Dealing With Variable Drug Exposure Due to Variable Hepatic Metabolism: A Proof-of-Concept Application of Liquid Biopsy in Renal Impairment

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Precision dosing strategies require accounting for between-patient variability in pharmacokinetics (PK), affecting drug exposure, and in pharmacodynamics (PD), affecting response achieved at the same drug concentration at the site of action. Although liquid biopsy for assessing different levels of molecular drug targets has yet to be established, individual characterization of drug elimination pathways using liquid biopsy has recently been demonstrated. The feasibility of applying this approach in conjunction with modeling tools to guide individual dosing remains unexplored. In this study, we aimed to individualize physiologically-based pharmacokinetic (PBPK) models based on liquid biopsy measurements in plasma from 25 donors with different grades of renal function who were previously administered oral midazolam as part of a microdose cocktail. Virtual twin models were constructed based on demographics, renal function, and hepatic expression of relevant pharmacokinetic pathways projected from liquid biopsy output. Simulated exposure (AUC) to midazolam was in agreement with observed data (AFE = 1.38, AAFE = 1.78). Simulated AUC variability with three dosing approaches indicated higher variability with uniform dosing (14-fold) and stratified dosing (13-fold) compared with individualized dosing informed by liquid biopsy (fivefold).

Further, exosome screening revealed mRNA expression of 532 targets relevant to drug metabolism and disposition (169 enzymes and 361 transporters). Data related to these targets can be used to further individualize PBPK models for pathways relevant to PK of other drugs. This study provides additional verification of liquid biopsy-informed PBPK modeling approaches, necessary to advance strategies that seek to achieve precise dosing from the start of treatment.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Liquid biopsy is established as an early diagnostic tool by monitoring genes associated with certain diseases in extracellular vesicles circulating in plasma. Genotyping can stratify patients into bands in relation to the activity of proteins relevant to drug pharmacokinetics.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Can liquid biopsy be used with pharmacokinetic models to predict individual drug exposure and enable precision dosing by reducing between-patient variability in exposure?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Individual liquid biopsy-informed pharmacokinetic models predicted drug exposure and reduced variability between patients by guiding specific dose adjustment.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Liquid biopsy-informed models provide a means for individual patient characterization and allow selection of the optimal dosing regimen from the start of treatment.

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Variability between patients in drug exposure and response is a major challenge for healthcare providers, leading in many cases to suboptimal patient outcomes.1 Severe adverse drug reactions or lack of efficacy invariably require cessation of drug therapy or changes in dosing regimens. Tailoring drug selection and dose to the requirements and characteristics of the individual patient within the framework of precision medicine has recently gained traction within the regulatory and clinical settings but has made very little progress due to the lack of technologies required to replace the ‘one-size-fits-all’ approach commonly practiced in health care.2 The bottleneck is highlighted by the requirement for defining the main patient characteristics that determine the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs in the body.3 Timely access to relevant patient characterization information is important, including information accessible in electronic health records, such as age, weight, sex, kidney function, relevant genotypes, concomitant medications, and disease severity, as well as relevant PK/PD biomarker data that can be used to adjust dosing regimens based on the individual’s expected pharmacological response.4 Combining these data into robust physiologically-based pharmacokinetic (PBPK) and quantitative systems pharmacology (QSP) models is expected to provide the necessary change to enable relevant drug/dose selection to meet the needs of individual patients.5,4

Recently, ‘liquid biopsy’ assays have been proposed to define an individual quantitative grade for a patient’s hepatic drug elimination capacity using plasma exosomes5,6 as an alternative to tissue biopsies. This approach offers several advantages, such as minimal invasiveness, wide accessibility, and the quantitative nature of the generated measurements,5,7,8 which have been the subject of recent debates.9–11 We previously reported methodological details of a liquid biopsy technique5 and demonstrated its utility in patient phenotyping by linking liquid biopsy measurements with in vivo activity for several clinically relevant probes in a cohort with cardiovascular disease.12 Whereas implementation of this characterization method with modeling platforms has been discussed previously,2,13 the feasibility of such approach has yet to be demonstrated. To explore the potential clinical application of these models in personalized dose adjustment, this study aimed to evaluate individual predictions of midazolam exposure using liquid biopsy-informed PBPK models in a cohort of patients with healthy renal function or different stages of chronic kidney disease (CKD), for whom variability in metabolic clearance has previously been reported.14 To achieve this aim, midazolam PK profiles for patients with healthy renal function or different stages of chronic kidney disease (CKD) were administered orally as part of a cocktail that also contained 375-μg dabigatran etexilate, 10-μg pitavastatin, 100-μg atorvastatin, and 50-μg rosuvastatin, following overnight fasting. Blood was drawn from patients pre-dose (assigned time 0 hour) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hours postadministration. Total drug concentration in plasma was measured at each time point for the 25 participants. Details of the clinical study have been published previously,14 and a summary of midazolam PK data is included in Table S1.

Clinical data

Prior to blood collection, a microdose of 10-μg midazolam was administered orally as part of a cocktail that also contained 375-μg dabigatran etexilate, 10-μg pitavastatin, 100-μg atorvastatin, and 50-μg rosuvastatin, following overnight fasting. Blood was drawn from patients pre-dose (assigned time 0 hour) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32, 48, and 72 hours postadministration. Total drug concentration in plasma was measured at each time point for the 25 participants. Details of the clinical study have been published previously,14 and a summary of midazolam PK data is included in Table S1.

Measurement of mRNA expression of enzymes and transporters in liquid biopsy

Plasma samples were processed to measure liver shedding and expression of cell-free RNA (cfRNA) of enzymes and transporters (Figure S1), as previously described.5,7,15 RNA expression levels were normalized to the total number of RNA reads in each sample and recorded in units of reads per million (RPM). The clinical study have been published previously,14 and the resultant CDNA (5 μL) was used in target amplification by PCR (17 cycles) using Illumina’s AmplicSeq™ Transcriptome Human Gene Expression Panel and AmplicSeq™ HiFi Mix, part of AmpliSeq™ Library PLUS (96 reactions). Library quality was assessed using a Fragment Analyzer 5200 System (Agilent), for a sharp peak between 265 and 275 bp. Libraries were quantified by qPCR using the KAPA Library Quantification Kit for Illumina (Roche). Amplicon libraries were purified and further amplified (seven cycles) and quantified by qPCR, followed by normalization, dilution (900 pM), and pooling into two pools (180 nM final concentration for sequencing). Pooled libraries were sequenced on a NovaSeq™ 6000 platform (Illumina) with 2 × 150 bp paired-end reads using NovaSeq™ 6000 S2 Reagent kit (300 cycles). Each clinical and control sample was prepared and analyzed once.

Sequencing data were processed using RNA Amplicon App 2.0.1 (Illumina) and the Burrows–Wheeler Aligner. Differential RNA expression analysis used the DESeq2 algorithm16 to generate mRNA expression data for the targets (enzymes and transporters) and liver-specific markers (APOA2, FGB, AHSG, HPX, SERPINC1, F2, CFHR2, F9, SPP2, MBL2, A1BG, TF, and C9) that make up the liver-to-plasma shedding factor used to offset variability in shedding of liver exosomes (Figure S2), as previously described.5,7 RNA expression levels were normalized to the total number of reads in each sample and recorded in units of reads per million (RPD). The shedding factor (SF) was computed for each sample and used for the normalization of the levels of target enzymes/transporters, as detailed previously.5

METHODS

Clinical samples and study design

The outline of the study is illustrated in Figure 1. Plasma samples from a cohort of participants with variable renal function (n = 25) were supplied by Merck & Co., Inc. (Rahway, NJ).14 The cohort included donors with

normal renal function (n = 6) and donors with mild (n = 6), moderate (n = 6), or severe (n = 7) renal impairment (RI). Blood was collected with prior informed consent. Patients had previously received oral midazolam (10 μg) as part of an oral microdose cocktail. No strong or moderate inhibitors or inducers of CYP3A4, CYP2C9, P-gp, OATP1B, or BCRP were administered concomitantly with the cocktail. Plasma samples (collected March to July 2018) were screened for quality (storage and freeze–thaw history) before selection and pooling for liquid biopsy assays. Demographic and clinical information for the 25 donors is summarized in Table 1. Healthy plasma from eight donors (age: 23–60 years; three females; collected in April 2018,15 used as QC and to establish baseline liquid shedding, was supplied by BioIVT (West Sussex, UK).

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Liquid biopsy data projection

RNA sequencing data were included in the analysis only for samples that had (i) sufficient cfRNA yield (> 1 ng/mL) and acceptable quality (DV200 score > 20%), (ii) visible cDNA peak at 265–275 bp, and (iii) sufficient transcriptome sequencing depth (total > 10 M reads). Liquid biopsy measurements were converted into projected hepatic abundance in units compatible with Simcyp® v21 R1 (Figure S3). Liquid biopsy data above the lower limit of quantification (LLOQ) were converted to abundance data using in-house relationships (correlations between liquid biopsy measurements and protein abundance in matched liver tissue approximated to linear equations), generating hepatic protein data in units of pmol/mg membrane protein. Because tissue abundance was projected from liquid biopsy, the data were compared against published ranges of enzymes and transporters in liver tissue. 

Individual renal impairment PBPK models

Virtual twin models (n = 25) were generated using the Simcyp® v21 R1 Population Simulator for each individual participant starting from the corresponding Simcyp® library population (for healthy, mild RI, moderate RI, and severe RI). Individualization was carried out based on individual demographic, clinical and enzyme/transporter expression data (of relevant pathways projected from liquid biopsy, Table S2), in addition to mean plasma protein binding data for each cohort (Table S3). The area under the curve (AUC0–inf) of midazolam plasma concentration–time profiles was compared between predicted and observed data in individual donors. 

Reduction in between-patient variability in midazolam exposure was assessed with stratified and individualized dosing relative to uniform dosing, as defined below.

- For uniform dosing, the cohort of virtual individuals was administered a routinely recommended standard oral dose, as per clinical prescribing information (5 mg).
- For stratified dosing, the same cohort was stratified into four groups based on clinical characteristics and status of renal impairment. The stratified dose was selected based on the CKD category in the Simcyp library.
**ARTICLE**

**Table 1 Summary of donor characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal renal function</th>
<th>Mild renal impairment</th>
<th>Moderate renal impairment</th>
<th>Severe renal impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 8 (50–71)</td>
<td>66 ± 10 (49–75)</td>
<td>67 ± 11 (50–77)</td>
<td>65 ± 7 (57–72)</td>
</tr>
<tr>
<td>Sex</td>
<td>3 females, 3 males</td>
<td>2 females, 4 males</td>
<td>4 females, 2 males</td>
<td>1 female, 6 males</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 ± 9 (151–174)</td>
<td>164 ± 12 (145–178)</td>
<td>165 ± 7 (160–178)</td>
<td>166 ± 12 (148–184)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83 ± 11 (62–93)</td>
<td>81 ± 10 (56–126)</td>
<td>85 ± 11 (72–104)</td>
<td>82 ± 12 (65–98)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 ± 2 (27–33)</td>
<td>29 ± 7 (23–39)</td>
<td>31 ± 4 (26–37)</td>
<td>30 ± 4 (25–37)</td>
</tr>
</tbody>
</table>

Drug exposure was assessed as the AUCₜₐₑₜₜ of the concentration–time curve and variability across the cohort was calculated as the fold range and coefficient of variation (%CV).

**Statistical analysis**

Data were presented as mean and SD, whereas CV was used to describe variability. Differences (in shedding or expression) between control and disease groups were assessed using ANOVA and post hoc paired t-tests. A P-value cutoff of 0.05 was considered for statistical significance. Agreement between predicted and observed AUC was assessed with average fold error (AFE) and absolute average fold error (AAFE), with values of AFE and AAFE close to 1 indicating lower bias in predictions.

**RESULTS**

**Measurement of enzymes and transporters in liquid biopsy**

Technical details of the liquid biopsy method were published previously.⁶ The yield of cfRNA was 3.93 ± 1.40 ng/mL plasma, with a quality (DV200) score of 44% ± 11%, indicating moderate yield and quality of extracted exosomal RNA. AmpliSeq™ aimed to sequence > 30,000 human RefSeq genes per sample, generating quantitative data for 30,343 ± 2,536 transcripts (range: 22,019–35,383 across 25 samples). The number of reads was 46.31 (±15.75) million reads per sample. Sequencing quality was excellent, with 93% of sequenced bases achieving a sequencing quality score (Q-score) of ≥ 30 (i.e., ≥ 99.9% base call accuracy). All samples passed QC at the RNA, cDNA, and sequencing levels. The data allowed quantification of mRNA expression of 532 genes related to drug PK (Figure 2a), which included 169 enzymes, 361 transporters, and the neonatal Fc receptor (FcRn) α- and β-subunits (Table S4), some of which were mapped to pathways relevant to the metabolism and disposition of the substrates in the cocktail (Figure 2b). Variability (%CV) in PK targets ranged from 23% to 293%, reflecting a range of moderate to high variability. Disease perturbation (CKD cohort, n = 19) from baseline expression was assessed for the PK targets, with a total of 95 targets (18%) exhibiting differential expression in renal disease (Table S4).

In addition to PK targets, liver-specific markers were measured to determine individual liver shedding into the bloodstream, which was used to normalize measurements of enzymes and transporters. Shedding was moderately but statistically significantly higher and more variable in CKD patients (6.35 ± 2.93 RPM; n = 19) than baseline in confirmed healthy donors, characterized previously (0.99 ± 0.38 RPM; n = 8; Welch’s t-test, P < 0.001) (Figure 2c). Differences were observed between shedding in CKD and previously reported levels in liver cancer and cardiovascular disease (Figure S2). No major differences were observed in liver shedding between different grades of renal impairment (Table 2).

**Projection of liquid biopsy data to tissue abundance**

Shedding-normalized mRNA expression data for CYP enzymes, UGTs, CES1/2, and transporters are summarized in Table 2 and Figure 3a. RNA expression across different stages of renal disease was characterized previously. The yield of cfRNA was 3.93 ± 1.40 ng/mL plasma, with a quality (DV200) score of 44% ± 11%, indicating moderate yield and quality of extracted exosomal RNA. AmpliSeq™ aimed to sequence > 30,000 human RefSeq genes per sample, generating quantitative data for 30,343 ± 2,536 transcripts (range: 22,019–35,383 across 25 samples). The number of reads was 46.31 (±15.75) million reads per sample. Sequencing quality was excellent, with 93% of sequenced bases achieving a sequencing quality score (Q-score) of ≥ 30 (i.e., ≥ 99.9% base call accuracy). All samples passed QC at the RNA, cDNA, and sequencing levels. The data allowed quantification of mRNA expression of 532 genes related to drug PK (Figure 2a), which included 169 enzymes, 361 transporters, and the neonatal Fc receptor (FcRn) α- and β-subunits (Table S4), some of which were mapped to pathways relevant to the metabolism and disposition of the substrates in the cocktail (Figure 2b). Variability (%CV) in PK targets ranged from 23% to 293%, reflecting a range of moderate to high variability. Disease perturbation (CKD cohort, n = 19) from baseline expression was assessed for the PK targets, with a total of 95 targets (18%) exhibiting differential expression in renal disease (Table S4).

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is illustrated in Figure 3b, reflecting limited differences in exosomal levels across CKD cohorts. In addition to consistent detection of CYP3A4 and CYP3A5, the typically pediatric CYP3A7 was quantified in 8/25 participants, in line with reported expression of this enzyme in up to 20% of adults.23,24 Compared with the liver-enriched carboxylesterase CES1, exosomal CES2 may have originated from multiple tissues, and therefore, data analysis focused on CES1. Converted protein abundance data are summarized in Table S2 and a heatmap of relative data is shown in Figure S4. Projected protein levels were generally in line with published meta-analyses.16–18 Approved liquid biopsy-derived protein abundance data (expressed in compatible units; see Methods) were

Table 2 Summary of RNA expression of target pathways relevant to drug metabolism and disposition measured in liquid biopsy-derived exosomes. The measurements were normalized to liver shedding and recorded in units of reads per million (RPM)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Normal renal function</th>
<th>Mild renal impairment</th>
<th>Moderate renal impairment</th>
<th>Severe renal impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td>0.080±0.11</td>
<td>0.011±0.009</td>
<td>0.010±0.009</td>
<td>0.021±0.019</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>1.613±1.593</td>
<td>1.847±1.142</td>
<td>1.739±0.869</td>
<td>2.719±0.76</td>
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<tr>
<td>CYP3A4</td>
<td>0.028±0.035</td>
<td>0.022±0.025</td>
<td>0.022±0.025</td>
<td>0.013±0.013</td>
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<tr>
<td>CYP3A5</td>
<td>0.023±0.015</td>
<td>0.017±0.013</td>
<td>0.009±0.007</td>
<td>0.018±0.018</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>0.008±0.006</td>
<td>0.005±0.002</td>
<td>0.009±0.006</td>
<td>0.003±0.001</td>
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<tr>
<td>UGT1A1</td>
<td>0.013±0.006</td>
<td>0.015±0.006</td>
<td>0.010±0.005</td>
<td>0.015±0.016</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>0.005±0.002</td>
<td>0.004±0.001</td>
<td>0.005±0.003</td>
<td>0.009±0.008</td>
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<tr>
<td>UGT2B7</td>
<td>0.011±0.003</td>
<td>0.006±0.002</td>
<td>0.014±0.013</td>
<td>0.003±0.001</td>
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<tr>
<td>CES1</td>
<td>0.321±0.236</td>
<td>0.401±0.461</td>
<td>0.384±0.32</td>
<td>0.347±0.339</td>
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<tr>
<td>CES2</td>
<td>6.863±2.342</td>
<td>6.451±1.214</td>
<td>5.956±1.155</td>
<td>6.16±1.335</td>
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<tr>
<td>ABCB1</td>
<td>0.827±0.885</td>
<td>0.882±1.131</td>
<td>0.396±0.481</td>
<td>0.53±0.525</td>
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<tr>
<td>ABCG2</td>
<td>1.740±1.683</td>
<td>1.419±2.179</td>
<td>0.665±0.532</td>
<td>0.821±0.52</td>
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<tr>
<td>SLC10A1</td>
<td>0.009±0.007</td>
<td>0.023±0.039</td>
<td>0.016±0.011</td>
<td>0.021±0.022</td>
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<tr>
<td>SLC10B1</td>
<td>0.073±0.136</td>
<td>0.114±0.137</td>
<td>0.017±0.02</td>
<td>0.017±0.02</td>
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<tr>
<td>SLC10B3</td>
<td>0.007±0.001</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
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<tr>
<td>SLC10B1</td>
<td>0.516±0.265</td>
<td>0.369±0.29</td>
<td>0.452±0.351</td>
<td>0.308±0.117</td>
</tr>
<tr>
<td>Liver shedding</td>
<td>7.20±4.96</td>
<td>5.20±3.33</td>
<td>7.65±3.69</td>
<td>6.51±1.34</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD; n, the number of samples where a reading above the limit of quantification was recorded; −, not determined.
incorporated into PBPK models (with demographic and renal function data) to generate individual virtual twin models for the participants.

Simulation of midazolam AUC using liquid biopsy data and uniform dosing

Liquid biopsy-informed virtual twin models were built to simulate individual midazolam plasma concentration–time profiles after a uniform dose (10 μg); comparison with clinical observations across the 25 individuals is shown in Figure 4a. Predictions of midazolam AUC were in agreement with observed data, with 76% of datapoints within twofold and 92% of datapoints within threefold of the observed AUC (Figure 4b). Average fold error (AFE) was 1.38 and absolute average fold error (AAFE) was 1.78 (Figure 4b), indicating high precision and accuracy of predictions. Compared with variability in observed midazolam AUC (CV 56% across the cohort), variability in AUC simulated using the individualized models was 72%. The use of default stratified models for different RI grades available in the Simcyp library predicted variability in midazolam AUC of 22%. The latter approach did not explain all of the observed variability (56%), which was better reflected by models individualized with CYP3A expression data.

Reduction of variability in midazolam exposure with personalized dosing

The generated virtual twin models were used to assess the reduction in variability with individualized dosing guided by liquid biopsy measurements (doses tailored to the specific characteristics of each participant). Individualized doses of midazolam ranged from 0.9 to 15.2 mg (mean: 4.1 ± 3.4 mg, Figure 5a) compared with stratified dosing based on CKD status (four doses, one for each group, Figure 5a) and uniform midazolam dosing based on routine clinical guidelines (5 mg). Variability in simulated midazolam AUC (Figure 5b) was large following uniform dosing (14-fold, CV = 72.4%) and stratified dosing (13-fold, CV = 72.7%), in contrast to individual dosing (fivefold, CV = 39.3%). Individualized dosing resulted in a 2.8-fold reduction in variability (or 1.8-fold reduction in %CV) compared with the one-size-fits-all approach (Figure 5b,c).

DISCUSSION

Clinical studies conducted in drug development routinely collect drug exposure/response data as well as some information on relevant intrinsic and extrinsic factors that may influence the drug’s PK/PD profile, focusing on characteristics mandated by labeling recommendation (e.g., in pediatrics) or advised by regulatory
Figure 4 (a) Predicted and observed midazolam plasma concentration-time profiles in individual donors with normal renal function or mild, moderate or severe renal impairment. (b) Predicted and observed AUC was compared for all groups in the cohort. Simulations were carried out using liquid biopsy-informed virtual twin models. In panel (a), the blue lines represent the average predicted concentration, and the gray lines represent the 5th and 95th percentiles, while the circles show the measured plasma concentrations from 0 to 24 h. In panel (b), the continuous line is the line of identity, and the dashed lines represent twofold difference in AUC. AFE and AAFE are the average and absolute average fold errors for the predicted AUC relative to the observed AUC across the group. RI, renal impairment.
guidelines (e.g., in hepatic and renal impairment). However, dose adjustment recommendation for groups of patients with such characteristics is often lacking. For example, Jadhav et al. reported that > 50% of drugs approved in 2015 had no dose recommendation information for patients with severe renal impairment, a trend that did not improve in subsequent years. Moreover, for the majority of drugs, robust information on pediatric dosing is often not available at the time of approval. Even in the cases where such recommendations are available, they tend to focus on one factor at a time, and the healthcare provider is expected to integrate the provided information in complex cases and to guess the appropriate dose adjustment for each case.

PBPK models that successfully predict changes in exposure due to enzyme/transporter modulation are expected to address these gaps in dose recommendation information, especially for disease/special populations and those with comorbidities. When used as part of clinical decision support systems, these models should allow integration of different factors to enable dosing recommendations to be made for complex patients. Patient-specific characterization of metabolic/transporter pathways required for bottom-up PBPK models may be afforded by pharmacogenomics or liquid biopsy assays. Such patient characterization should enable researchers to access data about individuals or groups of patients for whom such assessment was previously not feasible or possible (e.g., pregnancy).

Although genotyping can assign the patients into bins of expected phenotype, variability within each bin is often too high to support the use of genotyping as the ultimate predictor of metabolic or transporter activity. Moreover, some genotypic effects only show an impact under interaction with environmental factors. And finally, for many enzymes and transporters, there are no major genotypes that correlate with the wide variability in activity observed in the population (e.g., CYP3A4 and CYP1A2), and often a combination of factors are associated with PK variability or increased risk of side effects. Thus, any effective direct or indirect method that offers further insights into the level of active enzyme or transporter relevant to the PK or PD of a given drug would be of major benefit.

Liquid biopsy assays were applied in this study to a cohort of 25 patients with variable renal function, and measurements were normalized for variability in liver-to-plasma shedding, monitored using stable liver-specific markers. Shedding levels in the renal impairment cohort confirmed population-specific distribution, which we reported previously in healthy, cardiovascular disease, and liver cancer cohorts. Shedding is especially important to take into account when assessing enzyme/transporter expression in heterogeneous patient groups, such as the cohort in this study, or when the liver is under direct stress (e.g., liver cancer). Projected expression of target proteins in the liver based on liquid biopsy measurements can be used to inform PBPK or PBPK-PD models using quantitative liquid

Figure 5 Simulated variability in midazolam AUC with uniform, stratified and individualized dosing. (a) Dosing followed either standard recommended dosing (5 mg PO), stratified dosing for each group in the cohort guided by clinical characteristics, or individualized dosing guided by liquid biopsy. Predicted (b) AUC and (c) its variability were compared among the three dosing approaches across all groups in the cohort (normal, mild RI, moderate RI and severe RI). Simulations were carried out using the constructed liquid biopsy-informed virtual twin models. In panel (b), the whiskers represent the ranges, the boxes are the 25th and 75th percentiles, the lines are the medians and the + are the means. RI, renal impairment.
biopsy-tissue relationships, as defined previously. While other complementary data can be used to inform such models (e.g., pharmacogenomic and activity phenotype data), the advantage of implementing projected protein expression values in tissue from liquid biopsy is highlighted for drugs with multiple elimination pathways which have variable contributions of such pathways to overall drug exposure between individuals. Liquid biopsy covers a broad range of elimination pathways, allowing quantitative monitoring of a large number of PK targets (in this case, 169 enzymes, 361 transporters, and FcRn), in line with our previous reports. In addition, the screening is able to monitor a range of PD targets, reflecting between-patient variability and disease perturbation levels (e.g., variability in the midazolam target GABA-A receptor, GABRA1). PBPK-PD models should take this variability into account when considering the propagation of exposure variability into changes in drug effects. The work presented here demonstrates that the scope of liquid biopsy screening can extend beyond traditional PK profiling to assess FDA-approved drug targets, as highlighted previously in the context of cardiovascular disease.

One of the most promising applications of liquid biopsy is highlighted in enabling the delivery of precision dosing at the point of care. The utility of the individualization approach is reflected in the apparent lack of differences in mean expression across the cohorts, emphasizing that each patient should be taken as an individual rather than a patient from a cohort. To demonstrate the feasibility of model-informed precision dosing in patients with renal impairment, we developed, for the first time, liquid biopsy-informed virtual twin models to guide individual dosing of midazolam in patients with variable renal and hepatic function. Progressive renal impairment population models available in Simcyp were individualized based on demography (age/sex), kidney function (creatinine clearance), and quantitative characterization data of critical hepatic elimination pathways informed by liquid biopsy (e.g., CYP3A4/5, UGT1A4). Dose adjustment using liquid biopsy data led to a reduction in variability in midazolam AUC from 14-fold (uniform dosing) to 5-fold (personalized dosing), suggesting that a priori dose adjustment should enable similar drug exposure across patients to be achieved. This level of reduction in variability was not observed with stratified dosing based on CKD classification, which did not take into account individual changes in protein expression. In this case, the most clinically important hepatic enzyme for metabolic clearance, CYP3A4, exhibits highly variable activity, reflected to some extent in expression data, but not explained by pharmacogenomics. This is further reinforced by the observation that variability in predicted midazolam exposure based on CYP3A expression projected from liquid biopsy data (CV 72%) better reflected variability in observed midazolam exposure (56%) across the study cohort, compared with predictions based on demography and renal function (17%) or changes due to progressive disease (22%). Whereas the significance of the reported trends for modeling individual drug exposure is emphasized, the reliance on an opportunistic study, in this instance, can introduce some limitations to the generalizability of the findings, particularly in relation to the limited sample size of the cohort. In addition, modeling was based on hepatic expression of enzymes, renal function, and demographics in particular, without taking into account other factors, such as the individual intestinal component contributing to midazolam clearance or effects of uremic toxins on drug exposure. These limitations represent areas of refinement for the proposed individualized modeling strategy.

Patient characterization offered by liquid biopsy can also be integrated into pharma-statistical models and the choice of a PBPK model is not a mandatory element (see Darwich et al.). While this population PK approach may be more immediately applicable with direct drug measurements, liquid biopsy provides a superior long-term alternative to phenotyping, with the latter requiring many different assays for each drug whilst the former offering all measurements in a single assay. Liquid biopsy measurements, however, cannot reflect changes in protein activity that are not underpinned by changes in expression, such as the case of competitive inhibition. Such information on likely drug–drug interactions needs to be added by predicting the inhibitor’s PK and its impact on the function of a rate-limiting enzyme or transporter.

In conclusion, liquid biopsy-informed PBPK model projections of midazolam exposure and corresponding variability, described in this study, further support the application of liquid biopsy as a patient characterization method for precision dosing. The demonstrated reduction in variability in midazolam exposure using virtual twin models is a large step forward toward effective individualization of pharmacotherapy.

**SUPPORTING INFORMATION**

Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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**CONFLICT OF INTEREST**

A.R.-H. is an employee of Certara Ltd., which holds patents in the area of liquid biopsy and provides modeling and simulation platforms for academic and industrial centers. K.L.Y. and C.G. are employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc. All other authors declared no competing interests for this work.

**AUTHOR CONTRIBUTIONS**