Proteomics-informed PBPK modeling to Predict Systemic and Tissue Drug Concentrations in Rats

Sheena Sharma, MS Pharm, PhD

Primary affiliation – College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, WA
Current affiliation – QSP & PBPK, Clinical Pharmacology and Pharmacometrics, Bristol Myers Squibb, Lawrenceville, NJ

Rosa Webinar | August 16, 2023

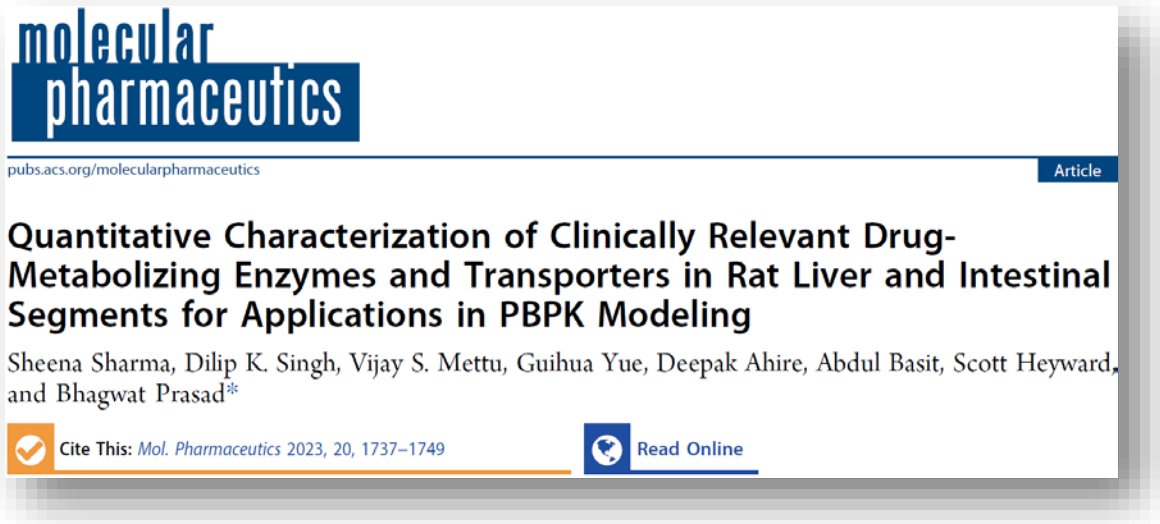
College of Pharmacy and Pharmaceutical Sciences
WASHINGTON STATE UNIVERSITY

Disclaimer

The presented work was conducted in Dr. Bhagwat Prasad's laboratory as part of my PhD dissertation and was funded by the College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane.

Acknowledgment

Dilip K. Singh, Vijay S. Mettu, Guihua Yue, Deepak Ahire, Abdul Basit, Scott Heyward, and Bhagwat Prasad


molecular pharmaceutics

pubs.acs.org/molecularpharmaceutics Article

Quantitative Characterization of Clinically Relevant Drug-Metabolizing Enzymes and Transporters in Rat Liver and Intestinal Segments for Applications in PBPK Modeling

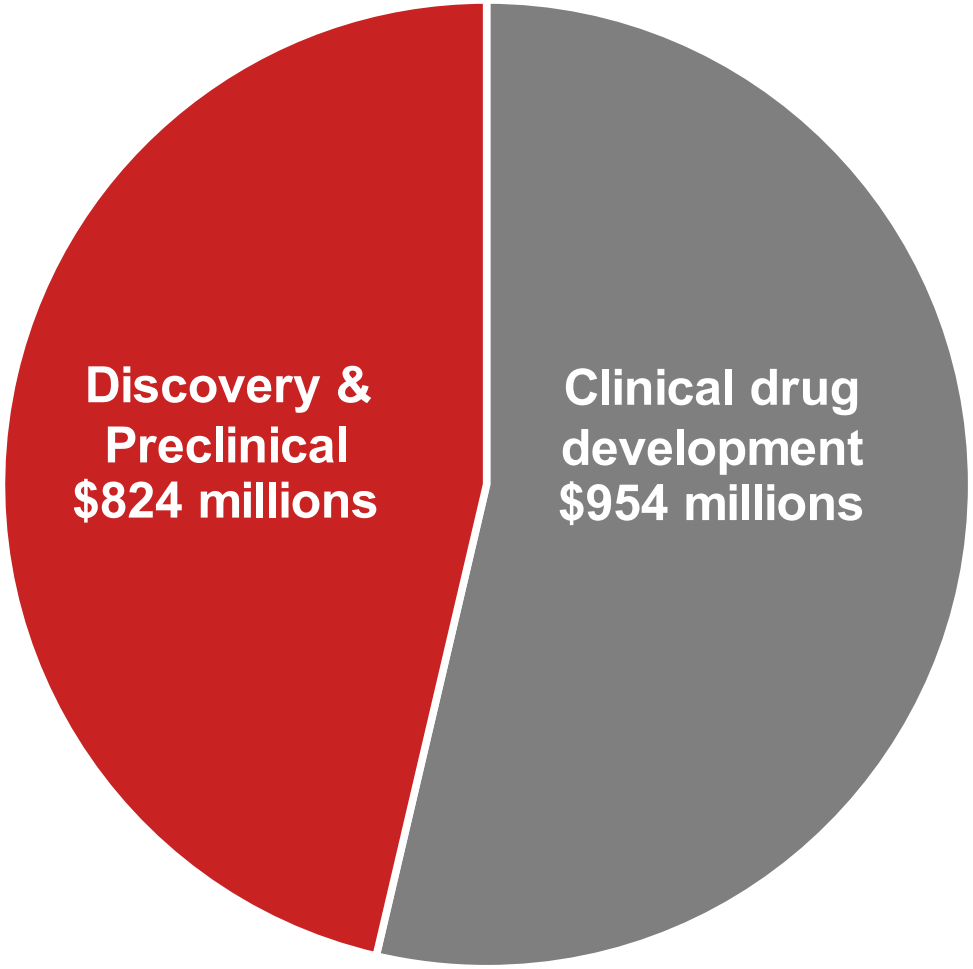
Sheena Sharma, Dilip K. Singh, Vijay S. Mettu, Guihua Yue, Deepak Ahire, Abdul Basit, Scott Heyward, and Bhagwat Prasad*

Cite This: Mol. Pharmaceutics 2023, 20, 1737−1749 Read Online

Agenda

1. Tissue drug concentration
2. Quantitative proteomics
3. Proteomics-informed PBPK modeling
4. Case study – prediction of tissue concentrations of digoxin
5. Conclusions

Drug development takes ~ 10 years and $2B

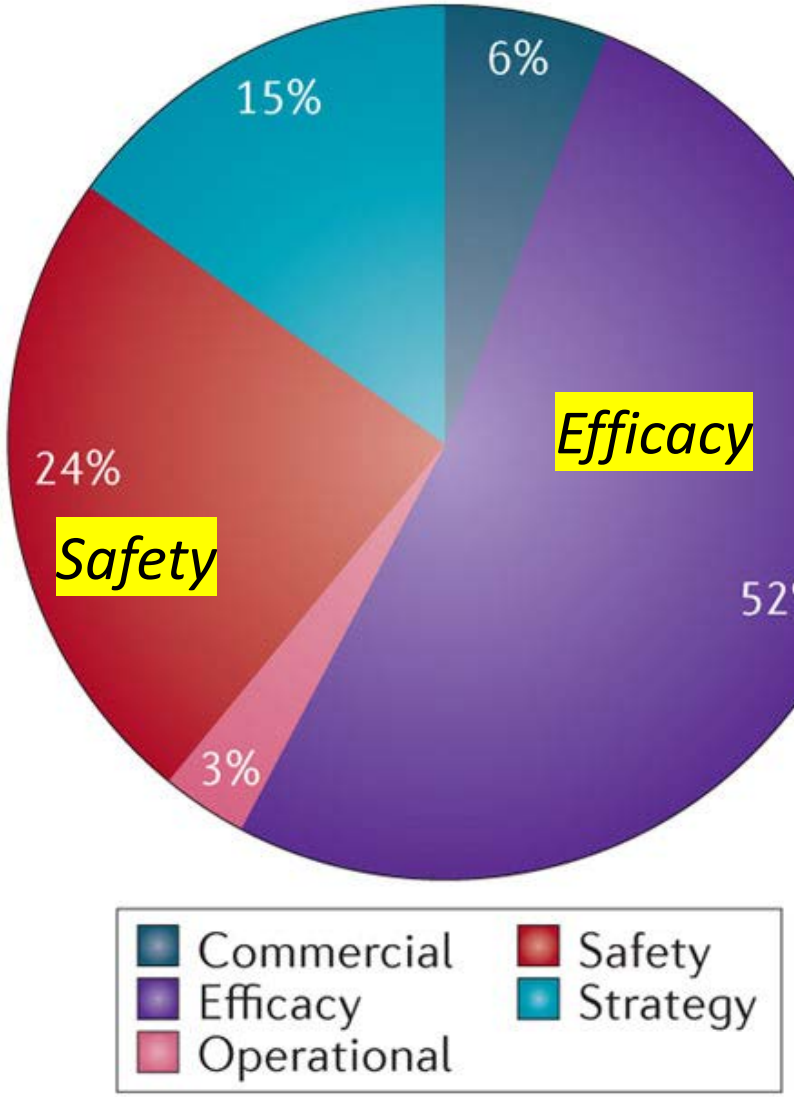


1 in 10 drugs is successful in the clinic!

Harrer et al., *Trends Pharmacol Sci (*2019). Paul et al., Nat. Rev. Drug Discov (2010).

Reasons of failing drugs in the market

Data between 2013-2015

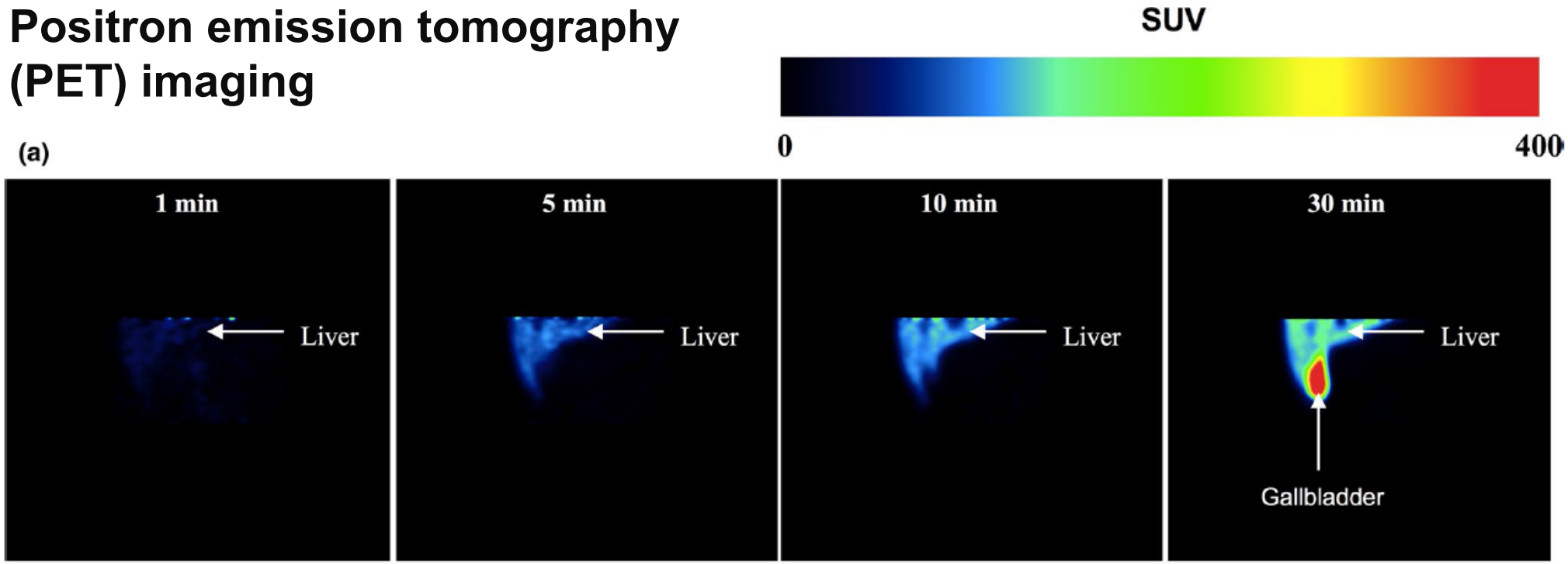


- 70% of drugs fail in the market due to lack of efficacy and safety concerns.
  - Partly because measuring drug concentrations in target tissues is often not feasible where efficacy or toxicity occurs
  - Low confidence in target occupancy profiles (key information for efficacy assessment)

Harrison, R., *Nat Rev Drug Discov* (2016).

Technique to measure tissue concentration of drugs

Positron emission tomography (PET) imaging

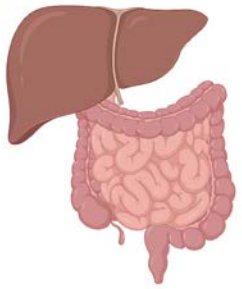


- This technique is costly and logistically challenging
- Highlights the need to predict tissue concentrations of drugs, especially when transporters are involved

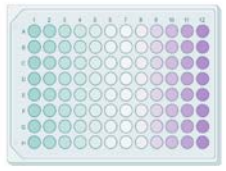
Billington et al., *Clin Pharmacol Ther* (2019).

Prediction of tissue concentration of drugs using in vitro assay, quantitative proteomics, and PBPK modeling

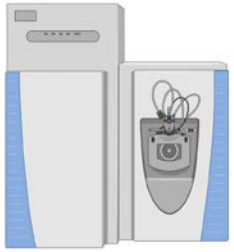
In vivo



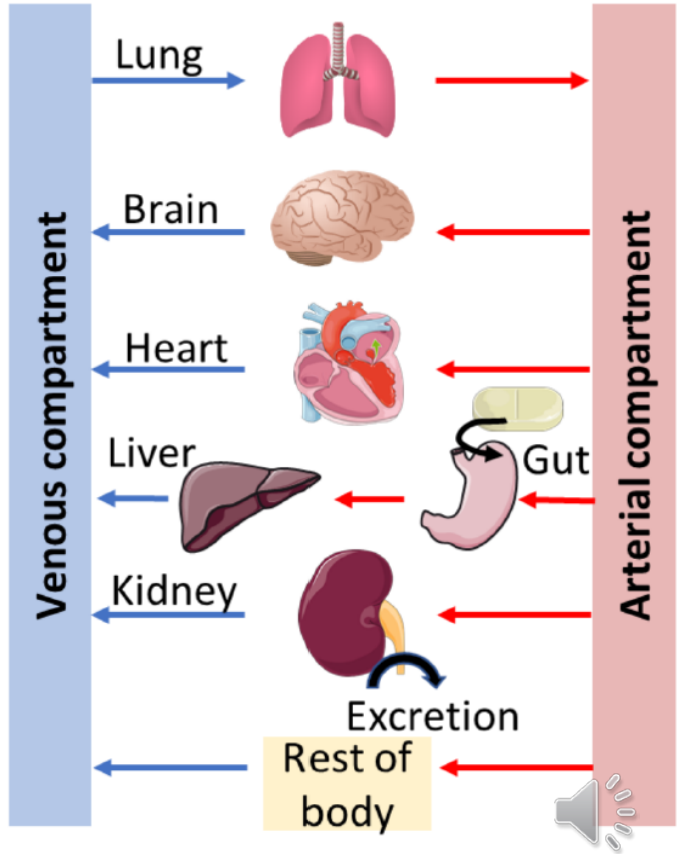
In vitro



$$\textit{Relative Expression Factor (REF)} = \frac{In\ vivo_{protein\ abundance}}{In\ vitro_{protein\ abundance}}$$



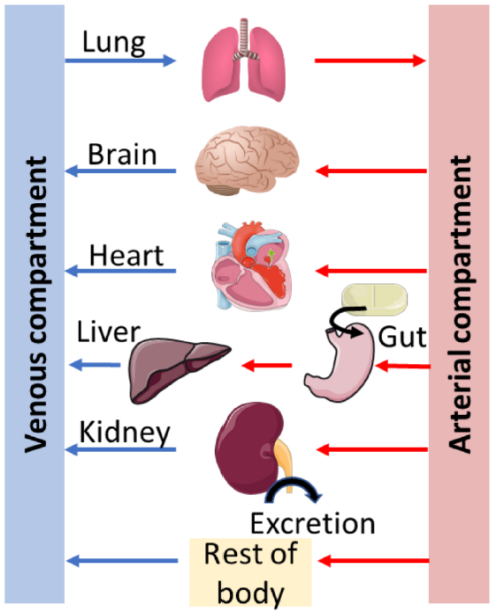
Quantitative targeted proteomics

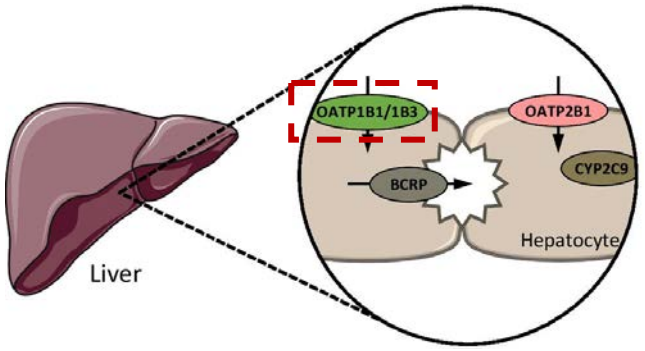


Storelli et al., *Pharmacol Ther* (2022).

Rosuvastatin's PBPK-PD Case Study

Effect of the input PD driving concentration: Plasma vs liver tissue

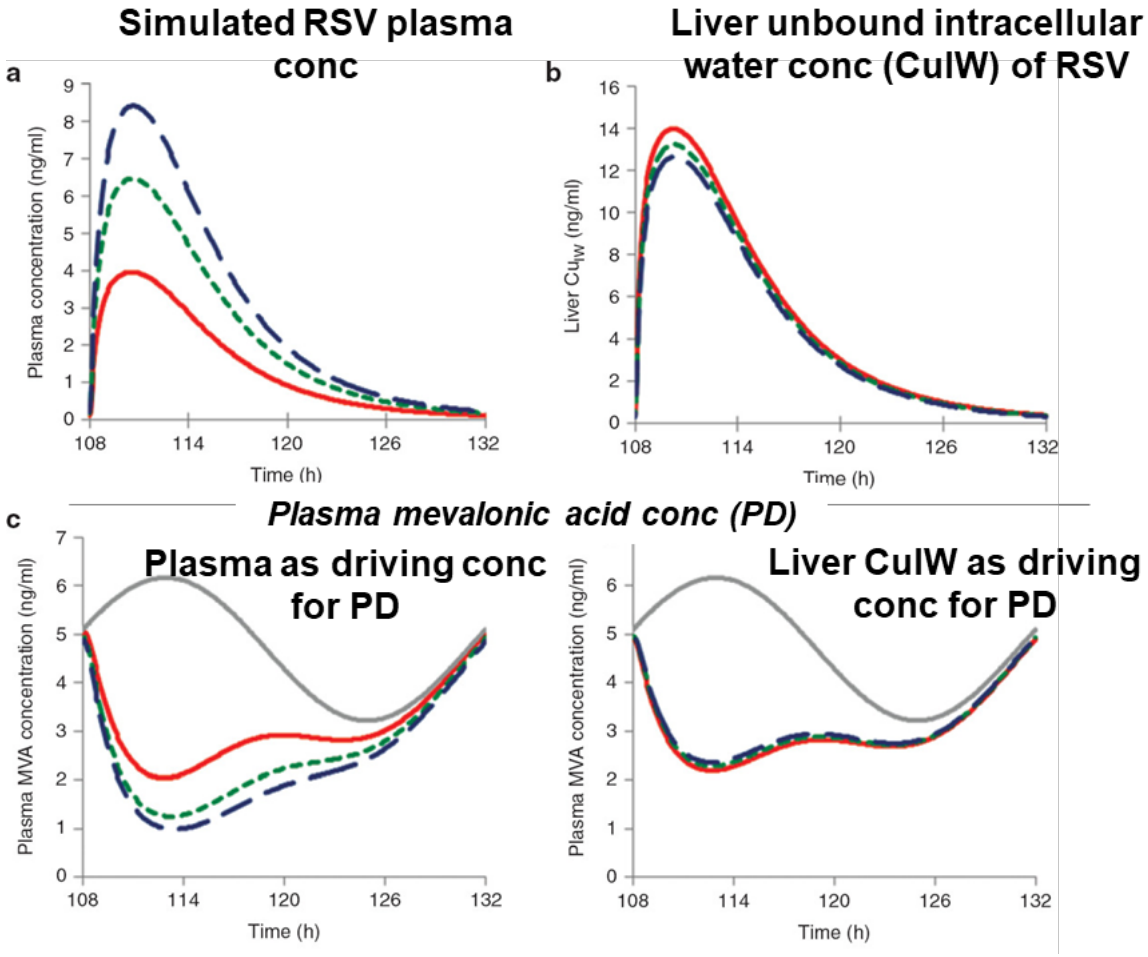




OATP1B1 genotypes

c.521TT — c.521TC — c.521CC — Baseline PD response

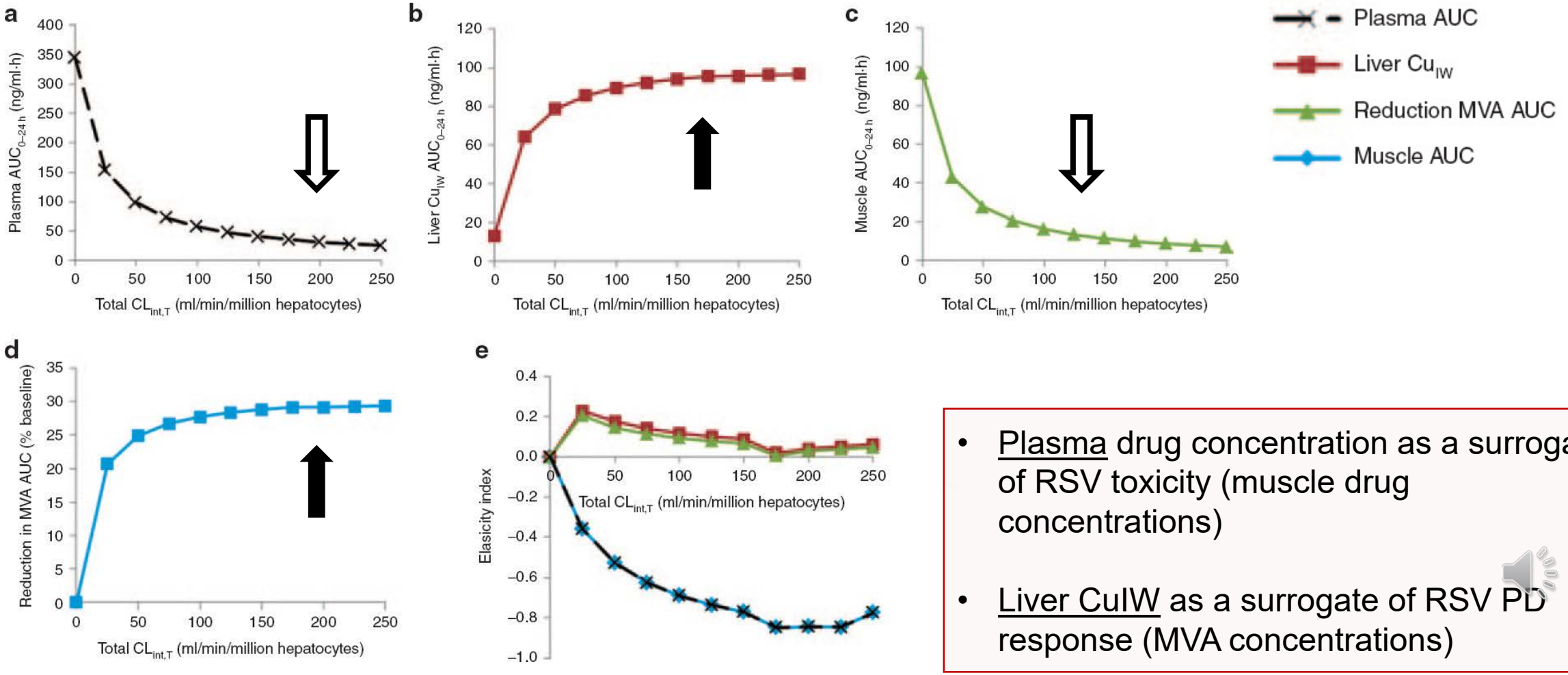
Reference; Extensive Transport | *Heterozygous; Intermediate Transport* | *Homozygous deficient; Poor Transport*



Rose et al., *CPT Pharmacometrics Syst Pharmacol* (2014).

Rosuvastatin's PBPK-PD Case Study

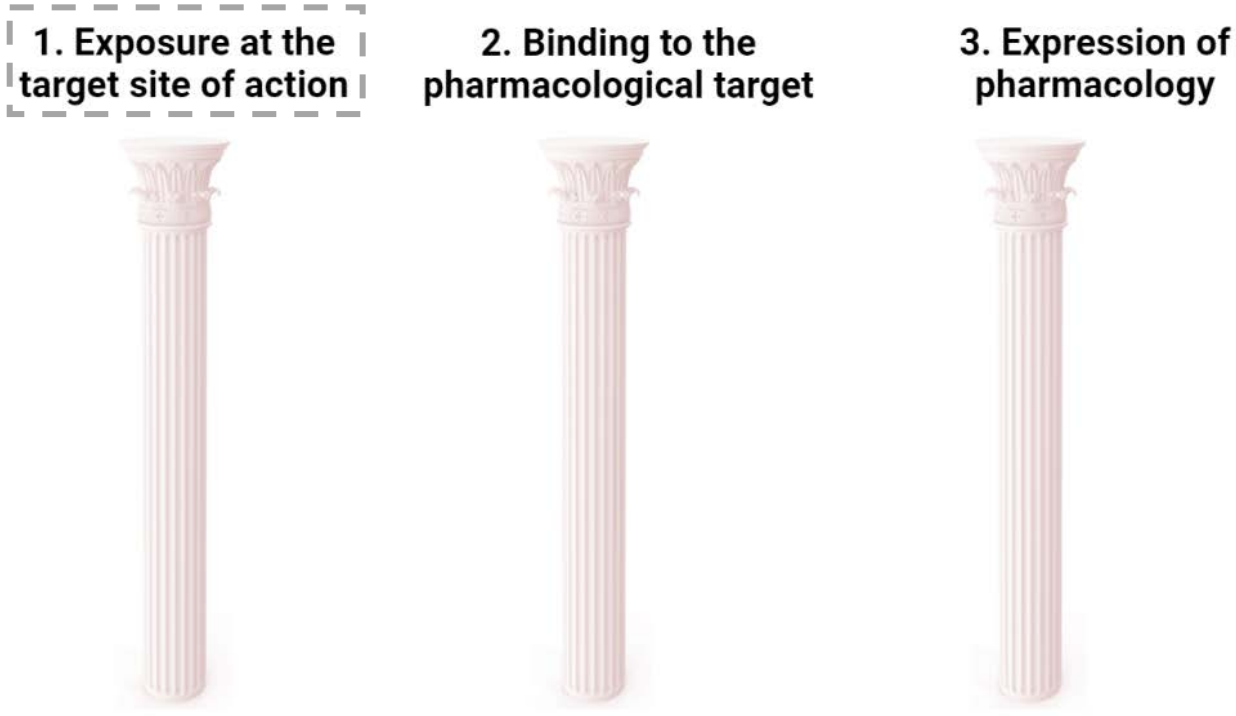
Sensitivity analysis - Influence of total uptake transporter intrinsic clearance ($CL_{int,T}$)

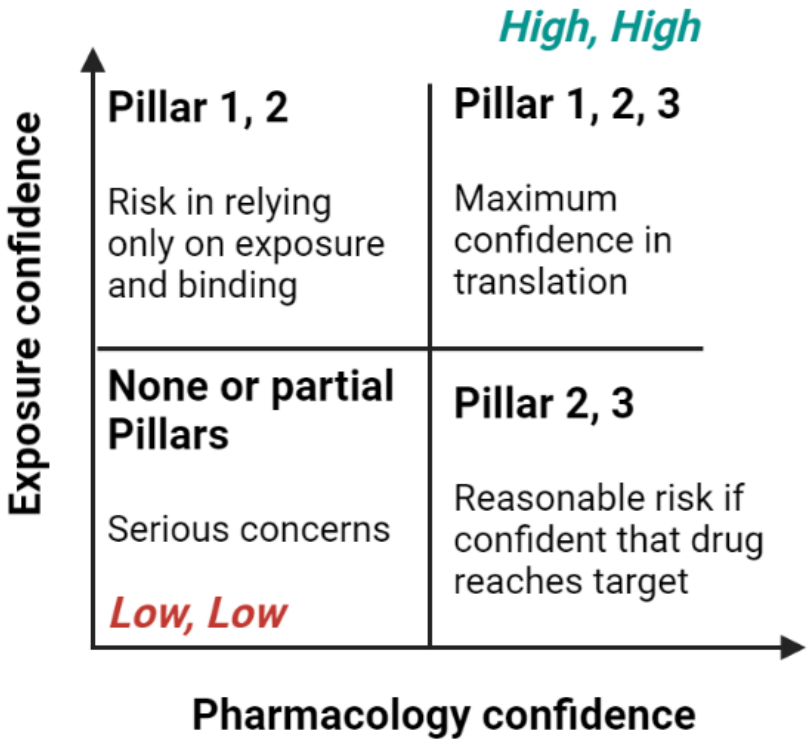


- Plasma drug concentration as a surrogate of RSV toxicity (muscle drug concentrations)
- Liver CuIW as a surrogate of RSV PD response (MVA concentrations)

Rose et al., *CPT Pharmacometrics Syst Pharmacol* (2014).

Navigating drug development: a three-pillar risk management matrix for mechanism testing and program progression





Morgan et al., *Drug Discov Today* (2012).

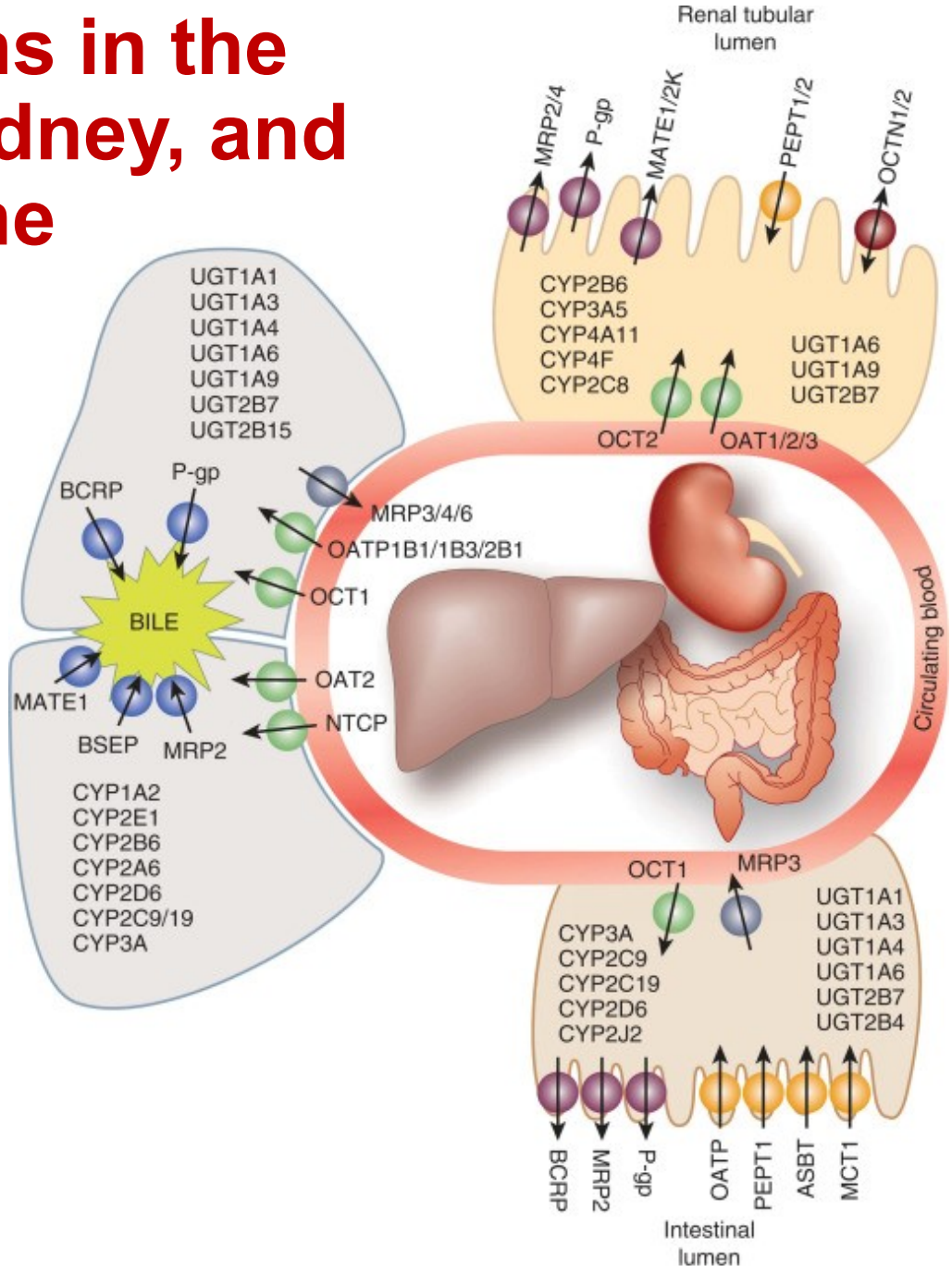
What data do we mostly have in the early phases of drug development?

1. In vitro systems and preclinical species such as mice, rat, dog, and monkey

2. Allometry scaling from preclinical species to extrapolate data to humans is challenging when drug is majorly metabolized and transported.

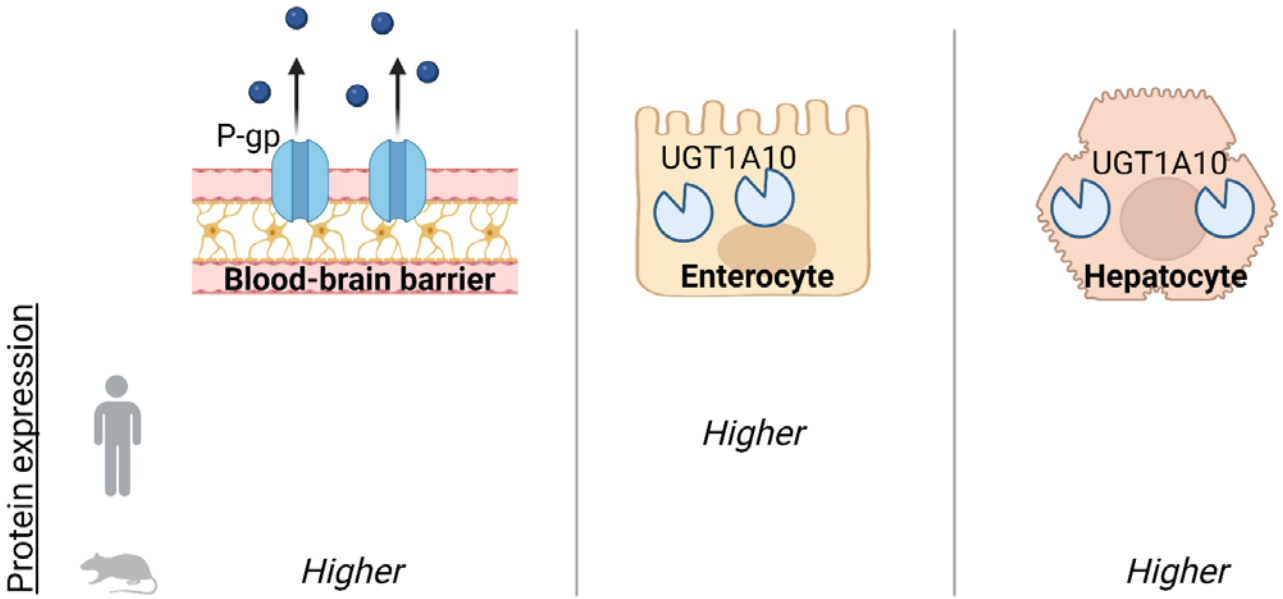
3. Differences in DMET protein abundance and orthology lead to interspecies variability in drug systemic and local PK (and therefore PD).

DMET proteins in the human liver, kidney, and intestine



Yeung et al., *Kidney Int* (2014).

Interspecies differences in DMET abundance - Challenges in extrapolating rodent PK data to humans



Differences in the observed efficacy and safety of drugs between humans and rats can be partly explained by physiological differences in the DMET protein abundance between the species.

1. JNJ-38877605 and SGX523 failed as clinical candidates because of nephrotoxicity in humans.
   - Higher aldehyde oxidase (AOX) abundance in humans compared to rodents, leading to the accumulation of AOX-mediated insoluble metabolites in kidneys

2. Rodents are not reliable in translating hepatotoxicity to humans.
   - Rodents failed to predict acetaminophen-induced liver injury, which can be partly explained by the inter-species differences in cytochrome P450 2E1 (CYP2E1)-mediated formation of hepatotoxic metabolite, N-acetyl-p-benzoquinone imine

3. Breast cancer resistance protein (Bcrp) abundance in the kidneys of rats is about 50-fold higher compared to human kidneys

Sharma et al., *Mol Pharm,* (2023); Basit et al., Mol. Pharm. 2020; Jaeschke et al., J Clin Transl Hepatol (2014); Sharma et al., Pharmaceutics (2023); Nakamura et al., Drug Metab Dispos (2008); Kutsukake et al., Drug Metab Dispos (2019); Verscheijden et al., Arch. Toxicol (2021).

How can animal data be translated to human considering the interspecies differences in DMET protein abundance?

- Physiologically based PK (PBPK) modeling is emerging as a reliable alternative to predict drug absorption and disposition including tissue drug concentrations.
- However, PBPK models require comprehensive data on drug- and physiology-specific parameters, including the abundance of DMET proteins.

The present study aimed to develop a repository of rat tissue quantitative proteomics data, which can then be used to predict systemic as well as tissue drug concentrations in rats prior to human studies by performing proteomics-informed PBPK modeling.

Regulatory agencies encourage the use of PBPK modeling!

FDA

The Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls

Guidance for Industry

Physiologically Based Pharmacokinetic Analyses — Format and Content

Guidance for Industry

EUROPEAN MEDICINES AGENCY

SCIENCE MEDICINES HEALTH

13 December 2018
EMA/CHMP/458101/2016
Committee for Medicinal Products for Human Use (CHMP)

Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation

Provisional Translation (as of February 2021)*

PSEHB/PED Notification No. 1221-1
December 21, 2020

To: Director of Prefectural Department of Health

Director of Pharmaceutical Evaluation Division,
Pharmaceutical Safety and Environmental Health Bureau,
Ministry of Health, Labour and Welfare
(Official seal omitted)

Guidelines for Analysis Reports Involving Physiologically based Pharmacokinetic Models

FDA modernization Act 2.0 – call to action?

Summary: S.5002 — 117th Congress (2021-2022)

All Information (Except Text)

Listen

There are 2 summaries for S.5002. Passed Senate (09/29/2022) Bill summaries are authored by CRS.

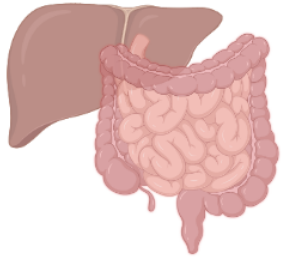
Shown Here:
Passed Senate (09/29/2022)

FDA Modernization Act 2.0

This bill authorizes the use of certain alternatives to animal testing, including cell-based assays and computer models, to obtain an exemption from the Food and Drug Administration to investigate the safety and effectiveness of a drug.

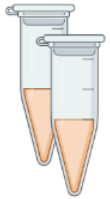
The bill also removes a requirement to use animal studies as part of the process to obtain a license for a biological product that is biosimilar or interchangeable with another biological product.

Workflow - Quantitative DMET proteomics



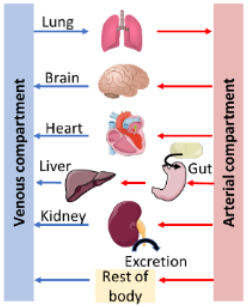
Sprague Dawley rat tissues (liver and regional intestine)

Protein isolation

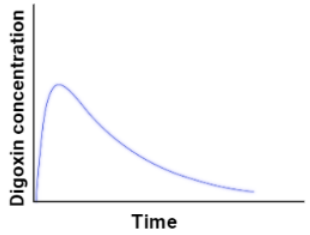


Trypsin digestion

PBPK modeling (PK-Sim)



Prediction of systemic and tissue concentrations of digoxin



Untargeted proteomics

Total protein approach (TPA)

$$[\text{Protein}]_x = \frac{\text{MS Response}_x}{\text{Total MS Response} \times \text{MW}_x}$$

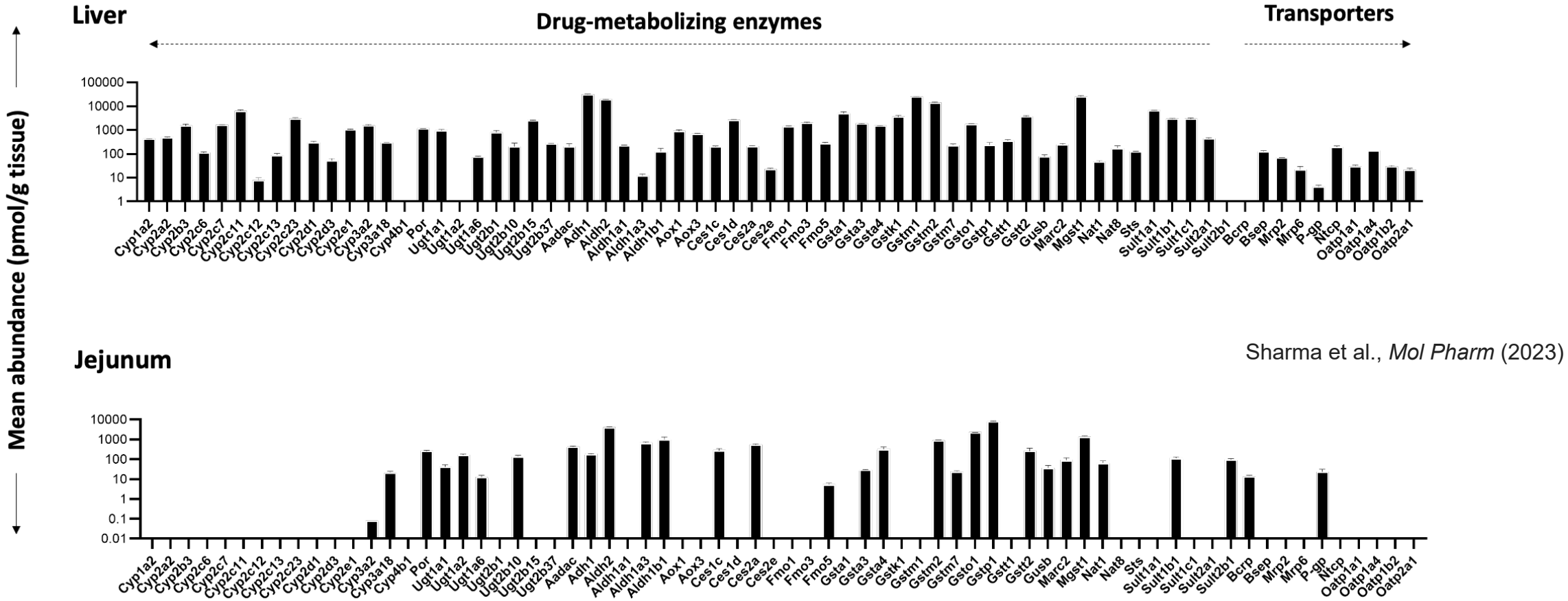
Quantifies all the proteins that are present

Targeted proteomics

Synthetic unlabeled peptides (calibrators)

Quantifies proteins of interest

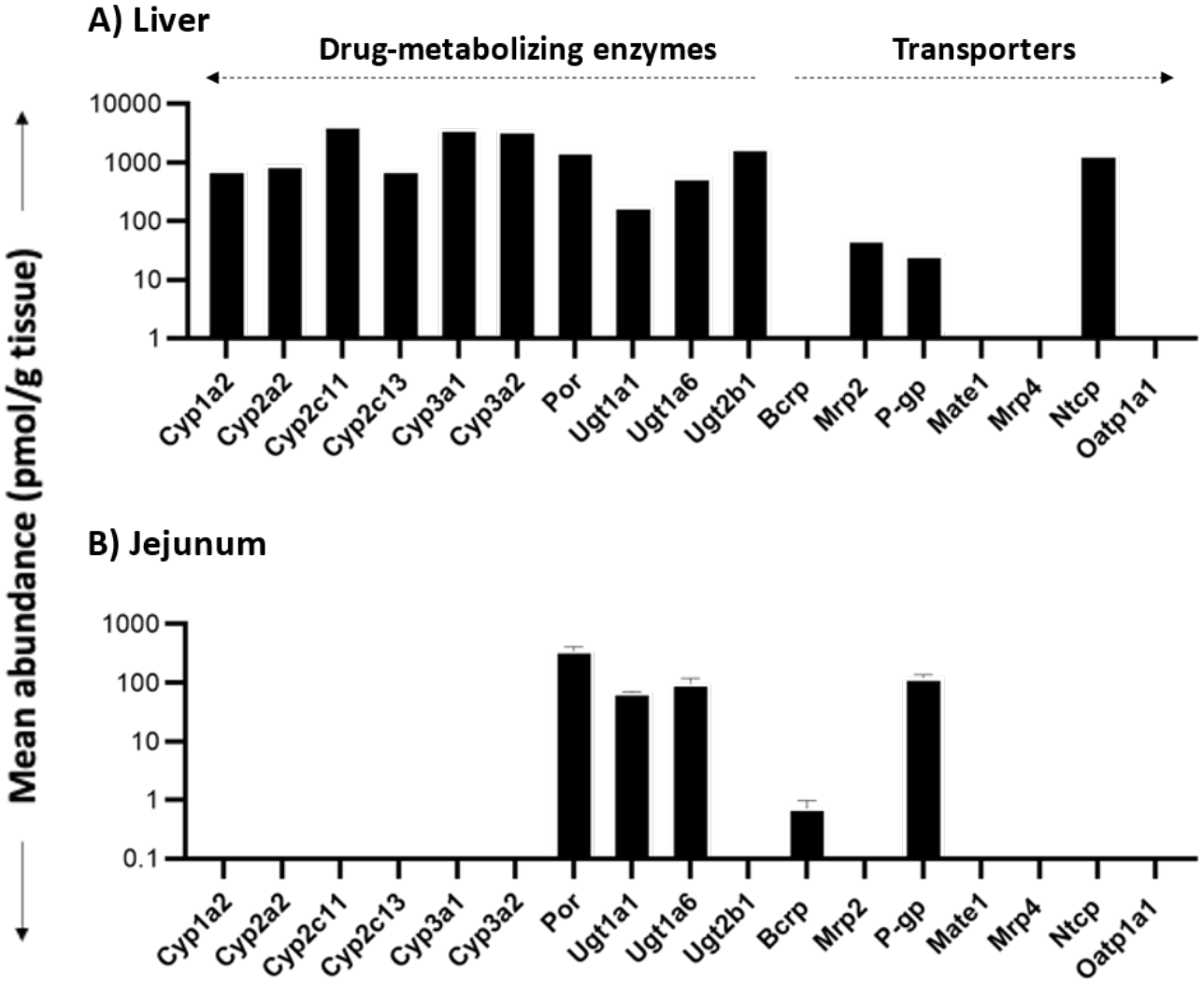
Untargeted (or global) proteomics map - DMET protein abundance across rat tissues



Sharma et al., *Mol Pharm* (2023)

- The global proteomics-based TPA was able to quantify 66 DMET proteins in the liver and 37 DMET proteins in the intestinal segments (duodenum, jejunum, ileum, and colon) of SD rats.
- Cyp and Ugt enzymes were mainly detected in the rat liver.
- P-gp abundance was higher in the intestine as compared to that in the liver.
- Bcrp was most abundant in the intestinal segments.
- Oatp 1a1, 1a4, and Mrp 2 and 6 were predominantly detected in the liver.

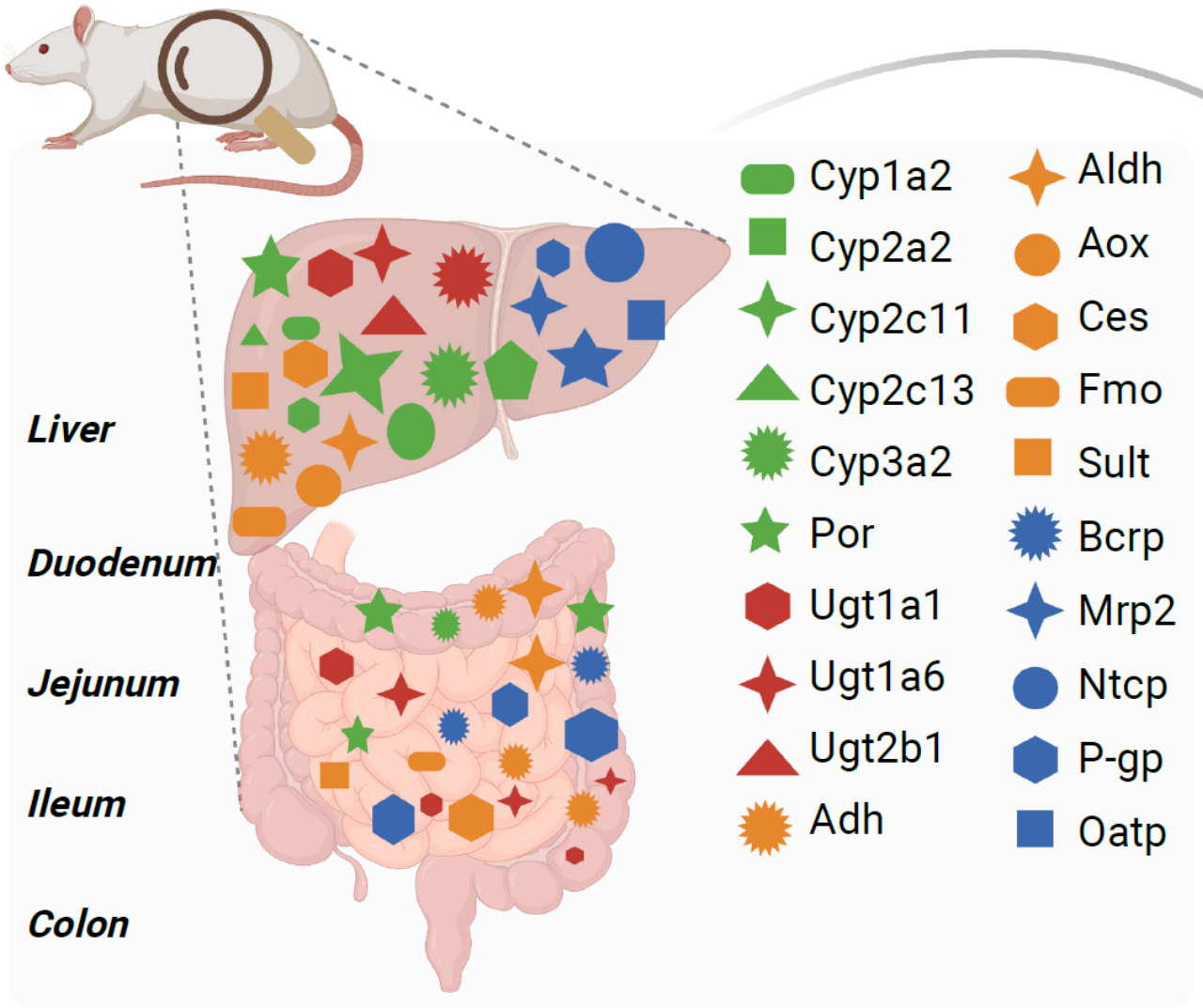
Targeted proteomics map - DMET protein abundance across rat tissues

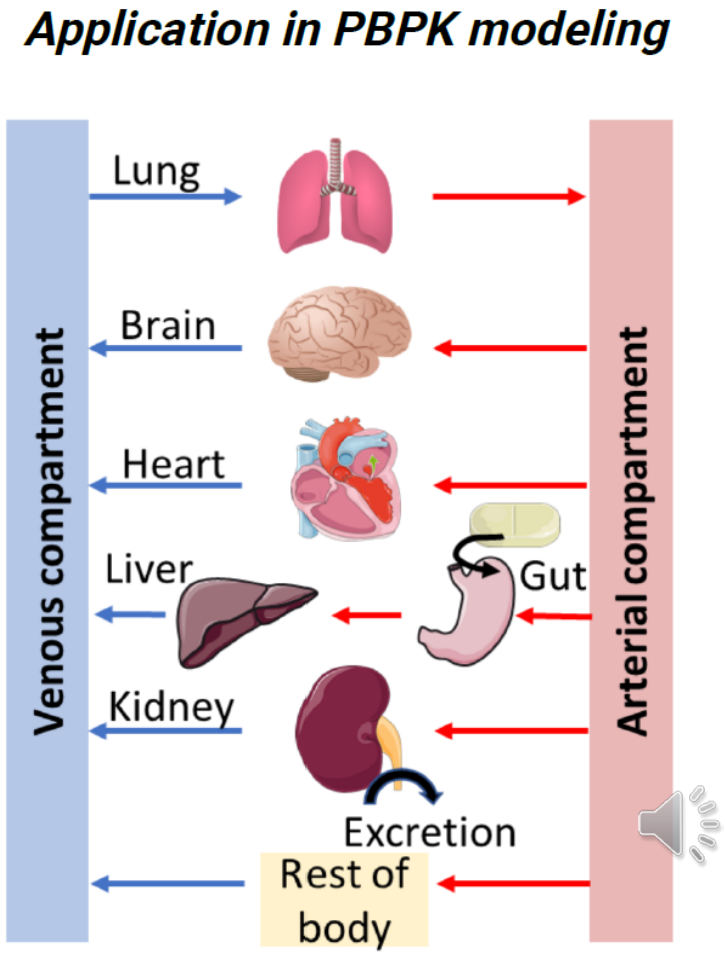


- The abundance of most DMET proteins determined by global and targeted proteomics analysis was within 3-fold, including Cyp1a2, Cyp2a2, Cyp2c11, Cyp3a2, Ugt2b1, and Mrp2.
- However, some proteins (e.g., Cyp2c13, Ugt1a6, Bcrp, Ntcp, and P-gp) showed more than 3-fold difference between proteomics methods.
- The proteins that showed larger differences in protein quantification by the global and targeted data were the low abundant ones.

Sharma et al., *Mol Pharm* (2023)

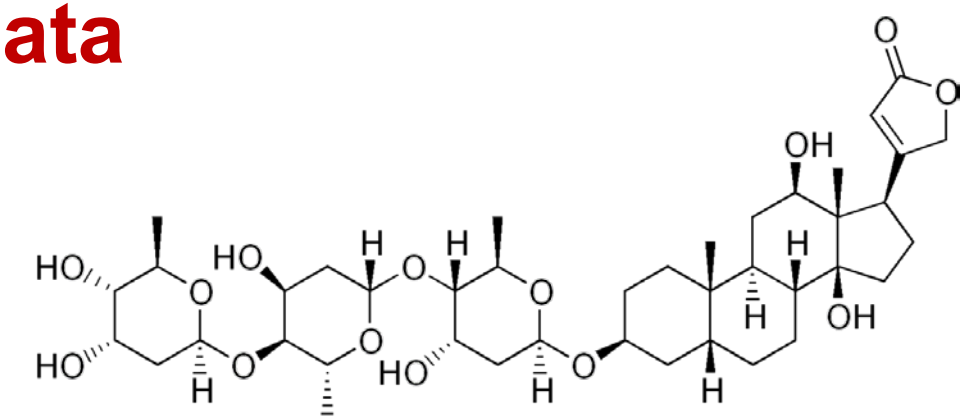
Integration of DMET abundance in PBPK modeling





Sharma et al., *Mol Pharm* (2023)

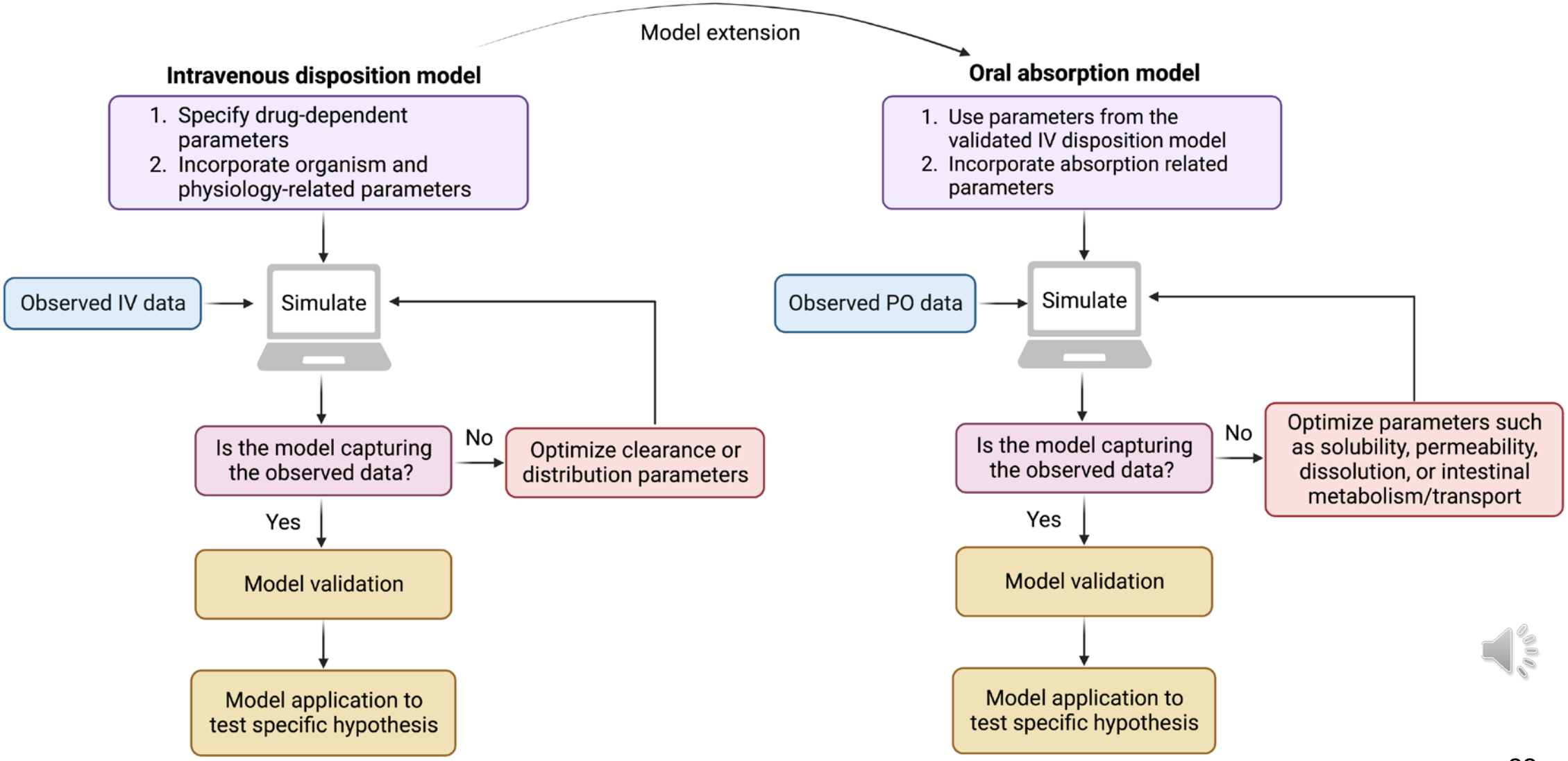
Digoxin as a model drug to show the utility of proteomics data



- Used for heart failure and arrhythmias
- Narrow-therapeutic index (0.8 to 2 ng/ml)
- Poorly water soluble (< 0.06 mg/ml) and permeable (effective permeability < 5.09×10^{-5} cm/s)
- Bioavailability ≈ 50-90%
- Substrate of an efflux transporter, P-gp

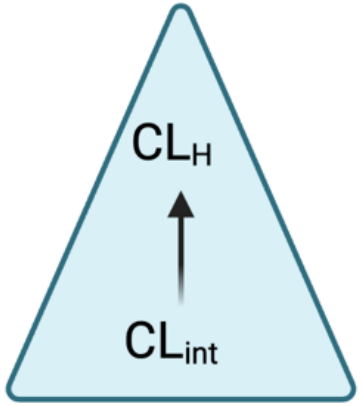
Angraal et al., *Am J Med* (*2019*)

Workflow - building a PBPK model



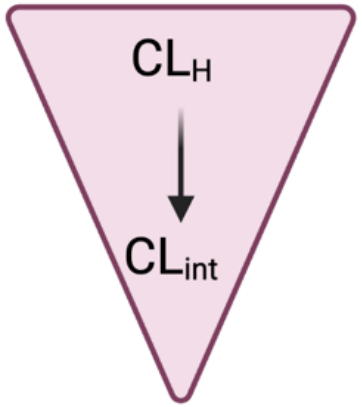
Approaches for building a PBPK model

Bottom-up



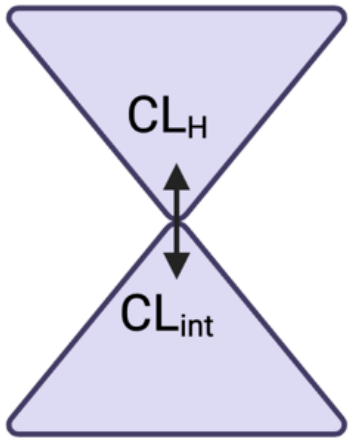
- Mechanistic
- Accounts non-linearity (K_m)
- Less accurate predictions

Top-down



- Accounts linearity (CL_{int})
- Supports clinical trial decisions
- Suitable for population PK analysis

Middle-out

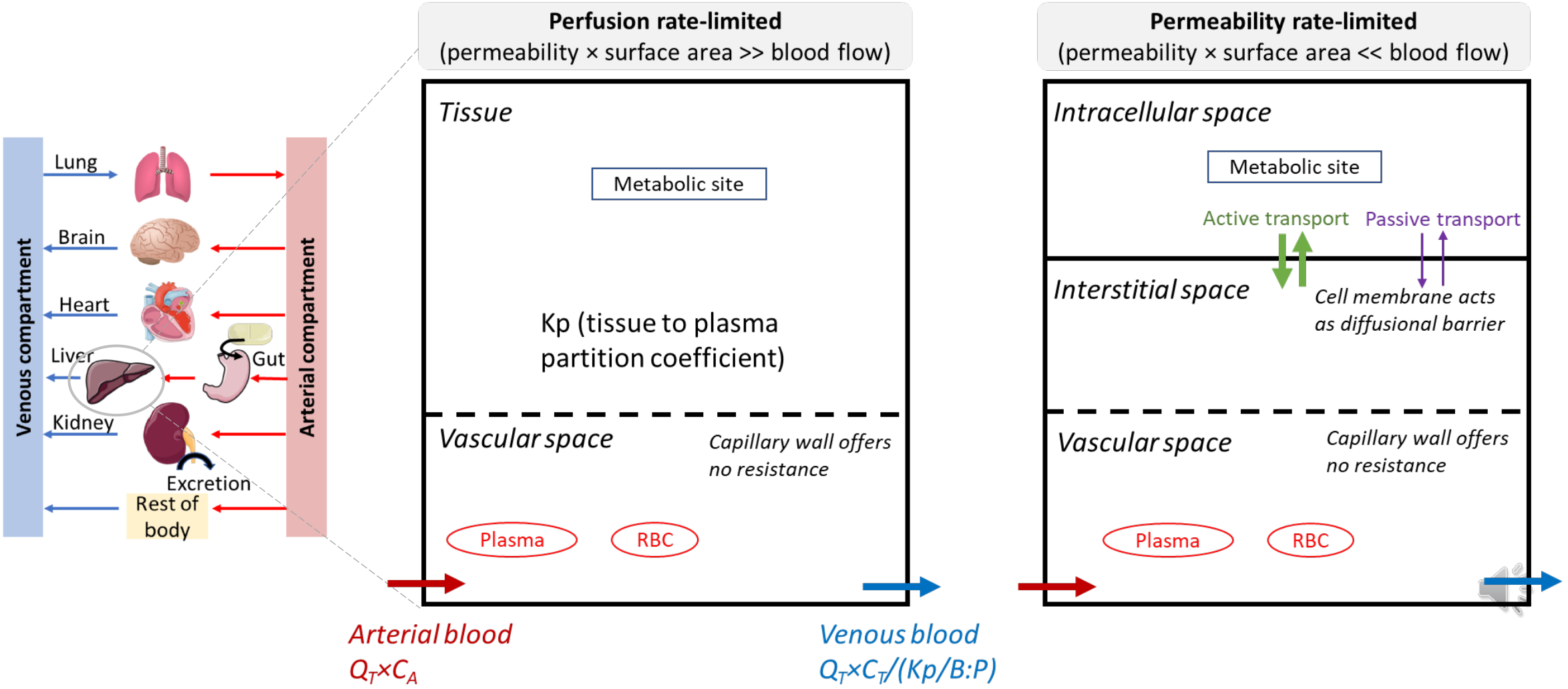


- Integrates both *in vitro* and *in vivo* data
- Semi-mechanistic and clinically relevant

Tsamandouras et al., *Br J Clin Pharmacol* (2015).

$CL_{int,}$: intrinsic clearance; $CLu_{int,H}$: in vivo unbound intrinsic hepatic clearance

Perfusion vs. permeability rate-limited tissue models



Jones et al., *CPT:PSP* (2013).

Validation criteria for PBPK modeling

Depends on the goal of the study!

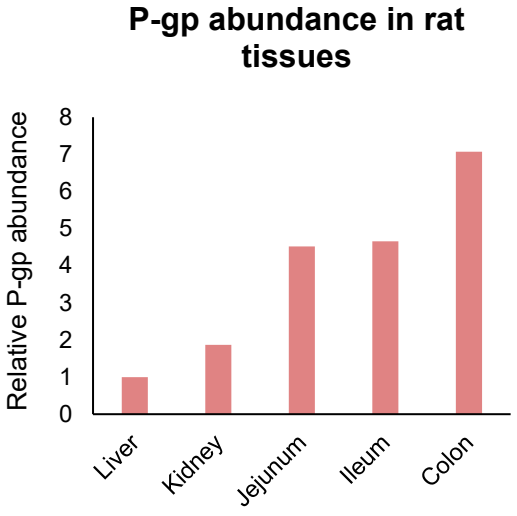
1. **Bioequivalence criterion**
    - Simulated to observed PK endpoint (AUC or C_{max}) within **0.85 to 1.25-fold**
    - Stringent and can be used for dosing regimen and waiver of clinical trials
2. **2-fold criterion**
    - Simulated to observed PK endpoint (AUC or C_{max}) within **0.5 to 2-fold**
    - Loose and can be used for trial design and sampling time-point collections

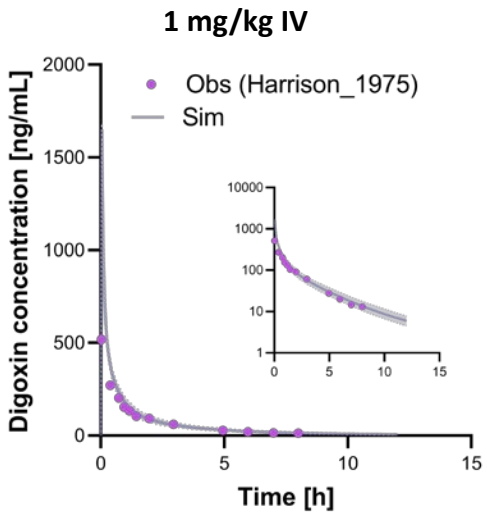
Proteomics-informed PBPK modeling of digoxin

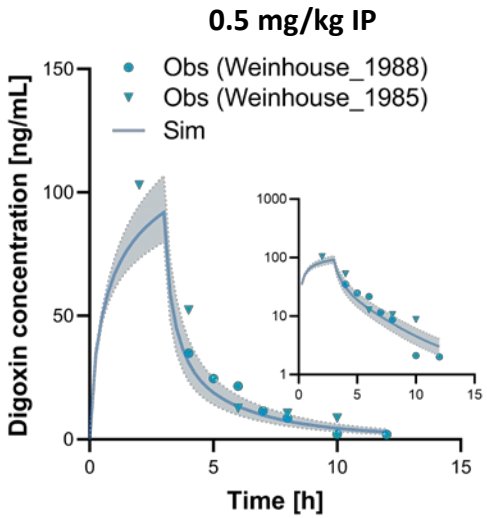
$$V_{max,tissue} = k_{cat} \times \text{relative abundance} \times [\text{abundance}]_{liver} \quad (\boldsymbol{Eq\ 1})$$

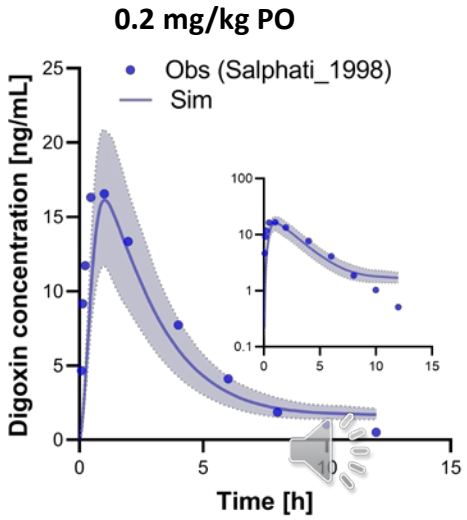
$$\text{Relative abundace} = \frac{[\text{abundance}]_{\text{intestine or kidney}}}{[\text{abundance}]_{liver}} \quad (\boldsymbol{Eq\ 2})$$

$$\text{Reference conc. (µmo l/l)} = \left(\frac{\text{pmol protein}}{\text{mg homogenate protein}} \times \frac{\text{mg homogenate protein}}{\text{g tissue}} \times \text{tissue density (g/l)}\right)/1000000 \quad (\boldsymbol{Eq\ 3})$$





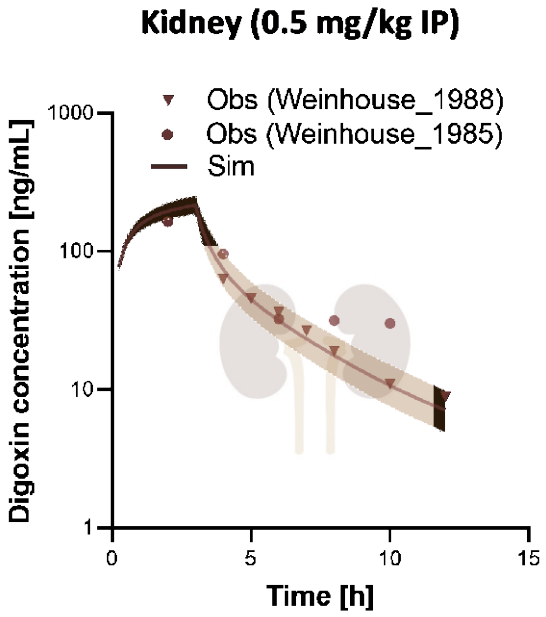


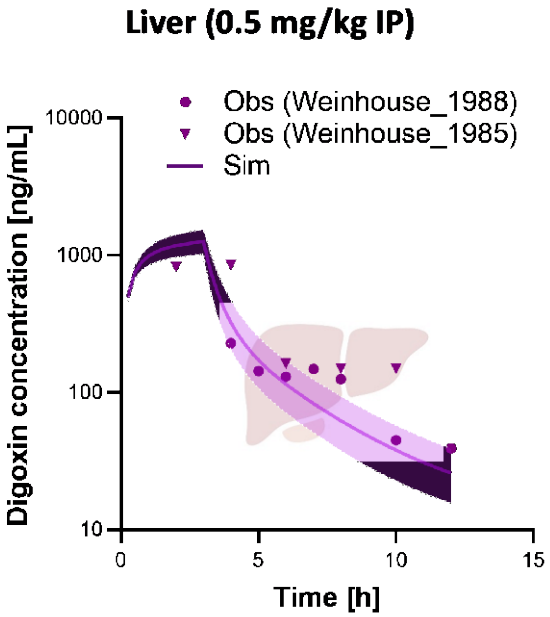


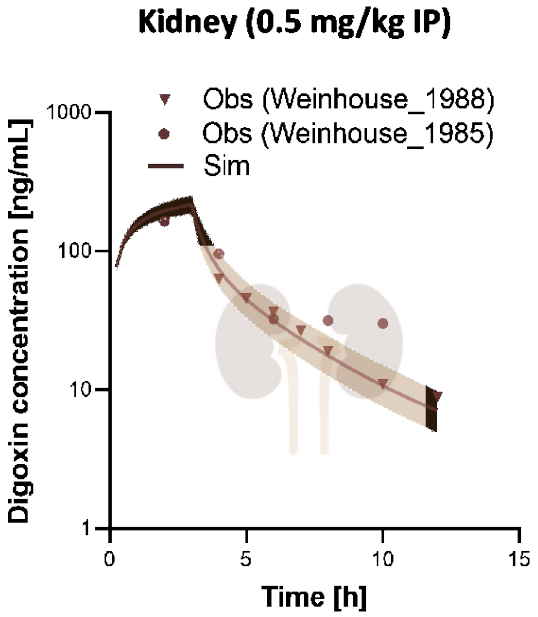
Sharma et al., *Mol Pharm, 2023*

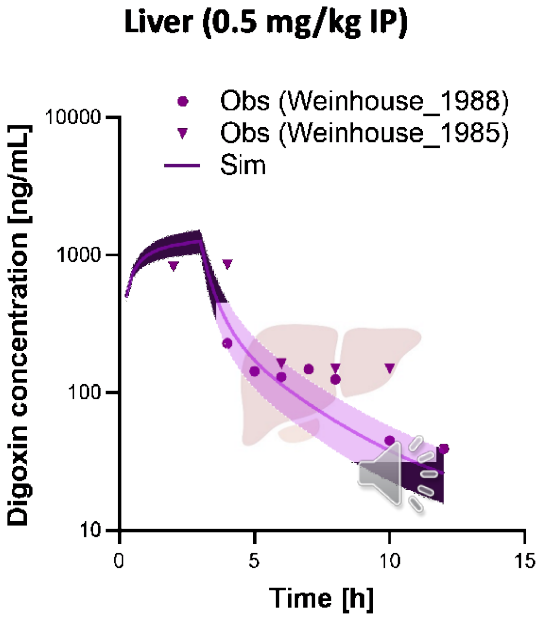
Simulations using PK-Sim v11

Predicting tissue concentrations of digoxin



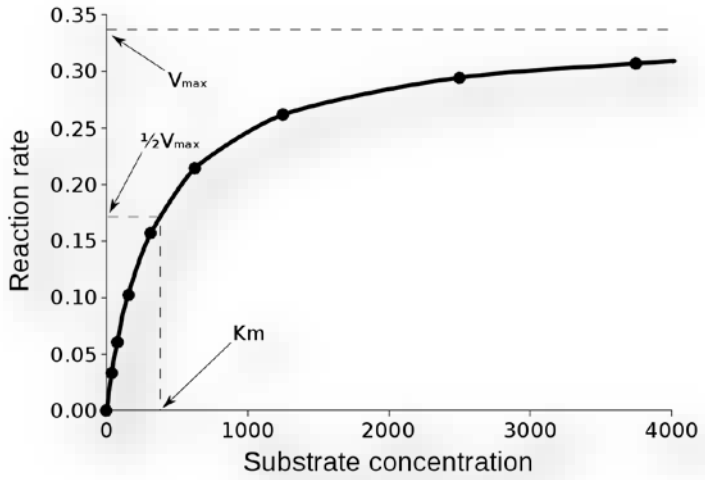




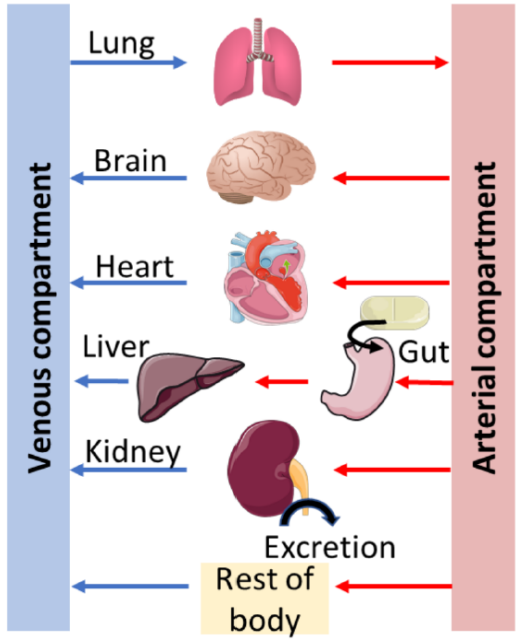


Sharma et al., *Mol Pharm (2023)* Simulations using PK-Sim v11

How to extrapolate the animal data to human using PBPK modeling?



$$\mathbf{CL_{int}} = \frac{\mathbf{V_{max}}}{\mathbf{K_m}} = \frac{\mathbf{k_{cat}} \times [\boldsymbol{abundance}]}{\mathbf{K_m}}$$



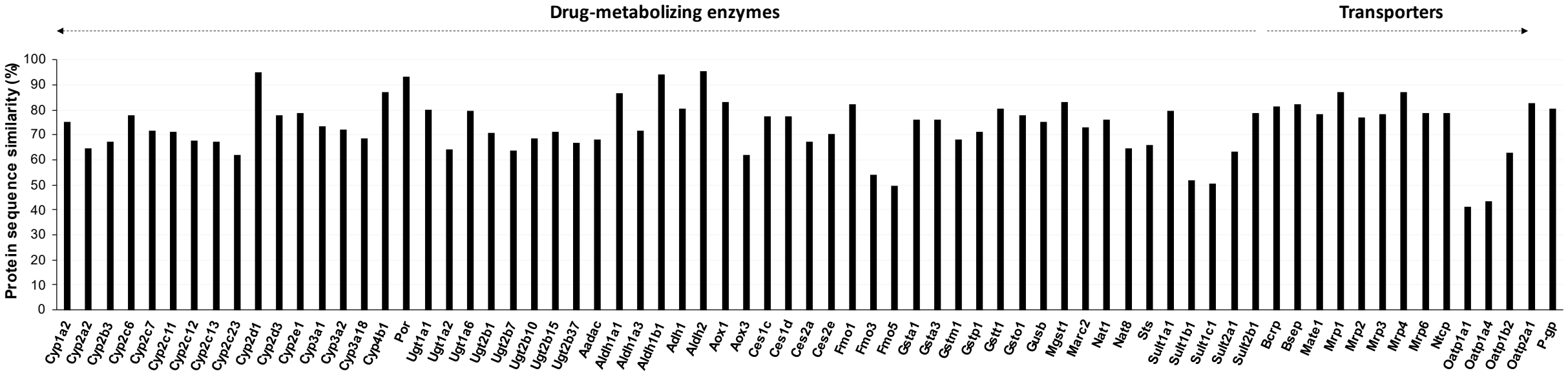
$$\mathbf{CL_{int,human}} = \mathbf{CL_{int,rat}} \times \frac{\mathbf{K_{m,rat}}}{\mathbf{K_{m,human}}} * \frac{[\mathbf{abundance}]_{\mathbf{human}}}{[\mathbf{abundance}]_{\mathbf{rat}}}$$

- *If the active sites of the proteins are not orthologous, it warrants information on Km*
- *k_{cat} : assumed constant across human and animal species*

Prasad et al., *J Pharm Sci* (2017).
Sharma et al., *Mol Pharm* (2023).

CL_{int} : intrinsic clearance; V_{max} : maximum reaction velocity; K_m : Michaelis-Menten constant; k_{cat} : enzyme catalytic activity

Percent sequence similarity - DMET proteins in *Rattus norvegicus* compared to *Homo sapiens*



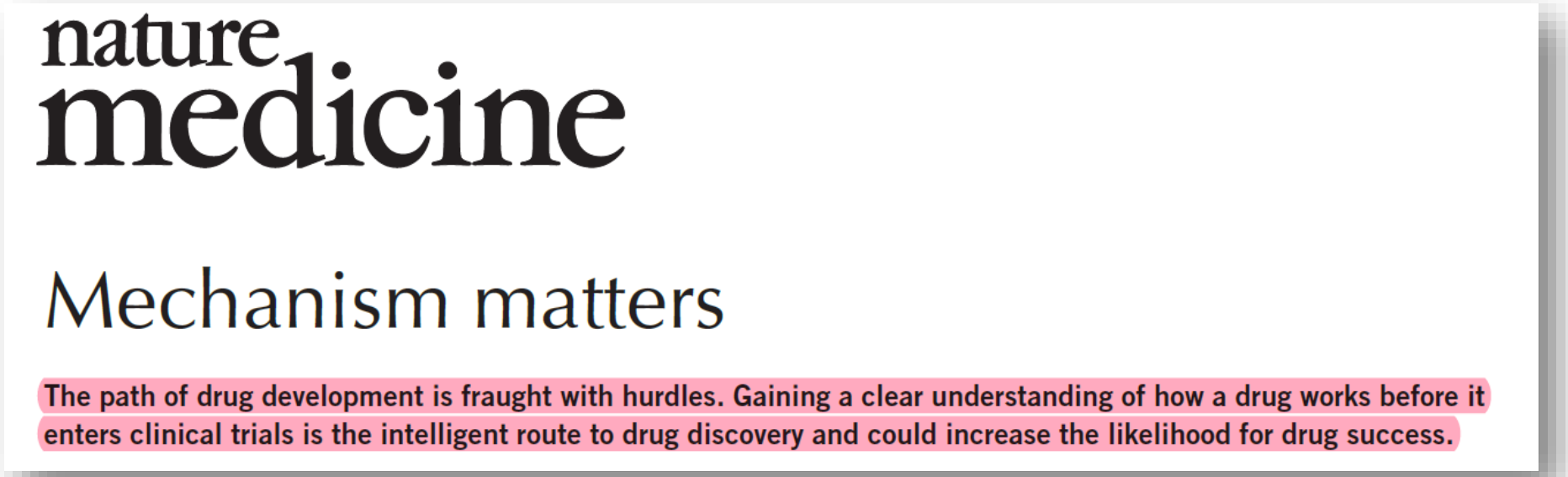
The average percent sequence similarity (orthology) of DMET protein sequences between *R. norvegicus* and *H. sapiens* was 70%.

Key takeaways

1. Tissue drug concentration is crucial for evaluating the drug efficacy and toxicity at the target tissue site but measuring this is often not feasible.
   - *It can be predicted by incorporating in vitro assay, quantitative proteomics, and PBPK modeling.*
2. As a proof of concept, a proteomics-informed PBPK model for digoxin was developed for rats, demonstrating its ability to predict tissue drug concentrations.
3. In drug development settings, this approach can be systematically applied by:
   - *obtaining DMET in vitro and proteomics data for the drug candidate*
   - *integrating this data into a PBPK model*
   - *using the model to predict tissue drug concentrations at the target site, thereby guiding dosage optimization and potential clinical trial outcomes.*

Changing the Era of PK-PD to PBPK-QSP Modeling

- Mostly PK-PD analysis is based on systemic drug concentration.
- PBPK-QSP modeling can
  - ✓enable PK-PD based on tissue concentration of drugs
  - ✓help to understand the mechanism of drug action at the tissue level
  - ✓establish confidence in target occupancy profiles


nature medicine

Mechanism matters

The path of drug development is fraught with hurdles. Gaining a clear understanding of how a drug works before it enters clinical trials is the intelligent route to drug discovery and could increase the likelihood for drug success.

Editorial - Nature Medicine (2010)