Recent Advances in Drug Transporter and Enzymology Sciences to Enable Drug Design

Larry Tremaine

NEDMDG Fall Meeting

September 13, 2017
The Parameters We Measure and the Predictions to Which They Contribute

- Predict Efficacious Concentration
  - Target $K_i$
  - $f_u,\text{target}
  - $K_{p,uu,\text{target}}$

- Predict PK
  - $f_u,\text{plasma}$
  - BL/PL

- Predict Variability
  - $f_{\text{met}}$

- Predict DDI
  - $f_{\text{transport}}$

- Predict Dosing Regimen

- Predict Safety
  - TK

- Predict Human Metabolites
  - Metabolite ID
  - CL$_{\text{int,txp}}$
  - CL$_{\text{int,met}}$
  - $f_u,\text{in vitro}$
  - CYP
  - CYP IC$_{50}$
  - Transporter IC$_{50}$
  - UGT IC$_{50}$
  - CL$_{\text{int,u}}$
  - $k_{\text{inact}}/K_i$
  - Animal
  - CL$_{\text{renal}}$

- The Parameters We Measure and the Predictions to Which They Contribute

Courtesy of S. Obach
<table>
<thead>
<tr>
<th>ADME Property</th>
<th>Measurements/Parameters</th>
<th>Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL - metabolic</td>
<td>Hepatic metabolism, reaction phenotyping</td>
<td>HLM, suspension HHEPs or HHEP relay +/- chemical inhibitors</td>
</tr>
<tr>
<td>DDI - victim</td>
<td>Fm, Ft</td>
<td>Chemical inhibitors with HHEPs, HLM</td>
</tr>
<tr>
<td>CL - renal</td>
<td>Ppb, active secretion</td>
<td>Rat SSS, OATs-HEK RAF</td>
</tr>
<tr>
<td>CL – hepatic uptake</td>
<td>Active uptake, passive uptake, transporter phenotyping</td>
<td>SCHH, PHH +/- inhibitors, OATPs-HEK RAF; NHP SSS</td>
</tr>
</tbody>
</table>
| Ceff, tissue distribution | Ppb; Cbu/Cpu  
Tissue exposure; fu,cell                                                                  | In silico; equilibrium dialysis; Pgp and BCRP-MDCK; Kpuu;            |
| CL – biliary          | Biliary excretion                                                                       | SCHH +/- Ca²⁺                                                        |
| DDI - perpetrator     | Competitive DDI  
TDI  
Induction                                                                                     | HLM  
HLM  
SCHH, PXR reporter assay                                            |
| Absorption - fafg     | Permeability; Efflux transporters; CYP3A metabolism                                      | RRCK (low canine Pgp expression)  
Pgp and BCRP MDCK  
HLM, preclinical species PK                                         |
Genesis of Predicting Human CL from In Vitro Data at Pfizer

NONSPECIFIC BINDING TO MICROSOMES: IMPACT ON SCALE-UP OF IN VITRO INTRINSIC CLEARANCE TO HEPATIC CLEARANCE AS ASSESSED THROUGH EXAMINATION OF WARFARIN, IMIPRAMINE, AND PROPRANOLOL

R. SCOTT OBACH
Department of Drug Metabolism, Pfizer Central Research
(Received April 25, 1997; accepted August 19, 1997)

Fig. (1). Examples of In Vitro Lability Data.
Symbols: Solid circles: the ideal data, a first-order decline in substrate concentration with time; Solid squares: an initial apparent rise in concentration, many times due to slow solubilization of a lipophilic substrate into the incubation matrix; Solid triangles: substrate is too stable to permit calculation of intrinsic clearance; Open diamonds: Substrate depletion that is slow but just within the limit where a reasonably reliable estimate of intrinsic clearance can be made (i.e. 15% depletion over the incubation time); Open triangles: substrate that is too rapidly consumed to permit reliable estimation of intrinsic clearance.

3. PREDICTING IN VIVO CLEARANCE

In order to predict in vivo clearance from in vitro data, two steps need to be followed: (a) The data must be scaled to the whole liver. (b) The scaled intrinsic clearance must be inserted into a model of liver clearance [4]. The in vitro degradation rate constant measured above is converted to an in vivo intrinsic clearance by dividing it by the protein concentration used in the incubation:

\[ CL'_{int} = \frac{k_{deg}}{mg \text{ microsomal protein per mL}} \]

(The term CL'_{int} refers to the intrinsic clearance of unbound substrate. It is equal to the measured total CL_{int} divided by the unbound fraction in the incubation. In high
PDM **CONSISTENTLY** re-evaluated our PK-predictions to improve and evolve our Science.

There has been many teams over the years:

- **1995 – 1998**
  - 19 Compounds
  - T₁/₂

- **2006 – 2010**
  - 115 Compounds
  - T₁/₂; CL/F

- **1998 – 2000**
  - 20 Compounds
  - T₁/₂

- **1998 – 2002**
  - 21 Compounds
  - Dose

- **1998 – 2005**
  - 50 Compounds
  - T₁/₂; CL/F

- **2007-2008**
  - 21 Compounds
  - Profile based on PBPK

---

Obach et al., JPET (1997) 283:46-58

*Courtesy S. Steyn*
No single method accurately predicts **ALL** compounds (**2006-2010 Data Set**)  
If several approaches agreed, then high “confidence in prediction”
Extended Clearance Classification System → Predictability

- Permeability cut off = $5 \times 10^{-6} \text{ cm/sec}$
- Molecular Weight cut off: 400 [Class 1]
- Ionization: Acid+Zwit vs Base+Neutral

Utilized physchem/ in vitro properties to classify compounds based on their rate determining clearance step

- Identified rate-determining clearance step using Pfizer/Novaartis dataset
- Obtained data from Obach et al. + other papers.

$N = 789$

Prevalence and importance of non-P450 metabolism

FDA–Approved Oral and Intravenous Drugs: 2006–2015

M Cerny *DMD* 2016, 44:1246–1252
Clearance Panel – Identify major CL mechanism, appropriate in vitro systems and generate rate constants

Empirical Classification based on Physiochemical Properties

ECCS Class 2 and 1A

ECCS Class 1B, 3 and 4

HHeps+/-ABT

HHeps Type of metabolism

Transporters Acids OATPs, OATs

Transporters Bases OCTs

CYP Dominated

No Metabolism

Non-CYP Dominated

rCYP CLint

UGT CLint

AO CLint

Other Conj.

Other Ox.

Hydrolysis

Tier 1

Tier 2
From Jan, 2016 – June 2017 (18 months) approximately 7000 compounds submitted to 4 hr suspension HHEP for metabolic CL

Approximately 1000 compounds have Clia <3 ul/min/million cells.
Extending Cl_{metabolic} Dynamic Range – Hepatocyte relay method for low clearance

\[ C_{corrected} = C_{n,total} \times \left( \text{recovery} \right)^n \times \left( \frac{\text{well volume}}{\text{transfer volume}} \right)^n \times \prod_{i=1}^{n} \left( \frac{C_{i-1,total}}{C_{i-1,supernatant}} \right) \]

Di et al., DMD (2012) 40:1860-1865
Di et al., DMD (2013) 42:2018-2023

<table>
<thead>
<tr>
<th>Assays</th>
<th>Incubation Time</th>
<th>CLint Detention Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 2a HHEP</td>
<td>4 Hours</td>
<td>7.5 mL/min/Kg</td>
</tr>
<tr>
<td>HHEP Relay</td>
<td>20 Hours – 0.5Mcells/ml</td>
<td>1.46 mL/min/Kg</td>
</tr>
<tr>
<td></td>
<td>20 Hours – 2.0Mcells/ml</td>
<td>0.36 mL/min/Kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Enzyme</th>
<th>In vivo</th>
<th>Relay Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>CYP3A, 2C19</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>CYP2C9</td>
<td>4.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Theophylline</td>
<td>CYP1A2</td>
<td>2.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Timolol</td>
<td>CYP2D6</td>
<td>36-49</td>
<td>14</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>CYP3A</td>
<td>5.9</td>
<td>4.6</td>
</tr>
<tr>
<td>S-Warfarin</td>
<td>CYP3A, 2C9</td>
<td>4.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Zolmitriptan</td>
<td>CYP1A2 and MAO</td>
<td>13</td>
<td>3.2*</td>
</tr>
</tbody>
</table>

* Under-predict due to extra-hepatic contributions of MAO
IVIVE for Metabolic CL using Global Lot of HHEPs

IVIVE for $CL_{b,obs} \leq 10 \text{ mL/min/Kg}$

- Most applicable clearance range for oral drugs.
- 70% of data within this cutoff (need high n’s for high confidence)
- Cutoff enables application of single scalar regardless of assay type.

Hepatocyte lot DCM apparent intrinsic clearance ($CL_{int,app}$) measures should be scaled by a factor of 1.0 regardless of assay conditions (4 hr suspension or relay)

_Hepatocyte lot DCM apparent intrinsic clearance ($CL_{int,app}$) measures should be scaled by a factor of 1.0 regardless of assay conditions (4 hr suspension or relay)_

*Courtesy S. Steyn*
Metabolic Phenotyping - Reporting Limitations of Substrate Depletion Assays (CYP)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Incubation time</th>
<th>Concentration</th>
<th>Lowest Reportable CLi,a,s</th>
<th>Measured CLint,a,s</th>
<th>Maximum Observable Inhibition</th>
<th>Current limit of parent depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM</td>
<td>60 min</td>
<td>0.75 mg/mL</td>
<td>7.0 mL/min/kg</td>
<td>150</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>HHEP</td>
<td>4 H</td>
<td>0.5e6 cells/mL</td>
<td>7.5 mL/min/kg</td>
<td>140</td>
<td>94%</td>
<td>99%</td>
</tr>
<tr>
<td>Standard Screening Assays</td>
<td>HLM</td>
<td>60 min</td>
<td>2.0 mg/mL</td>
<td>2.6 mL/min/kg</td>
<td>130</td>
<td>98%</td>
</tr>
<tr>
<td>HHEP</td>
<td>20 H</td>
<td>0.5e6 cells/mL</td>
<td>1.5 mL/min/kg</td>
<td>120</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>HHEP Relay</td>
<td>20 H</td>
<td>2e6 cells/mL</td>
<td>0.36 mL/min/kg</td>
<td>110</td>
<td>87%</td>
<td>93%</td>
</tr>
</tbody>
</table>

- Highlighted values represent compound CL required to enable capture of majority of CYP activity under given conditions (sum of ALL contributing isoforms)
  - i.e. 90% of one enzyme, 90% of 2 enzymes (40% CYP1, 50% CYP2)
- Increased sensitivity comes with increased reagent consumption and assay cost
Novel HHEP Relay - Reaction Phenotyping Assay

- Pre-incubation with Inhibitor
- Remove Inhibitor
- Incubate with Substrate
- Transfer Supernatant

- MBIs to avoid the impact of inhibitor depletion during long incubation
- MBIs were removed from incubation after inactivation to increase selectivity by minimizing reversible inhibition (CYP, AO, UGT, SULT, CES, Transporters)


Courtesy of L. Di
Experimental Conditions for HHEP Relay Phenotyping

<table>
<thead>
<tr>
<th>CYP</th>
<th>Pre-Incub. Time (min)</th>
<th>Inactivators and Concentrations</th>
<th>Substrates</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>15</td>
<td>1 µM Furafylline</td>
<td>Phenacetin</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>2B6</td>
<td>15</td>
<td>10 µM Phencyclidine</td>
<td>Bupropion</td>
<td>OH-Bupropion</td>
</tr>
<tr>
<td>2D6</td>
<td>15</td>
<td>1.8 µM Paroxetine</td>
<td>Dextromethorphan</td>
<td>Dextrorphan</td>
</tr>
<tr>
<td>2C8</td>
<td>30</td>
<td>100 µM Gemfibrozil Glucuronide</td>
<td>Amodiaquine</td>
<td>N-Desethylamodiaquine</td>
</tr>
<tr>
<td>2C9</td>
<td>30</td>
<td>15 µM Tienilic Acid</td>
<td>Diclofenac</td>
<td>4’-OH-Diclofenac</td>
</tr>
<tr>
<td>2C19</td>
<td>15</td>
<td>8 µM Esomeprazole</td>
<td>Mephenytoin</td>
<td>4’-OH-Mephenytoin</td>
</tr>
<tr>
<td>3A</td>
<td>15</td>
<td>25 µM Troleandomycin</td>
<td>Midazolam</td>
<td>1’-OH-Midazolam</td>
</tr>
<tr>
<td>3A4</td>
<td>15</td>
<td>2 µM CYP3cide*</td>
<td>Midazolam</td>
<td>1’-OH-Midazolam</td>
</tr>
<tr>
<td>Pan-CYP</td>
<td>30</td>
<td>1 mM ABT &amp; 15 µM Tienilic Acid</td>
<td>All Above</td>
<td>All Above</td>
</tr>
</tbody>
</table>

* Genotyped HHEP CYP3A5 EM

Courtesy of L. Di
HHEP Relay Phenotyping Assay Validation

**Reported $f_m$: 0.5 3A, 0.5 2C19**

**Relay $f_m$: 0.56 3A, 0.42 2C19**


Courtesy of L. Di
Increased Sensitivity of Metabolite Formation Assays (CYP)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Radiometric</th>
<th>Mass Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time</td>
<td>30 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Concentration</td>
<td>2 mg/mL pt HLM</td>
<td>2 mg/mL pt HLM</td>
</tr>
<tr>
<td>Substrate Conc</td>
<td>1 uM</td>
<td>1 uM</td>
</tr>
<tr>
<td>Quantitative limit</td>
<td>200 - 100000 dpm (0.2%)</td>
<td>0.1 nM (LLOQ)</td>
</tr>
<tr>
<td>Lowest Reportable CLint,a,s</td>
<td>0.03 mL/min/kg</td>
<td>0.0015 mL/min/kg</td>
</tr>
</tbody>
</table>

**Measured CLint,a,s**

<table>
<thead>
<tr>
<th>Measured CLint,a,s</th>
<th>Maximum Observable Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>0.4</td>
<td>99%</td>
</tr>
<tr>
<td>0.04</td>
<td>93%</td>
</tr>
<tr>
<td>0.01</td>
<td>70%</td>
</tr>
<tr>
<td>0.004</td>
<td>25%</td>
</tr>
<tr>
<td>0.002</td>
<td>25%</td>
</tr>
</tbody>
</table>

- Affords increased dynamic range due to differences in bioanalytical endpoint (change in metabolite response), decreased reagent usage.
- Availability of radiolabel compound offers additional opportunities for assessing low CL phenotyping.

Courtesy of A. Doran and S. Obach
An Overview of the Isolation Process


Metabolite Biosynthesis and NMR quantitation

Drug

Dose Animal or Incubate in Vitro

Obtain Matrix, Extract

Sample Containing Mixture of Drug-Related Material

Purify by Semi-Preparative HPLC

Calculate Concentration of Metabolite in the Sample - internal std, or -ERETIC, or -aSICCO

Fraction with Metabolite, Remove Solvent, Dissolve in Minimal Deuterated Solvent

1D H-NMR Spectrum

Sample in NMR solvent is used as a Standard Stock Solution of Metabolite to Calibrate the MS Response

Analyze Animal and Human Samples by HPLC-MS Quantitate Metabolite

WORLDWIDE RESEARCH & DEVELOPMENT
Transporters are important determinants of clearance (DDI) & exposure at the site of efficacy / toxicity

- Common target tissues: Brain, Liver, Kidney & Intestine
Active Renal Excretion
A relevant space with room for improvement

~ 20% of design effort in complex disposition space
High potential for exposure asymmetry (opportunity and/or liability)


Courtesy of M. Varma
Predicting $CL_{\text{renal}}$ via Single Species Scaling

Paine et al., DMD 2011, 1008

- No mechanistic description of renal reabsorption or active renal secretion

Correction for plasma protein binding

\[
CL_{\text{Human}} = CL_{\text{Species}} \times \frac{fu_{\text{Human}}}{fu_{\text{Species}}}
\]

Correction for plasma protein binding and kidney blood flow

\[
CL_{\text{Human}} = CL_{\text{Species}} \times \frac{fu_{\text{Human}}}{fu_{\text{Species}}} \times \frac{KBF_{\text{Human}}}{KBF_{\text{Species}}}
\]

Courtesy of M. Varma
Time-course of uptake by OATs-transfected HEK293 and wild-type cells

Mathialagan et al., DMD 2017, 409-417

OAT1  Substrate selectivity

--- OAT2  Substrate selectivity ---

--------- OAT3  Substrate selectivity ---------

RAF_{OAT_1} = 0.64
RAF_{OAT_2} = 7.3
RAF_{OAT_3} = 4.1
**CL_{renal} - OAT scaling via single transfecct cells**

Mathialagan et al., DMD 2017, 409-417

- Descriptions of both renal reabsorption and active renal secretion
Active Renal Excretion
Towards a fit for purpose approach to clearance prediction


Sumathy Mathialagan, Mary A. Piotrowski, David A. Tees, Bo Feng, John Litchfield, and Manthena V. Varma

\[ \text{RAF}_{\text{OATx}} = \frac{\text{in vivo } \text{CL}_{\text{int,sec}}}{\text{in vitro } \text{CL}_{\text{int,OATx}}} \]

\[ \text{CL}_{\text{int,sec}} = \text{CL}_{\text{int,OAT1}} \cdot \text{RAF}_{\text{OAT1}} + \text{CL}_{\text{int,OAT2}} \cdot \text{RAF}_{\text{OAT2}} + \text{CL}_{\text{int,OAT3}} \cdot \text{RAF}_{\text{OAT3}} \]

\[ \text{CL}_{\text{sec}} = Q_r \cdot \frac{f_{u,b} \cdot \text{CL}_{\text{int,sec}}}{Q_r + f_{u,b} \cdot \text{CL}_{\text{int,sec}}} \]

Mathialagan et al., DMD 2017, 409-417

Courtesy of M. Varma
Hepatobiliary Transport
A relevant space with room for improvement

~ 15% of design effort in hepatobiliary transport disposition space
High potential for exposure asymmetry (opportunity and/or liability)


\[ \text{CL}_{\text{int}} = (\text{CL}_{\text{bile}} + \text{CL}_{\text{met}}) \times \frac{\text{CL}_{\text{uptake}} + \text{CL}_{\text{passive}}}{\text{CL}_{\text{passive}} + \text{CL}_{\text{met}} + \text{CL}_{\text{bile}}} \]


Courtesy of M. Varma
In vitro tool – Primary human hepatocytes

Milestones

Suspension Heps (Oil spin method)

Sandwich-cultured human hepatocytes (SCHH)

Plated human hepatocytes (PHH)

Before 2004

Circa 2005

2011/12

2012-14

2014

2015-16

UNC/Pfizer collaboration

Bi et al. DMD 2006

SCHH-PBPK model

Jones et al. DMD 2012

Middle-out PBPK

Li et al. DMD 2012

SCHH-DDI M&S

Varma et al. 2012

Adopted from Sugiyama et al.

Courtesy of M. Varma
Mechanistic PBPK Modeling for Prediction of Hepatic Transporter-Mediated Disposition Using SCHH Data

Fig. 3. Simulated, fitted, and observed human intravenous plasma concentration-time profiles for pravastatin (A), cerivastatin (B), bosentan (C), fluvastatin (D), rosuvastatin (E), valsartan (F), and repaglinide (G). □, observed data; --- predicted data using the PBPK model; ----, fitted data.

<table>
<thead>
<tr>
<th>In vitro scaled and fitted sandwich culture human hepatocyte estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Pravastatin</td>
</tr>
<tr>
<td>Cerivastatin</td>
</tr>
<tr>
<td>Bosentan</td>
</tr>
<tr>
<td>Fluvastatin</td>
</tr>
<tr>
<td>Rosuvastatin</td>
</tr>
<tr>
<td>Valsartan</td>
</tr>
<tr>
<td>Repaglinide</td>
</tr>
<tr>
<td>Geometric mean</td>
</tr>
</tbody>
</table>

Superscript a represents the sum of SCI_{lat, u, act} and SCI_{lat, u, met} because for these three compounds both CL mechanisms are occurring and they cannot be uniquely identified in the fitting process.
**Hepatic Transporter Disposition - Current best practice (SCHH/PBPK)**

**v3** – Li (2014), *J Pharmacokinet Pharmacodyn, 41*(3), 197-209

- Trained with six ECCS 1 compounds devoid of biliary clearance
  - Use SCHH inputs with the following training set.
    - Bosentan, Cerivastatin, Fluvastatin, Glyburide, PF-05186462 (NAV1.7), & Repaglinide

- Population fit of clearance scaling factors (*SF*)
  - Log-normal inter compound variability on scaling factors (*SF*)
    - Account for imprecision in compound inputs
    - Provides a method to determine uncertainty in prospective predictions by bootstrapping the scaling factor distribution space

<table>
<thead>
<tr>
<th>Model</th>
<th><em>SFpass</em></th>
<th><em>SFact</em></th>
<th><em>SFmet</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>v3 (population)</td>
<td>0.35</td>
<td>27</td>
<td>0.19</td>
</tr>
<tr>
<td>90% CI</td>
<td>0.064-2.1</td>
<td>14-49</td>
<td>0.065-0.56</td>
</tr>
<tr>
<td>CV</td>
<td>110%</td>
<td>39%</td>
<td>66%</td>
</tr>
</tbody>
</table>

**Transporters in vitro HHEP systems do not represent in vivo physiological activity levels**

Courtesy of T. Maurer
Hepatic Transporter Phenotyping

IC$_{50}$ OF SELECTIVE INHIBITORS in HEK293 CELL LINES

Yi-An Bi ISSX 2017 poster
Challenging Class 1B compds: Montelukast case

Montelukast – recommended as substrate for CYP2C8 clinical DDI studies

Varma et al., CPT (2017) 101:406-415
PK prediction strategy

**Tier 1 PHH assay**

- Uptake inhibitable by Rif SV
- 0.1µM conc.*

- Yes
- No

**Renal CL**

- Class 3B
- Class 1B

**Cyno SS scaling**

- Yes
- No

**ECCS**

- Class 1B
- Class 3B

**Class 3B**

- No

**Class 1B**

- Yes

**Assume no active uptake in human for PK predictions**

**PBPK modeling**

- Multiple replicates for dose projections

- Phenotyping
  - Characterize transport mechanisms using HEK panel, selective inhibitors, etc.

- CLbile from SCHH
- Other ADME inputs from Enzymology and HDO

* Compounds with analytical sensitivity issues will be run at 1µM conc. as Tier 2.

**Courtesy of M. Varma**
OATP-HEK Uptake and steady state unbound tissue-to-plasma exposure of Glucokinase Activators PF-049 and PF-051 determined in rats post–4-day infusion. A Ghosh et al. DMD 2014;42:1599-1610

Glucokinase Activators

A Ghosh et al. DMD 2014;42:1599-1610
Drug Disposition and Trafficking in Cells – Factors Contributing to Asymmetric Distribution

Mitochondria: pH 8, -167 mV
Lysosomes: pH 4.7, +19 mV
Nucleus: pH 7.2, -9.2 mV

Lysosomes: pH 4.7, +19 mV
Nucleus: pH 7.2, -9.2 mV
Mitochondria: pH 8, -167 mV

Cytosol: pH 7.1, -38 mV
Medium/Blood pH 7.4

Courtesy of L Di
**Introduction of $K_{puu}$**

**In Vitro**

$$K_{puu} = \frac{\text{Free Cell}}{\text{Free Medium}}$$

**In Vivo**

$$K_{puu} = \frac{\text{Free Tissue}}{\text{Free Blood}}$$

**Pharmacology and ADMET**

**Cell-Based In Vitro**

**Steady State**

**Tissue exposure, PD, TI & DDI**

$$CL_{\text{int}} = \frac{CL_{\text{int}}'}{K_{puu}}$$

$$EC_{50} = EC_{50}' \times K_{puu}$$

$$IC_{50} = IC_{50}' \times K_{puu}$$

*Courtesy of L Di*
<table>
<thead>
<tr>
<th>Compounds</th>
<th>In Vivo Rat Liver-to-Plasma $K_{puu}$ (IV Infusion)</th>
<th>In Vitro Suspension Rat Hepatocyte $K_{puu}$</th>
<th>Fold Difference in Vivo $K_{puu}$/in Vitro $K_{puu}$</th>
</tr>
</thead>
<tbody>
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<td>Cerivastatin</td>
<td>29 ± 8.5</td>
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<td>Fluvastatin</td>
<td>44 ± 8.7</td>
<td>21 ± 1.5</td>
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<td>Rosuvastatin</td>
<td>57 ± 9.5</td>
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<td>Pravastatin</td>
<td>2.2 ± 1.5</td>
<td>2.9 ± 0.3</td>
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<td>5.7 ± 1.6</td>
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<td>PF-05187965</td>
<td>2.4 ± 0.6</td>
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Good IVIVE for Substrates of Oatps in Rat

*K Riccardi et al., DMD, (2017), 45:576-580*
Comparison of the Predictability of Human Hepatic Clearance for Organic Anion Transporting Polypeptide Substrate Drugs Between Different In Vitro—In Vivo Extrapolation Approaches

Saki Izumi 1, Yoshitane Nozaki 1,*, Takafumi Komori 1, Osamu Takenaka 2, Kazuya Maeda 3, Hiroyuki Kusuhara 3, Yuichi Sugiyama 4

Figure 2. Comparison of hepatic intrinsic clearance predicted by IVIVE with the observed in vivo overall hepatic intrinsic clearance. (a) Method I. Comparison of CL_{int,met} with CL_{int,all,vivo}. CL_{int,met} was calculated from the metabolic clearance in human liver microsomes and scaled to in vivo as described under Materials and Methods. The regression equations for Methods I and II were CL_{int,met} = 0.01 CL_{int,all,vivo} (R^2 = 0.65) and CL_{int,met} = 0.009 CL_{int,all,vivo} (R^2 = 0.75).
Drug Distribution to Brain - physiology of the central barriers

Courtesy of P. Trapa
Borst and NIH MDR1-MDCK Efflux Ratio Correlation with Rodent Brain Penetration (Cbu/Cpu)

Courtesy of B Feng
The two MDR1 assays do not correlate

Courtesy of B Feng
Key Properties for Borst vs. NIH MDR1 Cells
Translational Strategy for Brain Penetration

\[ \frac{C_{b,u}}{C_{p,u}} = \frac{1}{RAF_1 \times (ER_1 - 1) + RAF_2 \times (ER_2 - 1) + 1} \]

**Exptl. Parameters – Compound Specific**

- **binding:** *in vitro* equilibrium dialysis
- **intrinsic passive brain permeability:** *in silico*
- **active transport at the brain/choroid plexus:** analysis of *in vitro* cell lines (MDR1 and BCRP)

**Output**

- Extracellular fluid (ECF)
- Intracellular fluid (ICF)

**Key assumptions**

- **Steady-state**
- **Passive flux >> bulk ESF flow**
- **Only PgP (ER1) & BCRP (ER2)**

**Expression levels:** measured from tissues and cell lines or activity from IVIVC at steady state

**Physiology:** from literature

*Trapa PE et al. (2016) J Pharm Sci 105: 965 - 971*
Brain Penetration
Exhaustive translational model qualification

2 approaches
• Model based estimation against neuroPK data
• Relative protein expression

Courtesy of P Trapa
Efflux Transporter expression-level changes across species can impact distribution


**BBB Efflux Transporter Protein Abundance**

(from Uchida Y et al J. Neurochemistry 2011 and Ho K et al J. Pharm Sci 2011)

![Graph showing BBB Efflux Transporter Protein Abundance](image)

- Mouse
- NHP
- Human

![Graph showing predicted C<sub>b,u</sub>/C<sub>p,u</sub> in human](image)

- PGP substrate
- BCRP substrate

![Graph showing fraction effluxed by PGP](image)
• **Mechanistic Approach to Clearance IVIVE**
  – \( \text{CL}_{\text{hepatic}} \) mature but challenged by expanded chemical space
  – Incorporation of hepatic- and renal-transporter mediated CL

• **Phenotyping**
  – Lower and non-CYP metabolic CL require novel approaches to phenotyping
  – Drug transporter phenotyping is still evolving and lacks clinical markers

• **Drug Distribution**
  – Greater appreciation of intracellular pH, membrane potential and uptake and efflux transporters influencing tissue Kpuu and BBB permeation
Gaps and Horizon

- **IVIVE relationships are limited by lack of human PK**
  - Cliv; Vd; non-CYP PK; fafg; Cbu/Cpu
  - Consideration of preclinical species

- **Reagent / technology advances continually needed**
  - HHEP relay costs impacts capacity
    - Co-cultures, immortalized cell lines and microphysiological systems
  - Hepatic transporter cell model(s) needed with greater dynamic range
    - Can we identify an Industry-wide approach?

- **Non-traditional modalities will continue to increase**
  - E.G. – Antibody drug conjugates, biologics, gene therapy, parenteral delivery
# Acknowledgements

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<tr>
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Additional Thanks to Colleagues in Hit Design Lead Optimization, NonReg Bioanalytical and Comparative Medicine
Thank You for Your Attention
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Figure 1. Time profiles for the uptake of OATP substrate drugs in cryopreserved human hepatocyte suspensions (lot V88, pool of 30 donors). Uptake of atorvastatin (a), bosentan (b), cerivastatin (c), fexofenadine (d), fluvastatin (e), glibenclamide (f), irbesartan (g), nataglinide (h), pitavastatin (i), pravastatin (j), repaglinide (k), rosuvastatin (l), telmisartan (m), torasemide (n), and valsartan (o) over 60 minutes.