XenoTech
A BioIVT Company

Navigating the Transporter Wave of 2013: A Review of the Seven Recent ITC Publications on Drug Transporters

Greg Loewen, Andrea Wolff, Amanda Hays, Ph.D.

XenoTech Drug Transport Group

XenoTech – brief introduction

XENOTECH
A BioIVT Company

Main focus:
In Vitro products and services to predict clinical DDI as outlined in the FDA and EMA guidance documents on DDI

- Located in Lenexa, KS
- GLP - compliant CRO, founded in 1994 by Dr. Andrew Parkinson
- ~110 employees
- Global: Distributors in Europe, Japan, Korea, India, China and Singapore
- Partners include Sekisui Medical (Japan), Cypex (Scotland), Xenometrics (USA)
- July 2008 – XenoTech acquired by Sekisui (SMD)
- NEXT IND, Partnership of small CROs

XenoTech's In Vitro ADME-DMPK Core Service Capabilities

XENOTECH
A BioIVT Company

XT Consulting Department

Expert data review and study consultation

Drug Metabolism

Metabolic stability and species comparison

Metabolite characterization/ID

Reaction phenotyping (CYP & UGT)

Customized services

Enzyme Induction

In vitro studies in cultured hepatocytes (human and animal)

Ex vivo studies in animals

Toxicity & mechanistic studies

Bioanalytical

Non-GLP Bioanalysis

GLP and non-GLP in vitro study support

Enzyme Inhibition

Evaluate potential for direct, and metabolism-dependent inhibition(MDI or TDI)

Mechanistic studies (direct or MDI)

Non-CYP enzymes (*e.g.*, UGT, MAO, AO)

Transporters

FDA and EMA required transporters (7-10)

In vitro studies in mono-layer cell lines for uptake

Bi-directional assay for efflux transporters

Membrane-based vesicles and ATPase assays

Discovery and Late-Preclinical Support

Medium-throughput ADME screening studies

Definitive ADME and DDI studies to support design and necessity of clinical studies

XenoTech support groups

XenoTech is big and diverse enough to have independent support groups such as our analytical group, knowledge management and QA which add value to your study but we are small enough the groups work seamlessly to provide a high quality final product

- Follow recommended methods outlined in FDA and EMA guidance documents
- Review and discuss physiochemical properties and pk data to suggest most relevant studies
- GLP compliant facility - high standards of documentation and traceability
- Strong in house LC-MS/MS support – samples analyzed the same day
- Collaborate with our parent company in Japan – doubling our size and knowledge base
- Widest range of transporter assays available
- Fully licensed to use all cell lines and transporters
- Been involved in the transporter field since 2006

Drug Transport services at XenoTech

XENOTECH
A BioIVT Company

- All transporter assays requested by the FDA and EMA and we help you with study design
- MDCKII, LLC-PK1 and Caco-2 cells for transwell assays (P-gp and BCRP)
- Vesicles for efflux transporters (MRPs, BSEP, BCRP, P-gp etc)
- Transfected HEK293 and S2 cells for SLC (uptake) transporters, (Oocytes capability)
- Hepatocyte uptake and Lysosomal trapping assays
- Partner with Sekisui on certain assays and study design

Drug Transport group

XENOTECH
A BioIVT Company

Efflux

- MDR1 (P-gp) (human, mouse 1a/1b, rat 1a/1b, monkey, dog)
- BCRP (human and mouse)
- BSEP
- MRP2 (and MRP1, MRP3, MRP4, MRP5)
- MATE1
- MATE2K

Uptake

- OATP1B1 - 1B3 - 2B1 -1A2
- OAT1 – 2 – 3 – 4 - 7
- OCT1 – 2 - 3
- OCTN1 - N2
- NTCP1 - NTCP2 (ASBT)
- NPT1
- PEPT1 - 2
- URAT1
- OSTα/β
- LAT

Thanks to the ITC!

XENOTECH
A BioIVT Company

Lead Author	Title	Presenter
KM Giacomini	International Transporter Consortium Commentary on Clinically Important Transporter **Polymorphisms**	Amanda
KM Hillgren	**Emerging Transporters of Clinical Importance**: An Update From the International Transporter Consortium	Amanda
D Tweedie	Transporter Studies in Drug Development: Experience to Date and Follow-Up on **Decision Trees** From the International Transporter Consortium	Amanda
KLR Brouwer	***In Vitro* Methods** to Support Transporter Evaluation in Drug Discovery and Development	Greg
X Chu	**Intracellular Drug Concentrations** and Transporters: Measurement, Modeling, and Implications for the Liver	Greg
MJ Zamek-Gliszczynski	ITC Recommendations for Transporter **Kinetic Parameter** Estimation and **Translational Modeling** of Transport-Mediated PK and DDIs in Humans	Andrea
JC Kalvass	Why Clinical Modulation of Efflux Transport at the Human **Blood–Brain Barrier** Is Unlikely: The ITC Evidence-Based Position	Andrea

XENOTECH
A BioIVT Company

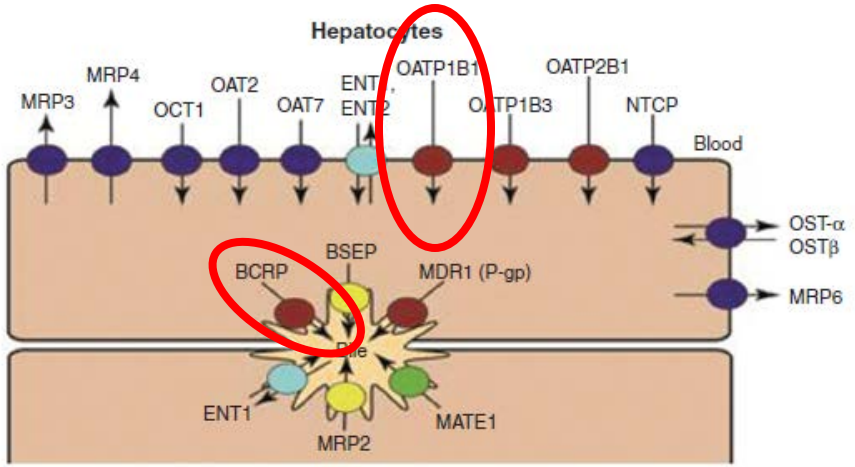
International Transporter Consortium Commentary on Clinically Important Transporter Polymorphisms

KM Giacomini[1], PV Balimane[2], SK Cho[3], M Eadon[4], T Edeki[5], KM Hillgren[6], S-M Huang[7], Y Sugiyama[8], D Weitz[9], Y Wen[10], CQ Xia[11], SW Yee[1], H Zimdahl[12] and M Niemi[13]; on behalf of the International Transporter Consortium

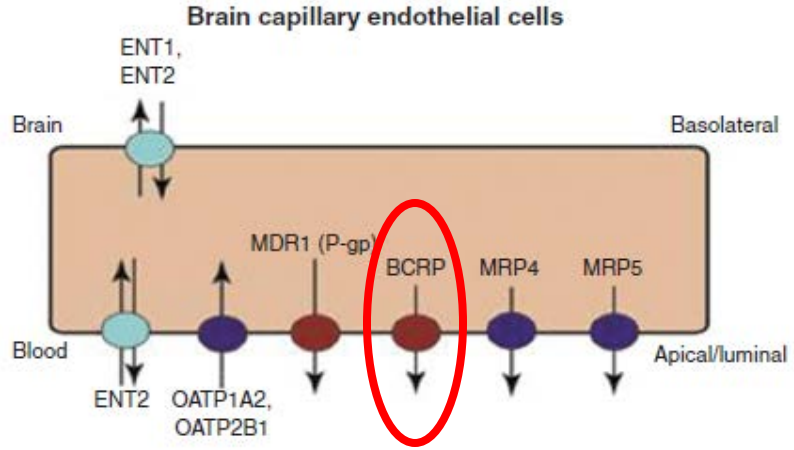
Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

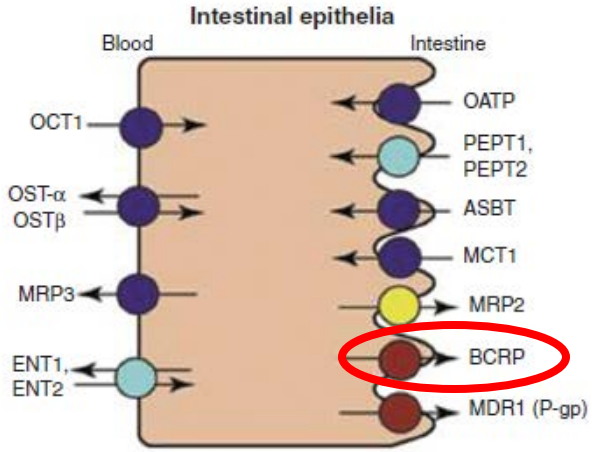
OATP1B1 and BCRP

- OATP1B1 (*SLCO1B1*)
  - c.521T>C, p.V174A
  - Increased statin plasma exposure



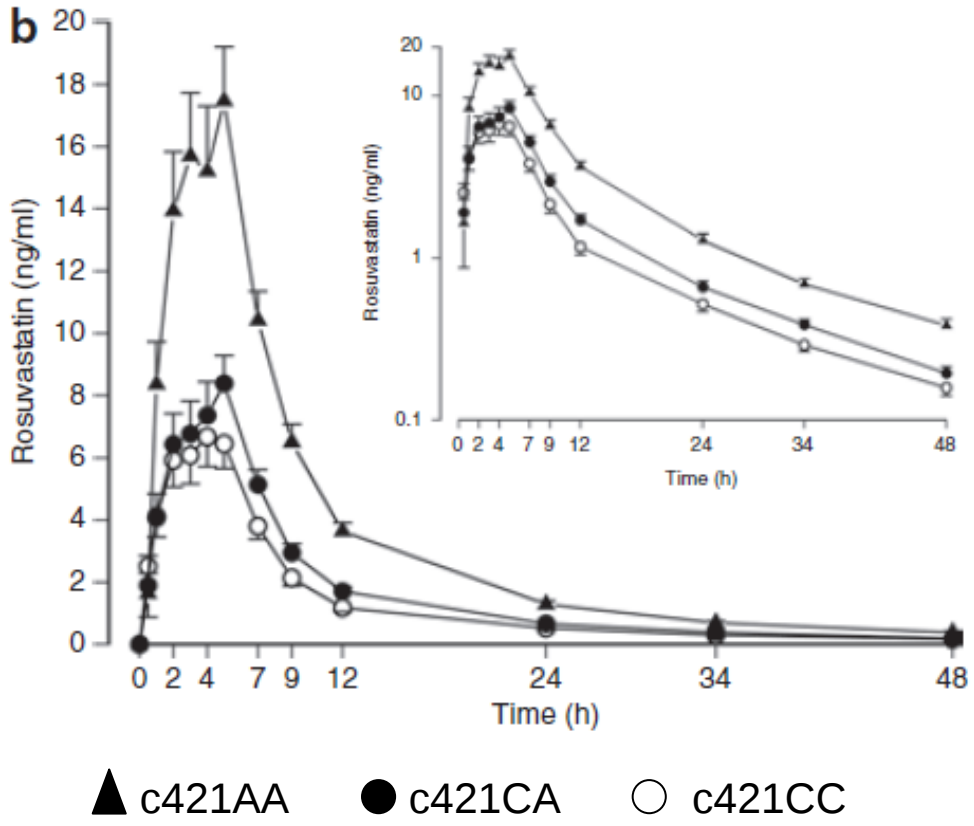
- BCRP (*ABCG2*)
  - c.421C>A, p.Q141K





Figures from Hillgren K.M. et al., Clin Pharm Ther *92:5*2-63. 2013

Clinically relevant transporter polymorphisms



Keskitalo, J.E. et al., Clin Pharmacol Ther *86: 197-203*. 2009

Summary: Recommendations for future drug transporter association studies

XENOTECH
A BioIVT Company

1. Conduct *in vitro* studies to determine whether a drug is a substrate of key transporters (i.e., OATP1B1 and BCRP).
2. Consider conducting a DDI study with a transporter inhibitor as recommended in the FDA guidance.
   - Small therapeutic window, AUC > 2
3. If a pharmacogenomic study is suggested, design well-powered studies that consider variants in drug transporters that have been shown to interact with the drug in *in vitro* studies.
4. Collect DNA samples from subjects enrolled in studies. Include sufficient numbers of subjects with variant alleles representative of various race/ethnic groups.
5. Associate SNPs with drug concentrations, response and safety.
6. Apply a multiscale systems pharmacology approach that incorporates all data (*in vitro* and *in vivo* human studies).

XENOTECH
A BioIVT Company

Emerging Transporters of Clinical Importance: An Update from the International Transporter Consortium

KM Hillgren[1], D Keppler[2], AA Zur[3], KM Giacomini[3,4], B Stieger[5], CE Cass[6] and L Zhang[7]; on behalf of the International Transporter Consortium

Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

Transporters involved in clinical DDIs

XENOTECH
A BioIVT Company

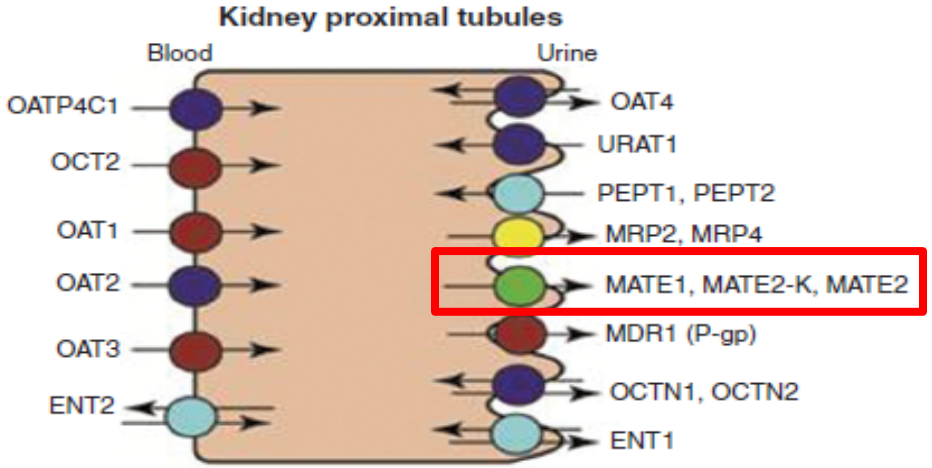
- P-gp (MDR1, *ABCB1*)
- BCRP (*ABCG2*)
- OATP1B1 (*SLCO1B1*)
- OATP1B3 (*SLCO1B3*)
- OCT2 (*SLC22A2*)
- OAT1 (*SLC22A6*)
- OAT3 (*SLC22A7*)

Emerging Transporters of Clinical Importance:

- MATE1 (*SLC47A1*)
- MATE2 (*SLC47A2*)
- MRP2 (*ABCC2*)
- BSEP (*ABCB11*)

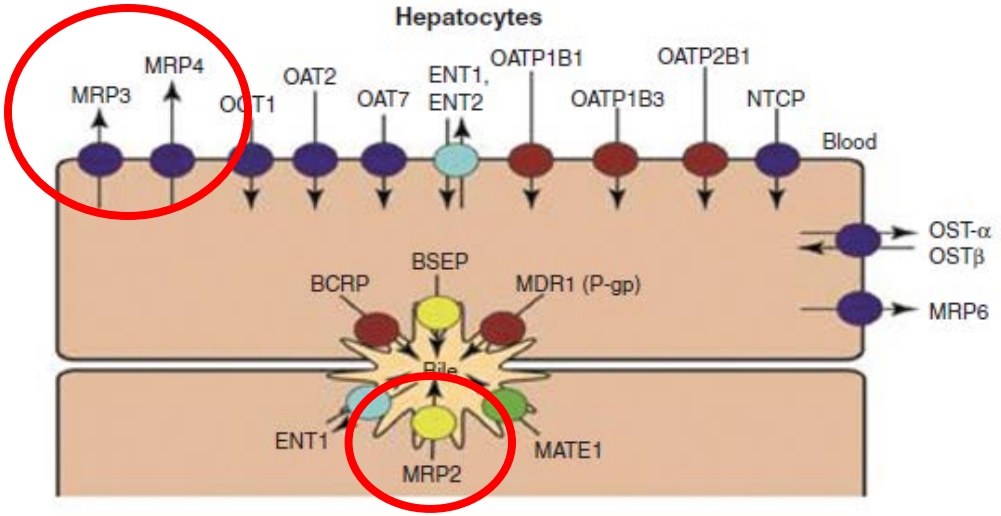
The emerging transporters MATE1 and MATE2K

- MATE1, MATE2, MATE2K.
- Expressed at the apical membrane of renal proximal tubules.
- Secrete cations and zwitterions into urine.
- Cation/H^+ antiporter in tandem with OCT2 (Metformin)
- Current evidence for clinical DDIs is strongest for MATE1 and MATE2K.
- MATE SNPs have been linked to clinical effects in metformin-treated subjects.
- Suggest *in vitro* models



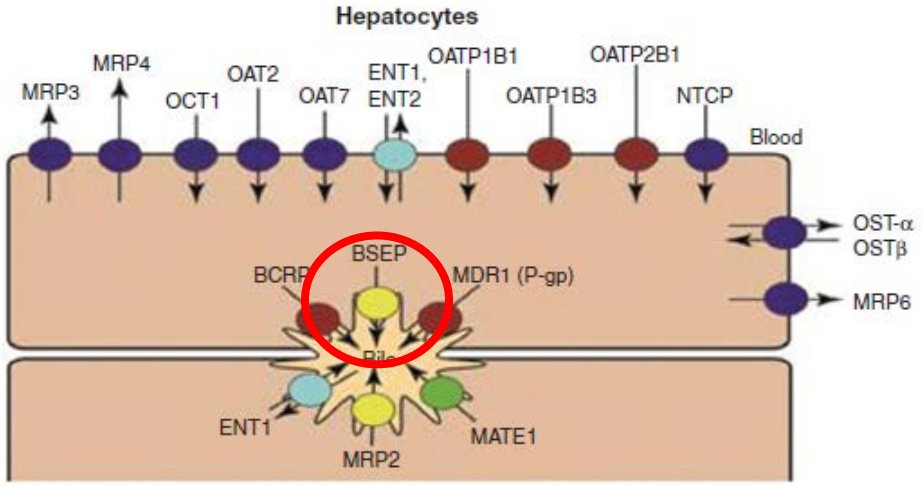
Multidrug Resistance Proteins: MRP2, MRP3 and MRP4

- MRP2, MRP3, MRP4.
- Unidirectional ATP-dependent efflux pumps.
- Hepatocytes, intestinal epithelia, kidney tubules.
- Anionic substrates, drugs and conjugates.
- A SNP with complete loss of MRP2 functionality contributes to hyperbilirubinemia (Dubin-Johnson Syndrome).
- Inside-out membrane vesicles



BSEP and hepatotoxicity

- Rate-limiting step of bile salt transport across hepatocytes.
- No backup system.
- SNPs cause progressive cholestatic liver disease.
- Unlikely involved in drug disposition, inhibition may contribute to adverse cytotoxic events.
- Isolated membrane vesicles.



hENTs and PepTs

XENOTECH
A BioIVT Company

- For certain drug classes, should consider:
  - hENT1 (anticancer nucleoside analogs)
  - Pept1 (oral dosed peptide-like drugs)

- Model systems:
  - Oocytes
  - Cell lines
  - Knock-out mice

Summary

XENOTECH
A BioIVT Company

- NMEs that are actively renally secreted are recommended for *in vitro* investigation as MATE1 or MATE2K substrates in addition to being investigated as OAT1, OAT3 or OCT2 substrates.

- If signs of cholestasis are seen, retrospective analysis of inhibition of MRPs and BSEP can help determine the mechanism of toxicity.

- hENT1 and Pept1 are examples of transporters that do not need to be routinely screened but can contribute to efficacy and distribution.

XENOTECH
A BioIVT Company

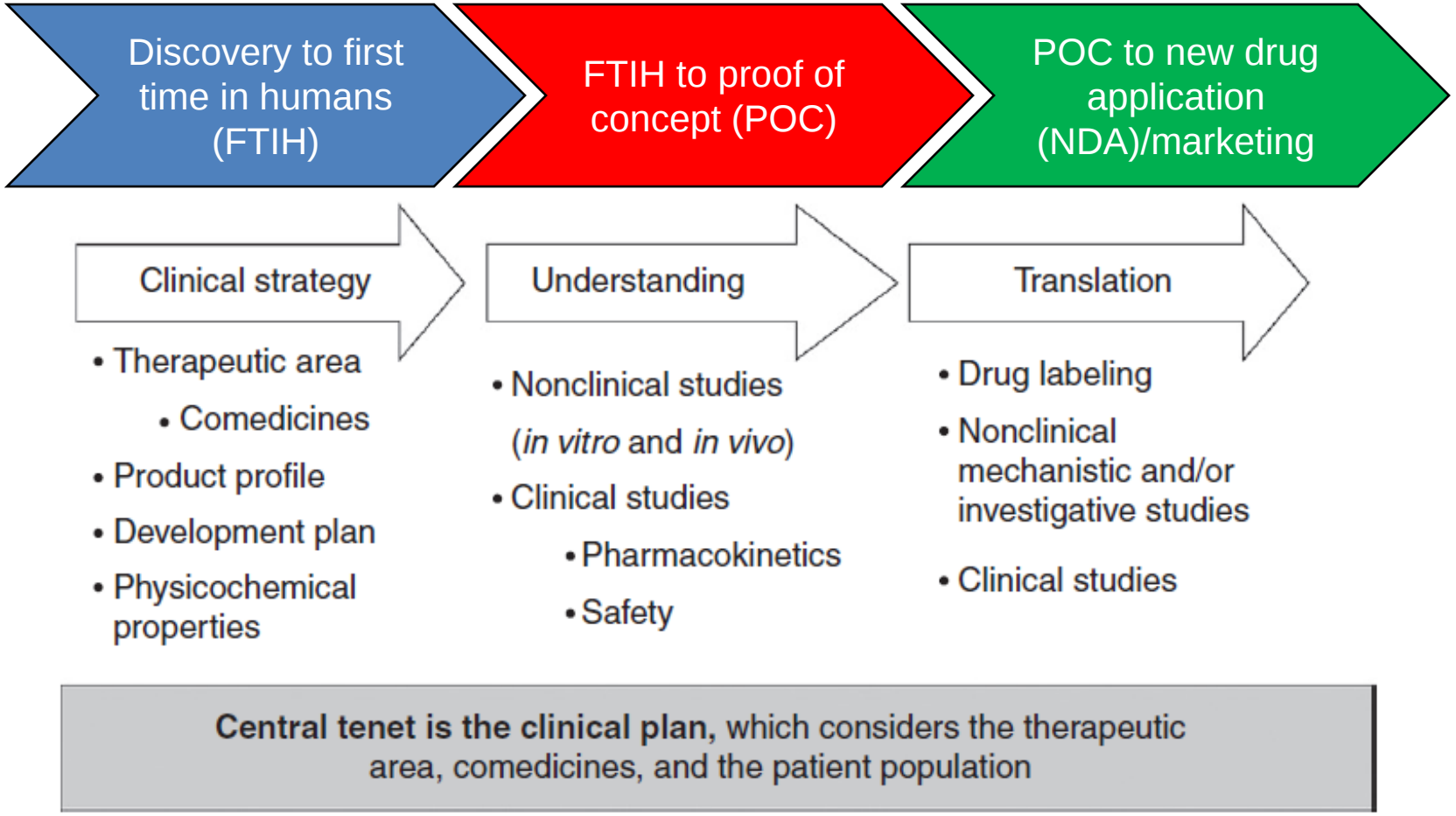
Transporter Studies in Drug Development: Experience to Date and Follow-up on Decision Trees From the International Transporter Consortium

D Tweedie[1], JW Polli[2], E Gil Berglund[3], SM Huang[4], L Zhang[4], A Poirier[5], X Chu[6] and B Feng[7]; On Behalf of the International Transporter Consortium

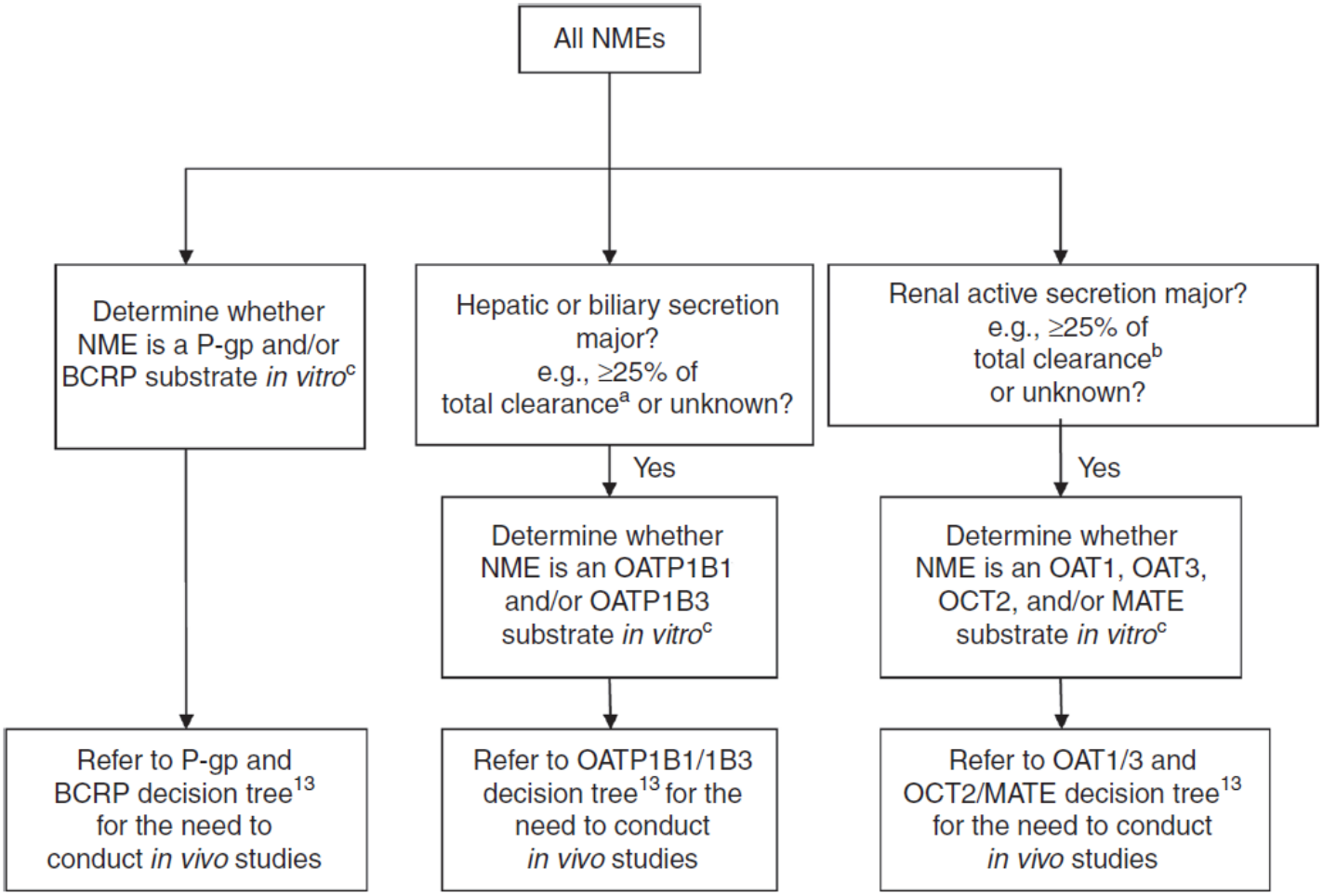
Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

Drug transporter assessment strategy

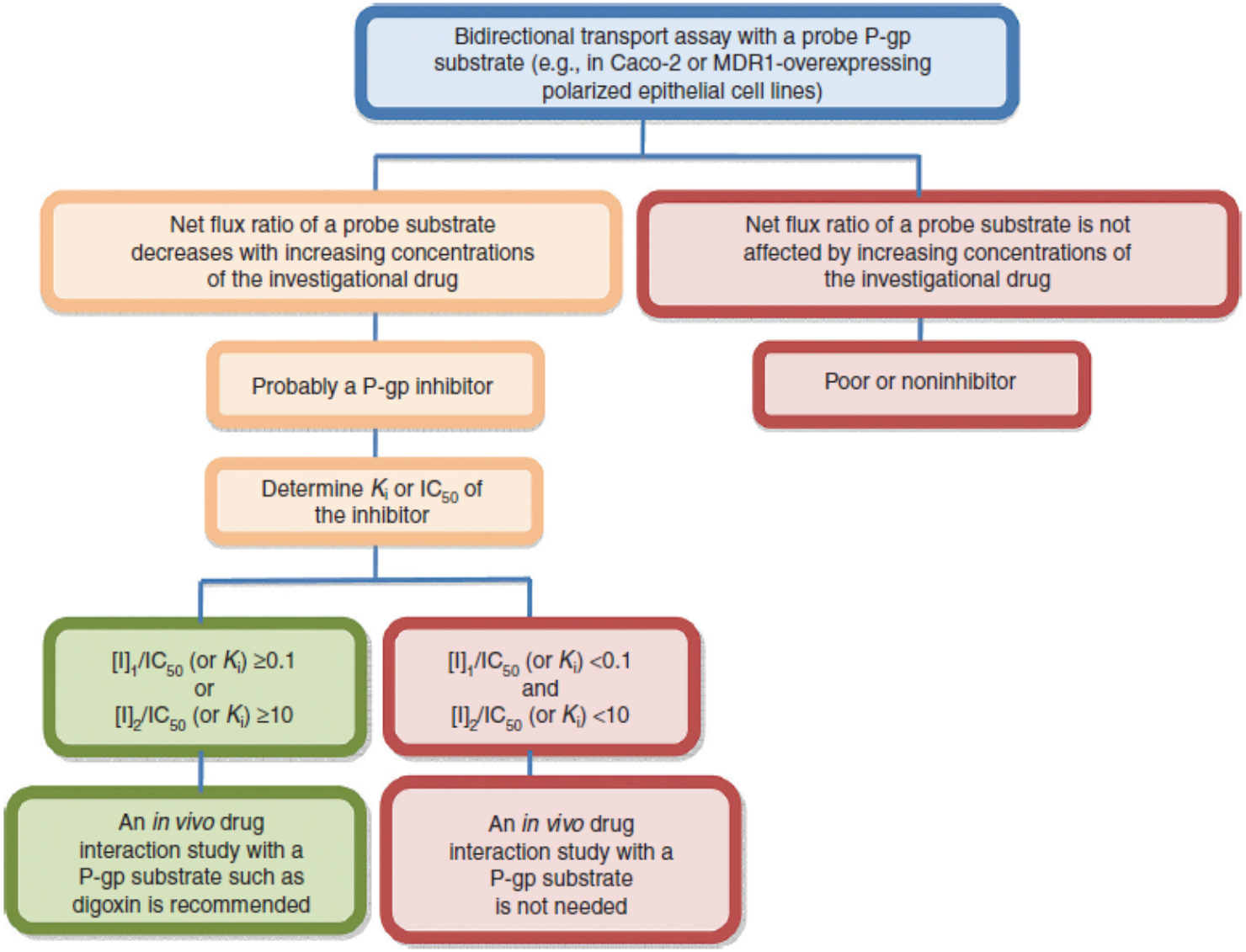
XENOTECH
A BioIVT Company



Evaluation of investigational drugs as substrates of transporters



P-gp/BCRP inhibition tree



Summary

XENOTECH
A BioIVT Company

- Flow chart presented for timing of Transport studies
- Discussion of the integration of transport studies into the clinical plan (*e.g.,* therapeutic area, co-meds, patient population, physicochemical properties, PK)
- Three decision trees presented
- Summary of approved compounds that 'failed' the FDA cutoff for clinical DDI study (table 1)
- Summary of PMR and PMC (table 2)
- Three case studies presented
  - Applying P-gp/BCRP inhibition decision trees
  - Complexity of DDI involving OATP transporters
  - Assessment of transporter-mediated DDIs for liver-targeting compounds

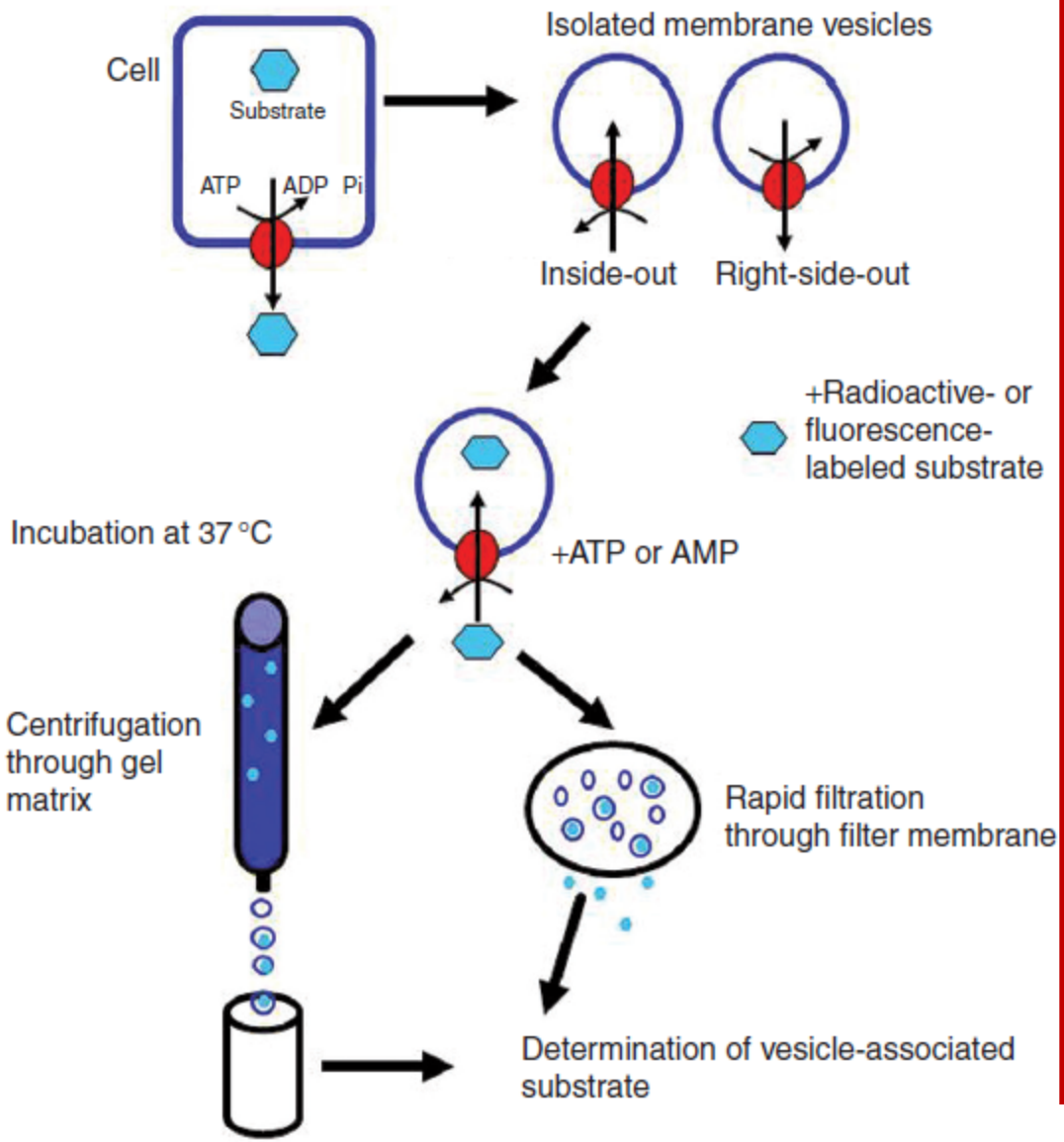
XENOTECH
A BioIVT Company

In Vitro Methods to Support Transporter Evaluation in Drug Discovery and Development

KLR Brouwer, D Keppler, KA Hoffmaster, DAJ Bow, Y Cheng, Y Lai, JE Palm, B Stieger and R Evers; on behalf of the International Transporter Consortium

Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

Vesicle assays



- ABC transporter expressing vesicles are prepared
  - From Spodoptera frugiperda insect cells (Sf9 or Sf21) infected with a cDNA containing baculovirus
  - cDNA-transfected mammalian cell lines; HeLa, V79 hamster, HEK293, MDCK, and MDCKII
  - Tissue including kidney and liver
- Homogenization method is very important
- Substrates and inhibitors listed in the article
- MRP2, BSEP most common , also MRPs, P-gp and BCRP

Recombinant Cell lines

XENOTECH
A BioIVT Company

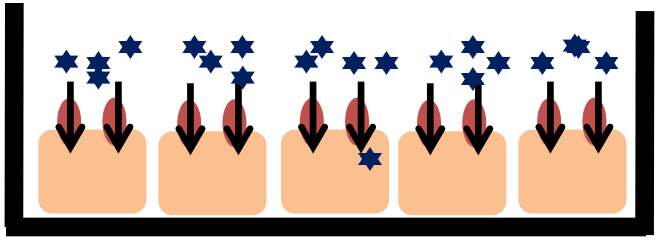
- Cells are transfected with full length cDNAs encoding a transporter of interest
  - Plasmids introduced into the host cell line either chemically, physically, or by retroviral transduction to create a stable transfection

  (and transiently transfected but not preferred)
- Uptake transporters (SLC):
  - HEK293, CHO, MDCKII, S_2, monolayer on a the bottom of cell culture plate
  - Oocytes: in suspension
- Efflux transporters (ABC)
  - LLC-PK1, MDCKII
  - Bidirectional transport or *transwell* assay

Uptake (SLC) transporter assays

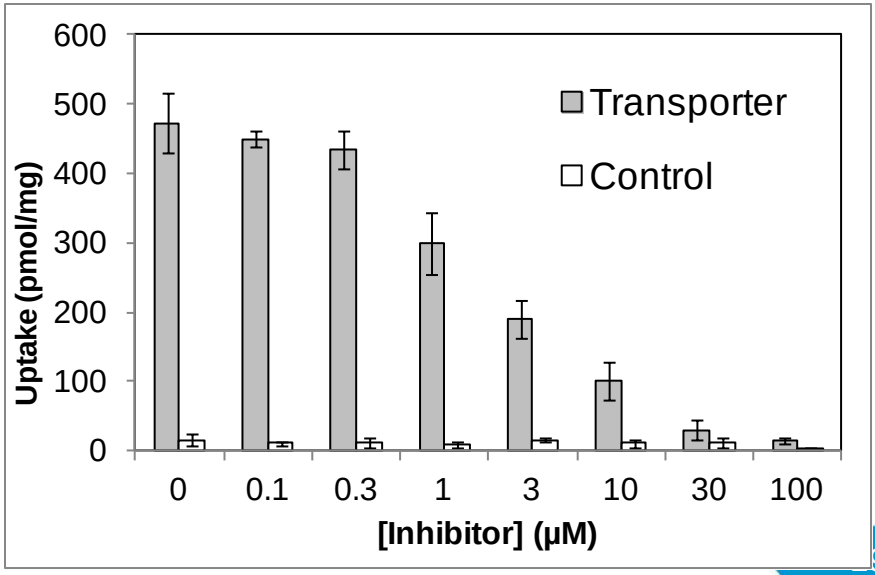
XENOTECH
A BioIVT Company

- **Test system:** transporter transfected cells grown in cell culture plates
- **Inhibition:** measure the effect of the test article on the accumulation of a probe substrate
- **Substrate:** measure the accumulation of the test compound in transfected and control cells
- **Hepatic:** OATP1B1, OATP1B3, OCT1
- **Renal:** OAT1, OAT3, OCT2, MATE1, MATE2K



Transporter expressing cells

Control cells



Vesicle and uptake transporter

XENOTECH
A BioIVT Company

Accumulation is rapid and kinetics should be studied in the linear range of time and protein concentration

Solubility and non-specific binding should be considered – solvents and BSA

No consensus on the best way to calculate inhibition constants for transporters **yet**

1. For competitive inhibitors, the IC_{50} value depends on the substrate concentration the *Ki does not*

2. **IC_{50} values will approach *Ki*** *if a substrate concentration* ***far*** **below the *Km*** *is used*

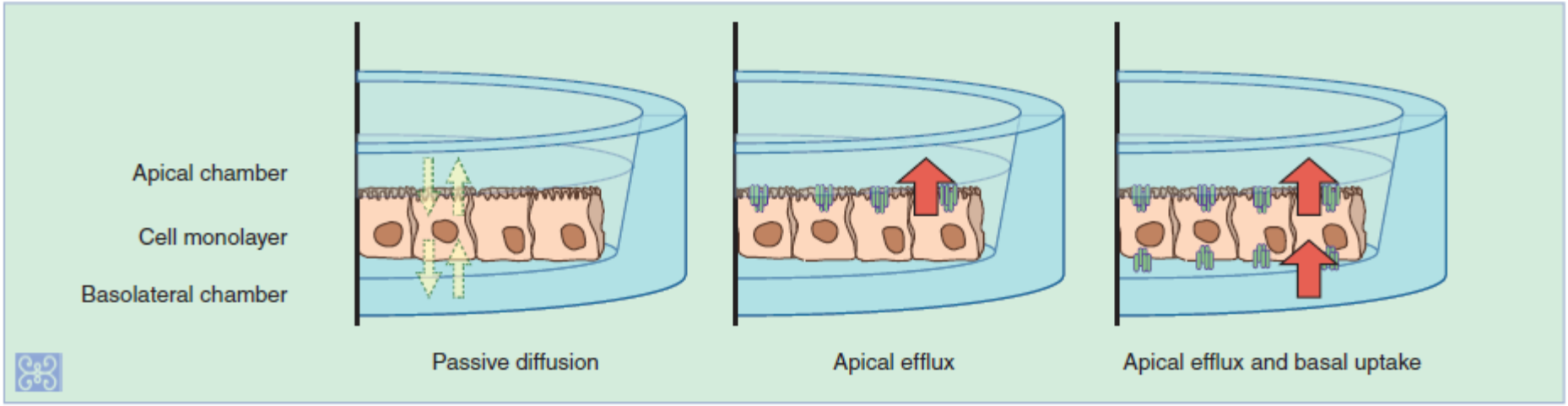
3. *If the* Cheng–Prusoff equation is used to estiamte the Ki the **probe substrate concentrations within twofold of the *Km*** *value.*

4. The Cheng–Prusoff equation assumes that the inhibition is competitive in nature

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

Bidirectional transport (transwell)

XENOTECH
A BioIVT Company



- Typically for P-gp and BCRP
- MCKDII, LLC-PK1 or Caco-2 cells
- Compound is loaded on the Apical (A) or Basolateral (B) side and permeability across the monolayer is measured and efflux ratio (ER) determined (BA ÷ AB)
- ER > 2 typically suggests active transport
- Difficult to correlate ER to extent of active transport *in vivo*

- Binding and metabolism can complicate data interpretation, recovery should be > 70%
- High permeable compounds; saturation Low permeable compounds uptake transporter
- Caco-2 can be used as a model for oral absorption
- **Inhibition:** several IC_{50} calculations available (Bentz-Lee paper)

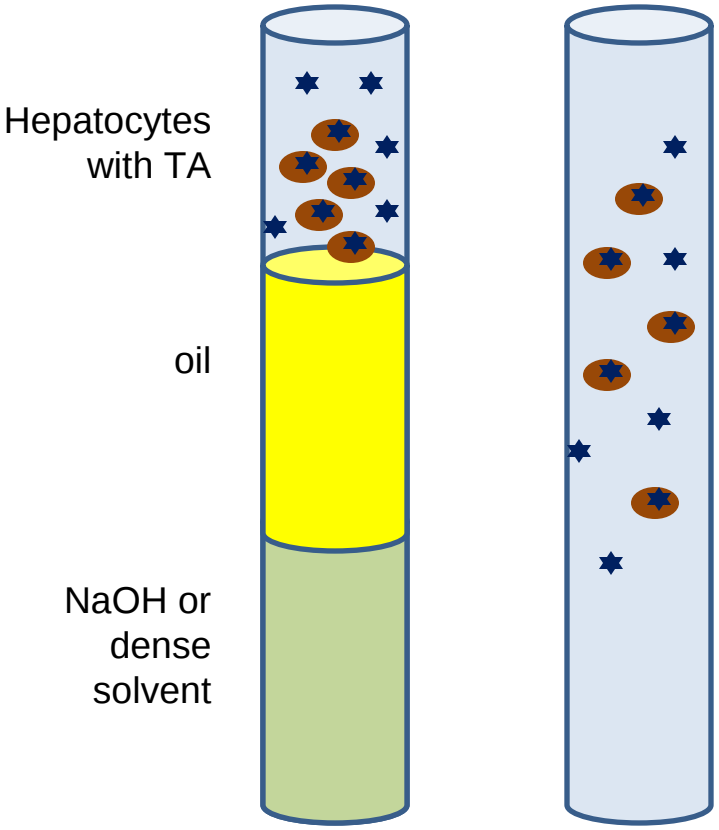
Hepatocytes

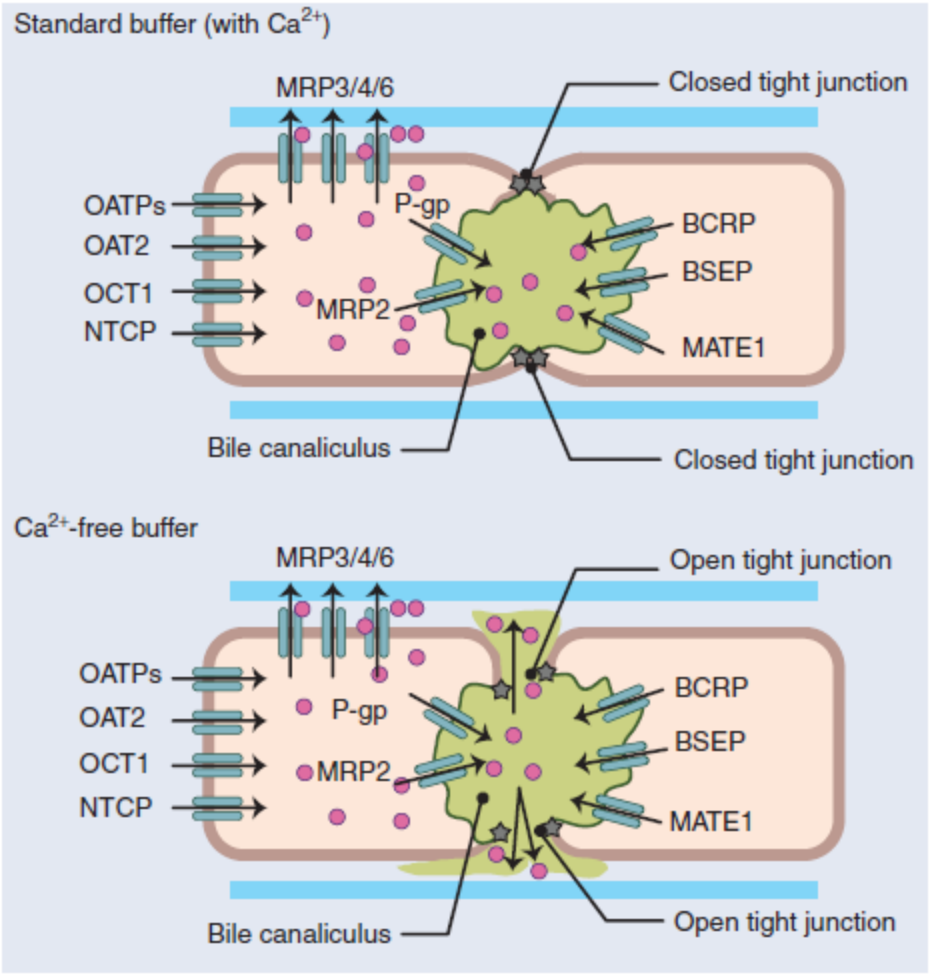
XENOTECH
A BioIVT Company

Oil spin

Loss of substrate

Sandwich culture hepatocytes (SHC)





Summary: Tables

XENOTECH
A BioIVT Company

- **Table 1:** Vesicle substrates and inhibitors
- **Table 2:** Cell system substrates and inhibitors
- **Table 3:** Commonly used equations for calculation of kinetic parameters in vesicles, cell lines, and hepatocytes
- **Table 4:** Applications, strengths, and limitations of various in vitro transporter assay systems
- **Table 5:** Integration of in vitro and in vivo data to determine the role of transporters in compound absorption, distribution, clearance, and DDIs
  - Scientific question:
  - Observations to support In vitro transporter investigations:
  - In vitro tools to address scientific hypothesis:
  - Outcome of in vitro experiments:
  - Potential follow-up studies:

XENOTECH
A BioIVT Company

Intracellular Drug Concentrations and Transporters: Measurement, Modeling, and Implications for the Liver

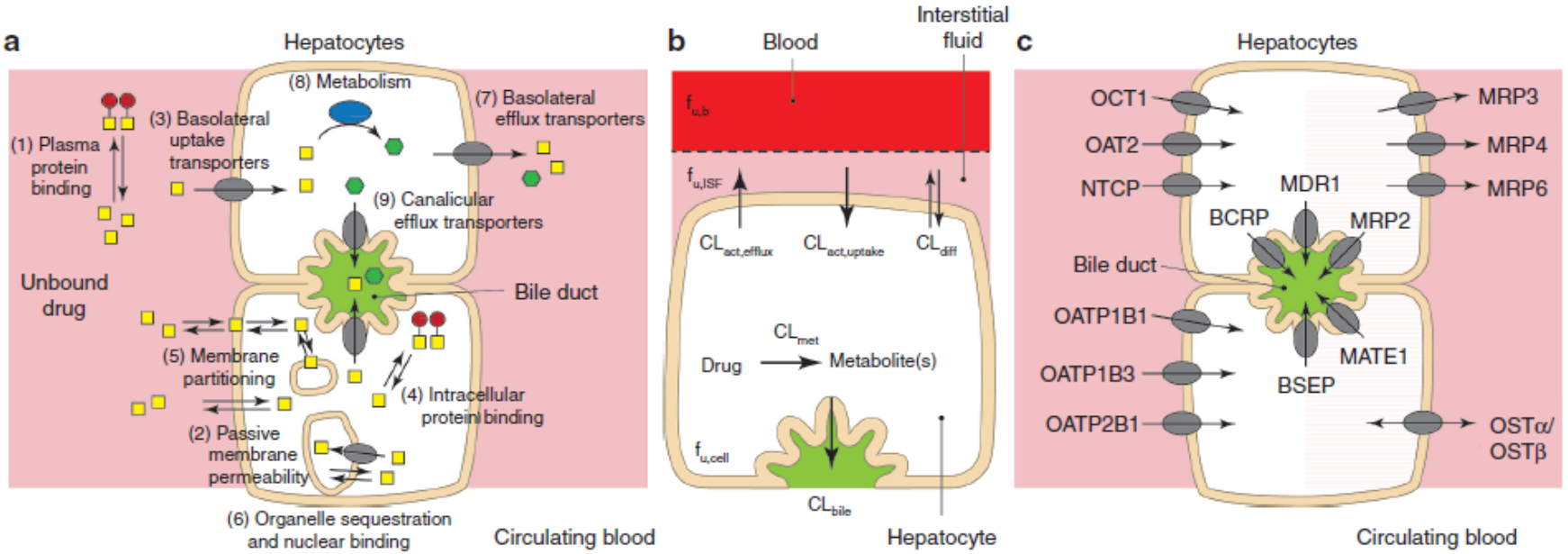
X Chu, K Korzekwa, R Elsby, K Fenner, A Galetin, Y Lai, P Matsson, A Moss, S Nagar, GR Rosania, JPF Bai, JW Polli, Y Sugiyama and KLR Brouwer; on behalf of the International Transporter Consortium

Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

Factors that influence intracellular drug disposition in the liver

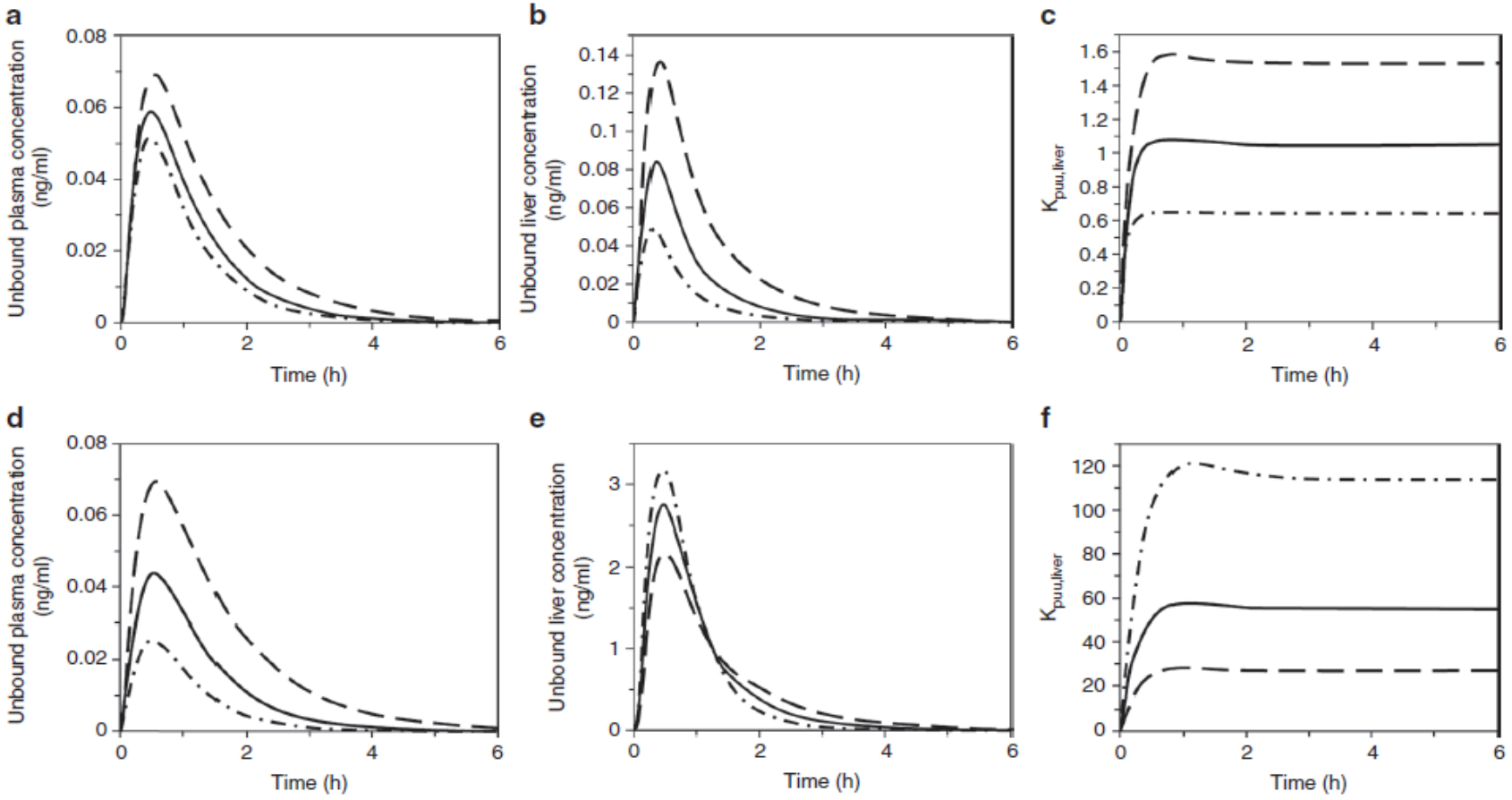
XENOTECH
A BioIVT Company

- *Blood flow, mixing of portal and venous blood*
- $K_{puu,liver}$ is defined as the steady-state liver-to-sinusoidal blood partition coefficient for unbound drug
- function of CL_{diff}, $CL_{act,uptake}$, $CL_{act,efflux}$, CL_{bile}, and Cl_{met}
- High permeability compunds: $K_{puu,liver}$ =1
- Metabolized or effluxed: $K_{puu,liver}$ <1
- Active uptake: $K_{puu,liver}$ >1
- Organelle or membrane distribution: $K_{puu,liver}$ >1



PBPK model

- *a – c: clearance by metabolism*
  - *Activity: solid- normal, dash -1/2x, dash dotted 2x*
- *d - f: clearance by uptake transporters*
  - *Activity: solid- normal, dash -1/2x, dash dotted 2x*



Summary of tables

XENOTECH
A BioIVT Company

- **Table 1:** Examples of studies in which membrane transporters have been shown to alter hepatocellular drug concentrations
  - MRP2, MRP3, BCRP, OCT1, OATP1B1, MATE1
- **Table 2:** *In vitro, in situ,* and *in vivo* models to estimate the intracellular concentrations of drugs and metabolites in the liver
  - Systems, Advantages, Disadvantages and examples of parameters estimated
  - Vesicles, recombinant proteins, hepatocytes, SCH, perfused liver, animal models, KO animals, Human *in vivo* studies
- **Table 3:** Direct and indirect methodologies for the estimation of intracellular drug concentrations
  - Direct or indirect method, analyte/matirx, detection method, utility/limitations/assumptions
  - Capillary electrophoresis, MSI: Nano-SIMS, MIMS, Raman microscopy, Nuclear microscopy, Microautoradiography, PET/SPECT imaging, PET imaging with simultaneous microdialysis, Bulk analysis
- **Table 4:** Summary of methodologies available to estimate intracellular fraction of unbound drug in cells
  - Method, Predictive equations developed for $f_{u,cell}$, Comments
  - Physiochemical properties, monolayer permeability, hepatoctye uptake and binding, transport kinetics, PBPK models

Summary: Current challenges

XENOTECH
A BioIVT Company

- Our understanding of intracellular drug disposition is rudimentary
  - e.g., trapping within the cell
- Currently available *in vitro and in vivo models have limited* capability to quantitatively predict the impact of transporters on intracellular drug concentrations
- Currently, there are no standardized, accepted methods to directly measure unbound intracellular drug concentrations
- Significant progress has been made in using modeling approaches to predict the effect of drug-metabolizing enzymes and transporters on the systemic exposure of drugs in preclinical species and humans

XENOTECH
A BioIVT Company

ITC Recommendations for Transporter Kinetic Parameter Estimation and Translational Modeling of Transport-Mediated PK and DDIs in Humans

MJ Zamek-Gliszczynski[1], CA Lee[2], A Poirier[3], J Bentz[4], X Chu[5], H Ellens[6], T Ishikawa[7], M Jamei[8], JC Kalvass[9], S Nagar[10], KS Pang[11], K Korzekwa[10], PW Swaan[12], ME Taub[13], P Zhao[14] and A Galetin[15]; on behalf of the International Transporter Consortium

Clinical Pharmacology & Therapeutics | Advanced online publication | APRIL 2013

Uptake transporters: 2 Step Method (Conventional)

XENOTECH
A BioIVT Company

- Single low substrate concentration, single incubation time point
- Cell systems
  - Linear rate conditions and time range, 4 and 37°C
  - Active uptake = Uptake at 37°C – Uptake at 4°C
  - Transporter mediated uptake determined with inhibitors
  - Limitations
    - Limited membrane fluidity at 4°C
    - Lack of specific of inhibitor
    - Substrate dependent inhibition (OATPs)
- Expression systems
  - Active uptake = Uptake in transfected cells – Uptake in control cells
  - Limitations
    - Assumes transport is an isolated process, Does not account for the bidirectional nature of passive diffusion, intracellular binding, metabolism, or active efflux
    - Data transformation required for parameter estimation
- Applicable for low permeable compounds

$$v = \frac{V_{max} \times C_{med}}{K_m + C_{med}} + CL_{diff} \times C_{med}$$

Uptake transporters: Mechanistic compartmental model

XENOTECH
A BioIVT Company

- Plated or suspended hepatocytes
  - Active transport, passive diffusion, and intracellular/ extracellular binding
- Multiple sub substrate conc. and times, 37°C
- Time points beyond time-linear range used to attain steady-state intracellular conditions
  - more accurate estimation of intracellular binding
- Assumes intracellular binding is not saturated
  - overestimation of the fraction unbound in cell ($f_{u,cell}$)
- Active efflux not considered; also internalization of efflux transporters
- Metabolism not incorporated; uptake data obtained in the presence of ABT should be used for CYP substrates
- Applicable for compounds that are not metabolized or only metabolized by CYP enzymes

$$\frac{dC_{cell}}{dt} = \frac{\frac{V_{max} \times C_{med,u}}{K_{m,u} + C_{med,u}} + CL_{diff} \times C_{med,u} - CL_{diff} \times C_{cell} \times f_{u,cell}}{V_{cell}}$$

$$\frac{dC_{med,u}}{dt} = \frac{-\frac{V_{max} \times C_{med,u}}{K_{m,u} + C_{med,u}} - CL_{diff} \times C_{med,u} + CL_{diff} \times C_{cell} \times f_{u,cell}}{V_{med}}$$

Uptake transporters and metabolism: Mechanistic compartmental model

XENOTECH
A BioIVT Company

- Plated or suspended hepatocytes
  - Active transport, passive diffusion, intracellular/ extracellular binding, metabolism
- Extended incubation times to obtain steady-state
- Both drug and metabolites are measured
- Data analysis is more complex but has less error
- Provides a mechanistic description of in vitro process
- Can account for canalicular efflux using sandwich-cultured hepatocytes
  - Single low substrate concentration, multiple time points
- Applicable for compounds that are metabolized by enzymes with no selective inhibitors (e.g., glucuronidation)
- Determination of uptake K_m and V_{max} is preferred to CL for IVIVE

$$\frac{dC_{cell}}{dt} = \frac{\frac{V_{max} \times C_{med,u}}{K_{m,u} + C_{med,u}} + CL_{diff} \times C_{med,u} - C_{cell} \times f_{u,cell} \times \left(CL_{diff} + CL_{met,u}\right)}{V_{cell}}$$

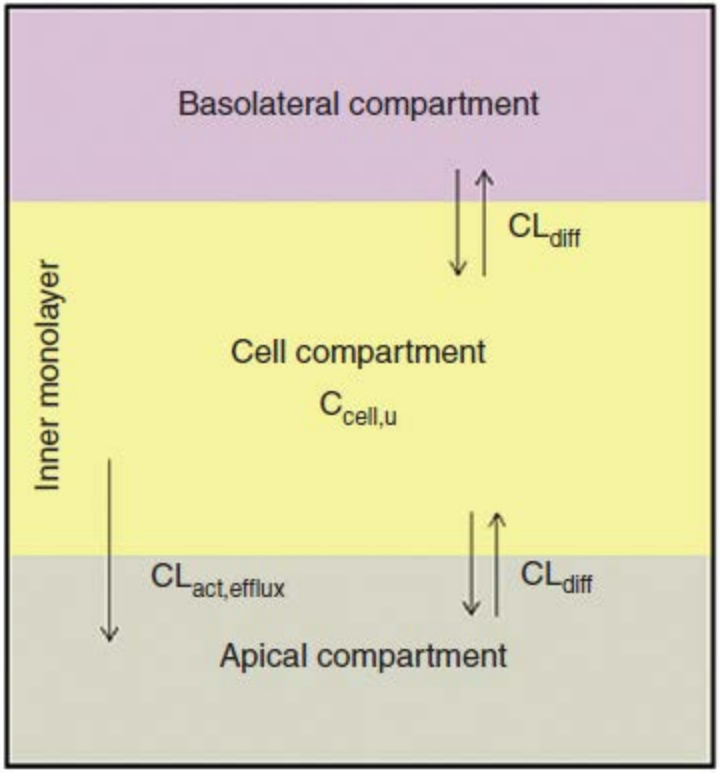
Efflux transporters: Vesicles

XENOTECH
A BioIVT Company

- Vesicles are best for low permeable compounds
- Commonly used for polar substrates (MRPs, MATES, BCRP)
- Inhibition of bile acid transport (BSEP)
- Not the test system of choice for P-gp and BCRP since these substrates are lipophilic and have higher permeability

Efflux transporters: Confluent cell monolayers (Model I)

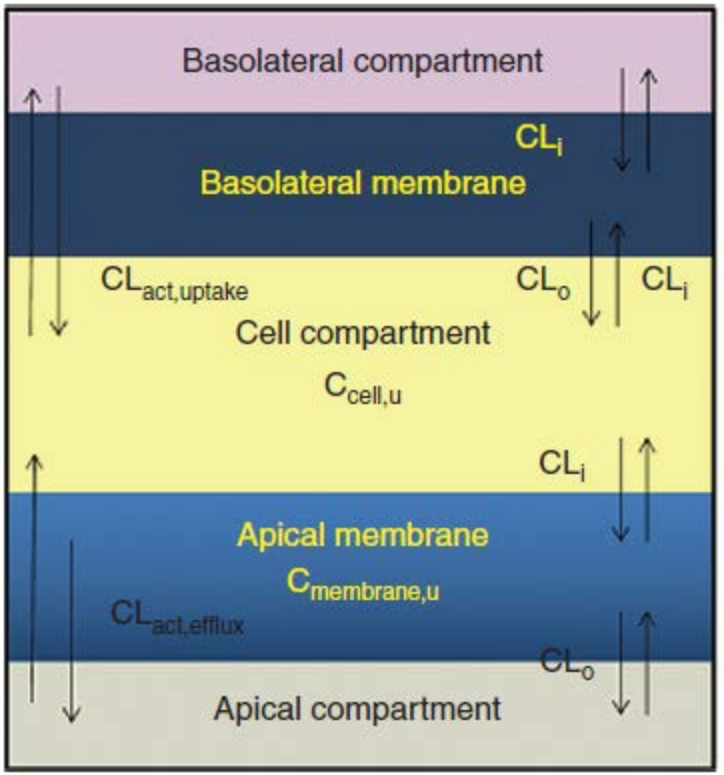
- 3 compartment model (apical, basal, cellular)
- Basolateral ER_B: transcellular passive permeability/$P_{app,A \rightarrow B}$
- Asymmetry ER_α:$P_{app,B \rightarrow A}$/$P_{app,A \rightarrow B}$
- Cellular ER_C :cellular concentration in absence of efflux/cellular concentration in presence of efflux
- ER values used to calculate K_m, V_{max} and IC_{50}
- Assumes minimal lag time in flux, similar passive permeability across the apical and basolateral membranes
- If these assumptions are not true, this model is not valid



$$K_m = \frac{K_{m,app}}{ER_{B,max} + 1} \quad \text{or} \quad K_m = \frac{2K_{m,app}}{ER_{A,max} + 3}$$

Efflux transporters: Confluent cell monolayers (Model II)

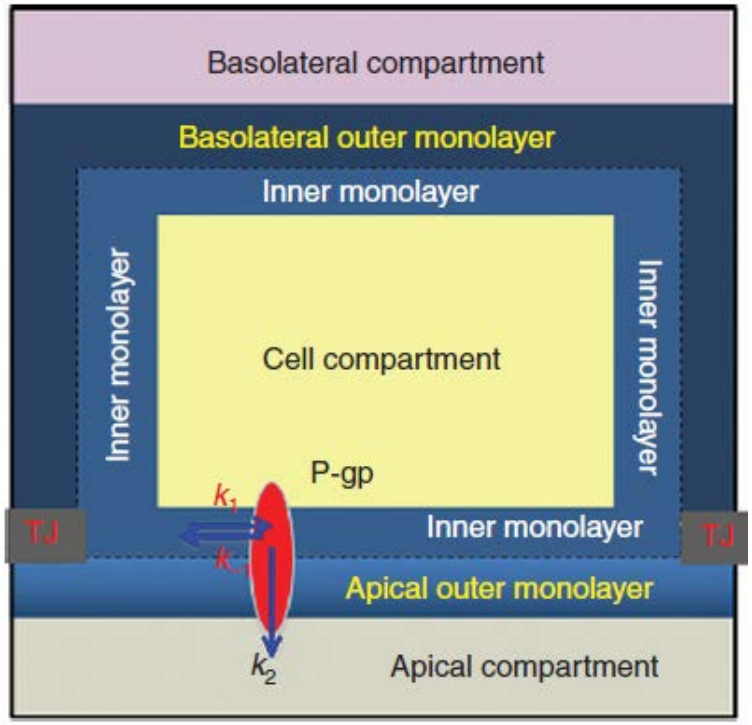
- 5 compartment model (includes apical and basolateral membrane compartments)
- Membrane-to-water partition coefficient (K_p) estimated in human liver microsomes
- K_m and IC_{50} estimated in terms of the unbound drug concentration in membrane and cytosol, respectively
- 3- and 5-compartment models predict similar intracellular concentrations following apical addition of substrate, but differ in prediction of intracellular concentrations for flux in the B→A direction
- Appropriate when appreciable lag flux time and for prediction of tissues with basolateral exposure to the drug (e.g., liver or kidney).
- Metabolism can also be incorporated into the five-compartment model



Efflux transporters: Confluent cell monolayers (Model III)

XENOTECH
A BioIVT Company

- Structural model: considers binding of substrate from within the inner leaflet of the apical membrane, with on [k_{on} $(M^{-1}s^{-1})$] and off [k_{off} (s^{-1})] rate constants and an efflux rate constant from P-gp into the apical chamber [k_{out} (M)]
- Estimates P-gp efflux active surface density, $T(0)$
- These parameters are used to calculate K_m and V_{max}
- Requires use of multiple initial drug concentrations and time points until steady state is reached between P-gp-mediated efflux into the apical chamber and passive permeability from the apical chamber back into the cytosol
- K_i can be calculated from the IC_{50}
- Accounts for basolateral transport



$$K_m = \frac{k_2 + k_{-1}}{k_1}$$

$$V_{max} = k_2 \times T(0)$$

Practical considerations for in vitro estimation of uptake and efflux parameters

XENOTECH
A BioIVT Company

- Probe concentration << K_m for IC_{50} = K_i (uptake transporters and efflux in vesicles)
- Method for K_i in polarized cell lines have not been agreed upon, IC_{50} is preferred in this test system
- Substrates requiring both basolateral uptake and apical efflux (e.g., digoxin) should use net value for inhibition to determine IC_{50}
- Evidence of substrate-dependent inhibition suggests transporters have multiple binding sites. Inhibition determined with relevant comedications and prototypical substrates.
- Preincubation may increase inhibition. With and without preincubation should be evaluated.
- Incubations should be conducted at relevant physiological pH

Knockout animal models

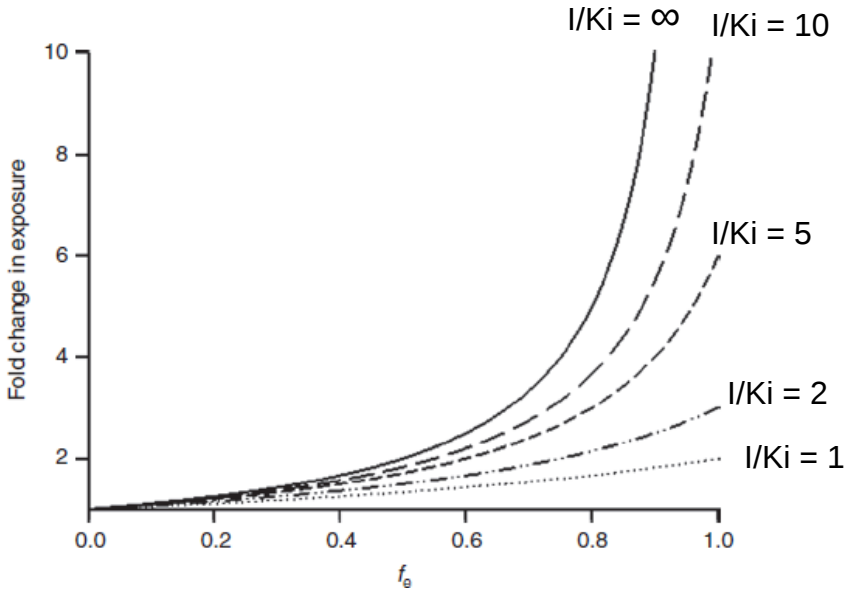
- With exception of OAT and MATE, all major transporters can be evaluated in knockout animals
- P-gp and BCRP data from knockout models: predict rate and extent of adsorption.
  - If complete adsorption, minimal in vivo efflux, may not need to conduct a clinical DDI study of intestinal efflux
  - May be useful for BBB predictions
- Fraction excreted (fe) method is preferred:

Knocked out pathway (substrate):

$$\text{fold}\ \ \Delta\ \ \text{exposure} = \frac{1}{1 - f_e}$$

Knocked out pathway (inhibition):

$$\text{fold}\ \ \Delta\ \ \text{exposure} = \frac{1}{\dfrac{f_e}{(1 + [I]/K_i)} + (1 - f_e)}$$



Knockout animal models

XENOTECH
A BioIVT Company

- Confirm compound exhibits similar pharmacokinetics between humans and knockout species
  - Species differences should be assessed in vitro to support translation
- Potential up- or down-regulation of transporters and drug-metabolizing enzymes may complicate interpretation

Limitations of static models for IVIVE

XENOTECH
A BioIVT Company

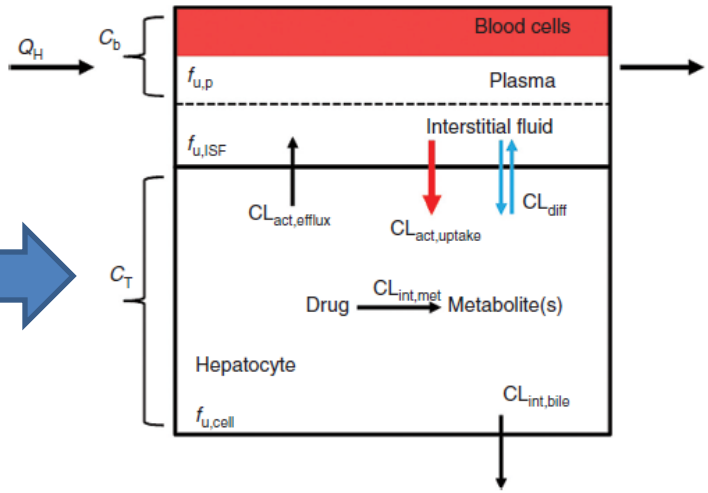
- Based on clearance concepts
- Assume a single transporter dominates victim drug adsorption, distribution and clearance
- Assume constant concentration of inhibitor or substrate during dosing
- May eliminate false negatives by assessing “worse-case scenario”, but can result in false positives

PBPK for prediction of transporter-mediated PK

XENOTECH
A BioIVT Company

- Incorporate permeability-limited tissue compartments to account for diffusional barriers
- Can be optimized with existing clinical data for improved predictability (“top-down” approach)
- Clinical data can bridge the gap in transporter IVIVE by generating empirical scaling factors (ESFs)
- Caveats: Interindividual variability, cellular system differences, difference in transporter expression, differences in test system vs tissue activity, allelic variants

Permeability-limited liver model



Summary

XENOTECH
A BioIVT Company

- IVIVE require information from numerous processes and organs
- Each has its advantages and limitations in translating data to the clinic
- Compartmental modeling is the best approach
- Full kinetic characterization of transporters is required
- Knockout animals provide valuable information and can improve the understanding of human PK (with caution)
- Expression and localization of transporters is needed
- More work is required to determine scaling factors

XENOTECH
A BioIVT Company

Why Clinical Modulation of Efflux Transport at the Human Blood–Brain Barrier Is Unlikely: The ITC Evidence-Based Position

JC Kalvass[1], JW Polli[2], DL Bourdet[3], B Feng[4], S-M Huang[5], X Liu[6], QR Smith[7], LK Zhang[8] and MJ Zamek-Gliszczynski[9]; on behalf of the International Transporter Consortium

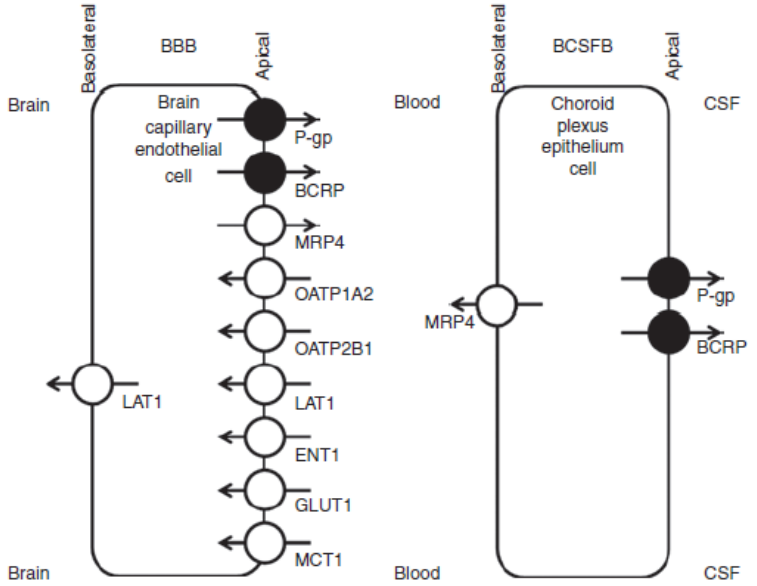
Clinical Pharmacology & Therapeutics | VOLUME 94 NUMBER 1 | JULY 2013

Blood Brain Barrier (BBB) Drug Transporters

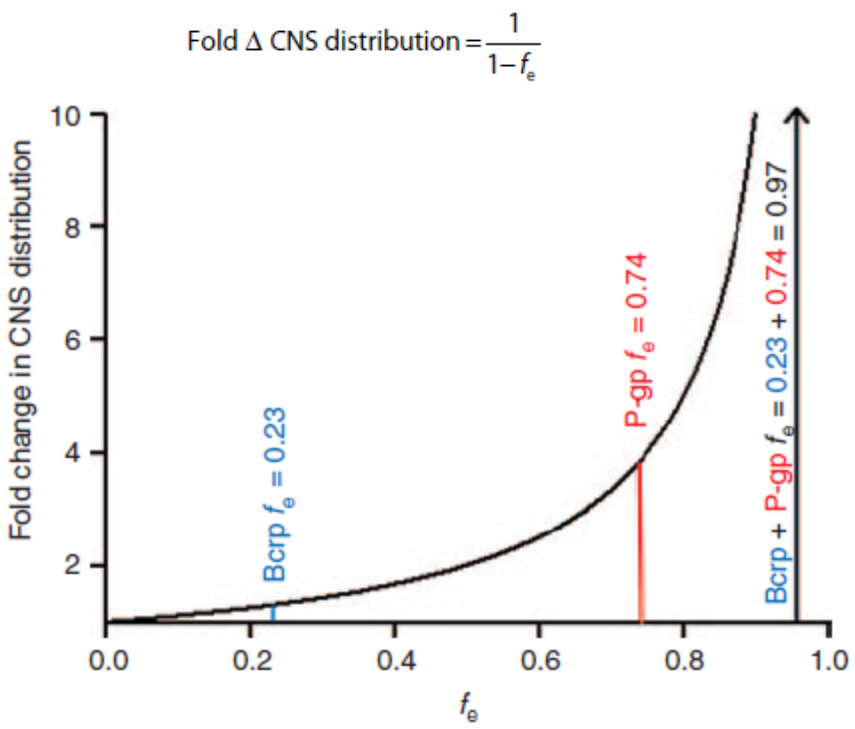
XENOTECH
A BioIVT Company

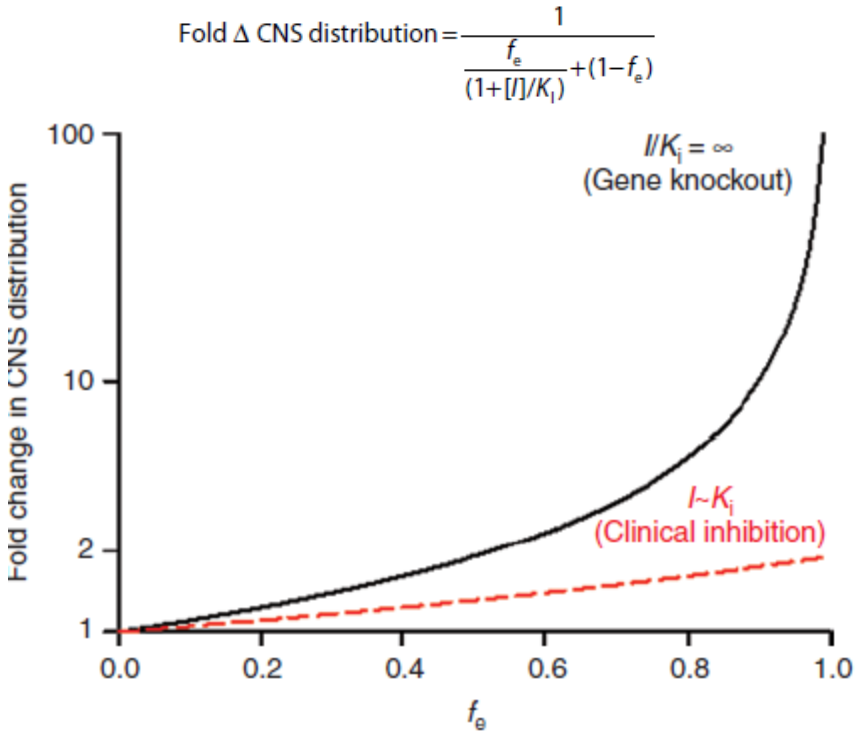
- BBB is tightest endothelium in human body
- ↑ lipophilicity ↑ passive permeability
- Efflux transporters limit CNS penetration
- Transporter function at blood-cerebrospinal fluid (CSF) barrier can function differently than BBB

- Example, P-gp and BCRP secrete substrates into CSF, but at BBB efflux into blood



P-gp/BCRP synergy (lapatinib)





- Knockout mice
  - Bcrp-knockout: f_e = 0.23 (1.3-fold)
  - P-gp-knockout: f_e = 0.74 (3.8-fold)
  - Both: f_e = 0.97 (33-fold)
  - Actual: 26-fold

- Mdr1a/1b/Bcrp$^{-/-}$ mice
  - Genetic ablation
- Clinical inhibition
  - Only weak inhibition is obtained (I = Ki)
  - Cyclosporin Cmax/Ki (P-gp) = 1.6
  - Efavirenz Cmax/Ki (BCRP) = 0.06
  - Largest DDI with lapatinib = 1.9-fold

Other transporters: MRPs and uptake transporters

XENOTECH
A BioIVT Company

- Multidrug-resistance proteins (MRPs)
  - Only MRP4 in human and rodent BBB
  - Limits penetration of adefovir and topotecan
  - Mrp4 knockout mice: < 2-fold increase in exposure
  - Risk of DDI is low
- Uptake transporters (LAT1, ENT1, MCT1, Glucose transporter 1, OATPs)
  - Competitive inhibition not likely due to Km >> Cmax
  - No clinical interactions

Methodologies

XENOTECH
A BioIVT Company

- CNS exposure is defined by Kp (Brain to plasma ratio)
- ↑ systemic drug conc, ↑ brain exposure
- Systemic DDI ≠ BBB DDI
  - brain distribution is not altered
- Models
  - Brain capillary endothelial cells
  - Noncerebral cell lines: MDCK and LLCPK1
  - Animal models (including knockout)
  - PET imagining
  - CSF concentration
  - CNS pharmacodynamic effects

Summary: Why BBB DDI is unlikely

XENOTECH
A BioIVT Company

- DDI predicted based on KO animal studies with complete loss of transporter function
- Complete inhibition of transporters at the BBB is not likely
  - Unbound Cmax ≤ Ki, P-gp and BCRP inhibition ≤ 50% (2-fold increase in exposure)
- No clinical induction of BBB P-gp reported
- No clinical correlation between P-gp function and CNS disorders

XenoTech
A BioIVT Company

Upcoming Events: September 2013

XenoTech will be attending the following meetings:

- **9/17** – IQPC: Clinically Relevant Drug Transporters **(London, UK)**

  Tom Zaleski, Ph.D. tzaleski@xenotechllc.com

- **9/30** – 10th International ISSX Meeting **(Toronto, Ontario, Canada) Booth # 301**

XENOTECH
A BioIVT Company

Thank You!

General Contact Information

XENOTECH
A BioIVT Company

XenoTech, LLC
16825 W. 116th St
Lenexa, KS 66219
(T) 913-438-7450
info@xenotechllc.com