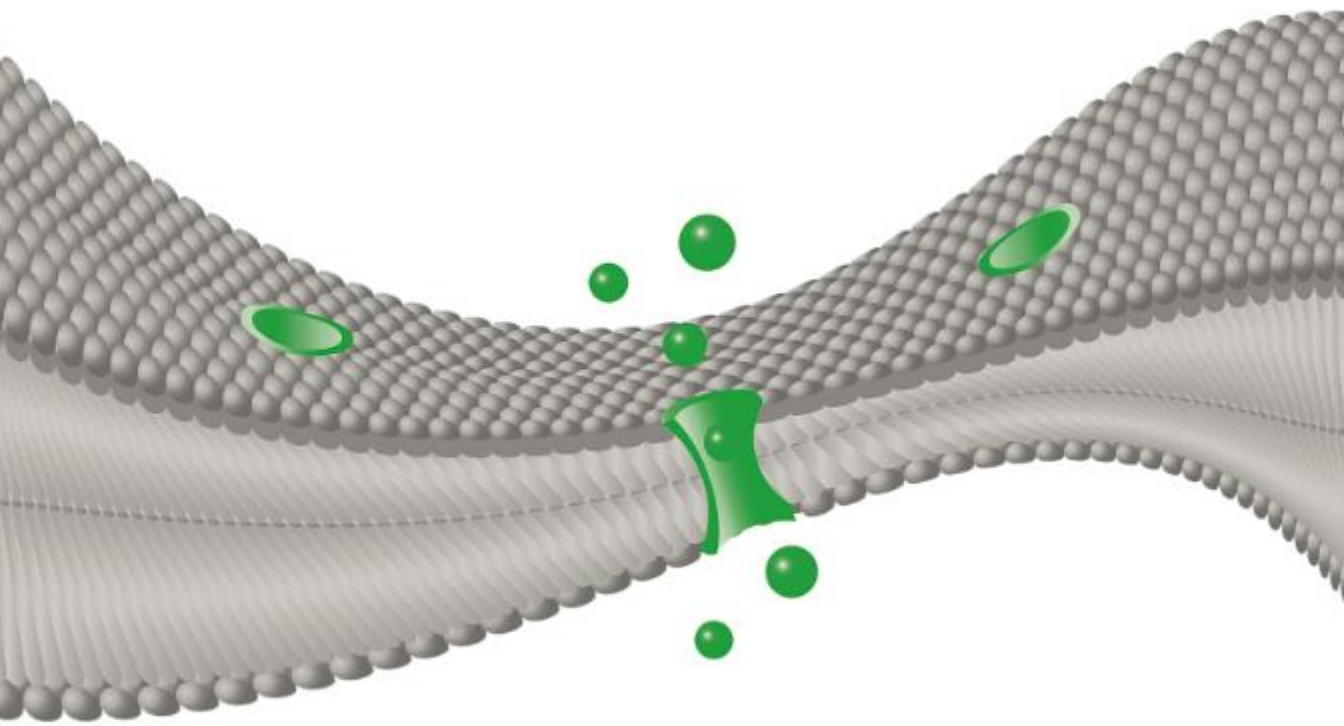
Transporter Reference Guide

2017 *In Vitro* DDI FDA Guidance | 4th Edition



ABSORPTION SYSTEMS®

Things To Know

Drug Metabolism

Enzyme Inhibition:

- Microsomal protein binding in liver microsomes should be a routine assessment in parallel with enzyme inhibition with liver microsomes
- Second substrate requirement for CYP3A4 unmentioned in new Guidance
- Data interpretation modifications:
  - Reversible CYP inhibition:
    Unbound plasma concentrations used
    Cut-off value (R value) changed from R > 1.1 to R ≥ 1.02
  - Time-Dependent Inhibition:
    R value updated from R > 1.1 to R ≥ 1.25

Enzyme Induction:

- Acknowledgement of the use of immortalized cell lines
- Endpoints for CYP induction:
  - mRNA continues to be the preferred endpoint
- Measure test compound concentrations at several time points on the last day of incubation

Reaction Phenotyping:

- The new FDA Guidance indicates two different methods:
  - Using inhibitors (chemical/drug or antibody) in human liver microsomes
  - Using individual human recombinant enzymes

Drug Transporters

Transporter Inhibition:

- MATE1 and MATE2K are now included
- Time-dependent inhibition for OATP1B1 and OATP1B3 addressed by pre-incubating the investigational drug prior to adding probe substrate

Transporter Substrate:

- MATE1 and MATE2K are now included if active renal secretion is ≥ 25% of total clearance
- *In Vitro* studies for P-gp and BCRP can be avoided if the investigational drug is BCS class I, unless there is a specific safety concern with distribution into particular tissues where these transporters are expressed

Protein Binding

- Accurate assessment of protein binding is critical due to the conservative nature of the updated FDA Guidance

Transporter Reference Guide
4th Edition, October 2018

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Exton, Pennsylvania, 19341
+1.610.280.7300
www.absorption.com

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Written requests for reprint or other permission should be emailed to:
marketing@absorption.com

Transporter Reference Guide

About Absorption Systems

Absorption Systems supports pharmaceutical and medical device companies in identifying and overcoming ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) barriers in the development of drugs and medical devices. The company's mission is to continually develop innovative research tools that can be used to accurately predict human outcomes or to explain unanticipated human outcomes when they occur. Absorption Systems' facilities are strategically located on both of the US coasts and Panama, and encompass nearly 65,000 sq. ft., servicing hundreds of customers throughout the world. For more information on the company's comprehensive contract services and applied research programs, please visit www.absorption.com.

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Introduction

Why evaluate the substrate and inhibitor potentials of the investigational drug for specific transporters and why does the FDA (and EMA) care?

Membrane transporters can have clinically relevant effects on the pharmacokinetics and pharmacodynamics of a drug in various organs and tissues by controlling its absorption, distribution, and elimination.

Transporter-mediated uptake or efflux of drugs and endogenous compounds may impact the efficacy and safety of the investigational drug and potential concomitant medications.

Several key transporters have the potential to interact with drugs in clinical use and the FDA recommends determining if the investigational drug is a substrate and/or an inhibitor of those key transporters.

Why use *in vitro* Assays?

In Vitro studies are part of an integrated approach to reduce unnecessary clinical studies.

Negative *in vitro* results can preclude the need for clinical drug-drug interaction (DDI) studies; in some cases it is also possible to waive clinical studies for compounds that do interact with P-gp and/or BCRP (find more information on biowaivers on page 36).

If clinical DDI studies are required, then *in vitro* results may serve as the basis for design of those studies. Similarly, the results of clinical DDI studies help to explain and refine predictive *in vitro* models or tools.

Timing of *in vitro* evaluation?

Because it is recommended to evaluate all investigational drugs as inhibitors of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K, and as substrates of P-gp and BCRP, it is suggested to investigate these transporters relatively early in drug development.

Furthermore, knowing if the investigational drug is a potential substrate or inhibitor of one of these transporters can help with potential clinical studies down the road.

Introduction

Why Absorption Systems?

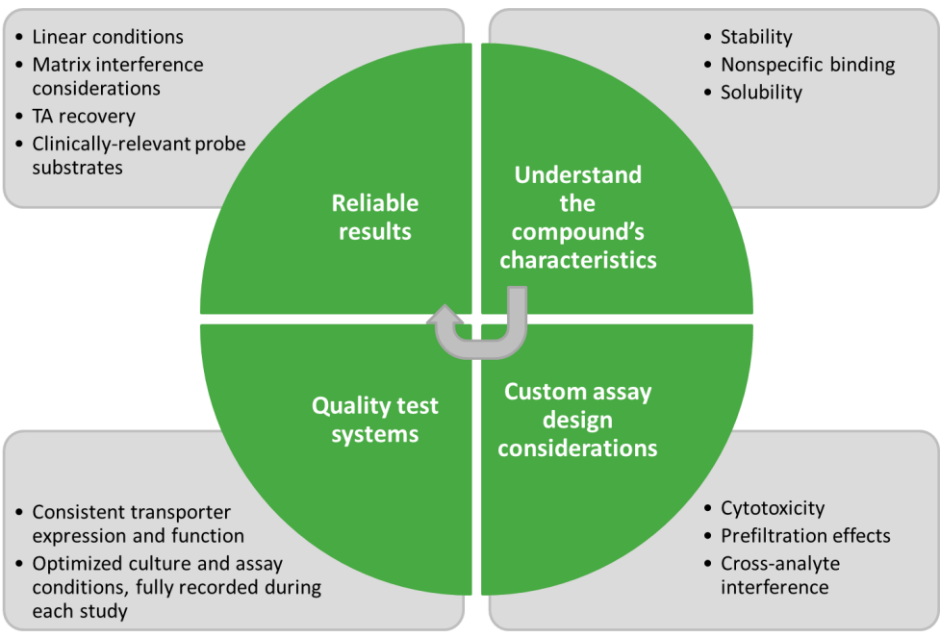
Absorption Systems has a deep understanding of the expectations of the FDA Guidance and EMA Guidelines in regards to *in vitro* transporter substrate and inhibitor potential investigations.

We have a rich knowledge base to support your drug investigation and we provide comprehensive data analysis and in-depth consultation on your non-clinical assay design.

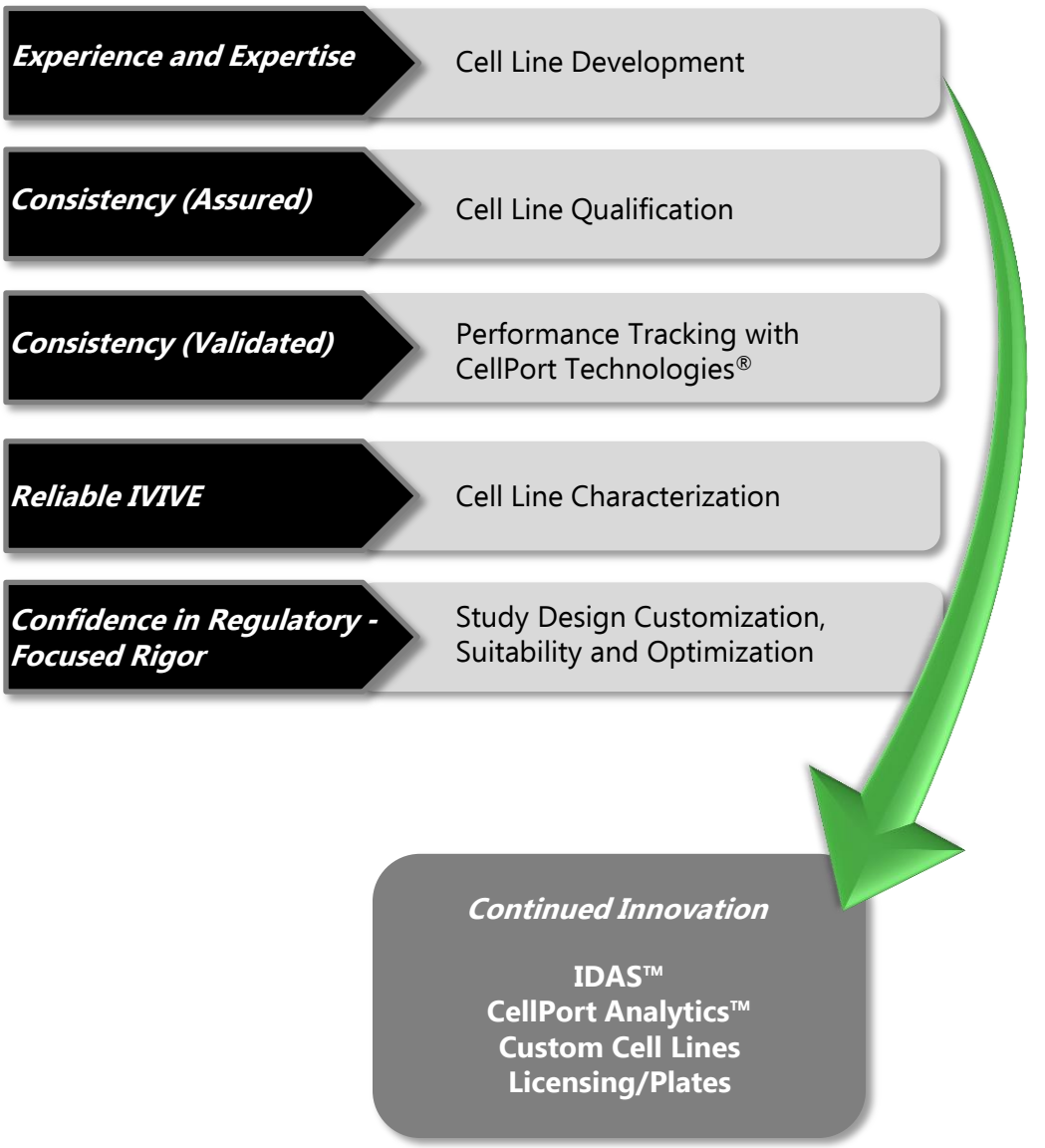
We have years of experience with *in vitro* transporter substrate and inhibitor potential assay development, and in working on different types of investigational drugs in terms of therapeutic area (potential co-medications), product profile, development plan, clearance route (hepatic vs. renal), chemical structure, and physicochemical properties.

CellPort Analytics™ technology allows us to provide a spectrum of validated assays for investigating all transporters recommended by both the FDA and EMA. Additionally, we offer in vitro assays for an array of other essential transporters, such as MRP2, PepT1, NTCP, OATP2B1, and OATP1A2.

Our Quality Approach



The Absorption Systems Difference



Introduction

Regulatory Perspective on Transporters

Transporter	Location	FDA Guidance 2017	EMA Guideline 2012	Absorption Systems Portfolio
P-gp	Ubiquitous	✔	✔	✔
BCRP	Ubiquitous	✔	✔	✔
BSEP	Liver		✔	✔
OATP1B1	Liver	✔	✔	✔
OATP1B3	Liver	✔	✔	✔
OCT1	Liver		✔	✔
OCT2	Kidney	✔	✔	✔
OAT1	Kidney	✔	✔	✔
OAT3	Kidney	✔	✔	✔
MATE1	Kidney	✔		✔
MATE2K	Kidney	✔		✔
MRP2	Ubiquitous			✔
PepT1	Ubiquitous			✔
NTCP	Liver			✔
OATP1A2	Intestine			✔
OATP2B1	Ubiquitous			✔
OATP2A1*	Ubiquitous			✔

*** Cell line under development**

Introduction

Regulatory Perspective on Transporters

Why is it Important?
• Most characterized of transporter proteins • Involved in test drug absorption and distribution
• Genetic polymorphisms are clinically relevant • Involved in test drug absorption and distribution
• May be important depending on the cholestatic potential of the test drug • Involved in test drug distribution and/or elimination
• Major role in statin disposition • Genetic polymorphisms are clinically relevant • Important in hepatic and biliary clearance • Involved in test drug distribution and/or elimination
• Major role in statin disposition • Important in hepatic and biliary clearance • Involved in test drug distribution and/or elimination
• Important in hepatic clearance • Involved in test drug distribution and/or elimination
• Known to work in concert with MATE transporters for renal excretion of compounds • Important in renal clearance • Involved in test drug distribution and/or elimination
• Important in renal clearance • Involved in test drug distribution and/or elimination
• Important in renal clearance • Involved in test drug distribution and/or elimination
• Important in renal clearance • Involved in test drug distribution and/or elimination
• Known to work in concert with OCT2 transporters for renal excretion of compounds • Important in renal clearance • Involved in test drug distribution and/or elimination
• Involved in test drug absorption and/or elimination
• Involved in test drug absorption
• Involved in test drug distribution and/or elimination
• Involved in test drug absorption
• Important in hepatic and biliary clearance • Involved in test drug distribution and/or elimination
• Important for efficacy of prostaglandin-related drugs • Involved in test drug distribution and/or elimination

What is CellPort Analytics™?

Introduction

Transporter-overexpressing cells are used in our facilities to assess *in vitro* drug-drug interactions with multiple cell lines that are cultured and used concurrently. It is of utmost importance to keep an error-free environment throughout the assay. Proprietary software, Cellport Analytics™, was developed to fulfill these goals.

Methods

A Plate Request Form is generated using Cellport Analytics™ and sent to the manufacturing group, where the corresponding cell lines are seeded (Figure 1). On the day of the assay, the transporter activity of each type of cells is verified. The QC results are recorded via Cellport Analytics™ which captures and summarizes all relevant information (cell-type seeded, cell passage, etc.) into a single data domain.

Results

The biggest advantage using Cellport Analytics™ is to identify any abnormalities in our transporter assays. Three representative hepatic panel transporters, OATP1B1, OATP1B3 and OCT1, were used as an example to demonstrate the workflow. Morphologically, the cells transfected with OATP1B1 and OATP1B3 are not different with those transfected with OCT1 (Figure 2). However, the fluorescent QC compounds used for the OATPs and OCT1 are distinct: FMTX for both OATP1B1 and OATP1B3 and ASP for OCT1 (Figure 3). While FMTX can be used to distinguish OATP1B1/1B3 and OCT1, it cannot differentiate between OATP1B1- and OATP1B3- transfected cells and therefore there has to be controlled and proper identification of the cells from seeding through assay execution. This is possible using the software by barcoding all aspects of the cell seeding and culturing process.

Conclusions

The balance of efficiency and error-free cell culture has been established successfully with Cellport Analytics™.

CELL LINE PLATING REQUEST FORM

Date of request: 09-Jun-2017 Request by: [redacted]

Date to plate: 26-Jun-2017 Customer Study #: 17TESTP1

Date plates needed: 28-Jun-2017

Special Instructions:

TOTAL NUMBER OF PLATES REQUESTED: 2

Fill in wells with corresponding numbers for how each plate should be made. Attach additional sheets if needed.

# of plates: 1

HEK-1B1-N	HEK-1B1-N	HEK-1B1-N	HEK-1B3-N	HEK-1B3-N	HEK-1B3-N
HEK-1B1-N	HEK-1B1-N	HEK-1B1-N	HEK-1B3-N	HEK-1B3-N	HEK-1B3-N
HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N
HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N	HEK-VC-N

# of plates: 1

HEK-OCT1-N	HEK-OCT1-N	HEK-OCT1-N			
HEK-OCT1-N	HEK-OCT1-N	HEK-OCT1-N			
HEK-VC-N	HEK-VC-N	HEK-VC-N			
HEK-VC-N	HEK-VC-N	HEK-VC-N			

Figure 1: Sample of plating requested, received and processed through CellPort Analytics™

What is CellPort Analytics™?

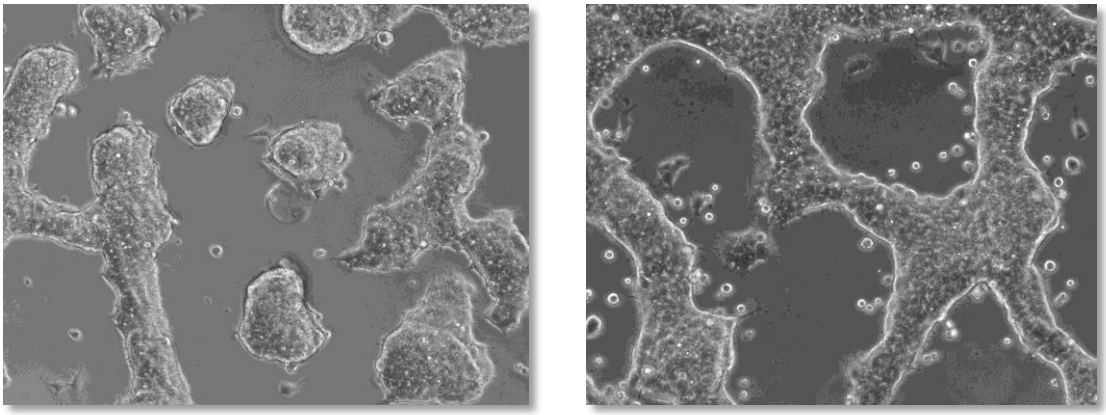


Figure 2: HEK cells transfected with the transporter proteins OATP1B1 and OCT1 do not appear morphologically different

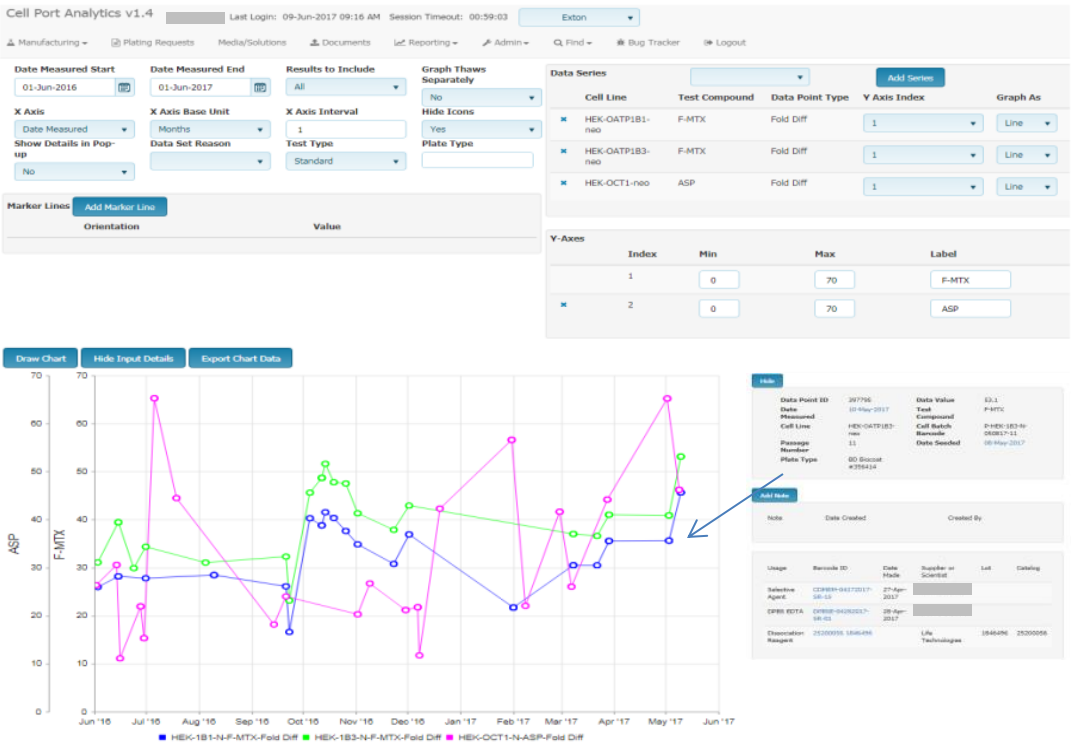


Figure 3: Screenshot of QC Data generated from CellPort Analytics™ software. (June 1, 2016 – June 1, 2017)

Services Portfolio for Substrate Assessments

Transporter	CellPort Test System
P-gp	**Caco-2;** human colon adenocarcinoma
	MDR1-MDCK vs. MDCK; canine kidney cells transfected with human P-gp
BCRP	**Caco-2;** human colon adenocarcinoma
	BCRP-MDCK vs. MDCK; canine kidney cells transfected with human BCRP
BSEP	**hBSEP-expressing vesicles;** membrane vesicles derived from recombinant baculovirus-infected insect cells
OATP1B1	**OATP1B1-HEK293;** human embryonic kidney cells transfected with OATP1B1*1A or OAT1B1*1B
OATP1B3	**OATP1B3-HEK293;** human embryonic kidney cells transfected with OATP1B3*1 or OATP1B3*2
OCT1	**OCT1-HEK293;** human embryonic kidney cells transfected with OCT1
OCT2	**OCT2-HEK293;** human embryonic kidney cells transfected with OCT2
OAT1	**OAT1-HEK293;** human embryonic kidney cells transfected with OAT1
OAT3	**OAT3-HEK293;** human embryonic kidney cells transfected with OAT3
MATE1	**MATE1-HEK293;** human embryonic kidney cells transfected with MATE1
MATE2K	**MATE2K-HEK293;** human embryonic kidney cells transfected with MATE2K
MRP2	**Caco-2 vs. CPT-M1;** proprietary P-gp knockdown Caco-2 cells
PepT1	**Caco-2;** induced human colon adenocarcinoma
NTCP	**NTCP-HEK293;** human embryonic kidney cells transfected with NTCP
OATP2B1	**OATP2B1-HEK293;** human embryonic kidney cells transfected with OATP2B1
OATP1A2	**OATP1A2-HEK293;** human embryonic kidney cells transfected with OATP1A2

CellPort Advantage
• Enables waiver of clinical DDI studies based on drug-specific factors
• Highly sensitive test system • Also predicts intrinsic brain permeation potential
• Enables waiver of clinical DDI studies based on drug-specific factors
• Highly sensitive test system
• Inside-out vesicles increase sensitivity of the test system through direct access to the transporter • Robust expression of the transporter in vesicles from over expressing cells
• Prevalent clinically relevant genetic variants
• Prevalent clinically relevant genetic variants
• Human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Specific transporter knockdown eliminates dependence on non-specific chemical inhibitors • Enables waiver of clinical DDI studies based on drug-specific factors
• Highly sensitive test system with induced PepT1 expression • Optimize oral exposure of peptide pro-drugs
• Human cell line • Stably transfected with well-characterized phenotype
• Human cell line • Stably transfected with well-characterized phenotype
• Human cell line • Stably transfected with well-characterized phenotype

Services Portfolio for Inhibitor Assessments

Transporter	CellPort Test System
P-gp	**Caco-2;** human colon adenocarcinoma
	MDR1-MDCK; canine kidney cells transfected with human P-gp
BCRP	**BCRP-MDCK;** canine kidney cells transfected with human BCRP
BSEP	**hBSEP-expressing vesicles;** membrane vesicles derived from recombinant baculovirus-infected insect cells
OATP1B1	**OATP1B1-HEK293;** human embryonic kidney cells transfected with OATP1B1*1A or OAT1B1*1B
OATP1B3	**OATP1B3-HEK293;** human embryonic kidney cells transfected with OATP1B3*1 or OATP1B3*2
OCT1	**OCT1-HEK293;** human embryonic kidney cells transfected with OCT1
OCT2	**OCT2-HEK293;** human embryonic kidney cells transfected with OCT2
OAT1	**OAT1-HEK293;** human embryonic kidney cells transfected with OAT1
OAT3	**OAT3-HEK293;** human embryonic kidney cells transfected with OAT3
MATE1	**MATE1-HEK293;** human embryonic kidney cells transfected with MATE1
MATE2K	**MATE2K-HEK293;** human embryonic kidney cells transfected with MATE2K
PepT1	**Caco-2;** induced human colon adenocarcinoma
NTCP	**NTCP-HEK293;** human embryonic kidney cells transfected with NTCP
OATP2B1	**OATP2B1-HEK293;** human embryonic kidney cells transfected with OATP2B1
OATP1A2	**OATP1A2-HEK293;** human embryonic kidney cells transfected with OATP1A2

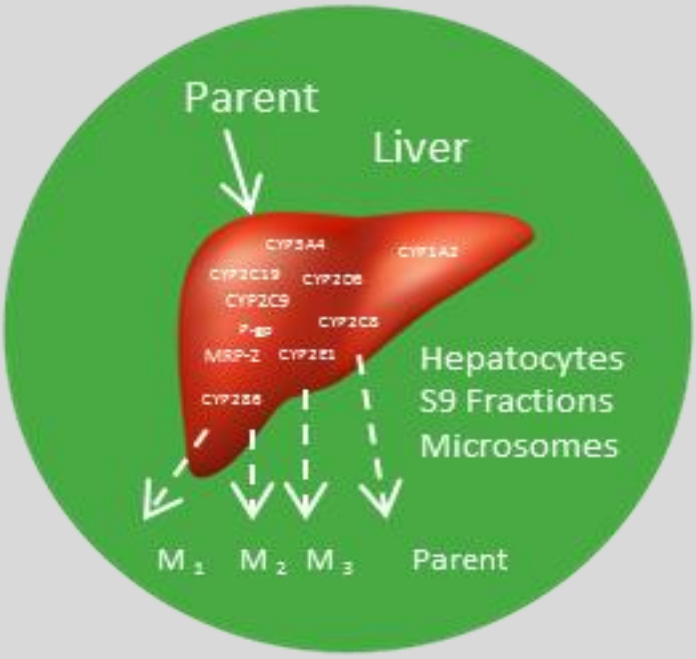
[a]Customized Preparation

CellPort Advantage
• Clinically relevant probe
• Clinically relevant probe
• Clinically relevant probe
• Inside-out vesicles allow low permeability compounds direct access to the transporter • Validated using clinically relevant inhibitors
• Prevalent clinically relevant genetic variants
• Prevalent clinically relevant genetic variants
• Human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Physiologically relevant human cell line • Stably transfected with well-characterized phenotype
• Highly sensitive test system with induced PepT1 expression
• Human cell line • Stably transfected with well-characterized phenotype
• Human cell line • Stably transfected with well-characterized phenotype
• Human cell line • Stably transfected with well-characterized phenotype

Drug Metabolism

Drug metabolism has been an area of research for many years, resulting in an appreciable understanding of the mechanisms behind enzyme-related clinical drug-drug interactions (DDIs). *In vitro* assays used today have been designed to investigate both the impact of a drug (perpetrator) on a metabolic enzyme, as well as the impact of an enzyme on a drug (victim), as it has been well-established that altering a drug's rate of clearance can result in dramatic, sometimes life-threatening, side effects. For example, mibefradil's inhibitory effects on CYP3A and CYP2D6 caused it to be withdrawn from the U.S. market one year after approval, when it was found to have lethal interactions with at least 25 other drugs including common antibiotics, antihistamines, and cancer drugs.

Through *in vitro* safety testing (phenotyping, inhibition, and induction), the liability of potential DDIs can be uncovered to guide the need for/design of appropriate *in vivo* drug interaction studies.



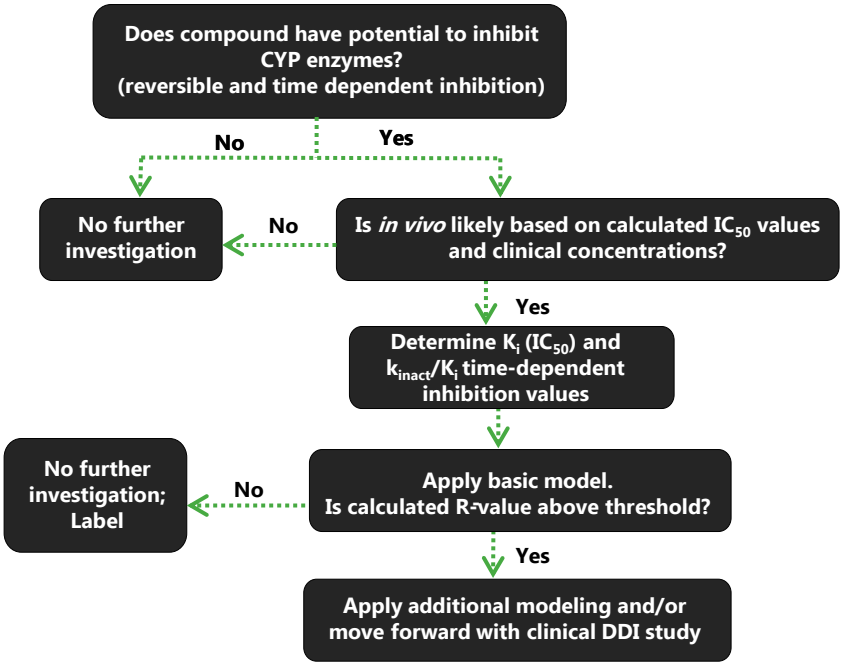
Drug Metabolism: Inhibition

Spotlight on Enzyme Inhibition

Knowing the mode of inhibition (reversible vs. time-dependent) for a given drug is important in the design of clinical DDI studies. Assessment of inhibition potential *in vitro*, combined with predictive modeling, can result in meaningful, actionable steps for the design and conduct of clinical DDI studies.

Proper administration (dose, route, and timing) of the substrate (victim) and perpetrator drugs is crucial, as it allows you to ensure that the maximum effect will be observed. Failure to properly design these clinical trials can result in underestimation of the interaction, which can lead to toxicity in the patient population and ultimately, the possible withdrawal of a product from the market.

Decision Tree for Enzyme Inhibition



(Adapted from 2017 FDA Draft Guidance for Industry: In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies)

Drug Metabolism: Inhibition

In Vitro Study Design and Considerations

Assessment	Enzymes/Isoforms*		Test Systems	Concentration Selection
Inhibition	CYP enzymes	CYP1A2,CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2J2, CYP2E1, and CYP3A	Human liver microsomes, recombinant enzymes, or hepatocytes	Range of concentrations based on maximal unbound plasma ($I_{max,u}$) and luminal (I_{gut})* concentrations

*Recommended by the FDA,, more available upon request
*Luminal concentrations (I_{gut}) are recommended for enzymes which are known to be expressed in intestinal enterocytes, such as CYP3A

In Vitro Data Application: Interpretation and Modeling

For interpretation and translatability of the *in vitro* data, the FDA has provided a number of basic models for reversible and time-dependent inhibition (TDI). As a crucial component to these models, they have defined reference value (R value) calculations and thresholds for each study type. Based on the calculated R value from the *in vitro* studies, important decisions can be made with regard to the necessity for and design of clinical DDI studies.

Reversible Enzyme Inhibition: Basic Model

For reversible inhibition, R_1 is defined as:

$$R_1 = 1 + I_{max,u}/K_i \text{ or } R_{1,gut} = 1 + I_{gut}/K_i$$

Site of Interaction	R value	DDI Prediction	Clinical DDI Study Recommended
Intestine	$R_{1, gut} \geq 11$	Likely	Yes
Systemic	$R_1 \geq 1.02$	Likely	Yes

$I_{max,u}$ = the maximal unbound plasma concentration ($C_{max,u}$)
I_{gut} = the intestinal luminal concentration calculated as the dose/250 ml
K_i = the unbound inhibition constant determined in vitro

Note: While an IC_{50} can be used to estimate the likelihood of a clinical DDI, inter-lab variability in these values can result in confounding predictions. K_i values are more definitive and are recommended when assessing the need for a clinical DDI study.
Note: In the cases where several CYPs score positive for inhibition, determination of the R value will allow rank-ordering of the DDI potential. If the innovator moves forward to perform an *in vivo* study with a sensitive substrate of the CYP with the largest R value, and *in vivo* results demonstrate no interaction, *in vivo* studies for CYPs with smaller R values can be waived. Please note, for metabolites which are present at ≥25% of the parent AUC, if inhibition is demonstrated and an R value calculated, this value CANNOT be rank-ordered vs. the R value of the parent compound to eliminate the need for a clinical DDI study.

Time-Dependent Inhibition: Basic Model

Time-dependent inhibition can be due to either the formation of more potent inhibitory metabolites or mechanism-based inactivation of the metabolizing enzyme(s). Incubations consist of the test compound with microsomes, with and without NADPH. Regardless of the nature of the interaction, TDI is indicated by a shift to a more potent IC_{50} value (a significant shift is defined at Absorption Systems as ≥1.5 fold) after incubation with the test compound and NADPH. In that case, the FDA recommends moving forward with defining the TDI parameters *in vitro* – i.e., determining the k_{inact} (maximal inactivation rate constant) and K_I (apparent inactivation constant) values. These values can then be used to determine the potential clinical liability of TDI using the sets of equations and thresholds below, based on the calculated R_2 value.

Drug Metabolism: Induction

$R_2 = (k_{obs} + k_{deg})/k_{deg}$ where $k_{obs} = k_{inact} \times 50 \times I_{max,u}/(K_I + 50 \times I_{max,u})$

Site of Interaction	R value	DDI Prediction	Clinical DDI Study Recommended
Systemic	$R_2 \geq 1.25$	Likely	Yes

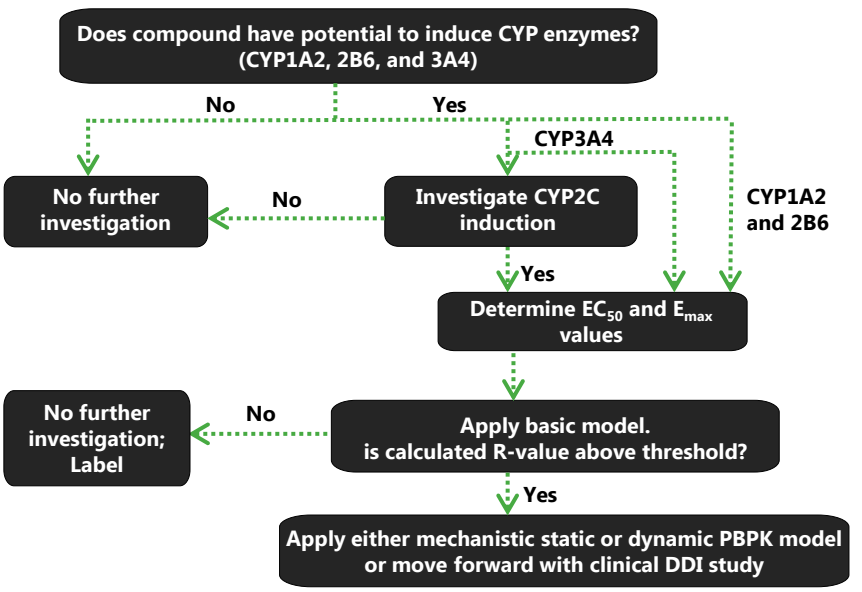
$I_{max,u}$ = the maximal unbound plasma concentration ($C_{max,u}$)

Note: Unlike reversible inhibition, the R_2 value for TDI is dependent on the rate constant of enzyme degradation, in addition to k_{inact}, K_I, and inhibitor exposure levels. The degradation constant may be taken from the scientific literature. If possible, the constant should be based on in vivo data. As degradation kinetics of each CYP has not been fully defined, where *in vitro* data suggests a TDI interaction (i.e. R_2 values which are higher than the thresholds), it is recommended to move forward with an *in vivo* DDI study.

Spotlight on Enzyme Induction

In response to a drug being introduced into a cell, certain enzymes may be expressed at a higher level. This response, known as enzyme induction, can result in acceleration of drug metabolism and greatly affect the concentrations of other drugs in the body. Note that the implications for drug efficacy depend on whether the victim drug is administered as a prodrug or as the active moiety: the former may become more efficacious while the latter become less so. Changes in the rate of metabolic clearance can present challenges to achieving therapeutic concentrations while avoiding toxicity. Due to the fact that induction is mainly caused by activation of gene transcription, the FDA recommends using changes in the mRNA level of the target gene as an endpoint when performing *in vitro* CYP induction studies.

Decision Tree for Enzyme Induction



(Adapted from 2017 FDA Draft Guidance for Industry: In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies)

If induction of CYPP3A is positive as determined by mRNA estimation, screening for induction of CYP2C and transporters (Pgp, OATs, MRP3), UGTs (1A1 and 1A2), and SULT (2A) may be considered.

Drug Metabolism: Phenotyping

In Vitro Study Design and Considerations

Assessment	CYP Isoforms	Test Systems	Concentration Selection
Induction	CYP1A2 (AhR), CYP2B6 (CAR), and CYP3A‡ (PXR)	Hepatocytes‡‡ (fresh or cryopreserved)	Concentrations based on maximal unbound plasma ($I_{max,u}$) and luminal (I_{gut})* concentrations

‡Activation of the nuclear receptor PXR results in the co-induction of CYP3A and CYP2C isoforms. A negative result for CYP3A eliminates the need to assess CYP2C induction. If induction studies are positive for CYP3A, CYP2C induction should be assessed *in vitro* or in *vivo*.
*Luminal concentrations (I_{gut}) are recommended for enzymes which are known to be expressed in intestinal enterocytes, such as CYP3A
‡‡Three (3) donors are recommended, although a positive result in one donor can be considered definitive.

In Vitro Data Application: Interpretation and Modeling
CYP Induction: Basic Model

For CYP induction, R_3 is defined as:

$$R_3 = 1/[1+(d \times E_{max} \times 10 \times I_{max,u})/(EC_{50} + (10 \times I_{max,u}))]$$

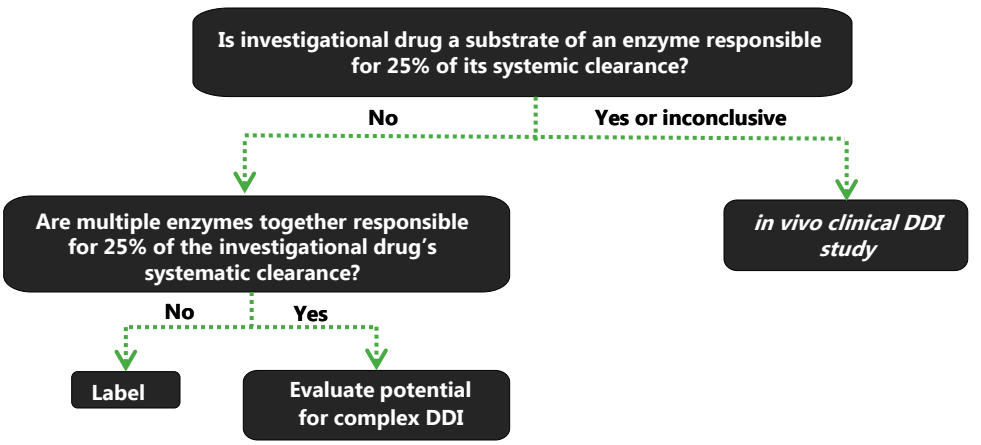
R value	DDI Prediction	Clinical DDI Study Recommended
$R_3 \leq 0.8$	Likely	Yes

$I_{max,u}$ = the maximal unbound plasma concentration ($C_{max,u}$); d is the scaling factor, for the basic model this can be assumed to be 1
Note: If 50% decrease in mRNA expression is observed, this may be an indication of gene down-regulation. If this decrease cannot be attributed to cell toxicity, it is recommended to investigate this phenomenon *in vivo*.

Spotlight on Enzyme Phenotyping

Drugs with a propensity for metabolism by a single metabolic pathway are at a greater risk for toxicity associated with drug-drug interactions. Phase I and Phase II enzymes involved in elimination pathways estimated to contribute to ≥ 25% of the overall clearance should be identified. Additionally, enzymes involved in formation and elimination of pharmacologically active metabolites should be identified.

Decision Tree for Enzyme Reaction Phenotyping



(Adapted from 2017 FDA Draft Guidance for Industry: In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies)

In Vitro Study Design and Considerations

Assessment	Enzymes/Isoforms			Test Systems***	Concentration Selection
Phenotyping	**Phase I enzymes**	**Major CYP isoforms**	CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A	Human liver microsomes Liver S9, cytosol, or other tissue fractions Fresh or cryopreserved hepatocytes Microsomes from cells expressing recombinant enzymes	Sufficiently low therapeutically relevant concentrations****
		Additional enzymes*	CYP2A6, CYP2J2, CYP4F2, CYP2E1, MAO, FMO, AO/XO		
	Phase II enzymes**		UGTs, SULT, and NAT		

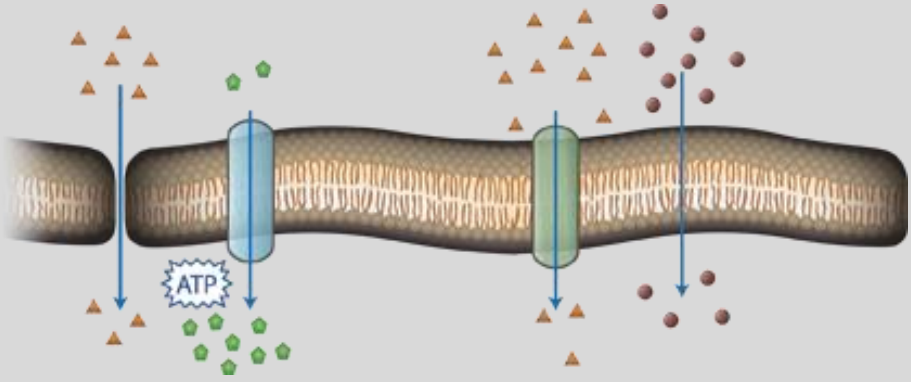
*Alternative enzymes should be assessed if Phase I metabolism is suspected but interactions with major isoforms cannot be demonstrated *in vitro*.
**To be assessed if a phase II reaction (e.g., glucuronidation) is a major pathway.
***Generally recommended to perform phenotyping in at least two parallel test systems.
****Concentrations should be sufficiently low as to not saturate the test system enzymes.

Drug Transporters

DDI Guidance:

Membrane transporters can have clinically relevant effects on the pharmacokinetics, pharmacodynamics, and safety of a drug in various organs and tissues by controlling its absorption, distribution, and elimination (Giacomini, Huang, et al. 2010; Giacomini and Huang 2013). In contrast to drug metabolizing enzymes that are largely expressed in the liver and small intestines, transporters are expressed in tissues throughout the human body and govern the access of endogenous and exogenous substances to various sites in the body. Transporters, in concert with metabolizing enzymes, can govern a drug's disposition and pharmacological action. Conversely, a drug can also modulate transporter expression or activity, resulting in altered disposition of endogenous (e.g., creatinine, glucose) or exogenous substances. Several transporters interact with drugs in clinical use (Giacomini, Huang, et al. 2010; Giacomini and Huang 2013), for example:

- P-glycoprotein (P-gp or Multi-drug Resistance 1 (MDR1) protein)
- Breast cancer resistance protein (BCRP)
- Organic anion transporting polypeptide 1B1/1B3 (OATP1B1/OATP1B3)
- Organic anion transporter 1/3 (OAT1/OAT3)
- Multidrug and toxin extrusion (MATE) proteins
- Organic cation transporter 2 (OCT2)

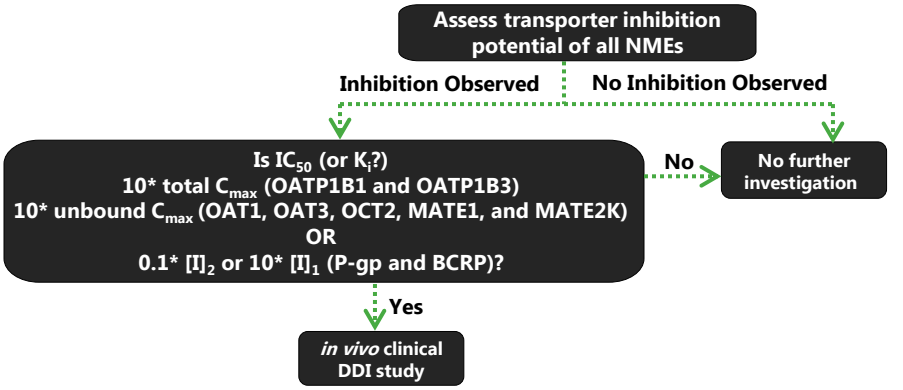


Drug Transporters: Inhibition

Spotlight on Drug Transporter Inhibition

The knowledge of drug transporters and their *in vivo* importance is rapidly evolving. Currently, the FDA recommends assessment of inhibition potential against nine transporters (P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2K) for all investigational drugs. *In vitro* assessment of transporter inhibition using well-defined test systems, combined with predictive modeling (basic or *in silico*), will aid in decision-making regarding the need for, and design of, clinical studies and product labeling.

FDA Decision Tree
Investigations of the Transporter-Inhibitory Potential of a Drug



(Adapted from 2012 FDA Draft Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, Figures A2, A4, and A6, pgs 66, 68, and 70 respectively)

Note: $[I]_1$ is defined as the total (free and bound) C_{max} following the highest proposed clinical dose. $[I]_2$ is defined as the dose/250 mL (luminal concentration).

Note: For OATP1B1 and OATP1B3, the FDA has also defined an R value. If the R value ≥ 1.25, it is recommended to move forward with an *in vivo* clinical study. If not, no further investigations are required.

In Vitro Study Design and Considerations

Assessment	Transporters			Test Systems	Concentration Selection*
Inhibition	Efflux	Intestinal	BCRP and P-gp	Transfected cell lines (overexpressing) Human-derived cell lines (relevant organ phenotype) Membrane vesicles	Range of concentrations based on unbound and total mean C_{max}, and intestinal concentrations
		Hepatic/Biliary**	BCRP and P-gp		
		Renal	MATE1, MATE2K, BCRP, and P-gp		
	Uptake	Hepatic	OATP1B1 and OATP1B3		
		Renal	OAT1, OAT3, and OCT2		

*Concentrations should be determined based on site of interaction.
**Though P-gp and BCRP are considered to be the most relevant, additional transporters, such as BSEP and MRPs, should be considered when appropriate (e.g., if cholestasis is observed, inhibition of BSEP should be assessed).

Drug Transporters: Inhibition

P-gp and BCRP Inhibitor Assessment		
Inhibitor Assessment	P-gp	BCRP
In Vitro Test System	Caco-2	Caco-2
	Cells overexpressing P-gp (e.g., MDR1-MDCK)	Cells overexpressing BCRP (e.g., BCRP-MDCK)
Assay Type	Bidirectional or unidirectional in Caco-2, and cells overexpressing P-gp or BCRP	
	Unidirectional in membrane vesicles overexpressing P-gp or BCRP	
Test Concentration Selection	As high as possible to maximize the inhibition effect but not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells	
	Starting with a concentration equal to dose of inhibitor/250 mL	
	The sponsor should evaluate approximately four to six concentrations and the sponsor should start with a high concentration of the test drug, at least an order of magnitude higher than the drug's clinically relevant concentration	
Criteria	If the investigational drug is administered orally, and the $I_{gut}/IC_{50} \geq 10$ where I_{gut} = dose of inhibitor/250 mL, the investigational drug has the potential to inhibit P-gp or BCRP	
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is an inhibitor, the sponsor should consider whether to conduct an *in vivo* study based on likely concomitant medications that are known P-gp or BCRP substrates in the indicated patient populations	

Drug Transporters: Inhibition

OAT1, OAT3, OCT2, MATE1, and MATE2K Inhibitor Assessment		
Inhibitor Assessment	OAT1, OAT3, and OCT2	MATE1 and MATE2K
In Vitro Test System	Transfected cells (e.g., HEK293)	Transfected cells (e.g., HEK293)
Assay Type	Drug uptake studies in the transfected cell line and in the parental or empty vector-transfected cell line	
Test Concentration Selection	As high as possible to maximize the inhibition effect but not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells	
	Starting with a high concentration, at least an order of magnitude higher than the drug's clinically relevant concentration	
	The sponsor should evaluate approximately four to six concentrations and the sponsor should start with a high concentration of the test drug, at least an order of magnitude higher than the drug's clinically relevant concentration	
	Start with $10 \times I_{max,u}$	Start with $50 \times I_{max,u}$
Criteria	$I_{max,u}/IC_{50}$ value is ≥ 0.1	$I_{max,u}/IC_{50}$ value is ≥ 0.02
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is an inhibitor of these renal transporters, the sponsor should consider whether to conduct an in vivo study based on whether the likely concomitant medications used in the indicated patient populations are known substrates of these renal transporters	

Drug Transporters: Inhibition

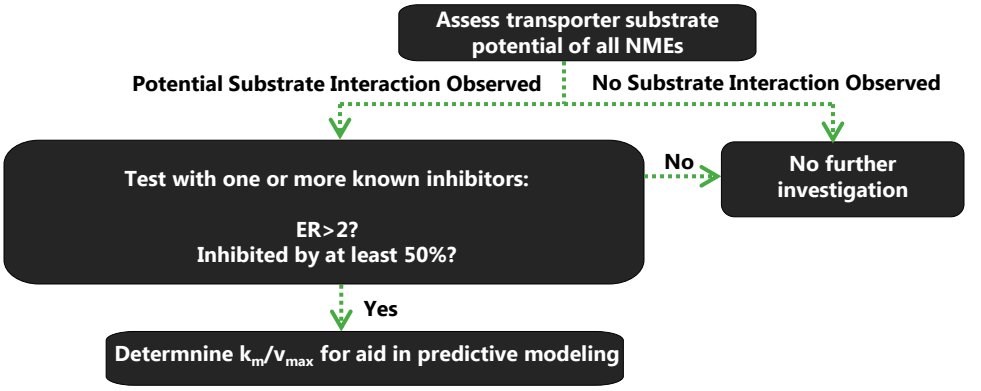
OATP1B1 and OATP1B3 Inhibitor Assessment	
Inhibitor Assessment	OATP1B1 and OATP1B3
In Vitro Test System	Transfected cells (e.g., CHO, HEK293, MDCK)
Assay Type	Drug uptake studies in the transfected cell line and in the parental or empty vector-transfected cell line
Test Concentration Selection	As high as possible to maximize the inhibition effect but not exceed the drug's solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells
	Starting with a high concentration, at least an order of magnitude higher than the drug's clinically relevant concentration
	The sponsor should evaluate approximately four to six concentrations and the sponsor should start with a high concentration of the test drug, at least an order of magnitude higher than the drug's clinically relevant concentration
	Start with 10×unbound $I_{in,max}$ or 25×$I_{max,inlet,\ u}$
Criteria	Unbound $I_{in,max}/IC_{50}$ value is ≥ 0.1 or $I_{max,inlet,u}/IC_{50}$ value is ≥ 0.04
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is an inhibitor of these renal transporters, the sponsor should consider whether to conduct an in vivo study based on whether the likely concomitant medications used in the indicated patient populations are known substrates of these renal transporters

Drug Transporters: Substrate

Spotlight on Drug Transporter Substrate Assessment

Currently, the FDA recommends assessment of substrate potential against nine transporters (P-gp and BCRP, OATP1B1 and OATP1B3 (if ≥25% of clearance is hepatic or biliary, or unknown), OAT1, OAT3, OCT2, MATE1, and MATE2K (if ≥25% of clearance is renal, or unknown) for all investigational drugs and their potential metabolites. *In vitro* assessment of transporter substrate potential using well-defined test systems, combined with predictive modeling (basic or *in silico*), will aid in decision-making regarding the need for, and design of, clinical studies and product labeling.

FDA Decision Tree
Investigations of the Transporter-Substrate Potential of a Drug



In Vitro Study Design and Considerations

Assessment	Transporters			Test Systems	Concentration Selection*
Substrate	Efflux	Intestinal	BCRP and P-gp	Transfected cell lines (overexpressing) Human-derived cell lines (relevant organ phenotype) Membrane vesicles	Range of concentrations based on unbound C_{max}
		Hepatic/Biliary**	BCRP and P-gp		
		Renal	MATE1, MATE2K, BCRP, and P-gp		
	Uptake	Hepatic	OATP1B1 and OATP1B3		
		Renal	OAT1, OAT3, and OCT2		

*Concentrations should be determined based on site of interaction.
**Though P-gp and BCRP are considered to be the most relevant, additional transporters, such as BSEP and MRPs, should be considered when appropriate

Drug Transporters: Substrate

P-gp and BCRP Substrate Assessment		
Substrate Assessment[a]	P-gp	BCRP
In Vitro Test System	Caco-2	Caco-2
	Cells overexpressing P-gp (e.g., MDR1-MDCK vs. MDCK)	Cells overexpressing BCRP (e.g., BCRP-MDCK vs. MDCK)
	Membrane vesicles overexpressing P-gp	Membrane vesicles overexpressing BCRP
Assay Type	Bidirectional in Caco-2 & cells overexpressing P-gp or BCRP	
Test Concentration Selection	Multiple concentrations to cover the range clinically relevant concentrations	
Criteria	Step 1: net flux ratio (or efflux ratio (ER)) of ≥ 2	
	Step 2: flux inhibited (e.g., ER decreases by at least 50% and/or net flux ratio = 1) by at least one known inhibitor at a concentration at least 10 times its K_i	
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is a substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known inhibitors in the indicated patient population	

Drug Transporters: Substrate

OAT1, OAT3, OCT2, MATE1 , & MATE2K Substrate Assessment		
Substrate Assessment	OAT1, OAT3, and OCT2	MATE1 and MATE2K
In Vitro Test System	Transfected cells (e.g., HEK293)	Transfected cells (e.g., HEK293)
Assay Type	Drug uptake studies in the transfected cell line and in the parental or empty vector-transfected cell line	
Test Concentration Selection	Multiple concentrations to cover the range of clinically relevant concentrations	
Criteria	Step 1: the ratio of the investigational drug's uptake in the cells expressing the transporter versus the drug's uptake in control cells (or cells containing an empty vector) is ≥ 2	
	Step 2: a known inhibitor of the transporter decreases the drug's uptake to ≤ 50% at a concentration at least 10 times its K_i or IC_{50}	
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is a substrate of one or more of these renal transporters, the sponsor should consider whether to conduct an *in vivo* study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known inhibitors of these renal transporters in the indicated patient populations	

Drug Transporters: Substrate

OATP1B1 and OATP1B3 Substrate Assessment	
Substrate Assessment	OATP1B1 and OATP1B3
In Vitro Test System	Transfected cells (HEK293)
Assay Type	Drug uptake studies in the transfected cell line and in the parental or empty vector-transfected cell line
Test Concentration Selection	Multiple concentrations to cover the range of clinically relevant concentrations
Criteria	Step 1: the ratio of the investigational drug's uptake in the cells expressing the transporter versus the drug's uptake in control cells (or cells containing an empty vector) is ≥ 2
	Step 2: a known inhibitor of the transporter decreases the drug's uptake to ≤ 50%
Impact on Potential *In Vivo* Study	If *in vitro* studies indicate that a drug is a substrate of one or more of these renal transporters, the sponsor should consider whether to conduct an *in vivo* study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known inhibitors of these renal transporters in the indicated patient populations

Concentration Selection

Concentration Selection for Transporter Evaluations

Important Considerations for *In Vitro* Transporter Assays:

Concentrations selected for substrate assessment should be based around the unbound plasma C_{max} of the test compound to ensure that results are clinically relevant. The concentration range should be approximately 100-fold.

Concentration Selection for Inhibitor Assessment

Concentrations selected for inhibitor assessment are based on the physiological site of interaction.

	Transporter	FDA	EMA
Efflux	Intestinal	0.1 x $[I]_2$	0.1 x $[I]_2$
	Renal	10x unbound C_{max} MATEs: 50x Unbound C_{max}	50x unbound C_{max}
	Hepatic	10x total C_{max}	50x unbound C_{max}
Uptake	Intestinal	0.1 x $[I]_2$	0.1 x $[I]_2$
	Renal	10x unbound C_{max} MATEs: 50x Unbound C_{max}	50x unbound C_{max}
	Hepatic	10x total C_{max}	25x $[I]_{u,inlet,max}$ (administered orally) or 50x unbound C_{max} (administered i.v.)

[I] : the concentration of drug in the intestinal lumen, defined as the highest dose divided by 250 mL
[I] u,inlet,max: the unbound hepatic inlet concentration of drug

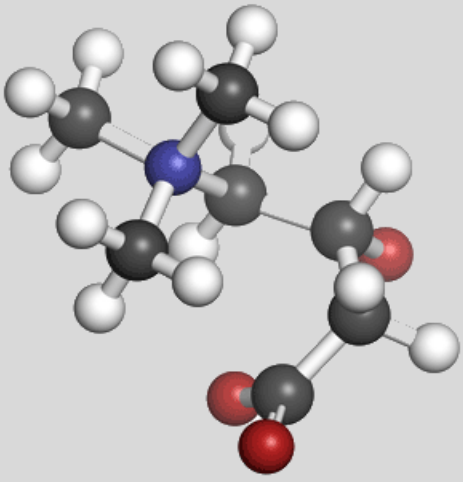
Evaluation of DDI Potential of Metabolites

Similar to the parent drugs from which they are generated, metabolites can also be involved in drug-drug interactions through changes in their own clearance or the clearance of other drugs. In order to truly understand the safety of the parent compound that is being administered, these downstream, metabolite-mediated liabilities must also be evaluated for all "major" metabolites. The FDA considers metabolites to be major based on the following:

1. Abundance: metabolites with an AUC ≥ 10% of the AUC of the parent drug
   - This criteria applies to the results of single, as well as multiple dosing pharmacokinetic studies (i.e., both must be considered if relevant to the patient population).
   - If a metabolite is less polar than the parent drug and $AUC_{metabolite} \geq 0.25 \times AUC_{parent}$, inhibition study of the metabolite is recommended.
   - If a metabolite is more polar than the parent drug and $AUC_{metabolite} \geq AUC_{parent}$, inhibition study of the metabolite is recommended.
2. Activity: metabolites whose *in vitro* activity contributes to ≥ 50% of the *in vivo* target pharmacological effect

Metabolites meeting this criteria should be evaluated for the following using the same guidelines used for the parent drug:

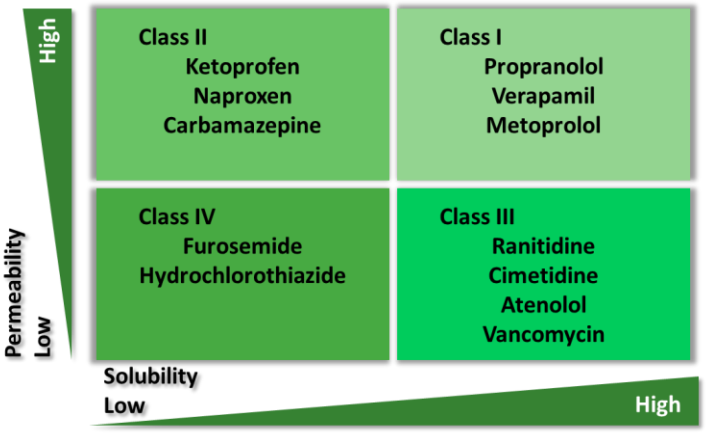
1. Enzymes contributing to formation and elimination
2. Enzyme inhibition and induction
3. Transporter involvement (substrate and inhibition)



Waiving Clinical Studies

Translating Cellular Science into Human Outcomes

Absorption Systems' Caco-2 cells are uniquely validated for both transporter interaction assessment and BCS. This test system may allow you to waive clinical DDI studies and/or human BA/BE studies based on certain drug-specific properties (high solubility, high permeability).



We Can Make *In Vivo* Interaction Studies Unnecessary.

According to the 2017 FDA BCS Guidance, compounds that are classified as Class I (highly soluble, highly permeable) are eligible for BCS biowaivers. For such compounds, the rate and extent of drug absorption is unlikely to be affected by drug dissolution and/or GI residence time, and *in vivo* bioequivalence studies (for new formulations, etc.) may be waived based on *in vitro* permeability and solubility data. Furthermore, for BCS Class I compounds, it is unlikely that absorption will be limited by efflux transporters. Thus, it may be possible to waive clinical DDI studies as well:

"If in vitro experiments demonstrate that an NME is a P-gp substrate, additional drug-specific factors may be considered before determining whether an in vivo drug interaction study is warranted. For example, the bioavailability of the BCS Class I or BDDCS Class I NMEs that are highly soluble, highly permeable, and highly metabolized may not be significantly affected by a co-administered drug that is a P-gp inhibitor, and thus, an in vivo interaction study may not be needed."

L. Zhang et al., "Predicting Drug-Drug Interactions: An FDA Perspective" (2009) The AAPS Journal

Absorption Systems has conducted over 100 *in vitro* BCS studies for classification and biowaivers over the last 10 years.

Waiving Clinical Studies

Translating Cellular Science into Human Outcomes
Here's an example of how and which clinical studies you could potentially waive by using Absorption Systems' dually validated Caco-2 test system:

P-gp Interaction Study

Substrate Assessment
- Caco-2

Inhibition Assessment
- Caco-2
- Probe Substrate: Digoxin

BCS Classification Study
- Caco-2

Possibly Waive
- Clinical P-gp Study
- Human BA/BE

BCRP Interaction Study

Substrate Assessment
- Caco-2

Inhibition Assessment
- CPT-P1
- Probe Substrate: Cladribine

BCS Classification Study
- Caco-2

Possibly Waive
- Clinical BCRP Study
- Human BA/BE

Glossary of Terms

ADME: An acronym for Absorption, Distribution, Metabolism, and Excretion (or Elimination).

Apparent Permeability: The apparent permeability coefficient (also known as P_{app}) is a parameter that is determined in an in vitro or ex vivo transport assay system. It is referred to as apparent permeability because its value represents the composite effects of traversing all permeation pathways across the test system and does not represent the permeability across any single barrier, such as the unstirred water layer, the cell membrane or the tight junctions between epithelial cells.

BCRP: An acronym for breast cancer resistance protein, product of the human ABCG2 gene and a member of the ABC (ATP-binding cassette) transporter superfamily. BCRP is expressed in the apical membrane of epithelial and endothelial cells in many tissues of the body, including intestine, liver, kidney, central nervous system (blood-brain barrier) and placenta. It was originally characterized as one of the gene products mediating resistance to cancer chemotherapeutic drugs; its involvement in drug disposition and drug-drug interactions was recognized more recently.

BCS: See Biopharmaceutics Classification System

Biopharmaceutics: The study of the effect of a drug's formulation and physicochemical properties on its absorption and disposition.

Biopharmaceutics Classification System (BCS): The Biopharmaceutical Classification System (BCS) was developed in the 1990s by a collaboration of academic, industrial and US FDA scientists in order to provide guidelines for the development of oral dosage forms. It was formalized in the US in 2000, when the FDA published the Guidance for Industry, Waiver of *In Vivo* Bioavailablity and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System.

Biowaiver: An exemption granted by the US FDA from conducting human bioequivalence studies when the active pharmaceutical ingredient(s) meet certain solubility and permeability criteria in vitro and when the dissolution profile of the dose form meets the requirements for an "immediate" release dose form. Biowaivers are based on the Biopharmaceutics Classification System (BCS) category of the active ingredient. Currently BCS class I and some class III compounds are eligible for biowaivers.

BSEP: An acronym for bile salt export pump, product of the human ABCB11 gene and a member of the ABC (ATP-binding cassette) transporter superfamily. BSEP is expressed in the canalicular membrane of hepatocytes in the liver, and is involved in the excretion of bile acids into the bile. When inhibited, it induces cholestasis as a result of transcellular accumulation of these bile salts.

Caco-2: Human colonic carcinoma cells. The Caco-2 cell line is widely used in in vitro assays to predict the absorption of candidate drug compounds across the intestinal epithelial cell barrier. The assay requires that the absorption rate (apparent permeability, or P_{app}) of a compound be determined approximately 21 days after Caco-2 cell seeding to allow the formation of a confluent monolayer and cell differentiation (appropriate localization of active transporters to the apical or basolateral plasma membrane).

Cytochrome P450 (CYP): A family of membrane-bound, heme-containing oxygenases that are active in a range of metabolic and biosynthetic pathways. They are involved in the metabolism (breakdown) of both endogenous and exogenous (xenobiotic) substrates, including drugs and environmental chemicals, and in the synthesis of metabolome components such as leukotrienes, prostacyclins, HETEs, retinoic acids, steroids and bile acids.

Drug-drug Interaction (DDI): A pharmacokinetic drug-drug interaction (DDI) involves two (or more) co-administered drugs, often referred to as the perpetrator and the victim: when co-administered with the perpetrator, the systemic exposure (C_{max} or AUC), tissue-specific exposure, or side effects (toxicity) of the victim drug are affected to an extent that is clinically significant. This type of DDI typically occurs when the victim drug is a substrate of a drug-metabolizing enzyme or transporter and the perpetrator drug is a substrate or inhibitor of the same enzyme or transporter. Qualitatively, the pharmacokinetic consequences of a metabolism-mediated DDI are fairly straightforward: enzyme inhibition can increase the exposure to a drug that is administered as the active species and can decrease the exposure to the active moiety of a pro-drug. Enzyme induction has the opposite effect. The consequences of transporter-mediated DDIs are much more difficult to predict; in fact, the clinical impact of a transporter-mediated DDI on a particular tissue or organ is often much more significant than the effect on systemic exposure. Whereas the risk of many clinical DDIs can be predicted qualitatively based on *in vitro* testing, reliable quantitative prediction of the magnitude of a clinical DDI is not yet possible in most cases.

Efflux: The process by which molecules (typically, drugs or other xenobiotics) are pumped out of cells. This process is monitored in laboratory assays using polarized epithelial cell lines such as Caco-2, which express several efflux pumps (e.g., P-gp, BCRP and MRP2) in the apical membrane and others in the basolateral membrane. Efflux is catalyzed by such efflux pumps for compounds that are substrates. The cell-based laboratory test systems are models of various cells in the body, which express many of the same efflux transporters as well as others.

Efflux Ratio: In *in vitro* and *ex vivo* assays, the efflux ratio is defined as the ratio of secretory to absorptive apparent permeability (P_{app}): (B-to-A P_{app}) (A-to-B P_{app}). The magnitude of this ratio is used as an indicator of active vs. passive transport through cells. At Absorption Systems, efflux is classified as significant when the efflux ratio is at least 2.0 and the B-to-A P_{app} is at least 1.0×10^{-6} cm/s.

HEK293: Continuous cell line derived from human embryonic kidney. HEK293 cells are frequently transfected with a vector containing one or more genes in order to create an *in vitro* model in which a protein of interest is over-expressed.

Investigational New Drug (also known as IND): The purpose of an IND application is to provide data showing that it is reasonable to begin tests of a new drug in humans. IND regulations are covered in 21 CFR 312. There are two categories of IND: commercial and research (non-commercial). An IND must contain information in three broad areas: animal pharmacology and toxicology studies; manufacturing information; and clinical protocols and investigator information. Once an IND is submitted, the sponsor must wait thirty calendar days before initiating any clinical trials. During this time, the FDA has an opportunity to review the IND for safety to ensure that research subjects will not be subjected to unreasonable risk. There are four types of IND:

- Company- (or institution-) sponsored IND: This is the traditional type of IND.

Glossary of Terms

- Investigator IND: Submitted by a physician who initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. A physician might submit such an IND to propose studying an unapproved drug, or an approved product for a new indication or in a new patient population.
- Emergency Use IND: Allows the FDA to authorize use of an experimental drug in an emergency situation that does not allow time for submission of an IND in accordance with the normal process. It is also used for patients who do not meet the criteria of an existing study protocol, or if an approved study protocol does not exist.
- Treatment IND: Used to facilitate access by patients to promising new drugs for serious or immediately life-threatening conditions while late-stage clinical trials (in which enrollment may be closed) are conducted and NDA review takes place.

LC-MS/MS: Liquid chromatography – tandem mass spectrometry. Mass spectrometry (MS) is an analytical technique to measure the mass-to-charge ratio (m/z) of ions. It is most generally used to find the composition of a physical sample by generating a mass spectrum representing the masses of the components of a sample. In addition to a number of broader applications, in drug development LC-MS/MS is generally used to quantify the amount of a compound in a sample using carefully designed methods (mass spectrometry is not inherently quantitative) and to determine the structure of a compound by analyzing its fragmentation pattern. In LC-MS/MS, molecules are partially resolved by rapid LC, followed by detection via tandem mass spectrometry (MS/MS). This mode of detection is exquisitely sensitive due to the operator's ability to tune the instrument to a particular m/z value to the exclusion of other molecular species that may be present.

MATE: An acronym for Multidrug And Toxin Extrusion transporter, product of the human SLC47 gene and a member of the SLC (solute carriers) transporter superfamily. MATE1 is primarily expressed in the kidney and also in liver. MATE2 and MATE2K are expressed only in the kidney, and are localized to the brush-border membranes (BBM) of the renal proximal tubules. The MATE family is proposed to play an important role in protecting the kidney from cationic toxins. So far, transport characteristics of the original hMATE2 remain unclear. MATE2K is currently the only functional isoform in the MATE2 subfamily.

MDR Protein: Multi-drug resistance protein 1 is the protein product of the human MDR1 gene and is a member of the ABC (ATP-binding cassette) superfamily of plasma membrane transporter proteins that pump drugs (such as some anti-cancer drugs) and other xenobiotics out of the cytoplasm of cells.

MDR1-MDCK: Madin-Darby Canine Kidney cells transfected with the human multi-drug resistance gene. Confluent monolayers made from these cells can be used to assess a test compound's potential as a P-gp substrate.

MRP2: Multidrug resistance-associated protein 2, a member of the MRP family of drug transporters and the product of the human ABCC2 gene. Previously known at CMOAT, MRP2 is expressed in the apical membrane of epithelial and endothelial cells in the liver, kidney, placenta, intestine, blood-brain barrier and many other tissues. It is also expressed in the apical membrane of Caco-2 cells.

OAT: An acronym for organic anion transporting, a family of solute-linked carrier (SLC) drug transporters. The major human OATs are OAT1 and OAT3, which are expressed in the kidney.

Glossary of Terms

OATP: An acronym for organic anion transporting polypeptide, a family of solute-linked carrier (SLC) drug transporters. The major human OATPs are OATP1B1, OATP1B3, and OATP2B1. OATP1B1 and OATP1B3 are expressed in the sinusoidal (basolateral) membrane of hepatocytes in the liver. A number of clinically significant, even fatal, increases in exposure to drugs have been reported involving the hepatic OATPs due to inhibition by co-administered drugs or pharmacogenetics (reduced expression or expression of a less active variant). As a result, drug developers and drug regulatory agencies now pay close attention to new drugs that are substrates of OATP1B1 and/or OATP1B3.

OCT: An acronym for organic cation transporter, a family of solute-linked carrier (SLC) drug transporters including OCT1 (product of the human SLC22A1 gene), OCT2 (SLC22A2) and OCT3 (SLC22A3). Expression of OCT1 is limited to the liver, OCT2 is expressed primarily in kidney (also brain, lungs, testes, etc.), and OCT3 is expressed in many tissues. The involvement of OCT2 in renal drug clearance and the fact that it has been implicated in more than one clinical drug-drug interaction has drawn the attention of drug developers and global drug regulatory authorities.

P-gp: P-gp is short for P-glycoprotein, a 170-kDa integral membrane transport protein present in a variety of types of mammalian cells. P-gp was first identified as a primary cause of multidrug resistance in tumors. Using ATP hydrolysis as an energy source, it transports its substrates, typically sterols or hydrophobic amines, into the gut, out of the brain, into urine, into bile, out of the gonads, or out of other organs. An understanding of P-gp and related transport proteins is key to designing strategies for the improvement of therapeutic efficacy of drugs that are their substrates and to anticipating their role in mediating drug-drug and drug-diet interactions.

Validated Cell Line: In the context of ADME assays, a validated cell line is a cell line that has been demonstrated to predict the clinical behavior of compounds, e.g., *in vivo* potency, efficacy or bioavailability.

Validation: Method validation is the process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent. It includes accuracy, precision, specificity, sensitivity, reproducibility, stability, predetermined limits for acceptance of data based on QC standards, and extensive documentation.

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