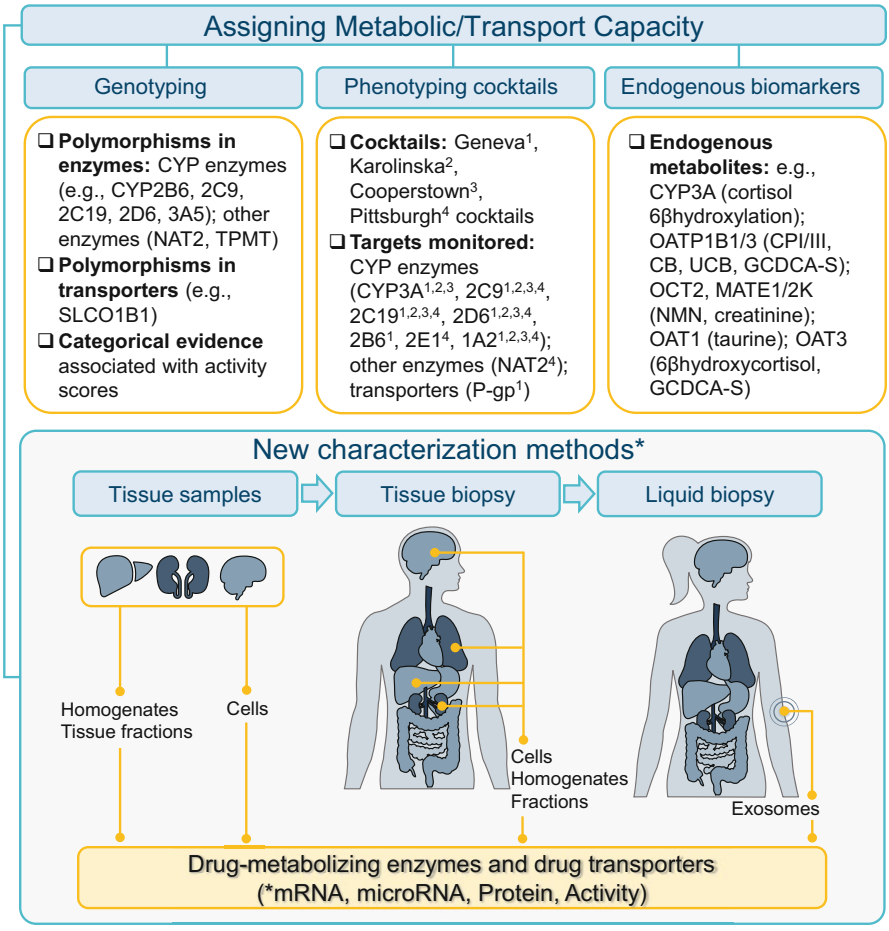

Fig. 6.4 Methods used for the characterization of drug-metabolizing and transporting pathways. Traditionally used methods include genotyping of polymorphic enzymes/transporters, characterization with specific probes (in cocktails administered orally) or the use of endogenous biomarkers for enzyme and transporter activity. More recent methods assess the expression/activity of enzymes and transporters in human samples (either from surgical surplus or post-mortem), in tissue biopsies (from individual patients), or in liquid biopsies (tissue-shedded exosomes). The measurements require modelling platforms for prediction of drug exposure and response. Abbreviations: *CB* conjugated bilirubin, *CPI/III* coproporphyrin I and III, *CYP* cytochrome P450, *GCDCA-S* glycochenodeoxycholate-3-O-sulphate, *MATE1/2 K* multidrug and toxin extrusion protein 1 and 2 K, *NAT2* N-acetyltransferase 2, *NMN* N1-methylnicotinamide, *OATP1B1/3* organic anion transporting polypeptide 1B1 (gene name SLCO1B1) and 1B3, *OAT1/3* organic anion transporter 1 and 3, *OCT2* organic cation transporter 2, *P-gp* P-glycoprotein, *TPMT* thiopurine methyltransferase, *UCB* unconjugated bilirubin. Under phenotyping cocktails, superscript numbers indicate the pathways each cocktail can monitor

is their limited scope which does not cover all relevant pathways of metabolism and transport for the range of clinically used drugs and the specificity of several substrates shows considerable overlap. Whereas tissue proteomics is able to address the quantitative nature of ADME/PD proteins for large sets of targets (a few thousand proteins in the same experiment), obtaining tissue from donors is fraught with ethical and logistic challenges. Hence, the recently introduced possibility of using liquid biopsy offers a more practical alternative for characterization of patients as an input compatible with PBPK models.

Liquid biopsies are biofluids sampled from a patient for diagnostic, companion diagnostic or therapeutic applications. Exosomes shedded by tissue into a biofluid offer a snapshot of the cellular biomolecular pool of macromolecules, which reflect the functional state of their tissue of origin (Fig. 6.5). The vesicles (30–150 nm in size) enclose DNA, (non-coding, messenger and micro) RNA, and (transmembrane and non-membrane) proteins, offering protection from degradation, and therefore longer half-lives of cargo molecules in systemic circulation [21]. 'Omics' analysis generates quantitative data for the cargo of extracted exosomes and the levels are linked to the abundance/activity of corresponding proteins in the liver or other organs. Several FDA-approved diagnostic oncology tests rely on liquid biopsy profiling with RNA or DNA sequencing to generate qualitative expression and mutation profiles of batteries of disease markers (e.g. the receptor tyrosine kinases, EGFR and ERBB2) [63]. Integration of quantitative transcriptomic and proteomic analyses into such assays is the next step in the development trajectory of current screening tests towards precision diagnostics and therapeutics. In addition to monitoring ADME proteins (PK variability), liquid biopsy can be used to define between-patient variability in receptors and other therapeutic targets (PD variability). Achour et al. [3, 4] demonstrated the possibility to monitor variability in the expression of over 500 ADME genes (171 enzymes, 362 transporters and the neonatal Fc receptor, FcRn) and over 80 FDA-approved drug targets after appropriate normalization for between-patient differences in the rate of shedding (defined based on expression of a set of tissue-specific stably expressed markers). Although not very well understood, exosome shedding is, in essence, a physiological process that is altered under pathological conditions, adding another parameter that modelling PK variability needs to contend with. Determination of such parameter becomes critical when the patient cohort includes a heterogeneous mix of diseases. The use of tissue-specific cell surface markers can help with purification, by immunoenrichment, of specific extracellular vesicles originating from the tissue of interest, e.g. asialoglycoprotein receptor 1, ASGR1, in the case of liver exosomes [76].

With sufficient validation and rapidly declining costs, the use of liquid biopsy will facilitate implementation of model-informed precision dosing owing to the inherent advantages of the technique; it is minimally invasive, quantitative (connecting exosomal profiles to tissue expression), and compatible with modelling platforms, such as Virtual Twins [31, 71]. Virtual Twins should incorporate detailed individual data, such as demographics, genotype, PK/PD expression grades (e.g. from liquid biopsy), and clinical scores (e.g. eGFR for renal function) into a generic PBPK

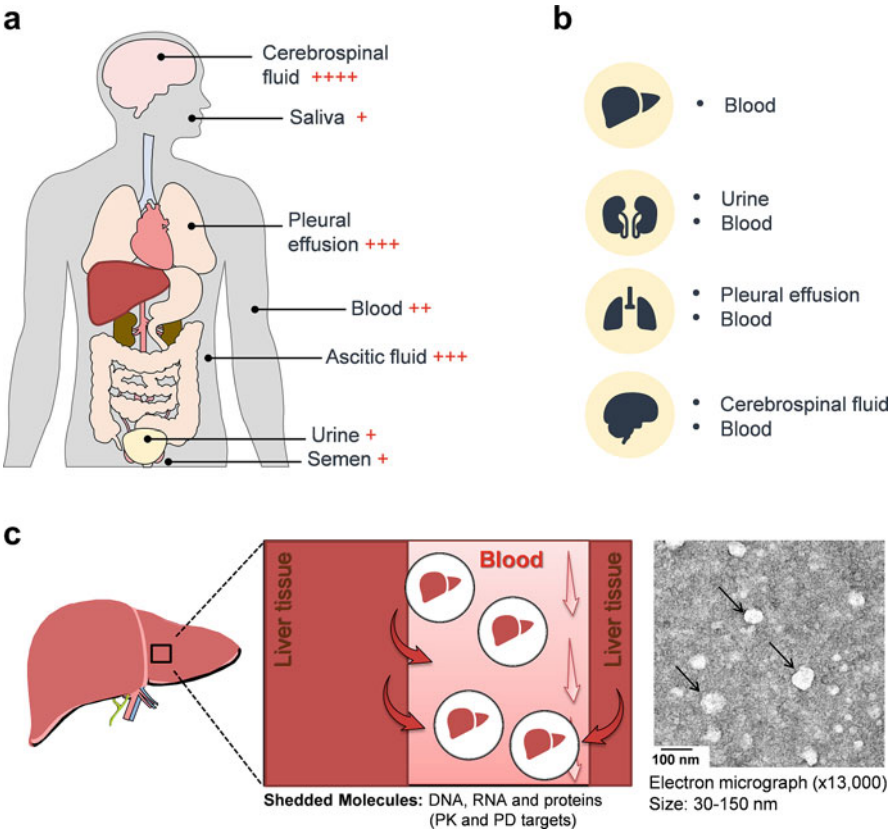

Fig. 6.5 Liquid biopsies, their nature, and attributes. (**a**) The anatomical origin and level of invasiveness of commonly sampled liquid biopsies (+, least invasive; ++++, most invasive, but all are less invasive than tissue biopsies). (**b**) Biofluids used to probe ADME/PD protein expression in liver, kidney, lung, and brain tissue as some of the main systems studied in PK/PD research. (**c**) Blood is the most widely used liquid biopsy with diagnostic, companion diagnostic and therapeutic applications. Tissue (liver) is perfused in blood and continuously sheds microvesicles (exosomes) into the systemic circulation. Molecules shedded include proteins and RNA (of PK and PD targets). The electron micrograph shows exosomes extracted from plasma (size range: 30–150 nm). Abbreviations: *ADME* absorption, distribution, metabolism and excretion, *PD* pharmacodynamics, *PK* pharmacokinetics

model of the cohort that the individual patient belongs to (Fig. 6.6). The use of liquid biopsy data with such modelling platforms opens the possibility of a priori selection of the optimal initial dose in a treatment regimen for an individual patient and allows identification of patients most likely to experience adverse events or lack of efficacy (for closer therapeutic monitoring). Achour et al. [4] demonstrated correlation with activity in a cohort of patients with cardiovascular disease monitored with the Geneva cocktail (for CYP1A2, CYP2B6, CYP2C9, CYP3A and P-gp), in support of findings by Rowland et al. [82] for CYP3A4 in a set of healthy volunteers before

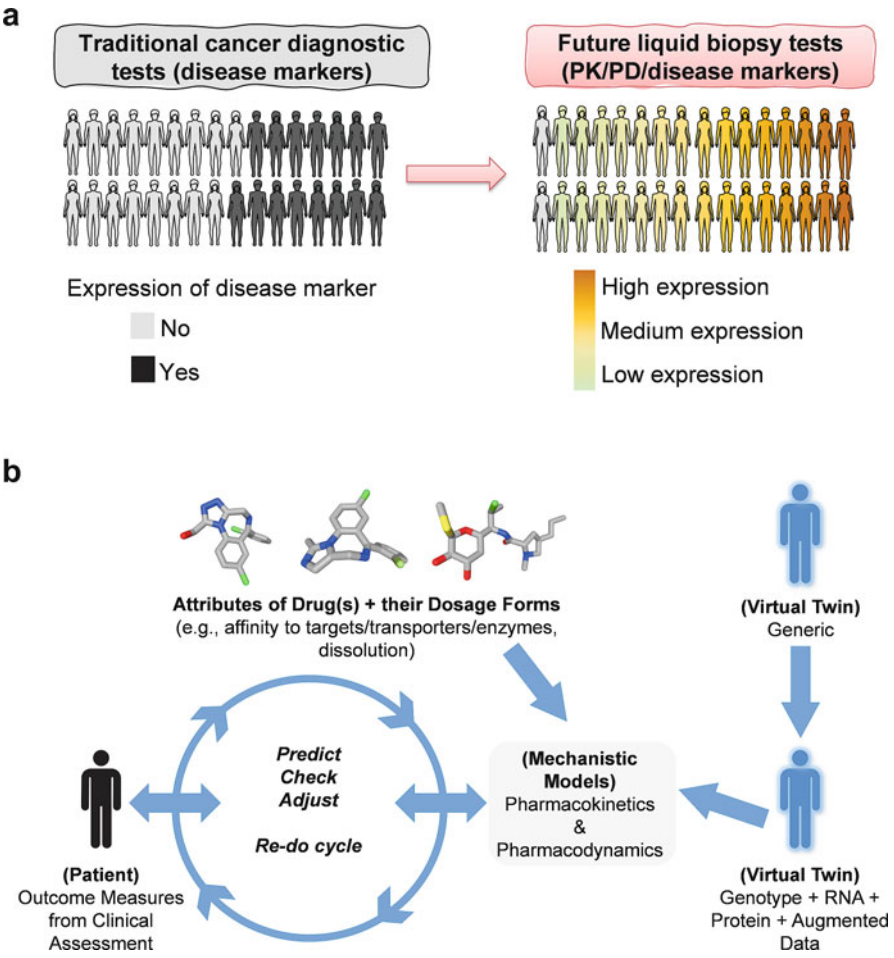

Fig. 6.6 The use of a liquid biopsy with modelling platforms for precision therapeutics. (**a**) Liquid biopsy can be used as a test for grading patients based on quantitative measurement of PK/PD targets while traditional tests (in oncology diagnostics) rely on qualitative evidence of the presence/absence of disease markers and the mutation profiles of such markers. (**b**) Quantitative data for PK and PD targets from liquid biopsy can be used to generate Virtual Twin models for individualized therapeutics. Abbreviations: *PD* pharmacodynamics, *PK* pharmacokinetics

and after induction. Early applications have focused on precision dosing and investigation of DDI potential [3, 76].

Despite its potential applications, liquid biopsy requires specialist expertise in isolation and purification of exosomes from biofluids, extraction of RNA and protein, and multi-omic analysis (genomics, RNAseq and proteomics). For this reason, the bulk of recent work has focused on assessment of enzymes and transporters in readily accessible systems, such as plasma exosomes [3, 27, 45, 56, 82],

while measurements of ADME targets in more challenging biofluids, such as urine [28] and cerebrospinal fluid, are lacking.

6.5 Modelling the Impact of Permutations of Various Comorbidities

The added value of PBPK becomes paramount when we consider combinations of factors that influence the fate of the drug, which are very difficult, if not impossible, to study in advance of the drug becoming available on the market. We take the example of DDIs as the case here. In 1999, Krayenbühl et al. [55] proposed that interpretation of interaction studies should focus not only on mean DDI effect but also observed and theoretically conceivable extremes. This initiated some efforts within the PBPK community to conduct virtual clinical studies involving large groups of virtual patients where various scenarios could be tested (the platform later became known as the Simcyp Population Based PBPK Platform) [52]. One of the essential elements of the system is its ability to run "what if" scenarios such as those shown in Fig. 6.7. "What if" scenarios take into account factors that affect the outcome of the interaction, e.g. genetics, renal/hepatic impairment, age, or combinations of these elements [79]. It took almost another decade before such facilities were put to practical use by some scientists. In 2012, researchers at the Office of Clinical Pharmacology (OCP) at the US FDA published a PBPK study that verified a previously reported case study [88] on DDI in renal impairment for telithromycin [97]. They went on then to prospectively project on the level of DDI for rivaroxaban

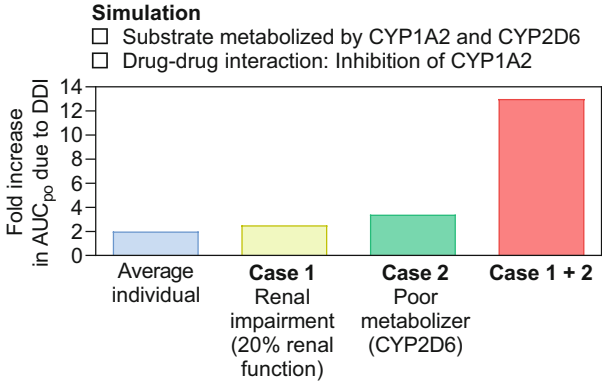

Fig. 6.7 "What if" scenarios simulated with PBPK modelling for a DDI between a substrate (metabolized by CYP1A2 and CYP2D6) and an inhibitor of CYP1A2. Scenarios examined the magnitude of interaction in renal impairment, CYP2D6 poor metabolizer genotype and a combination of the two. Abbreviations: AUC_{po} area under the plasma concentration-time profile after oral administration, *DDI* drug-drug interaction. (The concept of the figure is adopted from Rostami-Hodjegan and Tucker [79])

in renal impairment where no clinical data were available [46]. The study informed the label for the drug and was a guide to prescribers dealing with these rare occasions. Almost 10 years later, real-world data (RWD) analysis on retrospective information for rivaroxaban and associated side effects clearly demonstrated twofold higher incidence of bleeding in renally impaired patients who were receiving inhibitors of metabolic/transporter clearance (Grillo et al. [47]). The analysis was based on extracts from electronic health records (EHR) from HIPAA-compliant anonymized individual-patient-level data for 117 US institutions in the Cerner-Oracle RWD dataset for a 5-year period (2017–2021). One can postulate that such adverse effects could have been more frequent if the label did not contain the information on the combined impact of renal impairment and DDI. The example above is not unique and there are now many other cases where PBPK information has informed the drug label in the absence of clinical data. Table 6.2 shows a list of examples collated in an internal database by Certara. Similar but less comprehensive lists are published elsewhere [48, 50].

6.6 Conclusions and Future Use of PBPK for Model-Informed Precision Dosing

While the debate on the nature of PBPK models (Open Source Code versus Closed Source Code) continues [78], the use of closed-source systems has certainly accelerated applications in drug development. Achieving a similar success in model-informed precision dosing faces many hurdles and not just the lack of a user-friendly interface for PBPK. These are discussed by Darwich et al. [31] in the lines of creating virtual twins of patients [71]. However, the first critical step of such efforts is the faithful characterization of patients' phenotypes beyond genetic-based categorization. It appears that liquid biopsy, in conjunction of omics analyses, may just provide such capacity, if the technical aspects of such game-changing initiatives are addressed [4].

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Table 6.2 A representative list of approved drugs where PBPK simulations informed the drug label. The list is an internal database of more than 300 label claims for over 90 novel drugs based on the Simcyp Simulator (2009–2022). (Source: Certara Ltd.)

Oncology	**Agios**	Tibsovo (ivosidenib)	**Genentech**	Polivy (polatuzumab vedotin-piiq)	**Novartis**	Kisqali (ribociclib succinate)
	Amgen	Blincyto (blinatumomab)	**Genentech**	Rozlytrek (entrectinib)	**Novartis**	Rydapt (midostaurin)
	Amgen	Lumakras (sotorasib)	**Genentech**	Cotellic (cobimetinib)	**Novartis**	Tabrecta (capmatinib)
	Ariad	Alunbrig (brigatinib)	**Genentech**	Alecensa (alectinib)	**Novartis**	Scemlix (asciminib)
	Ariad	Iclusig (ponatinib)	**Incyte**	Pemazyre (pemigatinib)	**Novartis**	Vijoice (alpelisib)
	AstraZeneca	Lynparza (olaparib)	**Janssen**	Balversa (erdafitinib)	**Pfizer**	Bosulif (bosutinib)
	AstraZeneca	Tagrisso (osimertinib)	**Janssen**	Erleada (apalutamide)	**Pfizer**	Lorbrena (lorlatinib)
	AstraZeneca	Calquence (acalabrutinib)	**Lilly**	Verzenio (abemaciclib)	**Pharmacyclics**	Imbruvica (ibrutinib)
	Beigene	Brukinsa (zanubrutinib)	**Lilly**	Retevmo (selpercatinib)	**Sanofi**	Jevtana (cabazitaxel)
	BluePrint Medicines	Ayvakit (avapritmib)	**Loxo Oncology**	Vitrakvi (larotrectinib)	**Seattle Genetics**	Tukysa (tukatanib)
	Celgene	Inrebic (fedratinib hydrochloride)	**Novartis**	Jakavi (ruxolitinib)	**Spectrum**	Beleodaq (belinostat)
	Daiichi Sankyo	Turalio (pexidartinib)	**Novartis**	Odomzo (sonidegib)	**Takeda**	Exkivity (mobocertinib)
	Eisai	Lenvima (lenvatinib)	**Novartis**	Farydak (panobinostat)	**Verastem**	Copiktra (duvelisib)
	EMD Serono	Tepmetko (tepotinib hydrochloride)	**Novartis**	Zykadia (certinib)		
Rare Disease	**Auriana**	Lupkynis (voclosporin)	**Intercept**	Oclavia (obeticholic acid)	**Sanofi Genzyme**	Cerdelga (eliglustat tartrate)
	Akarx	Doptelet (avatrombopag maleate)	**Kadman**		**Vertex**	Symdeko (tezacaftor/ivacaftor)

(continued)

Table 6.2 (continued)

				Rezurock (belumosudil mesylate)		
	AstraZeneca	Koselugo (selumetinib)	**Merck**	Welireg (belzutifan)	**Vertex**	Trikafta (elexacaftor/ivacaftor/tezacaftor)
	Genentech	Enspryng (satralizumab)	**Mirum**	Livarti (maralixbat)	**Mitsubishi Tanabe**	Dysval (valbenazyne)
	Genentech	Evrysdi (risdiplam)	**Novartis**	Isturida (osilodrostat)		
	Global Blood Therapeutics	Oxbryta (voxelotor)	**PTC Therapeutics**	Emflaza (deflazacort)		
Central Nervous System	**AbbVie**	Qulipta (atogepant)	**GW Research**	Epidiolex (cannabidiol)	**Lilly**	Rayvow (lasmiditan succinate)
	Alkermes	Aristada (aripiprazole)	**Idorsia**	Quviviq (daridorexant)	**Novartis**	Mayzent (siponimod fumaric acid)
	Alkermes	Lybalvi (olansapine/samidorphan)	**Janssen**	Ponvory (ponesimid)	**UCB**	Briviact (brivaracetam)
	Eisai	Dayvigo (lemborexant)	**Kyowa Kirin**	Nourianz (istradefylline)	**AbbVie**	Rinvoq (upadacitinib)
Infectious Disease	**Gilead**	Remdesivir (veklury)	**Merck**	Prevymis (letermovir)	**Tibotec**	Edurant (rilpivirine)
	Viiv	Cabenuva kit (cabotegravir/rilivirine)	**Merck**	Pifeltro (doravirine)	**Novartis**	Egaten (triclabendazole)
	Janssen	Olysio (simeprevir)	**Nabriva**	Zenita (lefamulin acetate)		
Gastroenterology	**AstraZeneca**	Movantik (naloxegol)	**Shionogi**	Symproic (naldemedine)	**Shire**	Motegrity (prucalopride)
	Helsinn	Akynzeo (fosnetupitant/palonosetron)	**Phathom**	Voquezna Triple Pak		
Cardiovascular	**Actelion**	Opsumit (macitentan)	**Johnson & Johnson**	Xarelto (rivaroxaban)	**Pfizer**	Revatio (sildenafil)

	BMS	Camzyos (mavacamten)	**Novartis**	Entresto (sacubitril/valsartan)	**Bayer/Merck**	Verquvo (vericiguat)
Other	**Agios**	Pyrukynd (mitapivat)	**Lilly**	Olumiant (baricitinib)	**Merck**	Steglatro (ertugliflozin)
	Galderma	Aklief (trifarotene)	**AbbVie**	Orilissa (elagolix)	**Takeda**	Livtenicity (maribivar)
	Lilly	Mounjaro (tirzepadide)	**Janssen**	Invokana (canagliflozin)	**Peloton/Merck**	Welireg (ertugliflozin)

Data are presented in the order: pharmaceutical company (in bold), commercial name, and generic name of the drug (in parentheses). The last group denoted 'other' therapeutic classes includes metabolic disease, anaemia, post-transplant treatment, diabetes mellitus, inflammation, and dermatology

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