Utility of Quantitative Proteomics for Enhancing the Predictive Ability of Physiologically Based Pharmacokinetic Models Across Disease States

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Abstract
Disease states such as liver cirrhosis and chronic kidney disease can lead to altered pharmacokinetics (PK) of drugs by influencing drug absorption, blood flow to organs, plasma protein binding, apparent volume of distribution, and drug-metabolizing enzyme and transporter (DMET) abundance. Narrow therapeutic index drugs are particularly vulnerable to undesired pharmacodynamics (PD) because of the changes in drug PK in disease states. However, systematic clinical evaluation of disease effect on drug PK and PD is not always possible because of the complexity or the cost of clinical studies. Physiologically based PK (PBPK) modeling is emerging as an alternate method to extrapolate drug PK from the healthy population to disease states. These models require information on the effect of disease condition on the activity or tissue abundance of DMET proteins. Although immunoquantification-based abundance data were available in the literature for a limited number of DMET proteins, the emergence of mass spectrometry-based quantitative proteomics as a sensitive, robust, and high-throughput tool has allowed a rapid increase in data availability on tissue DMET abundance in healthy versus disease states, especially in liver tissue. Here, we summarize these data including the available immunoquantification or mRNA levels of DMET proteins (healthy vs disease states) in extrahepatic tissue and discuss the potential applications of DMET abundance data in enhancing the capability of PBPK modeling in predicting drug disposition across disease states. Successful examples of PBPK modeling that integrate differences in DMET proteins between healthy and disease states are discussed.

Keywords
disease states, liver disease, PBPK modeling, PBPK, pharmacokinetics and drug metabolism, quantitative proteomics, special populations

Disease states such as cirrhosis, chronic kidney disease (CKD), certain cancers, gastrointestinal (GI) disorders, autoimmune diseases, and diabetes can lead to altered drug disposition and pharmacokinetics (PK) by influencing the rate and extent of drug absorption, blood flow to organs, plasma protein binding, apparent volume of distribution, and the abundance of drug-metabolizing enzyme and transporter (DMET) proteins. Failure to account for the altered physiology in disease states can lead to undesired pharmacodynamics (PD), that is, drug toxicity or lack of drug efficacy, especially in case of narrow therapeutic index drugs. For example, appropriate dose adjustments are warranted in patients with hepatic or renal dysfunction for drugs belonging to anti-infectives (amikacin, gentamicin, and vancomycin), anticonvulsants (levetiracetam, lacosamide, zonisamide, primidone, phenobarbital, czipagibe/retigabine, oxcarbazepine, eslicarbazepine, ethosuximide, and felbamate), and anticancer drugs (bendamustine, cyclophosphamide, daunorubicin, doxorubicin, epirubicin, erlotinib, etoposide, idarubicin, imatinib, lenalidomide, and methotrexate). Cirrhosis results in a 3.8-fold increase in the area under the plasma concentration-time curve (AUC) of zidovudine. Similarly, end-stage renal disease (ESRD) is associated with a 72% decrease in the AUC of selumetinib. Other pathophysiological conditions such as inflammation and diabetes can also affect drug metabolism and transport as well as the concentration of plasma binding proteins; however, the effects of these conditions on the PK of drugs are not well studied.

The United States Food and Drug Administration (FDA) and European Medicines Agency acknowledge the potential impact of disease states on drug PK and PD and recommend clinical studies for investigational drugs in patients with hepatic and renal dysfunction. However, it is both ethically and logistically challenging to measure such changes by performing clinical trials in disease populations. For example, Child-Pugh classes A, B, and C of hepatic

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dysfunction and mild, moderate, and severe stages of renal dysfunction exert variable and multifactorial effects on the absorption, distribution, metabolism, and excretion (ADME) processes. Such changes require a large number of study subjects to achieve statistical power. Moreover, the deterioration of organ function during a longitudinal clinical study poses significant challenges in PK data interpretation. Thus, the complexity and the cost of clinical studies prohibit systematic evaluation of disease effects on PK of drugs. Physiologically based PK (PBPK) modeling is an alternate method to predict drug PK in disease states to substitute or reduce the number of clinical trials. PBPK models mechanistically explain drug PK by integrating fundamental ADME parameters (eg, solubility, permeability, in vitro drug metabolism, transport, etc.) with physiological changes associated with a disease state. However, PBPK modeling of disease effect requires comprehensive data on altered physiology. In particular, information on the effect of disease state on DMET activity or tissue abundance is essential for predicting drug metabolism and transport.

The evaluation of DMET activity is challenging because the selective probe substrates are not available for most of these proteins. To address this limitation, DMET abundance data can be used as a surrogate for the activity to measure altered drug disposition in disease state (Figures 2 and 3). Assuming the Michaelis-Menten model, DMET abundance data can be used to extrapolate drug intrinsic clearance (CL) from healthy to disease states, as illustrated in equation 1.

\[
CL_{\text{disease}} = CL_{\text{healthy}} \times \frac{DMET_{\text{disease}}}{DMET_{\text{healthy}}} \tag{1}
\]

For example, the disease-dependent changes in liver cytochrome P450 3A4 (CYP3A4) abundance can be integrated into PBPK models to predict changes in hepatic clearance of CYP3A4 substrates. Because clearance is an additive parameter, if more than 1 DMET protein is involved in drug disposition, individual metabolic and transporter clearance can be integrated to predict total intrinsic clearance. In addition, the decrease in functional organ volume (ie, milligrams of S9 fraction/microsomal protein per gram of organ) in disease state should be considered in PBPK modeling. Moreover, with increasing use of polypharmacy in the disease state, DMET abundance-based PBPK modeling can be used for the prediction of complex drug-drug interactions (DDIs). Thus, multifactorial changes in ADME mechanisms associated with a disease state can be integrated into PBPK modeling.

We compiled the available information on the impact of disease conditions on drug disposition and mechanisms thereof with a focus on the potential effect on DMET abundance. The available quantitative proteomic data on hepatic DMET proteins are curated and discussed here. However, because of the lack of DMET proteomics data in extrahepatic tissue, immunoquantification or mRNA levels of DMET proteins (healthy vs disease states) were compiled from these tissues to supplement the hepatic data. Successful examples of PBPK modeling in drug-disease and drug-drug-disease interactions as well as limitations and future directions of proteomics-informed PBPK modeling are discussed.

Basics of Quantitative Proteomics
Quantitative proteomics uses liquid chromatography-tandem mass spectrometry (LC-MS/MS) for selective
quantification of surrogate peptides of proteins of interest. The technique has emerged as a powerful approach in the last decade for the quantification of DMET proteins because of its sensitivity, reproducibility, and high throughput efficiency, thus addressing several limitations of the conventional methods (Table 1).

The basic workflow of quantitative proteomics is presented in Figure 4. This protocol can be used for DMET protein quantification in a variety of biological matrices, namely, tissues, cell lysates, microsomes, cytosol, S9 fraction, membrane extracts, biological fluids (e.g., plasma and urine), and recombinant enzyme systems. Accurate and reproducible quantification of DMET proteins requires selection of sensitive surrogate peptides from a nonmembrane region of a protein using in silico or experimental approaches. An ideal surrogate peptide should be high MS-responsive,
Table 1. Comparison of the Techniques Used to Quantify DMET Transcript or Protein Abundance Level

|                     | RT-PCR | Immunoquantification | Western Blotting | Quantitative Proteomics |
|---------------------|--------|----------------------|------------------|------------------------|
| Translational use   | ++     | ++                   | ++               | +++                    |
| Selectivity         | +++    | ++                   | ++               | +++                    |
| Sensitivity         | +++    | ++                   | ++               | +++                    |
| Linearity and dynamic range | +++    | ++                   | ++               | +++                    |
| Reproducibility     | ++     | +                    | +                | +++                    |
| Accuracy            | +      | +                    | +                | +++                    |
| Multiplexing        | +++    | +                    | +                | +++                    |
| Structural information (eg, PTM) | −      | Essential            | Essential        | +++                    |
| Antibody need       | Not applicable | Essential            | Essential        | Not required           |

Color codes green, yellow, and red indicate poor, moderate, and excellent scores, respectively. PTM, posttranslational modification; RT-PCR, reverse-transcription-polymerase chain reaction.

Figure 4. Quantitative DMET proteomic workflow for characterization of DMET proteins in tissues, cells, biofluids, and recombinant models.

stable, and reproducible (no ragged ends) and should not possess residues that are prone to changes because of posttranslational modifications (PTMs) and single-nucleotide polymorphisms (SNPs). The biological samples are digested using protease enzymes such as trypsin, LysC, chymotrypsin, or their combination, followed by desalting and peptide enrichment. The enriched peptide sample is injected on an LC column for separation before MS analysis. Different data acquisition techniques (targeted, data-dependent [DDA], and data-independent acquisition [DIA]) and data analysis tools (eg, Skyline and MaxQuant) can be used for protein quantification. Targeted proteomics is typically performed using the multiple reaction monitoring method, which uses the detection of preselected surrogate peptides. In DDA, both precursor ions (survey scan) and product ions are used for the quantification of DMET proteins, whereas in the DIA
Table 2. Effect of Hepatic and Kidney Disease Conditions on DMET Protein Activity and Drug Pharmacokinetics

| Drug                        | Disease State | Effect on DMET Protein Activity | AUCR (AUC_{disease}/AUC_{healthy}) |
|-----------------------------|---------------|---------------------------------|------------------------------------|
| Hepatic diseases            |               |                                 |                                    |
| Chlormethiazole\[133]       | Cirrhosis     | CYP2A6, -3A4/5                  | 17                                 |
| Eluxadoline\[24]           | Severe HI     | UGTs, OAT1B1, OAT3, MRP2        | 13.7                               |
| Ritonavir\[25]             | HI            | CYP3A, 2D6                      | 12.8                               |
| Paritaprevir\[25]          | HI            | CYP3A4                          | 10.5                               |
| Fibanserin\[34]            | Mild HI       | CYP2C19, -3A4                   | 4.5                                |
| Dasabuvir\[35]             | HI            | CYP3A                           | 4.1                                |
| Everolimus\[35]            | HI            | CYP3A4                          | 4.1                                |
| Repaglinide\[36]           | Liver cirrhosis | CYP3A4, -2CB, OAT1B1          | 4                                   |
| Zidovudine\[37]            | Alcoholic and HCV liver cirrhosis | UGT2B7                  | 3.8                                |
| Lamotrigine\[38]           | Alcoholic and HCV liver cirrhosis | UGT1A3, 1A4                  | 3                                   |
| Midazolam\[8]              | NASH          | CYP3A4                          | 2.4                                |
| Morphine\[139]             | Alcoholic and HCV liver cirrhosis | CYP3A4                  | 2.1                                |
| Alectinib\[40]             | HI            | P-gp, CYP                       | 1.6                                |
| Mirabegron\[41]            | HI            | CYP2D6, 3A4                     | 1.6                                |
| Metoprolol\[42]            | Liver cirrhosis | CYP2C19, UGT1A1, BCRP          | 1.6                                |
| Selumetinib\[43]           | NASH          | OAT1B, MRP2, MRP3               | 1.4                                |
| Morphine\[39]              | NASH          | MRP2, MRP3, OAT1B               | 1.3                                |
| 3-glucuronide\[44]         | NASH          | OAT1B, BCRP                     | 1.3                                |
| Rosuvastatin\[45]          | NASH          | P-gp, CYP                       | 0.6                                |
| Solithromycin\[46]         | HI            | P-gp, BCRP                      | 0.4                                |
| Ombitasvir\[25]            | RI            | OAT1, OAT3                      | 20                                 |
| Renal diseases              |               |                                 |                                    |
| Avibactam\[48]             | Severe RI     | OAT1, OAT3                      | 20                                 |
| Sugammadex\[47]            | Severe RI     | Renal excretion                 | 17.2                               |
| Mirabegron\[41]            | RI            | P-gp, CYP                       | 1.7                                |
| Piivastatin\[48]           | CKD           | OATP                            | 1.4                                |
| Repaglinide\[36]           | CKD           | CYP2C8, OATP                    | 1.3                                |
| Rosiglitazone\[49]         | CKD           | CYP2C8                          | 0.8                                |
| Pioglitazone\[50]          | CKD           | CYP2C8                          | 0.8                                |
| Selumetinib\[12]           | ESRD          | CYP2C19, UGT1A1, BCRP           | 0.7                                |

CKD, chronic kidney disease; ESRD, end-stage renal disease; HCV, viral hepatitis; HI, hepatic impairment; NASH, nonalcoholic steatohepatitis; RI, renal impairment.

approach, all product ions of detectable peptide precursors are detected in a biological sample with the help of high-speed MS instruments, for example, Orbitrap and Q-TOF.\[20]\]

Targeted proteomics is the most commonly used method for DMET protein quantification.\[20]\] Synthetic unlabeled (light) or stable isotope-labeled (SIL or heavy) peptides are used as calibrators for the absolute peptide quantification. Synthetic SIL peptides are used to monitor LC separation and MS analysis for addressing experimental variables in protein quantification, for example, retention time shift, matrix effect, and sample evaporation. After data acquisition, different vendor-specific or open-source software is used for the data analysis. We have previously discussed principles, methodology, and applications of quantitative DMET proteomics in a variety of biological matrices through a series of publications.\[20-22\]

This article discusses the potential applications of DMET abundance data in enhancing the capability of PBPK modeling across disease states, with a focus on the utility of quantitative DMET proteomics data.

Impact of Disease Conditions on Drug Disposition and the Potential Role of Quantitative Proteomics

Hepatic Dysfunction

Hepatic dysfunction is one of the major pathologies, affecting 4.5 million Americans including 13\% of deaths in 2017 alone.\[23]\] Depending on the severity of the hepatic impairment, the disease stages can be classified based on Child-Pugh A, B, and C scores. These 3 classes of hepatic impairment are associated with variable PK of drugs (Table 2), which are governed by the effect of disease condition on (1) blood and bile flow rates, (2) plasma-binding protein levels (albumin and alpha-1-acid glycoprotein), and (3) drug metabolism and transport (Figure 1). Although blood or bile flow rate and drug-binding plasma protein levels decrease with disease severity, the abundance and activity of
DMET proteins can be affected in a nonmonotonic fashion by a variety of hepatic diseases. A few representative hepatic diseases that can affect drug disposition are alcoholic liver disease, cholestasis, primary biliary cirrhosis/cirrhosis, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), viral hepatitis (hepatitis C virus [HCV]), hepatocellular carcinoma, liver transplantation, primary sclerosing cholangitis, and autoimmune hepatitis.

Reported studies on the effect of hepatic diseases on DMET protein activity and drug PK are summarized in Table 2. The overall impact of disease state on drug PK depends on (1) disease severity, (2) mechanisms of hepatic drug elimination, and (3) fraction metabolized (\(f_{\text{m}}\)) or transported (\(f_{\text{t}}\)) by individual DMET proteins. In general, there is an increase in the AUC of drugs (1.27- to 13.7-fold) in patients with hepatic impairment. For example, eluxadoline, a substrate of UDP-glucuronosyltransferase (UGT), organic anion-transporting polypeptide 1B1 (OATP1B1), organic anion transporter 3 (OAT3), and multidrug resistance-associated protein 2 (MRP2) showed a 13.7-fold increase in the AUC in severe hepatic impairment relative to a healthy state. Paritaprevir, a CYP3A4 substrate, is affected greatly (>10-fold increase in the AUC) in patients with hepatic impairment compared with healthy controls. Other drugs that show >2-fold increase in the AUC in hepatic disease conditions are repaglinide, zidovudine, lamotrigine, ritonavir, dasabuvir, everolimus, and fibanserin. For a few drugs (ombitasvir and solithromycin), a decrease in the AUC is reported with increasing disease severity, likely because of the change in the DMET abundance in healthy versus disease states, for example, upregulation of P-glycoprotein (P-gp, \(ABCB1\)) and breast cancer resistance protein (BCRP, \(ABCG2\))-mediated efflux transport (Table 3).

The emergence of quantitative proteomics technique has led to deeper investigations of the changes in hepatic abundance of DMET proteins in several hepatic disease conditions, as summarized in Table 3. These investigations rely on the quantification of proteins in the banked tissue samples. We previously used quantitative proteomics for characterization of interindividual variability in DMET proteins because of age, sex, genotype, and cirrhosis. In general, there is a decrease in the abundance of DMEs such as CYPs, UG Ts, and alcohol dehydrogenases in different hepatic disease states, which decreases the overall hepatic drug metabolism; however, the magnitude of change among different DMEs is highly variable. Unlike DMEs, the drug transporter level can be decreased or increased with variable magnitude in healthy versus hepatically impaired subjects. A recent quantitative proteomics study showed that OATP2B1 (\(SLC21A9\)), sodium taurocholate cotransporting polypeptide (NTCP, \(SLC10A1\)), and organic cation transporter 1 (OCT1, \(SLC22A1\)) are decreased (>2-fold change), and BCRP, P-gp, MRP3 (\(ABCC3\)), and MRP4 (\(ABCC4\)) are increased in patients with liver dysfunction. The levels of OATP1B1 (\(SLCO1B1\)), OAT2 (\(SLC22A7\)), bile salt export pump (\(ABCB1\)), MRp1 (\(ABCC1\)), and MRP2 (\(ABCC2\)) showed an inconclusive trend with increasing liver dysfunction (Table 3). Quantitative DMET proteomics in banked tissues from patients with hepatic dysfunction will provide necessary data for PBPK modeling of hepatic dysfunction.

**Kidney Dysfunction**

The kidney, one of the major organs of drug elimination, is a primary organ for the metabolism of ~30% of marketed drugs. In the United States, more than 37 million people live with chronic kidney disease (CKD). CKD is associated with high blood pressure, urinary tract obstruction, glomerulonephritis, structural abnormalities, diabetes, and autoimmune disorders. The altered glomerular filtration rate in CKD can directly affect the renal elimination of drugs. Varying degrees of water loading, protein binding, and altered activity of kidney transporters are other mechanisms that influence drug disposition. For example, avibactam, a substrate of OAT1 (\(SLC22A1\)) and OAT3 (\(SLC22A8\)) showed a 20-fold increase in AUC in severe renal impairment (RI) likely because of decreased transporter abundance or activity. We previously applied quantitative proteomics for characterization of interindividual variability in kidney transporters in adults and children, which can be further extended to quantify disease effects. Noteworthily, CKD also affects the nonrenal clearance of drugs. CKD leads to the accumulation of metabolic waste products such as urea, uremic toxins, creatinine, decrease in calcium, and vitamin D clearance and increased potassium, parathyroid hormone, and blood phosphate levels. Such waste products can influence hepatic drug elimination by affecting the abundance and activity of hepatic DMET proteins. Yeung et al reported reduced nonrenal clearance and increased oral bioavailability of 74 drugs in CKD patients, some of which are listed in Table 2. Table 4 summarizes a compilation of the effects of extrahepatic disease states on DMET abundance, plasma proteins, and blood flow to drug disposition organs. Because the extrahepatic DMET proteomics data are not reported, we made an effort to compile any available data on DMET abundance or activity because of kidney dysfunction. Nolin et al reported downregulation of CYP genes and mRNA in several CKD animal models, but there has been no supporting evidence reported in humans. Uremic sera from CKD patients also inhibited oxidative metabolism of losartan
### Table 3. Effect of Hepatic Disease States on DMET Proteins, Plasma Proteins, and Blood Flow (Q)

| Disease                              | Effect                                                                 |
|--------------------------------------|------------------------------------------------------------------------|
| ALD (unknown CPS)                    | DMEs: CYP3A4 (1.88), CYP2C9 (1.54), CYP2D6 (2.85), CYP2E1 (1.73), CYP1A2 (1.82), CYP2A6 (1.77), CYP2C8 (1.58), POR (1.60), UGT1A4 (1.91), UGT1A6 (1.96), UGT2B7 (1.85), UGT2B15 (1.48), CES1 (1.75), CES2 (1.13), ADHIA (1.79), ADHIB (1.76), ADHIC (1.74), ALDHIA1 (1.43) Plasma proteins: albumin (1), alpha-1-acid glycoprotein (1) |
| ALD (CPS B)                          | Transporters: P-gp (1.234), BSEP (1.65), MRP1 (1.45), MRP2 (1.89), MRP3 (1.12.68), OAT2 (1.81), OATP1B1 (1.74), OATP1B3 (1.86), OATP2B1 (1.73) |
| ALD (CPS C)                          | Transporters: P-gp (1.544), BSEP (1.55), MRP1 (1.44), MRP2 (1.95), MRP3 (1.29.18), NTCP (1.78), OCT1 (1.71), OCT3 (1.61), OAT2 (1.87), OATP1B1 (1.69), OATP1B3 (1.74), OATP2B1 (1.81) Cholestasis (unknown CPS) Transporters: MRP3 (1.200), NTCP (1.200), OATP MRP2 (1.180), NTCP (1.80), OCT1 (1.66), OCT3 (1.83) Plasma proteins: Albumin (1), alpha-1-acid glycoprotein (1) |
| PBC/cirrhosis (unknown CPS)          | Plasma proteins: albumin (1), alpha-1-acid glycoprotein (1) |
| PBC (CPS A)                          | Q: QPortal vein (1), Qhepatic arterial (1) |
| PBC (CPS B)                          | Q: QPortal vein (1), Qhepatic arterial (1) |
| PBC (CPS C)                          | Q: QPortal vein (1), Qhepatic arterial (1) |

| Disease                              | Effect                                                                 |
|--------------------------------------|------------------------------------------------------------------------|
| Nonalcoholic fatty liver disease (unknown CPS) | DMEs: CYP3A4 (1.90) Plasma proteins: albumin (1), alpha-1-acid glycoprotein (1) |
| Nonalcoholic steatohepatitis (unknown CPS) | Transporters: NTCP (1.400), OATP1B1 (1.400), OATP1B3 (1.900), MRP2, MRP3, MR4, OAT alpha and beta, P-gp, MRP3 (1.100), MRP4 (1.200), BCRP (1.100), MRP2 (1.100), OAT2 (1.30) Plasma proteins: albumin (1), alpha-1-acid glycoprotein (1) |
| HCV (unknown CPS)                    | DMEs: CYP3A4 (1.77), CYP2C9 (1.58), CYP2D6 (1.35), CYP2E1 (1.59), CYP1A2 (1.77), CYP2A6 (1.68), CYP2C8 (1.54), POR (1.49), UGT1A4 (1.76), UGT1A6 (1.95), UGT2B7 (1.84), UGT2B15 (1.54), CES1 (1.64), CES2 (1.08), ADHIA (1.41), ADHIB (1.51), ADHIC (1.56), ALDHIA1A1 (1.35), Transporters: MRPI (1.100), MRP3 (1.100-1900), MRP4 (1.200), P-gp (1.1900) Plasma proteins: albumin (1), alpha-1-acid glycoprotein (1) |
| HCV (CPS A)                          | Transporters: P-gp (1.270), BSEP (1.67), MRP1 (1.76), MRP2 (1.88), MRP3 (1.63.4), BCRP (1.90), NTCP (1.62), OCT1 (1.67), OCT3 (1.62), OAT2 (1.23), OATP1B1 (1.63), OATP1B3 (1.51), OATP2B1 (1.80) HCV (CPS B) Transporters: P-gp (1.448), BSEP (1.66), MRP1 (1.144), MRP2 (1.58), MRP3 (1.31.7), BCRP (2.22), NTCP (1.31), OCT1 (1.64), OCT3 (1.62), OAT2 (1.33), OATP1B1 (1.40), OATP1B3 (1.58), OATP2B1 (1.68) |
| HCV (CPS C)                          | Transporters: P-gp (1.652), BSEP (1.72), MRP1 (1.99), MRP2 (1.40), MRP3 (1.668), NTCP (1.74), OCT1 (1.87), OCT3 (1.91), OAT2 (1.31), OATP1B1 (1.49), OATP1B3 (1.66), OATP2B1 (1.73) Hepatocellular carcinoma (unknown CPS) Transporters: OATP1B1 (1.17), OATP2B1 (1.17), OCT1 (1.17), BCRP (1.17), BSEP (1.17), P-gp (1.17) |
| PSC (CPS A)                          | Transporters: P-gp (1.2017), BSEP (1.122), MRP2 (1.77), MRP3 (1.440), NTCP (1.169), OCT1 (1.445), OCT3 (1.494), OAT2 (1.157), OATP1B1 (1.8), OATP1B3 (1.147), OATP2B1 (1.5) |
| PSC (CPS B)                          | Transporters: P-gp (1.1000), BSEP (1.35), MRP2 (1.82), MRP3 (1.531), NTCP (1.13), OCT1 (1.50), OCT3 (1.280), OAT2 (1.67), OATP1B1 (1.27), OATP1B3 (1.66), OATP2B1 (1.15) |
| PSC (CPS C)                          | Transporters: P-gp (1.3090), BSEP (1.39), MRP1 (1.62), MRP2 (1.81), MRP3 (1.390), NTCP (1.24), OCT1 (1.26), OCT3 (1.546), OAT2 (1.109), OATP1B1 (1.4), OATP1B3 (1.44), OATP2B1 (1.33) |
| AIH (CPS A)                          | Transporters: P-gp (1.2446), BSEP (1.44), MRP1 (1.61), MRP2 (1.80), MRP3 (1.758), BCRP (1.119), NTCP (1.10), OCT1 (1.27), OCT3 (1.494), OAT2 (1.48), OATP1B1 (1.23), OATP1B3 (1.57), OATP2B1 (1.9) |
| AIH (CPS B)                          | Transporters: P-gp (1.2687), BSEP (1.50), MRP1 (1.11), MRP2 (1.87), MRP3 (1.718), BCRP (1.96), NTCP (1.58), OCT1 (1.64), OCT3 (1.423), OAT2 (1.16), OATP1B1 (1.58), OATP1B3 (1.10), OATP2B1 (1.55) |

ADH, alcohol dehydrogenase; AIH, autoimmune hepatitis; ALD, alcoholic liver disease; BSEP, bile salt export pump; CPS, Child-Pugh score; HCV, hepatitis C virus; OST, organic solute transporter; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Protein abundance data obtained through LC-MS/MS.

*The protein abundance data in the literature are either reported as pmol of membrane protein or pmol/g of liver tissue.

*Unknown CPS indicates that the Child Pugh score is not identified.
Table 4. Effect of Extrahepatic Disease States on DMET and Plasma Proteins

| Disease State and Subtype | Effect on Drug DMEs, Transporters, and Protein Binding | Methodology | Tissue/Biological Fluid |
|--------------------------|-------------------------------------------------------|-------------|-------------------------|
| Kidney dysfunction       | ↓: CYP3A5 (27%)                                        | WB          | Kidney                  |
|                          | ↓: Albumin                                            |             | Blood                   |
| Uremia                   | ↑: AAG                                                |             |                         |
| Cancer                   | ↓: CYP1B1 (150%), CYP2D6 (43%), CYP2E1 (25%), CYP2S1 (64%), CYP2U1 (186%), CYP3A5 (133%), CYP51 (183%) | Immunohistochemistry | Colon                  |
|                          | ↑: MRPI (386%)                                        | WB          | Colon                   |
|                          | ↑: CYP3A4 (538%)                                      | WB          | Colon                   |
|                          | ↑: CYP3A4 (50%), EH (60%)                             | WB          | Colon                   |
| Colon cancer             | ↓: P-gp (13%) (colon)                                 |             | Colon, rectum           |
|                           | ↑: CYP3A4 (9%), P-gp 13% (colon)                      |             |                         |
| Colonic adenoma          | ↓: MRPI (251%)                                        | WB          | Colon                   |
|                           | ↑: CYP2C8 (85%), CYP3A4 (69%), CYP3A5 (54%)           | WB          | Colon mucosa            |
| Esophageal squamous-cell carcinoma | ↓: CYP3A4 (57%), CYP3A5 (20%), CYP2E1 (36%)       | WB          | Esophageal tissue       |
| Non-small cell lung cancer  | ↑: CYP1A1/1A2 (66%), EH (46%), β glucuronidase (7%)  | WB          | Lung                    |
|                           | ↑: GSTπ (96%), sulfatase (190%)                       |             |                         |
| Cancer                   | ↓: Albumin (14%)                                      |             | Blood                   |
|                           | ↑: AAG (51%)                                          |             |                         |
| Prostate cancer          | ↑: MRPI (47%)                                         | WB          | Prostate                |
| AML                      | ↑: P-gp (27%), MRPI (9.2%)                            | Flow cytometer | Bone marrow            |
| Osteosarcoma             | ↑: CYP1A1 (26%), CYP2B6 (21%), CYP2E1 (48%), CYP3A4 (39%) | Immunohistochemistry | Osteosarcoma cells     |
| Breast tumor             | ↑: GSTα (96%), sulfatase (190%)                       |             | Breast tissue           |
| Autoimmune and inflammatory diseases | ↓: CYP3A4 (78%), P-gp 85% (colonic) | WB          | Colon and ileum        |
| RA                       | ↓: Albumin (21%)                                      |             | Blood                   |
| SLE                      | ↓: AAG (128%)                                         |             | Blood                   |
| Crohn’s disease          | ↓: CYP3A4 (45%) (ileum)                               | WB          | Colon and ileum        |
|                         | ↓: CYP3A4 (78%), P-gp 85% (colonic)                   |             |                         |
|                         | ↓: CYP3A4 (16%)                                       |             |                         |
|                         | ↑: AAG (71%)                                          |             |                         |
| Inflammatory bowel disease | ↑: P-gp (124%)                                       | Flow cytometry | Colon                   |
| Ulcerative colitis       | ↓: MDR1 (20%), MRPI (6%), MRP3 (11%), MCT1 (66%)     | LC-MS       | Intestinal mucosa       |
|                         | ↓: OATP2B1 (100%)                                     |             |                         |
|                         | ↑: CYP3A4 (78%)                                       |             |                         |
| Diabetes                 | ↑: CYP3A4 (83%)                                       | WB          | Duodenum                |
| Celiac disease           | ↑: CYP2E1 (100%)                                      | WB, RT-PCR  | Lymphocyte, erythrocytes, and liver |
| IDDM                     | ↑: CYP3A4 (38%), GST (21%), UGT1A1 (42%), UGT1A9 (45%), UGT2B7 (65%), CYP3A5 (40%) (liver) |             |                         |

(Continued)
in the acute renal failure rat model. Similarly, Taburet et al showed inhibition of tolbutamide and midazolam metabolism (probe substrate of CYP2C9 and CYP3A4, respectively) in human liver microsomes after incubation with uremic plasma from ESRD patients.

CKD can also affect the PK of drugs by altering enzyme- and transporter-mediated disposition. For instance, OATP1B activity is reduced up to 60% in CKD, which can potentially alter the clearance of nonrenally eliminated drugs. Joy et al reported a 40% reduction in the oral clearance of fexofenadine and an increase in plasma half-life from 3.4 to 11 hours in patients suffering from CKD. Fexofenadine is a substrate of P-gp and OATPs, which are likely to be affected by CKD. Similarly, ESRD patients showed a decrease in both oral bioavailability and systemic clearance (by ~35%) of erythromycin, likely because of altered expression or activity of CYP3A4, P-gp, and OATPs. Although it is difficult to obtain liver tissue samples from patients with kidney dysfunction, quantitative proteomics can be used to investigate changes in DMET proteins in animal and in vitro hepatic models representing kidney dysfunction (eg, the effect of the uremic toxin on DMET abundance in human hepatocytes). Quantitative proteomics can also be used to characterize microphysiological systems (eg, kidney or liver on a chip) that are capable of recapitulating in vivo physiology including organ-to-organ communication.

Cancer
In 2019 alone, >600,000 deaths were reported because of cancer in the United States, which include 24% from lung and bronchus cancer, 8% from colon and rectum cancer, 8% from pancreatic cancer, 7% from breast cancer, and the rest from other types of cancer. For safe and effective anticancer treatment, it is important to characterize factors affecting the selectivity of an anticancer molecule (ie, cytotoxicity toward cancer cells versus normal cells) and the duration of action. DMET abundance in the tumor cell can regulate drug resistance or cytotoxicity, which is one of the primary causes of chemotherapy treatment failure. Yang et al reported a significant increase in MRPI expression in colorectal adenocarcinoma (386% increase) and colonic adenoma (251% increase), which led to the poor prognosis for patients with colon cancer. The overexpression of P-gp in osteosarcoma (30-fold increase) produced P-gp-facilitated cisplatin resistance in humans. Various CYPs are also overexpressed in various cancers. Kumarakulasingham et al reported overexpression of CYP1B1 (150%), CYP2D6 (43%), CYP2E1 (25%), CYP2S1 (64%), CYP2U1 (186%), CYP3A5 (133%), and CYP51 (183%) in colon cancer that is associated with poor prognosis. The overexpression of efflux transporters, that is, P-gp, MRPI, and BCRP, is a common mechanism of drug resistance. Altered plasma protein levels and changes in blood flow also influence the ADME of anticancer agents. Although cancer is generally associated with an increase in DMET protein abundance in tumor cells, the heterogeneous nature of different cancer types and their severity can also influence protein levels in a nonmonotonic fashion. Esophageal squamous-cell carcinoma results in the downregulation of CYP3A4, -3A5, and -2E1 enzymes by 57%, 20%, and 36%, respectively, in esophageal tissue. Waziers et al observed a moderate decrease in CYP3A4 levels (~50%) in human large intestine tumors compared with normal samples. The overexpression of P-gp in tumor tissue is associated with drug resistance and lack of efficacy that lead to cancer relapse. For example, the overexpression of efflux transporters on tumor plasma membrane affects the release of cytotoxic payloads of antibody-drug conjugates (ADCs) and leads to multidrug resistance in cancer. Liu et al recently quantified multidrug resistance proteins in the crude membrane fraction of different tumor cell lines by targeted quantitative proteomics, which showed high expression of P-gp, MRP2, and MRP4 in HepG2 and Hep3B2 cells, whereas BCRP

### Table 4. Continued

| Disease State and Subtype | Effect on Drug DMEs, Transporters, and Protein Binding | Methodology | Tissue/Biological Fluid |
|--------------------------|--------------------------------------------------------|-------------|-------------------------|
| Brain disorders          | AD<sup>167</sup>                                       | ↑: BCRP (87%), MRP4 (100%) ↓: P-gp (25%) | WB          | Hippocampus             |
|                          | Epilepsy<sup>95</sup>                                 | ↑: P-gp (150%), MRP2 (220%), MRP3 (60%), MRP5 (160%), BCRP (17%) ↓: P-gp (80%) | WB          | Hippocampus             |
|                          | Creutzfeldt-Jakob disease<sup>168</sup>               |             | Immunohistochemical      | Cortex and white matter |

GST, glutathione S-transferase; MCT, monocarboxylate transporter; RT-PCR, reverse-transcription-polymerase chain reaction; WB, Western blotting. Quantitative values were extracted from data figures or tables.

Cancer
In 2019 alone, >600,000 deaths were reported because of cancer in the United States, which include 24% from lung and bronchus cancer, 8% from colon and rectum cancer, 8% from pancreatic cancer, 7% from breast cancer, and the rest from other types of cancer. For safe and effective anticancer treatment, it is important to characterize factors affecting the selectivity of an anticancer molecule (ie, cytotoxicity toward cancer cells versus normal cells) and the duration of action. DMET abundance in the tumor cell can regulate drug resistance or cytotoxicity, which is one of the primary causes of chemotherapy treatment failure. Yang et al reported a significant increase in MRPI expression in colorectal adenocarcinoma (386% increase) and colonic adenoma (251% increase), which led to the poor prognosis for patients with colon cancer. The overexpression of P-gp in osteosarcoma (30-fold increase) produced P-gp-facilitated cisplatin resistance in humans. Various CYPs are also overexpressed in various cancers. Kumarakulasingham et al reported overexpression of CYP1B1 (150%), CYP2D6 (43%), CYP2E1 (25%), CYP2S1 (64%), CYP2U1 (186%), CYP3A5 (133%), and CYP51 (183%) in colon cancer that is associated with poor prognosis. The overexpression of efflux transporters, that is, P-gp, MRPI, and BCRP, is a common mechanism of drug resistance. Altered plasma protein levels and changes in blood flow also influence the ADME of anticancer agents. Although cancer is generally associated with an increase in DMET protein abundance in tumor cells, the heterogeneous nature of different cancer types and their severity can also influence protein levels in a nonmonotonic fashion. Esophageal squamous-cell carcinoma results in the downregulation of CYP3A4, -3A5, and -2E1 enzymes by 57%, 20%, and 36%, respectively, in esophageal tissue. Waziers et al observed a moderate decrease in CYP3A4 levels (~50%) in human large intestine tumors compared with normal samples. The overexpression of P-gp in tumor tissue is associated with drug resistance and lack of efficacy that lead to cancer relapse. For example, the overexpression of efflux transporters on tumor plasma membrane affects the release of cytotoxic payloads of antibody-drug conjugates (ADCs) and leads to multidrug resistance in cancer. Liu et al recently quantified multidrug resistance proteins in the crude membrane fraction of different tumor cell lines by targeted quantitative proteomics, which showed high expression of P-gp, MRP2, and MRP4 in HepG2 and Hep3B2 cells, whereas BCRP
and MRP4 were abundant in H226 cells. Furthermore, MRP4 was overexpressed in N87 cells, and BCRP, P-gp, MRP3, and MRP4 were overexpressed in KM-H2 cells. Absolute quantification of these efflux transporters allowed optimum payload dose selection of ADCs for ensuring clinically efficacious doses at the target site.70 Recently, Schwenger et al successfully predicted PK changes in a virtual oncology population using PBPK modeling integrated with a meta-analysis-based reduction in the abundance of CYP1A2, CYP2C19, and CYP3A4 enzymes.72

Autoimmune and Inflammatory Diseases
Inflammation may alter bioavailability and clearance of drugs by influencing the DMET abundance.73 Decreased CYP1A activity in theophylline metabolism results in low metabolism, which is possibly triggered by an increase in infection-induced inflammatory cytokines.74 A recent clinical study by Caris et al showed decreased OATP1B1 activity in the livers of rheumatoid arthritis (RA) patients using fluvastatin as a probe substrate.75 Inflammatory bowel disease alone, which includes ulcerative colitis and Crohn’s disease, affects about 1.4 million people in the United States.76,77 GI disorders, for example, Crohn’s disease, ulcerative colitis, and celiac disease, lead to changes in the normal physiology of the GI tract by causing inflammation and changes in intestinal surface area, thickness of the intestinal wall, intestinal pH, mucosal enzymes, intestinal microflora, gastric emptying time, and DMET levels.5 Holt et al reported a reduction in acetaminophen absorption in patients with Crohn’s disease and celiac disease, likely because of slower gastric emptying time. The inflammatory conditions associated with different GI disorders can also lead to a decrease in plasma albumin levels and an increase in the production of alpha-1-acid glycoprotein (AAG).78 Sandle et al reported malabsorption of propranolol in the proximal jejunum in celiac disease.79 DMET proteins in the GI tract play important roles in oral drug absorption and first-pass metabolism. GI tissue expresses a variety of DMEs (CYP1A1, -1B1, -2C, -2D6, -2E1, -2J, -3A4/5, UGT1A1, -1A3, -1A4, -1A6, -1A9, -2B4, -2B7, -2B15, -2B17, sulfotransferase [SULT1A1/1B1], and carboxylesterases [CES1/2]) and transporters (P-gp, BCRP, MRP1-3, monocarboxylate transporter-1, apical sodium-dependent bile acid transporter, peptide transporter [PEPT1/2], OATP2B1, equilibrative nucleoside transporter 1/2, organic solute transporter α/β, and OCT1).80,81 A significant decrease in CYP3A4 (<70%) expression was reported in Crohn’s disease and celiac disease.82 PEPT1 (SLC15A1) expression increases in Crohn’s disease or ulcerative colitis because of aggravated mucosal inflammation.5 Moreover, ulcerative colitis is associated with a decreasing trend in the intestinal efflux transporters, that is, P-gp > MRP3 > MRP1 and an increase in OATP2B1 observed in colonic mucosa.5 The potential mechanisms of decreased expression of DMET proteins involve protein downregulation by proinflammatory cytokines, such as interleukin 6 (IL-6), interferon gamma (INF-γ), tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β).83,84 Vee et al reported that exposure of TNF-α and IL-6 in human hepatocytes is associated with changes in mRNA expression of NTCP, OATP2B1, P-gp, MRP2, MRP3, and BCRP.85 DMET protein abundance and the mechanisms of their regulation can be investigated using quantitative proteomics.

Diabetes
Diabetes is one of the most prevalent public health problems in the world. In 2018 alone, >34 million were suffering from diabetes in the United States, including 1.4 million children and adults with type 1 diabetes, as per the American Diabetes Association.86 The diabetic condition affects protein, lipid, and carbohydrate metabolism and biochemical pathways including those associated with drug biotransformation.87,88 Diabetes can affect drug PK by altering absorption (prolonged gastric emptying time), distribution (glycation of albumin), metabolism (differential abundance of DMET proteins), and excretion (diabetic nephropathy).89 Higher blood glucose leads to glycation and conformational changes in albumin in diabetic patients, which can increase the free fraction of drug in the blood. Sotaniemi et al showed that total hepatic CYP content is elevated in type 1 diabetes, whereas it is suppressed in type 2 diabetes.90 Moreover, the effect of diabetes on DMET proteins is tissue specific and nonmonotonic across proteins.89,91 A decrease in CYP3A4 and an increase in CYP3A5 are reported in the diabetic liver patients.91 Some conflicting data also exist regarding the impact of disease states on DMEs. Fletcher et al reported elevated levels of CYP2E1 in lymphocytes; however, Pucci et al reported that the activity and expression of circulating CYP2E1 do not change in diabetic patients.92,93 Most of the phase 2 enzymes (glutathione S-transferases, and UGT1A1, -1A9, and -2B7) are decreased in the liver with diabetes.94 Furthermore, diabetic nephropathy, which is a common comorbidity, can also affect drug clearance as discussed above (Section 3.2). Quantitative DMET proteomics data on the effect of diabetes are not available, but the technique can be applied for investigating changes in DMET proteins including PTMs (eg, protein glycation). However, because diabetes is associated with secondary diseases and concomitant use of multiple drugs, it is important to gather and document...
comorbidities and medication use while collecting tissues for quantitative proteomics.

Other Diseases
In brain disorders, the effectiveness of drug therapy is often reduced because of the resistance, which is caused by increased expression of efflux transporters (eg, P-gp) at the blood-brain barrier (BBB). Quantitative proteomics can selectively and precisely quantify BBB transporters, such as P-gp, BCRP, and MRPs. Also, variable transporter expression plays a crucial role in the etiology and pathogenesis of different brain diseases such as Alzheimer’s disease, epilepsy, Creutzfeldt-Jakob disease, and Parkinson’s disease. For effective treatment of central nervous system disorders, a drug must reach a target site in the brain. Many drug molecules are substrates of efflux transporters expressed at the blood-cerebrospinal fluid barrier, which can be affected in disease states. For example, epilepsy is associated with a significant increase in P-gp, MRP2, and MRP5 (>140%), and Alzheimer’s disease is associated with an increase in the expression of BCRP (87%) and MRP4 (100%) and a decrease in P-gp (25%) as shown in Table 4. Such changes can be studied using quantitative proteomics and integrated into dynamic PBPK models for predicting the blood:brain ratio of drug and metabolites. Quantitative proteomics offers significant advantages compared with mRNA and conventional immunoblotting methods in characterization of translation, translocation or trafficking, and modification of proteins. Mechanisms of transporter expression and function can involve translocation of protein through the Golgi apparatus. The addition of bulky polar carbohydrate chains (glycosylation) can alter protein function. Glycosylation can also affect protein conformation, thus affecting protein folding both directly and indirectly. N-glycosylation and activity of drug transporters can be affected by hepatic dysfunction such as NASH and NAFLD. Filippo et al observed that the substitution of asparagine at the glycosylation site (N57, N64, and N91) of carnitine/organic cation transporter by glutamine (Q) decreased the carnitine transport. Powell et al reported impaired function of OATP1B3 in human hepatocytes because of the activation of protein kinase C (PKC) phosphorylation sites in OATP1B3. Activation of PKC results in a rapid decrease in OATP1B3 activity without affecting the surface or total protein level. Both glycosylation and phosphorylation of DMET proteins can be characterized by proteomics. Often, membrane drug transporter expression is not exclusively localized in the plasma membrane. Kumar et al developed a biotinylation-based quantitative proteomics method for the quantification of cell surface proteins. Thus, both transporter trafficking and PTM can be characterized using quantitative proteomics.

Potential Applications of Quantitative Proteomics in Understanding Mechanisms of Disease Affecting Drug Disposition
Existing reports show substantial evidence that inflammatory markers (eg, IL-6, INF-γ, TNF-α, and IL-1β) are the key regulators in the downregulation of DMET proteins. Although published studies use mRNA quantification and nonclinical studies, quantitative proteomics offers significant advantages by allowing selective and multiplexed quantification of proteins including transcription factors for a better mechanistic understanding. The complete process of production and maintenance of cellular proteins requires a sequential series of interconnected events, including transcription of mRNA, processing, and degradation of RNA to translation, translocation, modification (folding), and monitored destruction of the proteins. Quantitative proteomics offers an advantage compared with mRNA and conventional immunoblotting methods in characterization of translation, translocation or trafficking, and modification of proteins.

Proteomics-Informed PBPK Modeling in the Disease State
The use of PBPK modeling is emerging in the prediction of drug disposition in special populations...
including disease states.\textsuperscript{112,113} PBPK modeling was successfully used to make clinical decisions for 110 investigational drug applications submitted to the FDA from 2008 to 2017. Of these, 10\% were related to disease states in which hepatic impairment accounted for 6\% and the rest 4\% to RI.\textsuperscript{114} However, PBPK modeling is data hungry, and an accurate PK prediction through the modeling approach depends on the quality of data pertaining to drug-specific physicochemical (eg, solubility, ionization, and lipophilicity) and system-specific physiological (eg, blood flow, tissue volume, protein binding, and DMET abundance) properties (Figure 3).\textsuperscript{115} In particular, a differential abundance of DMET proteins between disease and healthy states is the key information that should be integrated with physiological changes for successful prediction of drug PK using PBPK modeling. The abundance of DMET proteins, which can be differentially affected during disease progression, should be quantitatively characterized for the development of PBPK models. Nevertheless, there are a few successful examples of proteomics-informed PBPK modeling being used for accurate prediction of drug PK. Incorporation of UGT2B7 proteomics data significantly improved the PK predictions of zidovudine and morphine in cirrhosis.\textsuperscript{1} Likewise, integration of UGT1A4 abundance information in the PBPK model of lamotrigine improved the model performance in both alcoholic and HCV cirrhosis.\textsuperscript{26} With guidance on PBPK modeling, the FDA is encouraging new drug applications to use PBPK modeling as a tool to make informed decisions regarding the need for clinical trials in different disease states. PBPK modeling was successfully used to quantitatively predict drug-drug-disease interactions for rivaroxaban.\textsuperscript{116} Vildhede et al recently quantified the protein abundance of major drug transporters in healthy, steatosis, and NASH liver samples using quantitative proteomics and successfully used these data to predict the PK of morphine (by OCT1) and its glucuronide metabolites (by MRPs and OATP1B), \textsuperscript{99m}Tc-mebrofenen (by MRPs and OATP1B), and rosvustatin (OATP1B and BCRP).\textsuperscript{117} Thus, quantitative proteomics offers great promise in characterizing DMET proteins in the disease state, and such data can be integrated into PBPK modeling for successful prediction of drug disposition; however, significant efforts are required to develop biobanks having well-characterized tissues.

Other considerations in PBPK modeling in the disease state: There are a variety of pathophysiological conditions that alter intra-tissue hemodynamics and thus may compromise tissue function. For instance, alteration in the regional blood flow significantly impacts hepatic and renal function. Conditions like nephrectomy, ureteric obstruction, renal artery stenosis, diabetes, and ischemia and reperfusion injury can severely alter regional renal blood flow (~20\% and 25\% of total cardiac output), resulting in impaired glomerular filtration.\textsuperscript{118} Likewise, alteration in liver blood flow can drastically impair liver function. High-extraction-ratio drugs (ER\textsubscript{H} > 0.7) are sensitive to blood flow changes, and hence their clearance from the body could be decreased or increased depending on the magnitude and direction (increase or decrease) of change in the corresponding blood flow to the liver. A PBPK model that integrated hemodynamic changes in hepatic and renal blood flow in chronic heart failure successfully explained the PK of carvedilol (ER\textsubscript{H} = 0.7).\textsuperscript{119} Similarly, the volume of distribution is a direct function of plasma protein binding that can be altered in a disease state. Also, an increase in total body water and tissue perfusion leads to an increased volume of distribution. Disease states affecting transporter function in large organs (liver, kidney, heart, and brain) can significantly influence the volume of distribution.\textsuperscript{120}

Quantitative Proteomics in DDI Predictions in Disease States

With an increasing number of chronic disease conditions (Figure 5) and multimorbidities, polypharmacy is common in the older population.\textsuperscript{121,122} In the United States, 33\% of prescription drugs are used by 13\% of the population older than 65 years of age.\textsuperscript{122} On average, 82\% of adults living in the United States are taking at least 1 medication per week. The polypharmacy is associated with drug-disease interactions, unpredictable DDIs, and adverse drug events. For example, in RA, CYP3A4 activity is significantly reduced compared
with in healthy individuals because of high IL-6 levels, which can alter the exposure of concomitant CYP3A4 substrates. A single subcutaneous injection of sarilumab, a humanized monoclonal antibody, led to a decrease in the C\text{max} and AUC of simvastatin and β-hydroxy-simvastatin acid.\(^7\) The changes in simvastatin PK are primarily because of inhibition of IL-6 binding to membrane-expressed IL-6 receptor, thus causing elevated CYP3A4, which leads to increased first-pass metabolism without affecting the half-life. Quantitative proteomics can simultaneously quantify IL-6, IL-6 receptor, and CYP3A4 in a sample. Moore et al reported a complex DDI between rivaroxaban with erythromycin in subjects with mild to moderate RI.\(^19\) Sixty-six percent of rivaroxaban dose is eliminated after metabolism (via CYP3A4/5 and CYP2J2), and the remaining is eliminated unchanged in urine, which partly involves P-gp and BCRP. Erythromycin, an inhibitor of CYP3A and P-gp, led to an increase in rivaroxaban exposure in renally impaired patients in a disease-severity-dependent fashion, that is, the AUC of rivaroxaban increased by 76% in mild RI patients and by 99% in moderate RI patients after coadministration with erythromycin. This variability in DDI magnitude is likely because of the differential effect of disease severity on the abundance or activity of DMET proteins, resulting in a variable fraction metabolized (f\text{m}) or transported (f\text{t}).

A hypothetical example of a complex DDI in the disease state is illustrated below using a drug that is a substrate of CYP3A4 and CES2 (eg, irinotecan; equations 2 and 3). It is assumed that the drug is metabolized by the 2 enzymes (f\text{m} = 0.5) to an equal extent in healthy individuals.

1. Healthy (ie, normal levels of CYP3A4 and CES2)

\[ \text{CL}_{\text{total}} = \text{CL}_{\text{CYP3A4}} + \text{CL}_{\text{CES2}} \]
\[ = \text{CL}_{\text{total}}(f_{\text{m,CYP3A4}} + f_{\text{m,CES2}}) \quad (2) \]

2. Disease state (ie, CYP3A4 is decreased by 80%, but CES2 is not changed)\(^1\)

\[ f_{\text{m,CYP3A4}} = 0.5 \times 0.2 = 0.1; \quad \text{and} \]
\[ f_{\text{m,CES2}} = 1 - 0.1 = 0.9 \quad (3) \]

Because DDI magnitude depends on the f\text{m} (Figure 6), which is increased from 0.5 to 0.9 in the disease state, CES2 inhibition can change the AUC of the victim drug from 2-fold to 10-fold in the healthy versus disease population.

Furthermore, the metabolic clearance of high ER\text{H} drugs (ER\text{H} > 0.7) depends on the blood flow, whereas the metabolic clearance of low ER\text{H} drugs (ER\text{H} < 0.3) is primarily controlled by intrinsic clearance. Thus, decreased intrinsic clearance of a high ER\text{H} drug in the disease state can make it susceptible to DDIs by enzyme inhibition by shifting it to the low ER\text{H} category.

**Challenges in Proteomics-Informed PBPK Modeling Across Disease States**

Although quantitative proteomics and the use of DMET abundance data in PBPK modeling are promising, the following limitations should be considered while applying these data and methodologies.
• Nonavailability of samples: Tissue samples are generally not available from living donors. Moreover, samples representing different stages of disease severity are difficult to obtain because of the invasive sample collection. For a majority of disease conditions that can affect DMET proteins, relevant tissue samples are not available. Organ-to-organ communication is a central mechanism in human physiology, and impairment of one organ can affect other organs, leading to additional complications in the sample collection. For example, kidney impairment can affect hepatic DMET proteins, but it is not possible to obtain liver tissue samples from these patients.

• Sample integrity: Most tissue samples representing the disease state are collected postmortem, which has the potential to cause handling-related variability (eg, protein degradation) because of inconsistency in sample procurement.

• Multimorbidity and medication use: Organ dysfunction is associated with multimorbidity and the use of multiple medications. Both these factors are significant confounders in characterizing the effect of a particular disease state on DMET protein abundance.

• Small sample size and volume: Because of the high variability from factors other than disease effect (eg, genotype, age, sex, and medication use), characterization of disease effect on DMET protein abundance requires a large number of samples to achieve statistical power. Furthermore, a small volume of biopsy samples (~5 mg) also restrains the comprehensive characterization of DMET proteins because of the low abundance of these proteins.

• Interlaboratory variability in quantitative proteomics methodology: Variability in the quantification results of DMET proteins generated in different laboratories can be caused by unharmonized methodology. Such technical variability can confound accurate estimation of biological or interindividual variability, which results in inaccurate PBPK prediction.

• Poor detection: DMET proteins are generally membrane-bound and of low abundance. This prohibits the selection of high MS-responsive peptides and their quantification by LC-MS/MS.

• Other confounding factors: Factors other than protein abundance such as protein folding, PTM, subcellular localization, and trafficking can be influenced by disease state.

Conclusions, Recommendations, and Future Directions

Quantitative proteomics has proven to be a precise, selective, multiplexed, high-throughput, and cost-effective technique for characterization of interindividual variability in DMET proteins in tissues and cells from humans and nonclinical species. Several groups use quantitative proteomics as a routine tool for characterization of interindividual variability, which could be extended to disease states if tissue samples are available from the patients. Such data can be used in PBPK modeling for better prediction of drug metabolism and PK. Furthermore, quantitative proteomics can be used in better characterization of mechanisms of DMET protein expression, PTM, and localization or trafficking in disease states using animal and in vitro models. In particular, simultaneous quantification of DMEs, transporters, transcriptional factors, and other modulators such as cytokines and their receptors can be achieved using this technique. Taken together, LC-MS/MS-based quantitative proteomics allows a comprehensive characterization of abundance and regulation of DMET proteins across disease states, which provides mechanistic information for the development of PBPK models. Such PBPK models will be important in designing data-informed clinical trials and precision drug therapy in disease states.

Considering the complexity and severity of disease states, there is a need for high-quality tissue repositories to support proteomics-informed PBPK modeling. In particular, sample collection should adopt a harmonized method that includes gathering detailed demographic information such as disease stage and severity, comorbidities, and medications used as well as age and sex of tissue donors. Interlaboratory variability in DMET abundance must be considered before applying these data in PBPK modeling. Noninvasive proteomics that uses biofluids (liquid biopsies) has a great potential to address the above-mentioned limitations. For instance, exosomes are a kind of membrane vesicle that are secreted from cells and perform many biological functions particularly in cell-to-cell communication. Furthermore, several studies suggest that biological entities like proteins and microRNA can serve as invaluable biomarkers. In particular, hepatic exosomes, which are mainly secreted from the liver, can be isolated to represent hepatic DMET protein activity. Rowland et al recently used exosomes as a surrogate of CYP3A4 induction by rifampicin. Further successful application of this technique to other DMET proteins will be crucial for broader applications of quantitative proteomics in translational ADME research.

Conflicts of Interest

All authors declare no competing interests for this work.

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