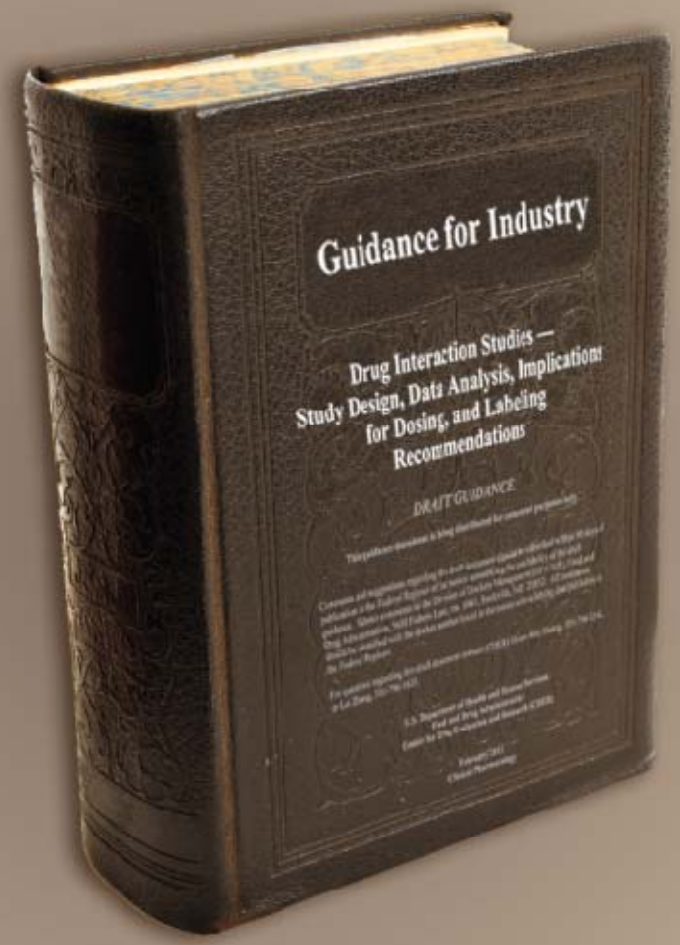

Clarity on Transporters:

Navigating the 2012 FDA Draft Guidance

ABSORPTION® SYSTEMS

PRECLINICAL TESTING FOR DRUGS,
BIOLOGICS AND MEDICAL DEVICES

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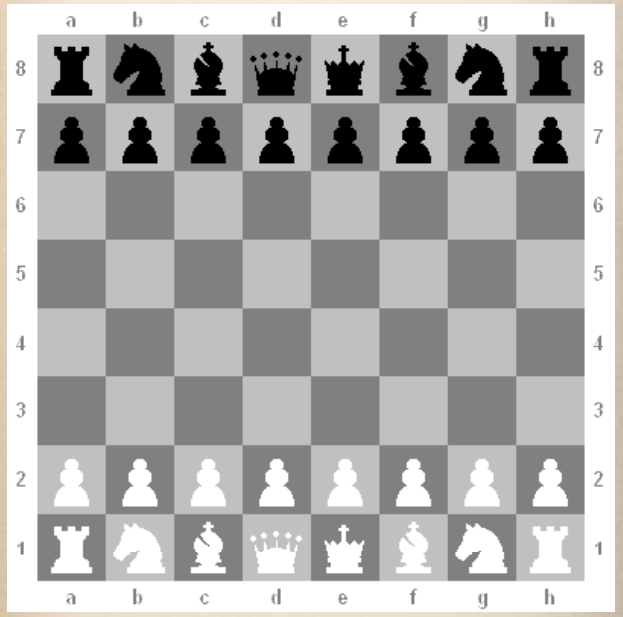
Through the Looking Glass



Preparation

Execution

Keys to Navigating
Transporter Science

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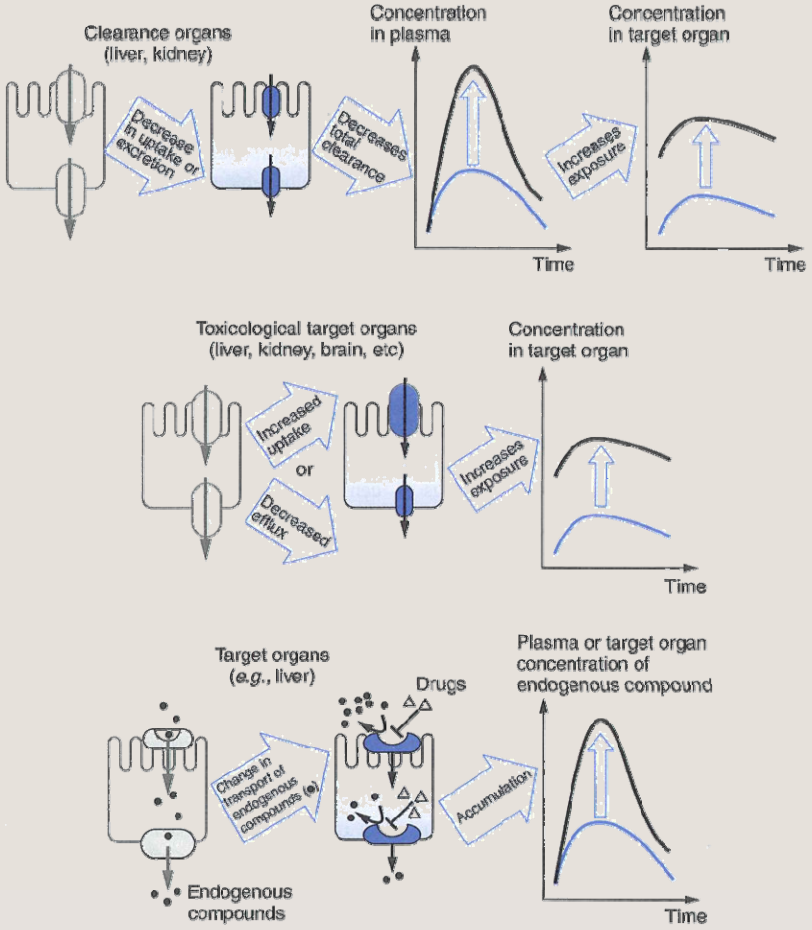
Clinical Significance of Drug Transporters

Why DDIs Occur

- Altered Pharmacokinetics
- Altered Pharmacodynamics



Source: Giacomini, KM and Sugiyama, Y. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 41-70. McGraw-Hill. New York 2006

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P-gp by 2006

The Jabberwocky…

Fearsome

Misunderstood

Can be Harmless

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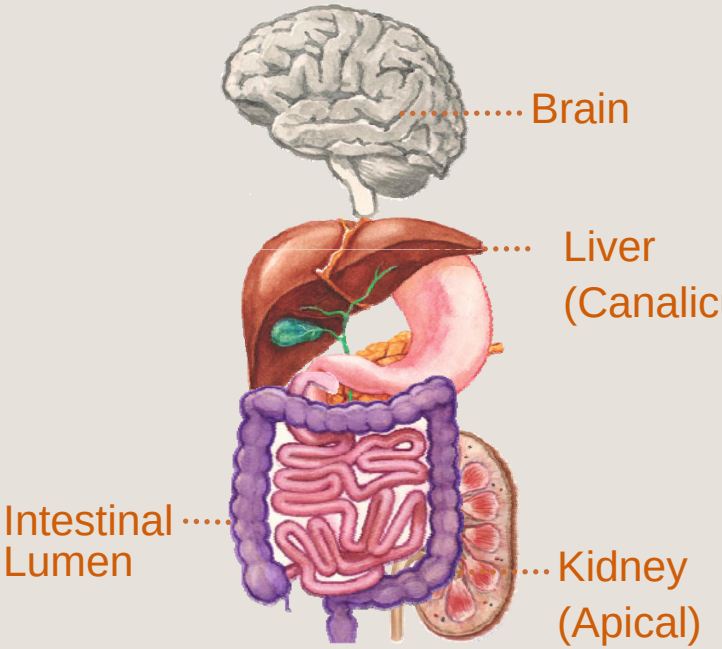
Why P-gp?



- Increased frequency in identification of substrates and inhibitors
- Clinical DDI mediated by P-gp
  - Digoxin and quinidine
  - Fexofenadine and ketoconazole (erythromycin)
  - Colchicine and clarithromycin
- Withdrawals/reapprovals
- Increased PMCs and PMRs

Adapted from Zhang L et al., Mol Pharmaceutics 2006; 3(1):62-69

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Regulatory Response: 2006 Draft Guidance

Lewis Carrol, Through the Looking Glass

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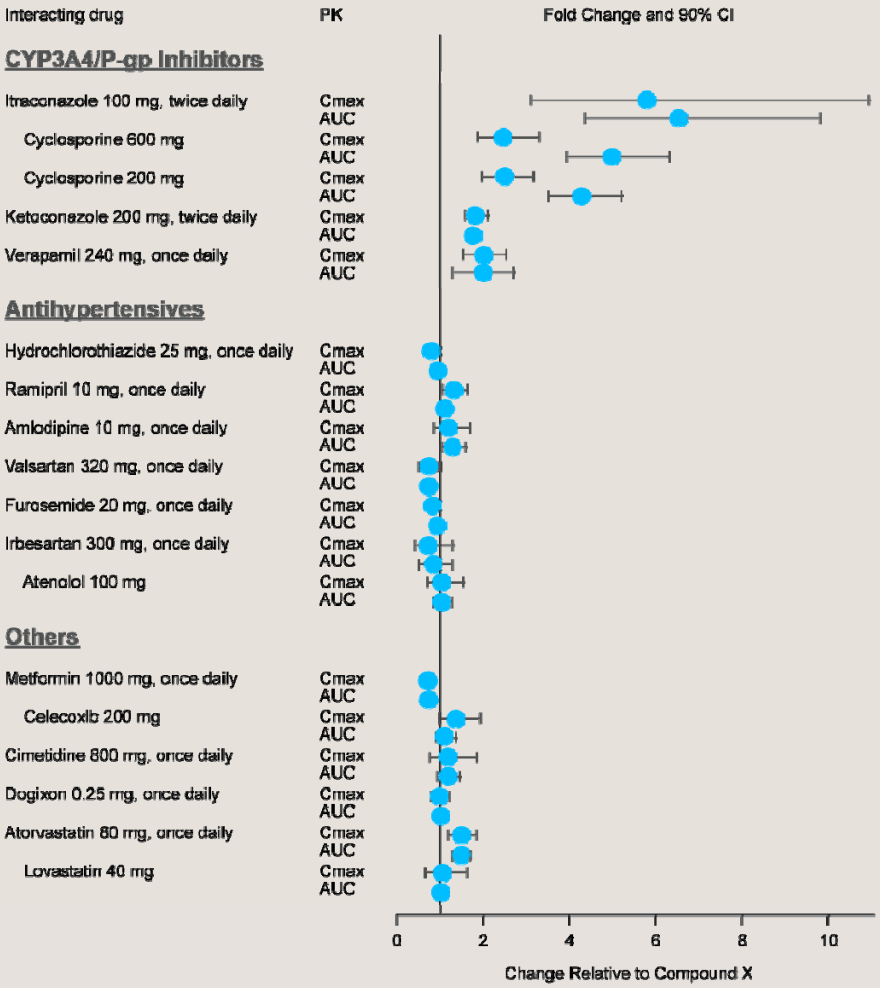
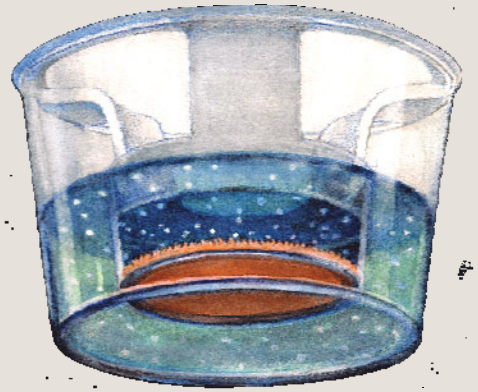
Evolving Outlook: A New Dimension





"In many cases, negative findings from early in vitro and early clinical studies can eliminate the need for later clinical investigations."

FDA Draft Guidance, Sept. 2006 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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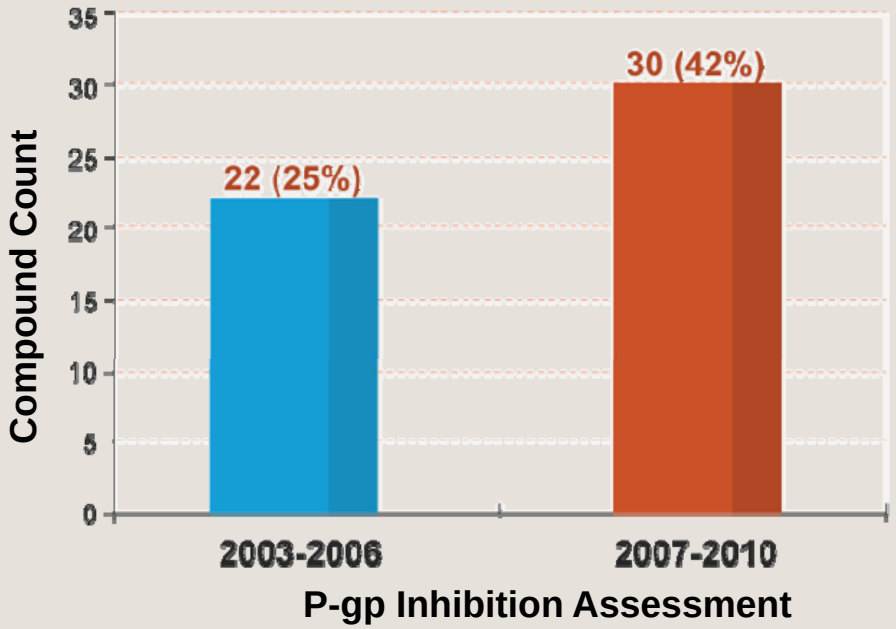
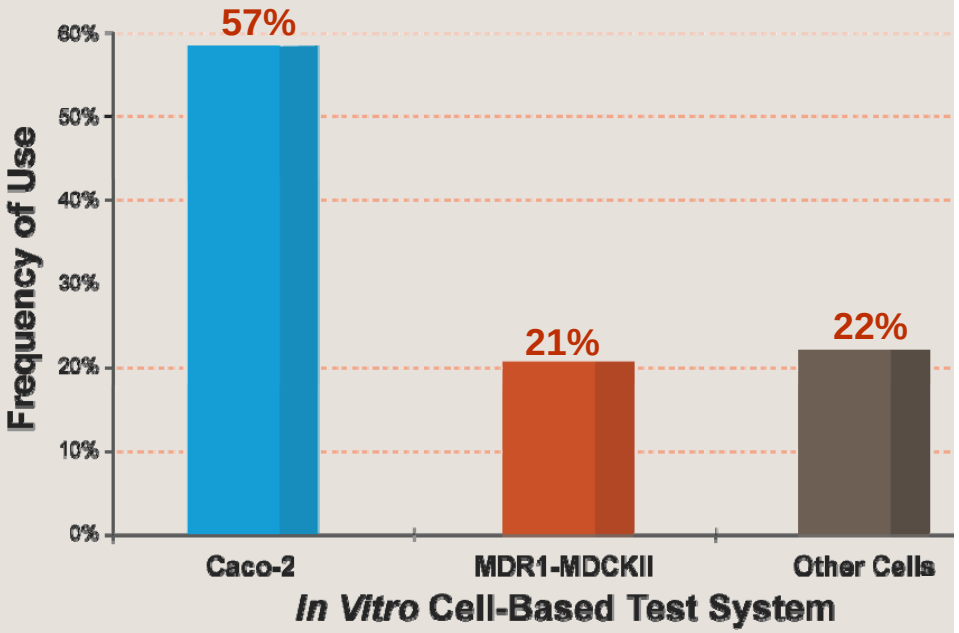
P-gp: 2006-2012

Rising Prevalence of Testing



Frequency of Tools



Agarwal S, et al, J Clin Pharm XX(X) 1-6

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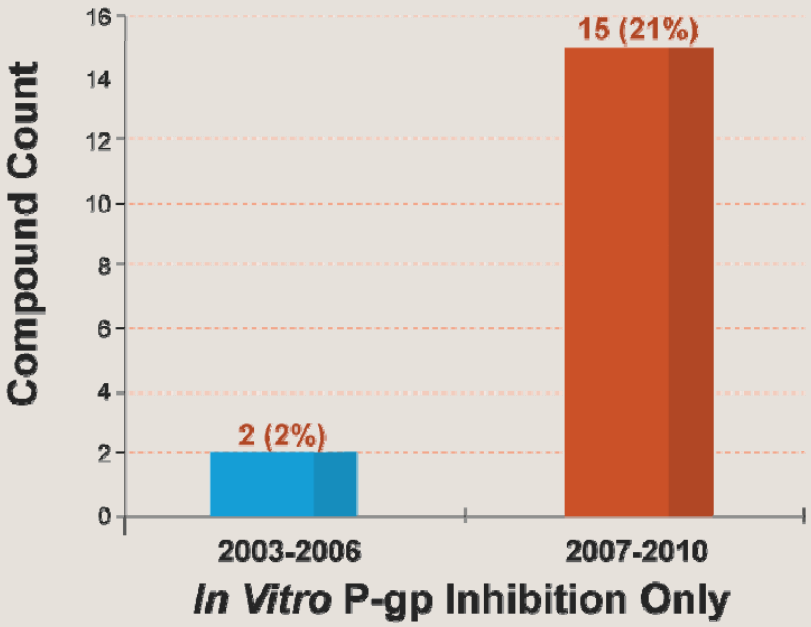
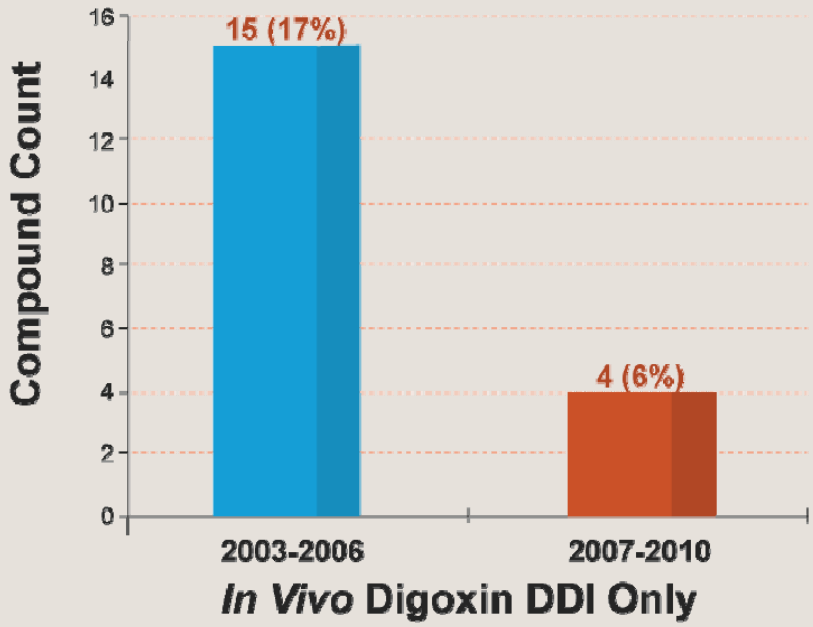
P-gp: 2006-2012





- Reliable Classification
- Potential for Clinical Exceptions

Agarwal S, et al, J Clin Pharm XX(X) 1-6

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In Vitro Choices

Inhibitor
Non-specific Binding
Ko143
CsA
Vectoria
FTC
Assay Format
Solubility
1
Substrate
Basolateral
Time Points
Apical
Digoxin
Uptake
Vesicles
Test Systems
Compound Characteristics
Cladribine
E3S
Caco-2
Knockdown
Rosuvastatin
MDR1-MDCK
Fexofenadine

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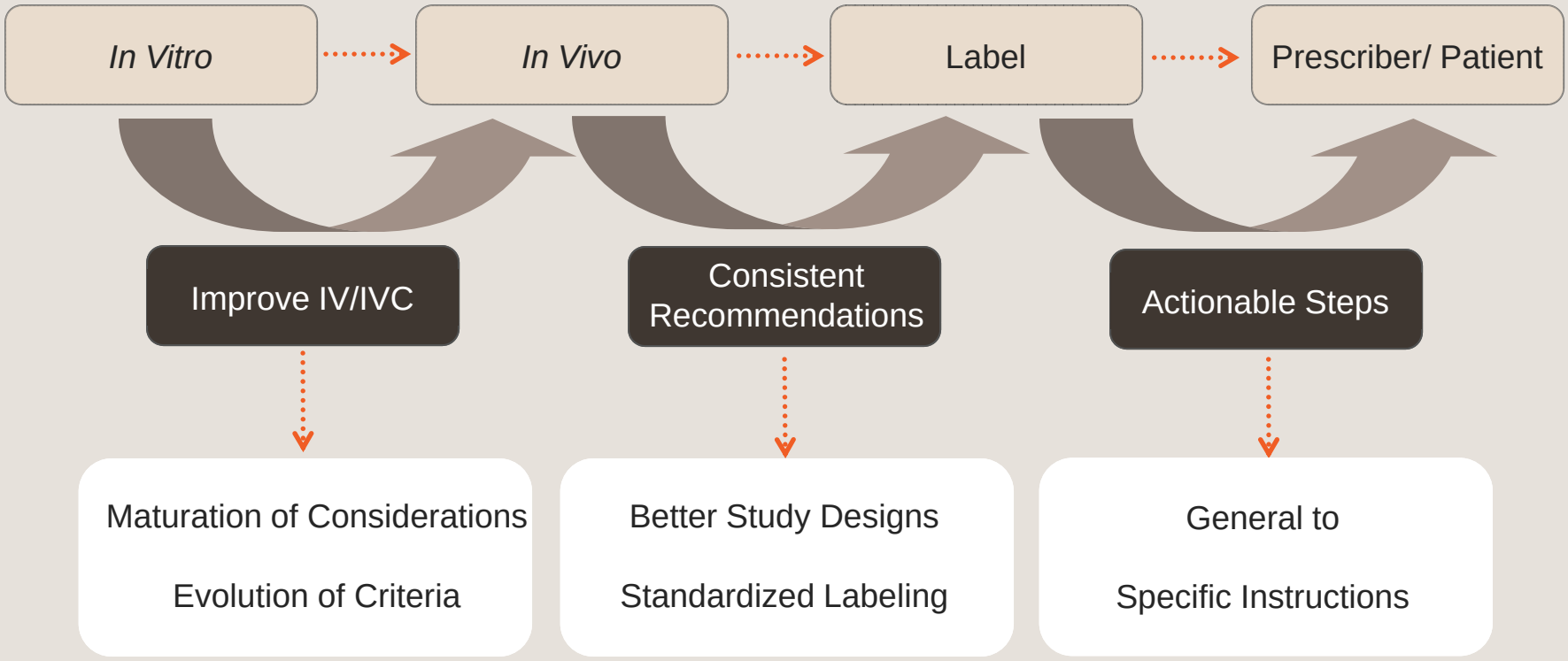
P-gp: 2006 - 2012

- **Improving Translatability**



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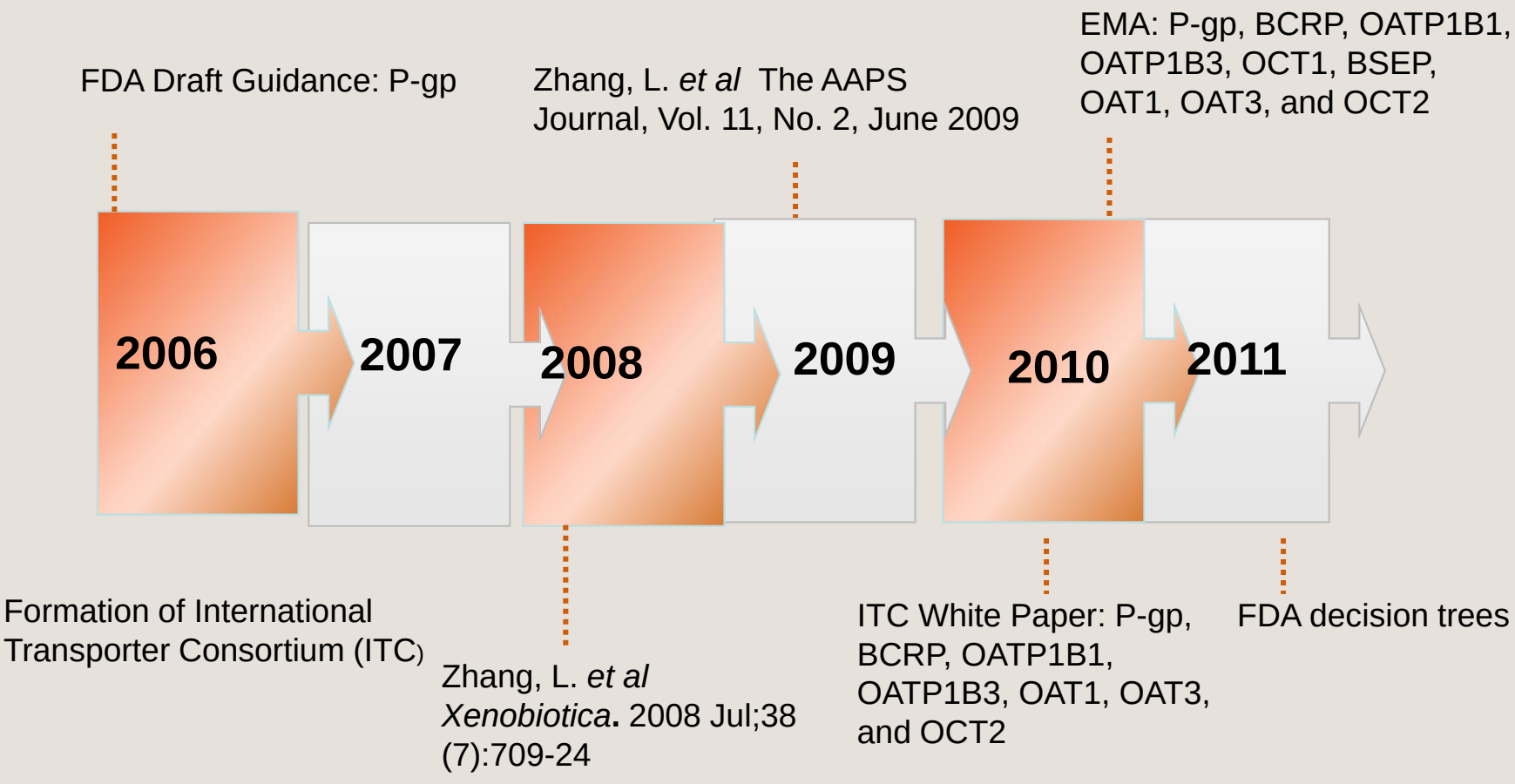
Ongoing Research: 2006 - 2012



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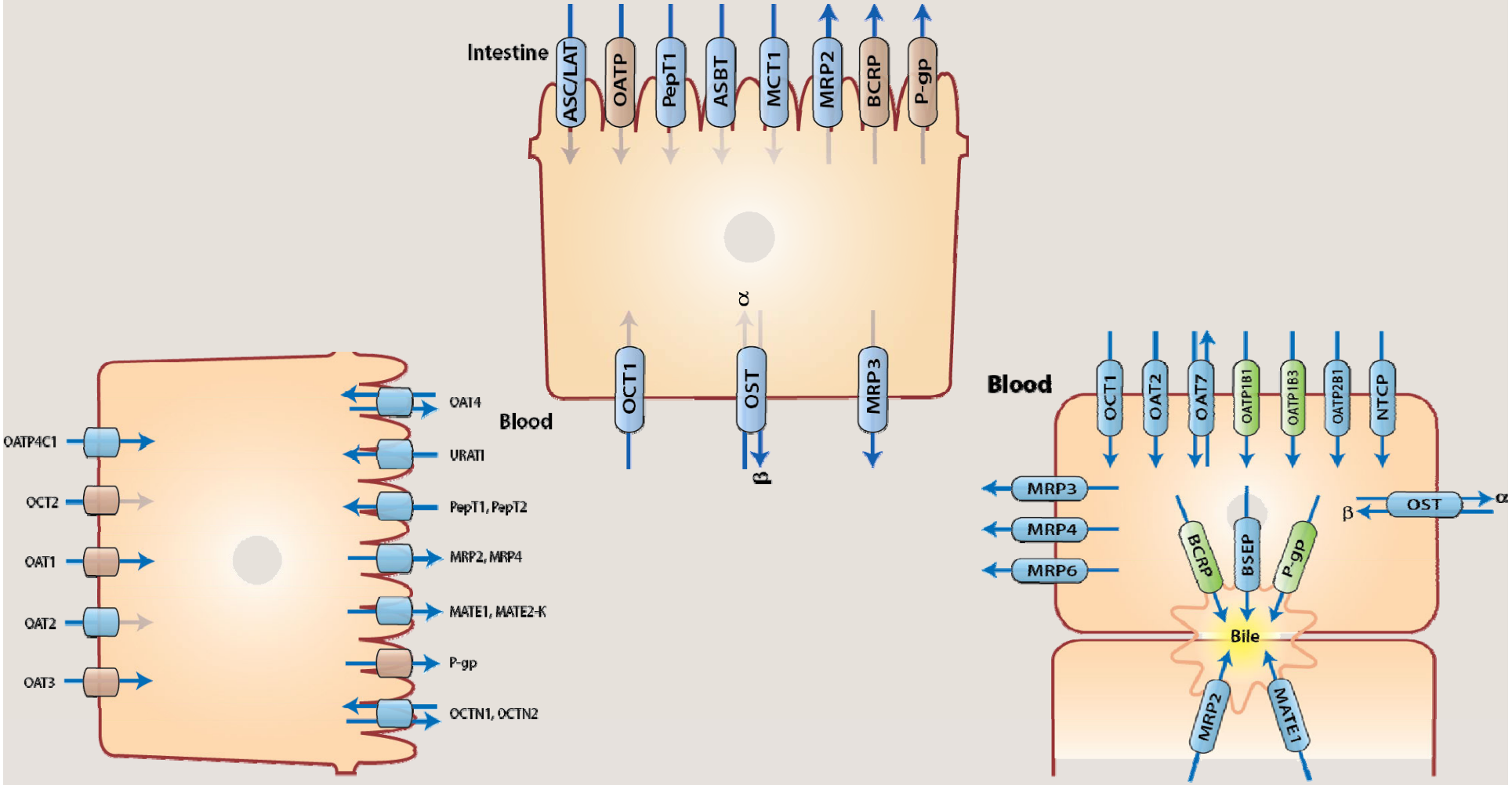
Rising Prominence: 2006 - 2012



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Why Hepatic and Renal Transporters?

Hepatic		
Compound	**Clinical Consequence**	**Transporter**
Lopinavir/Ritonavir	5-48 fold increase in Bosentan Exposure	OATP1B1
Cyclosporine	7.1 fold increase in Rosuvastatin Exposure	OATP1B3

Renal		
Compound	**Clinical Consequence**	**Transporter**
Cimetidine	1.5 fold increase in Pindolol Exposure	OCT2
Probenecid	3.6 fold increase in Cephradine Exposure	OAT1
Probenecid	2.9 fold increase in Furosemide Exposure	OAT3

FDA Draft Guidance, Feb. 2012 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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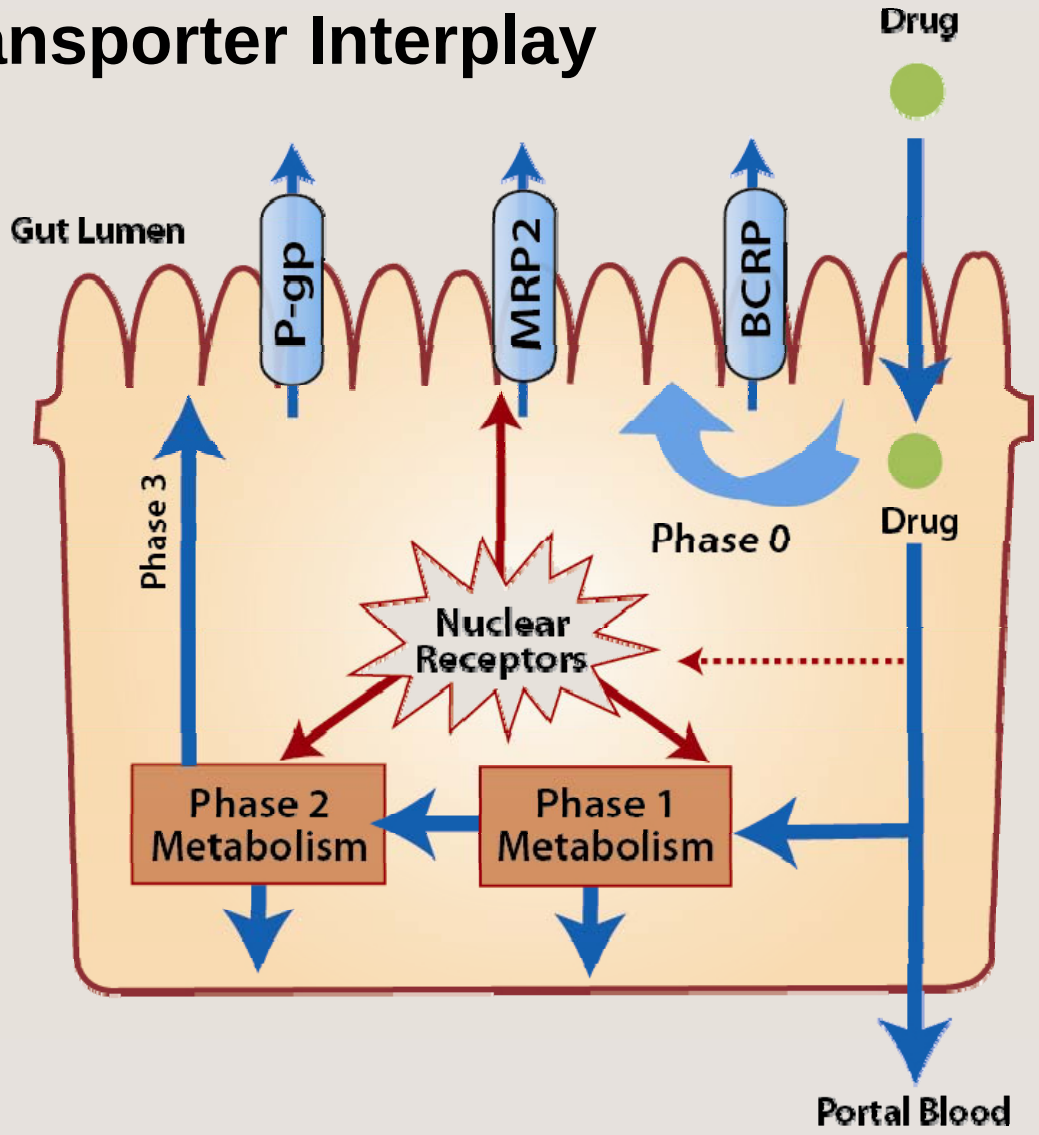
Enzyme-Transporter Interplay



Adapted from Gut 2003; 52: 1788-95

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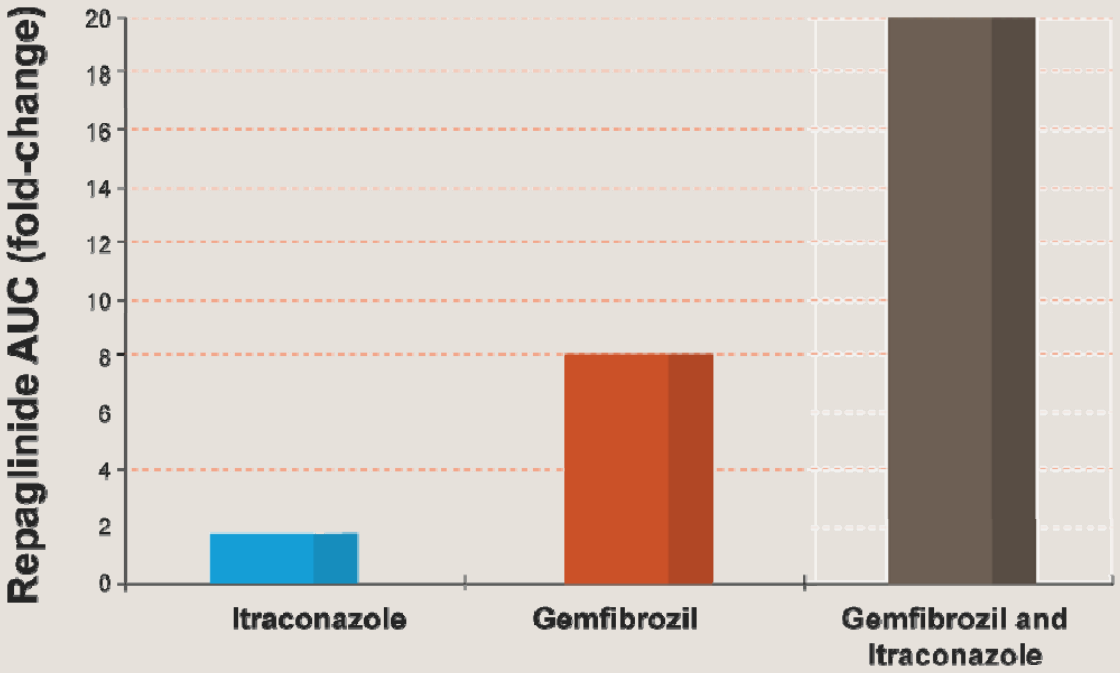
Repaglinide: What We Knew



Drug-Drug Interactions (2003)

"In vitro data indicate that repaglinide metabolism may be inhibited by antifungal agents like ketoconazole and miconazole, and antibacterial agents like erythromycin (cytochrome P-450 enzyme system 3A4 inhibitors). **Drugs that induce the cytochrome P450 enzyme system 3A4 may increase repaglinide metabolism;** such drugs include rifampin, barbiturates, and carbamezapine."

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Niemi al, Diabetologia. 2003 Mar;46(3): M et 347-51

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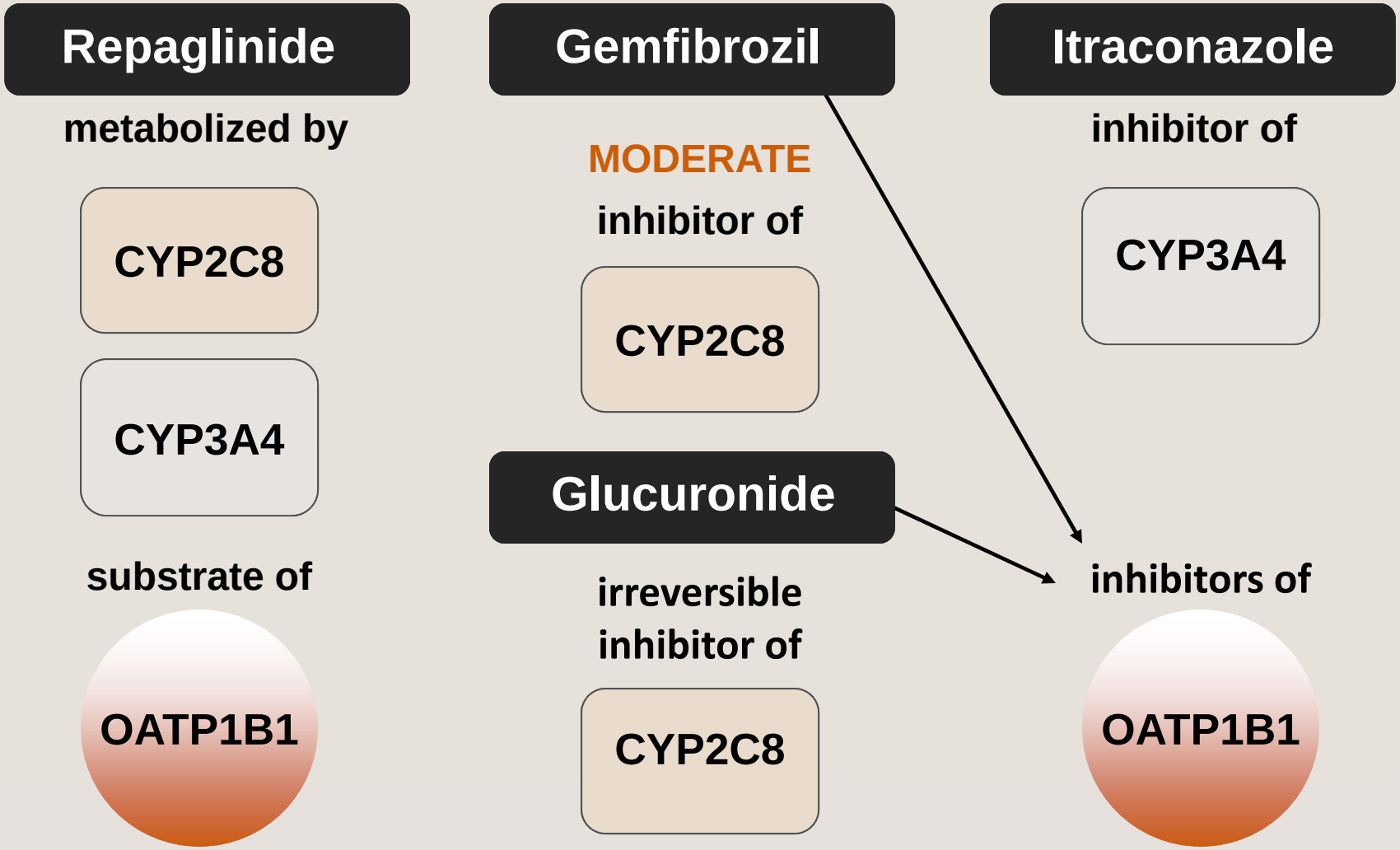
Complex DDI: CYPs and Transporters



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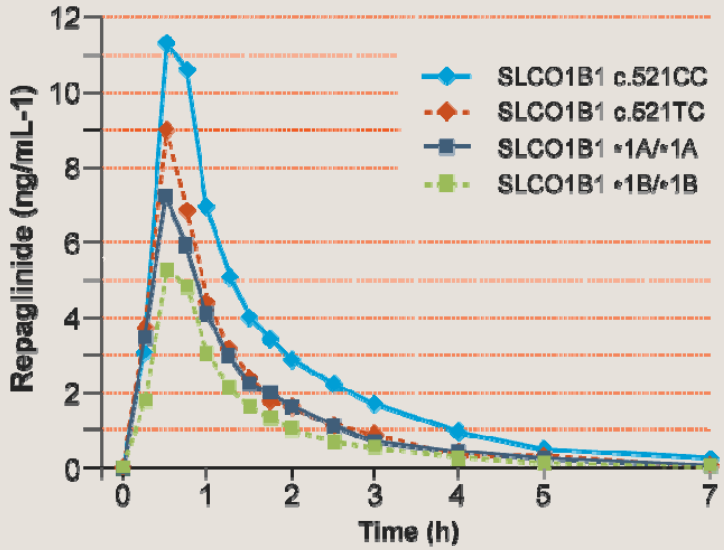
Repaglinide: What We Now Know

Comparative PK with Various Genotypes



- Concurrent inhibition of enzyme and transporter
- Increased inhibition of drug elimination by use of inhibitors of more than one enzyme that metabolizes the drug.
- Metabolite-mediated DDI

Drug-Drug Interactions (June 2009)

Repaglinide appears to be a substrate for **active hepatic uptake transporter (organic anion transporting protein OATP1B1). Drugs that inhibit OATP1B1** (e.g. cyclosporine) **may likewise have the potential** to increase plasma concentrations of repaglinide.

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A Kalliokoski and M Niemi, BJP, 2009

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Parallel Drug Transporter Work: 2006 - 2012

Emerging 7 Transporters

- P-gp
- BCRP
- OATP1B1
- OATP1B3
- OAT1
- OAT3
- OCT2

Complex DDI:

- Concurrent inhibition and induction
- Parallel inhibition of transporter and metabolism pathways
- Increased inhibition with co-dosed inhibitors
- Metabolite-mediated DDI
- Inhibition of non-polymorphic enzymes in poor metabolizers
- Inhibition in subjects with organ impairment

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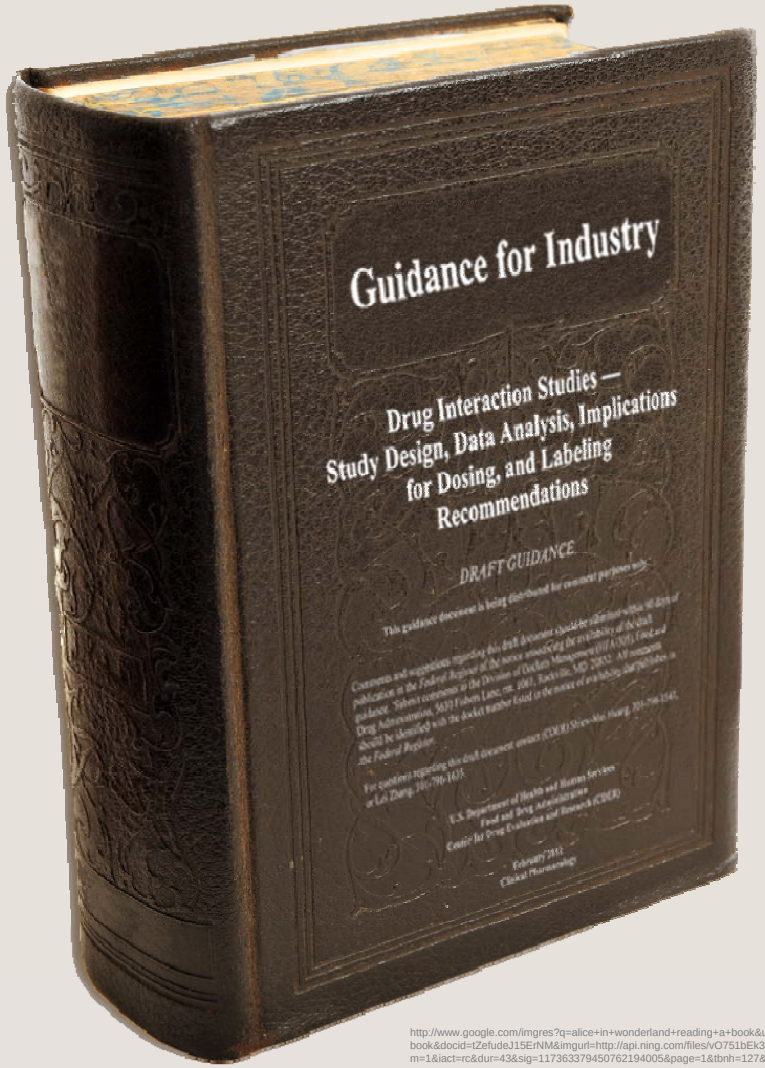
Drug Transporters: circa 2012



"It seems very pretty, but is hard to understand."

-Alice, Through the Looking Glass, Lewis Carroll

http://www.google.com/imgres?q=alice+in+wonderland+reading+a+book&um=1&hl=en&qscrl=1&nord=1&rlz=1T4ADRA_enUS463US465&biw=1051&bih=601&tbm=isch&tbnid=3Wy3RdxpD424zM:&imgrefurl=http://www.mymodernmet.com/profiles/blogs/find-your-book&docid=tZefudeJ15ErNM&imgurl=http://api.ning.com/files/vO751bEk3JxlxOH2lS4sjdp1neGMYAVYXNAf7mA13JCKH6u1zUG3XfvJcUlDdPhaO*Dh7bEbowzsWJirjL1Vu9Ne3dmNj0qF/GandhiBookstoresalice.jpg&w=721&h=481&ei=FhKZT9HBEMjf0QGhrKSDCg&zoom=1&iact=rc&dur=43&sig=117363379450762194005&page=1&tbnh=127&tbnw=181&start=0&ndsp=18&ved=1t:429,r:0,s:0,i:67&tx=54&ty=44

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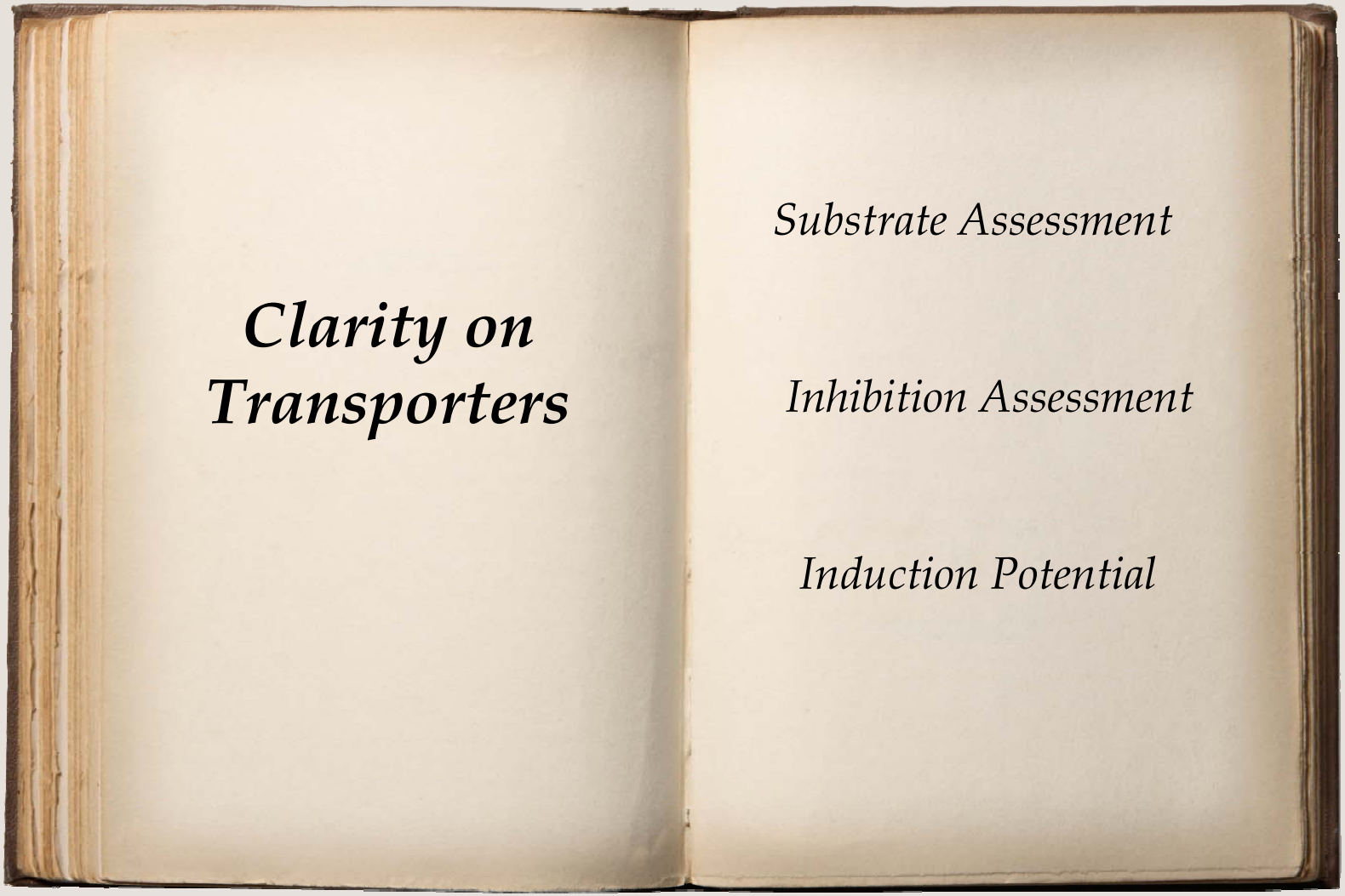

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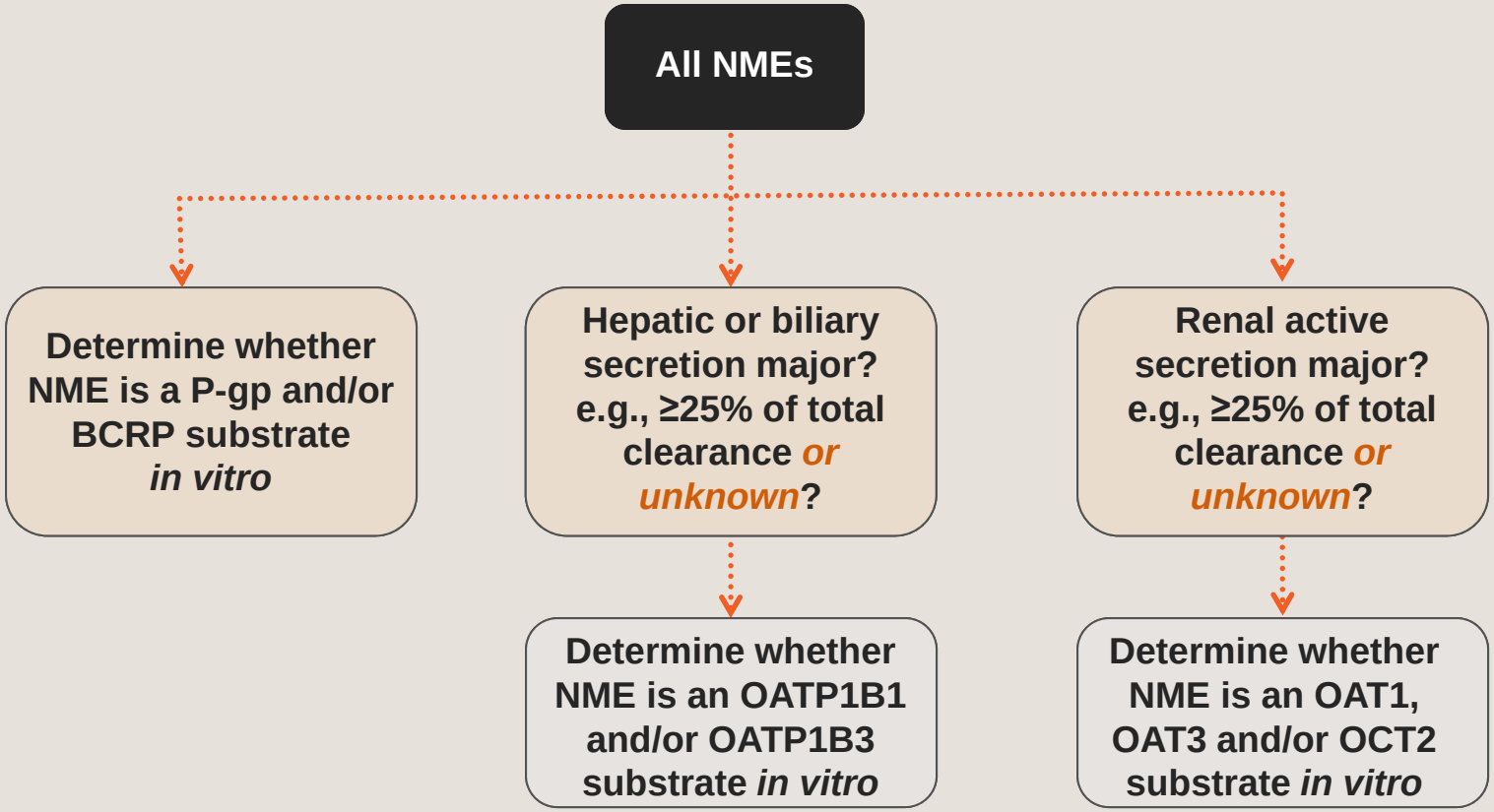
Evaluation of Substrate Potential



Other transporters (e.g. MRP2) may need to be studied based on knowledge of other drugs in the same therapeutic class.

FDA Draft Guidance, Feb. 2012 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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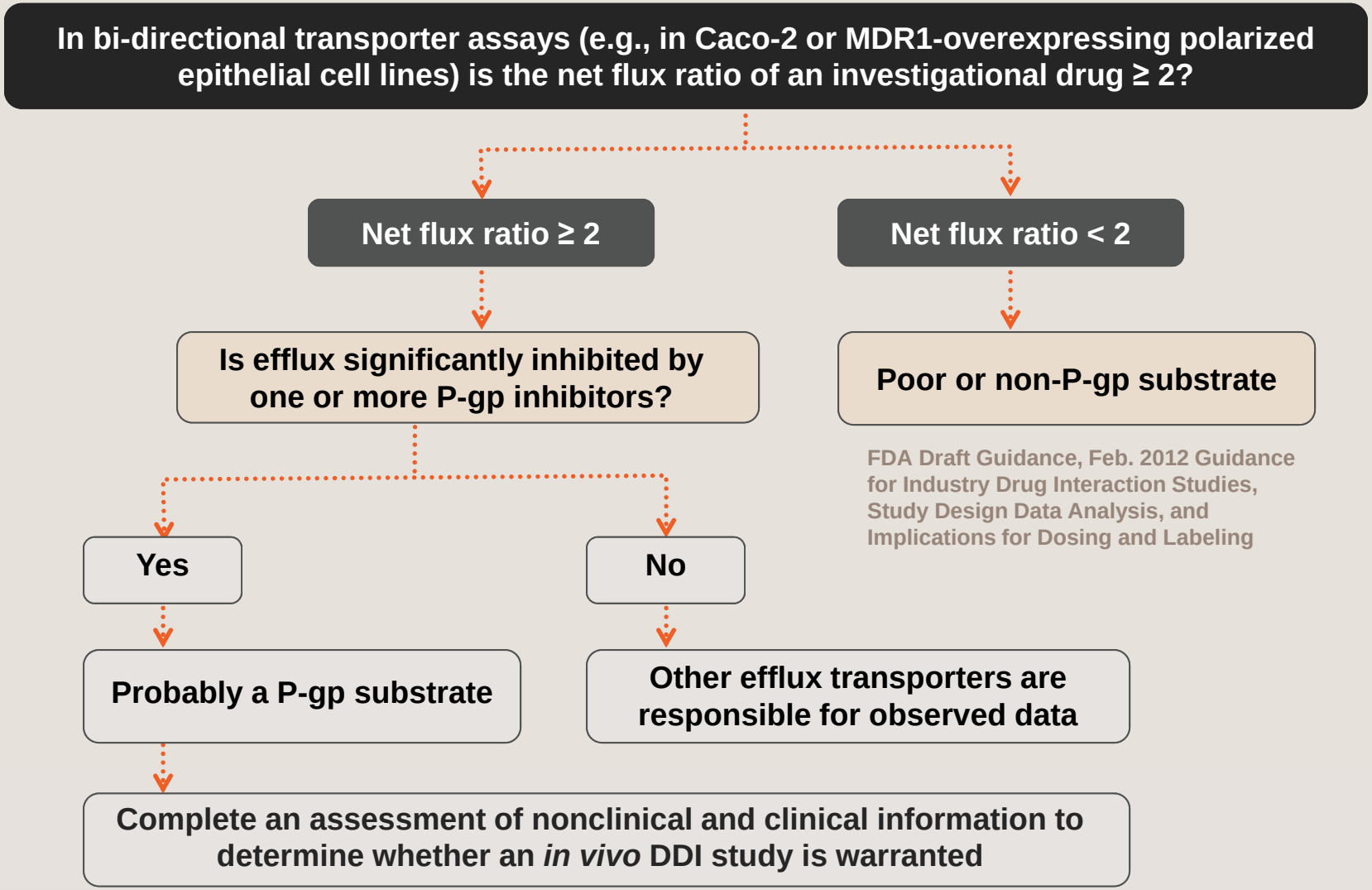
P-gp and BCRP Substrate



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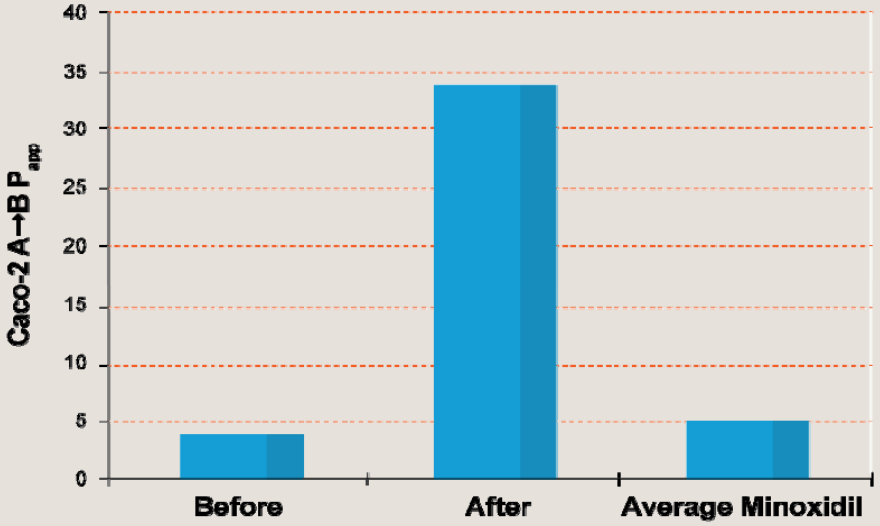
Considerations: Test Compound Characteristics

Non-Specific Binding

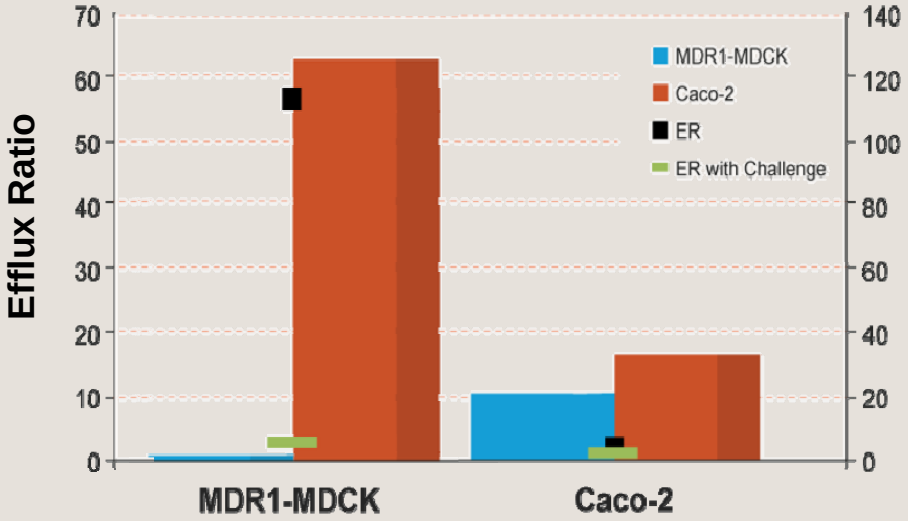
Compound A



- 1% BSA
- Pre-incubation

Intrinsic Permeability

Compound B



- Favorable drug-specific property
- Potentially enables biowaiver

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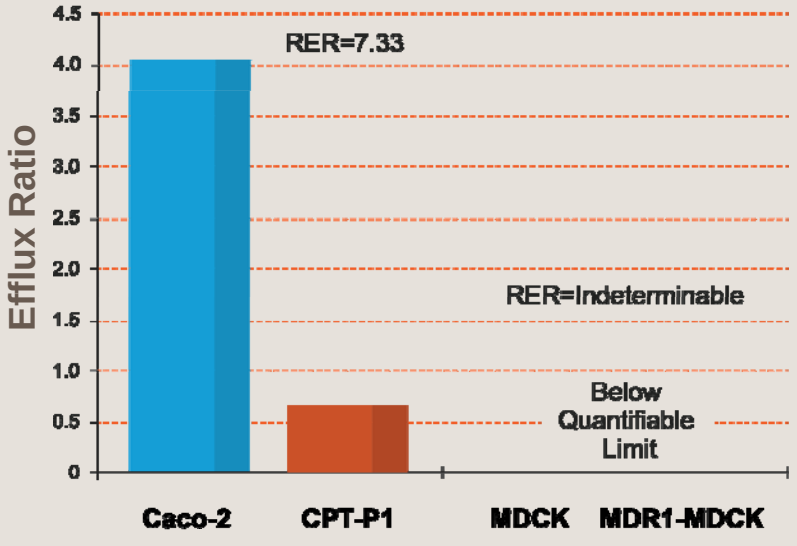
Considerations: Test System Choices

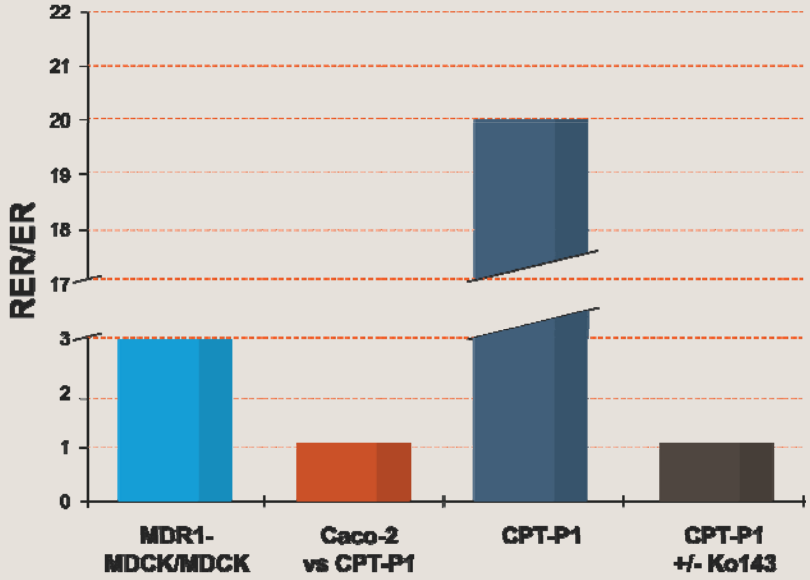
Relevant Organ Phenotype

Fexofenadine



- Multiplicity of transporters
- Clinical P-gp probe substrate

Cladribine



- Clinically relevant interaction with BCRP

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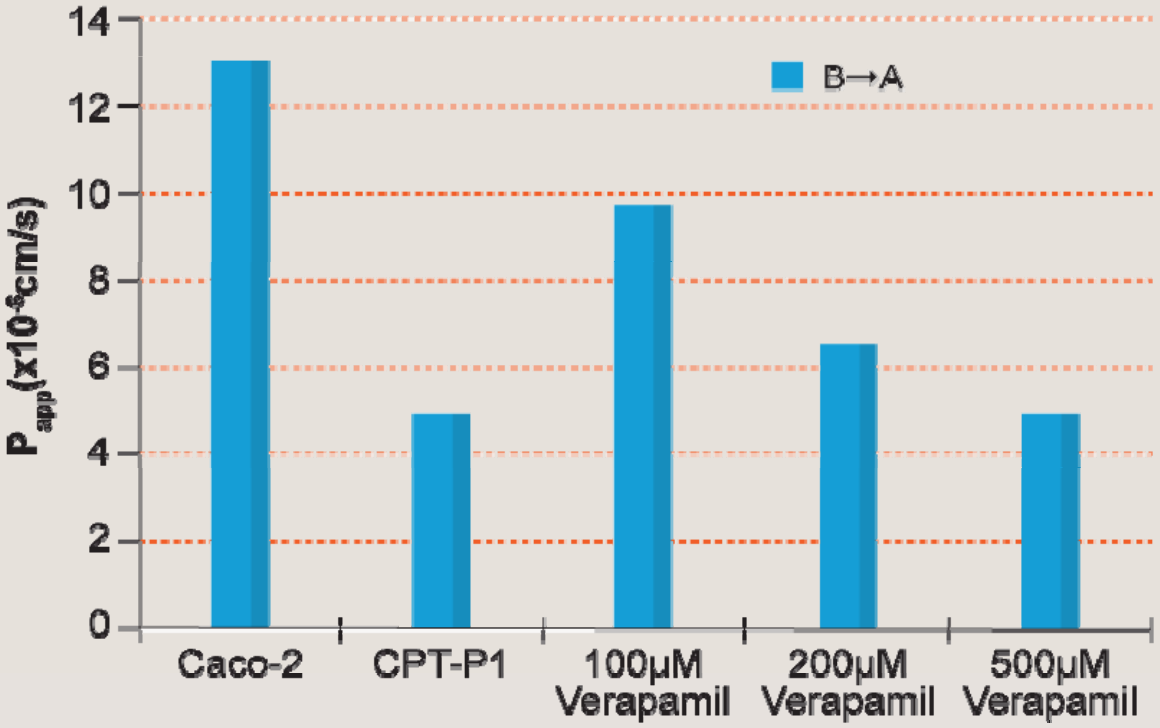
Considerations: Design Elements

Reagents vs. Knockdown

Rosuvastatin



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Clinical Waiver of Transporter Studies

FDA Perspective

*"For drugs that are **highly permeable** and **highly soluble**, the intestinal absorption is **not a rate-limiting step**, and, therefore it may be appropriate to **exempt** such drugs from the **in vivo evaluation** with a P-gp or BCRP inhibitor."*

$$J_e = J_c + J_m$$

$$\downarrow \quad \text{when } J_m >> J_c$$

$$J_e = J_m$$

J_e : effective flux
J_c : carrier-mediated flux
J_m : passive flux

FDA Draft Guidance, Feb. 2012 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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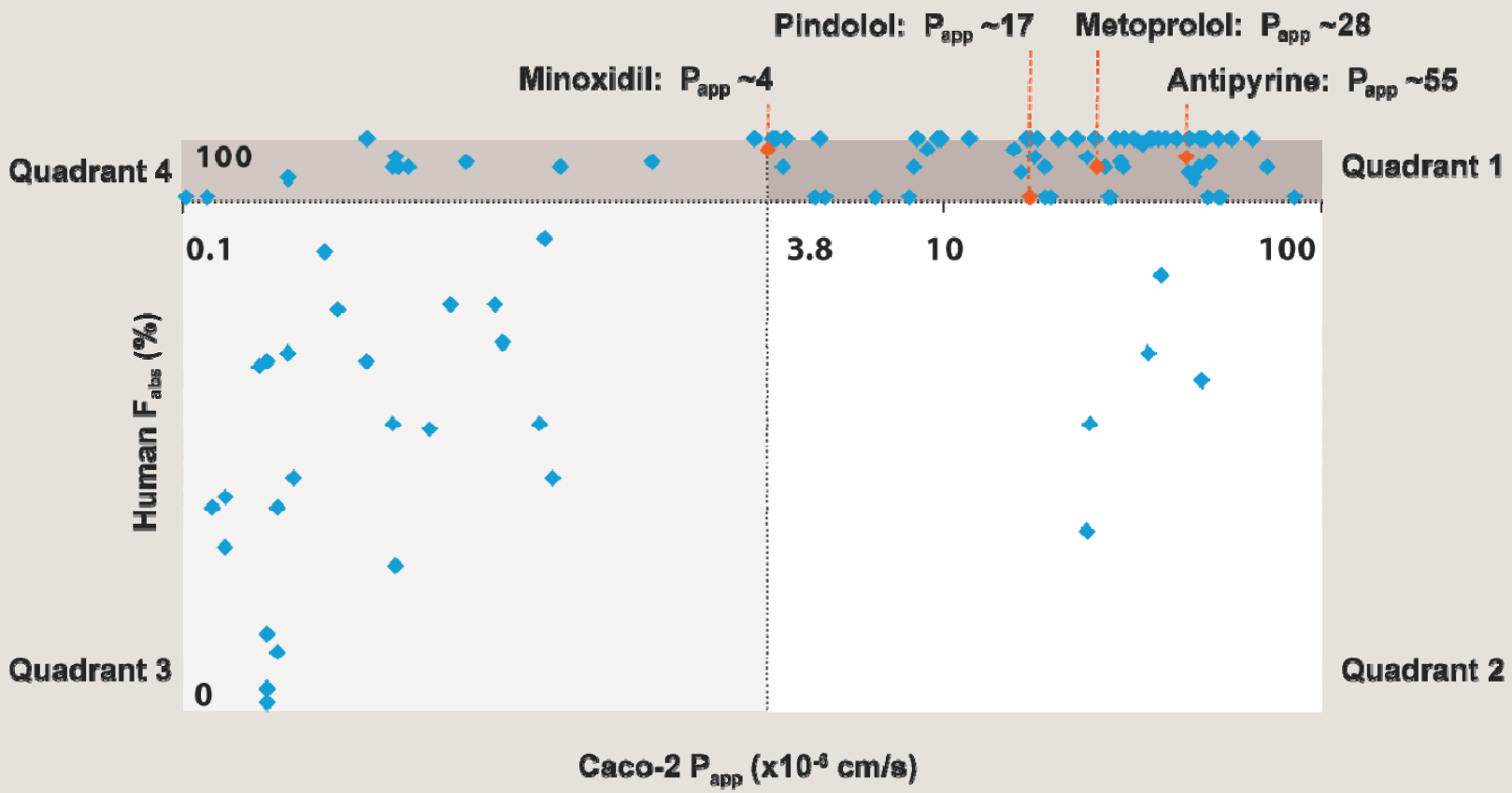
Exceptions: Consider the Rate-Limiting Step



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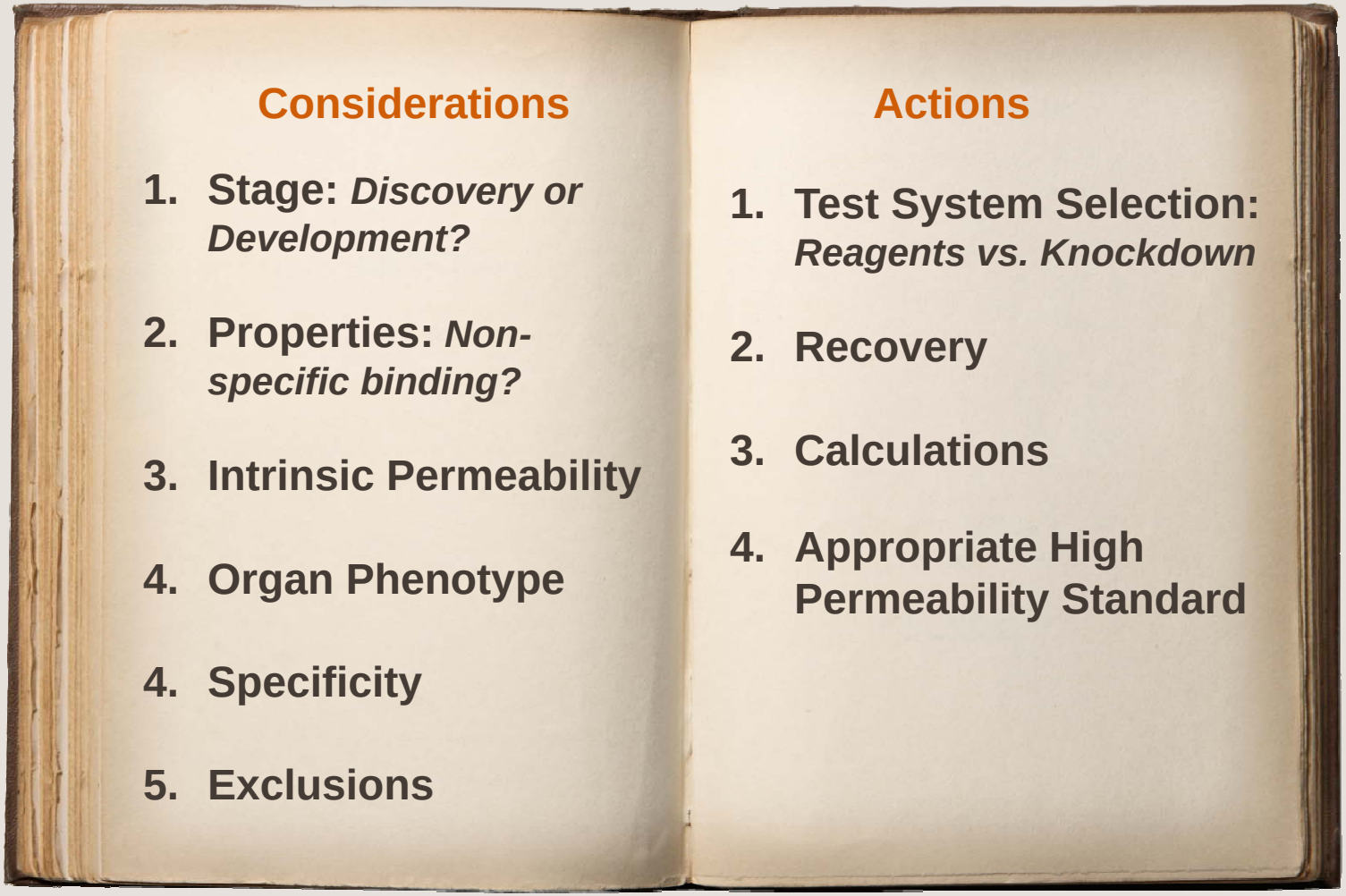
Best Practices



Considerations

1. Stage: *Discovery or Development?*
2. Properties: *Non-specific binding?*
3. Intrinsic Permeability
4. Organ Phenotype
4. Specificity
5. Exclusions

Actions

1. Test System Selection: *Reagents vs. Knockdown*
2. Recovery
3. Calculations
4. Appropriate High Permeability Standard

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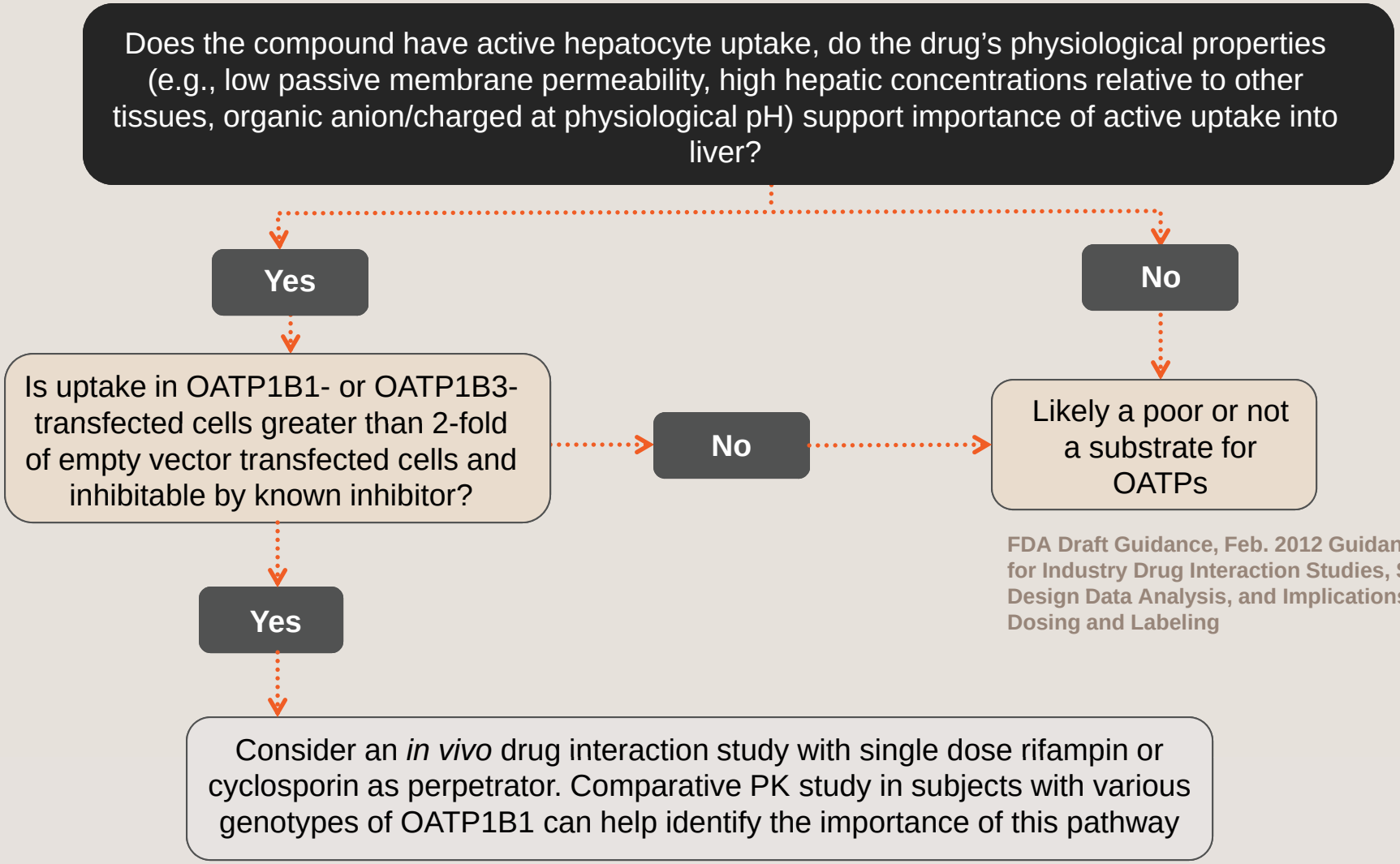
OATP1B1 and OATP1B3 Substrate



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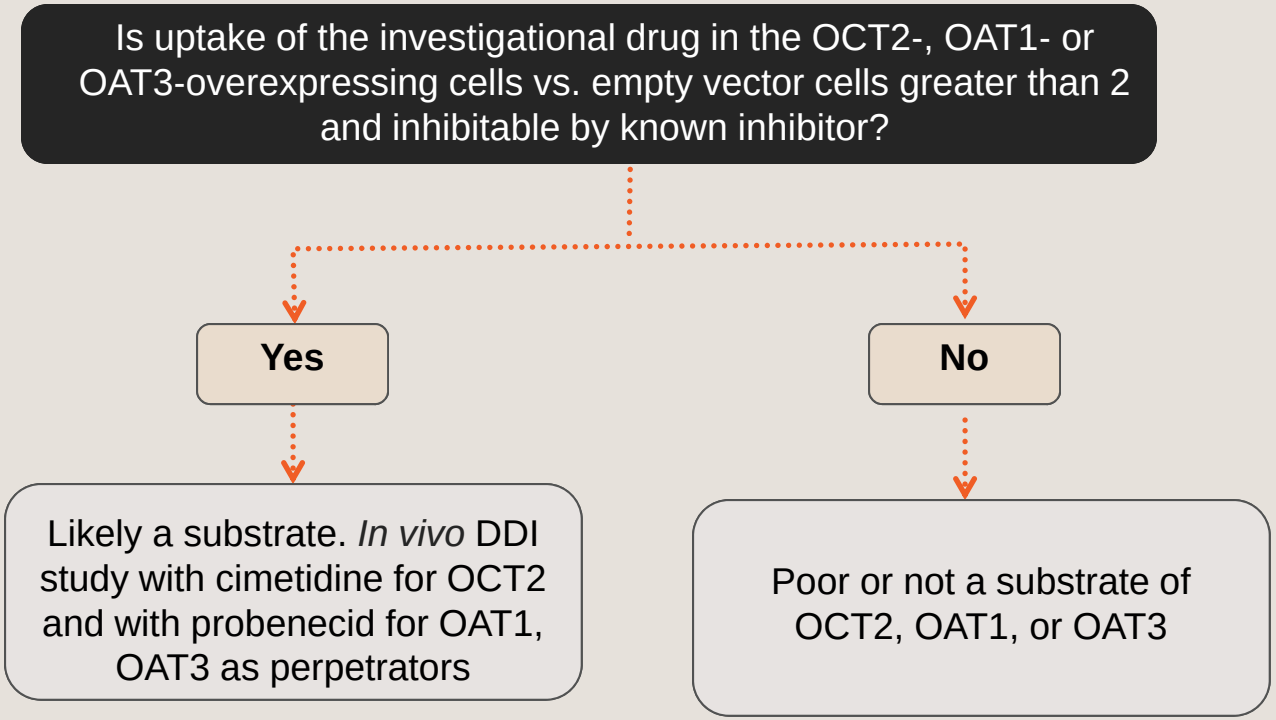
OAT1, OAT3, and OCT2 substrate



FDA Draft Guidance, Feb. 2012 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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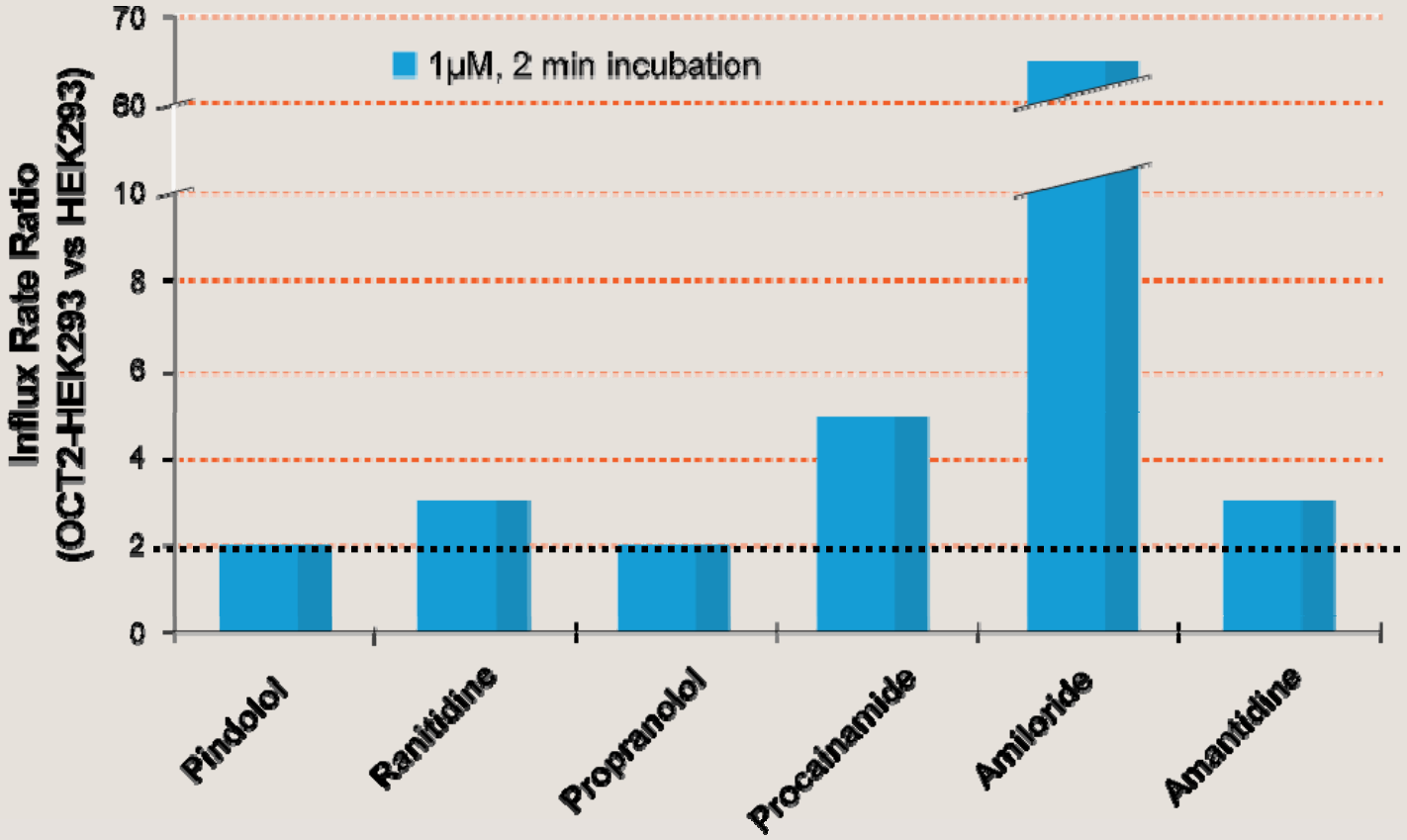
Considerations: Pre-Defined Conditions



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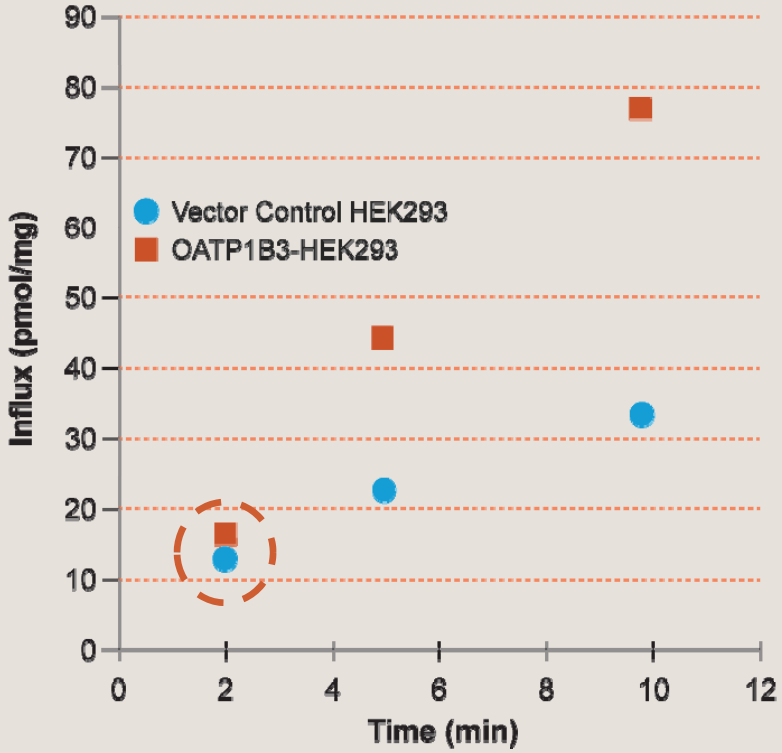
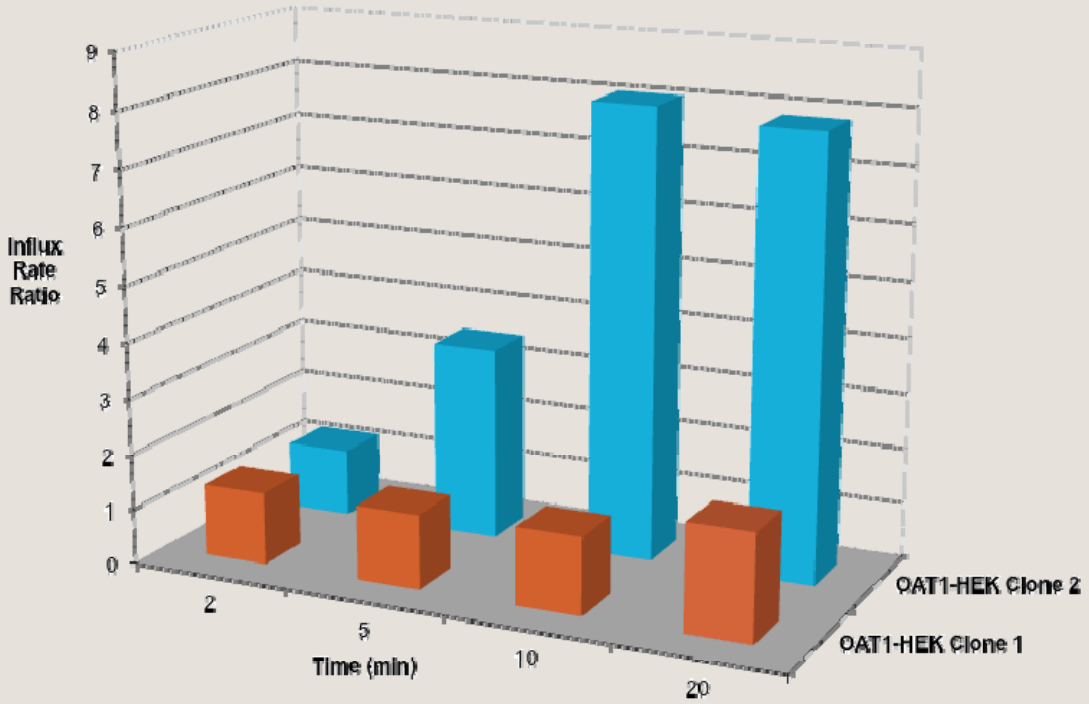
Considerations: False Negatives





- Non-specific binding
- Transporter properties
- Robustness of transfection

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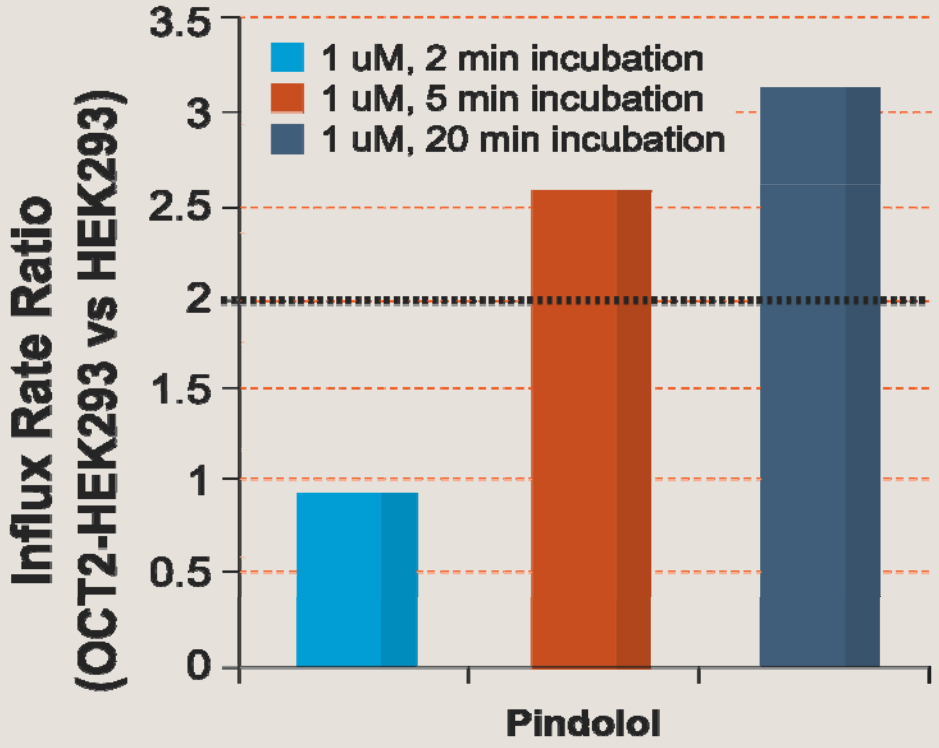
Considerations: Matrix Evaluation



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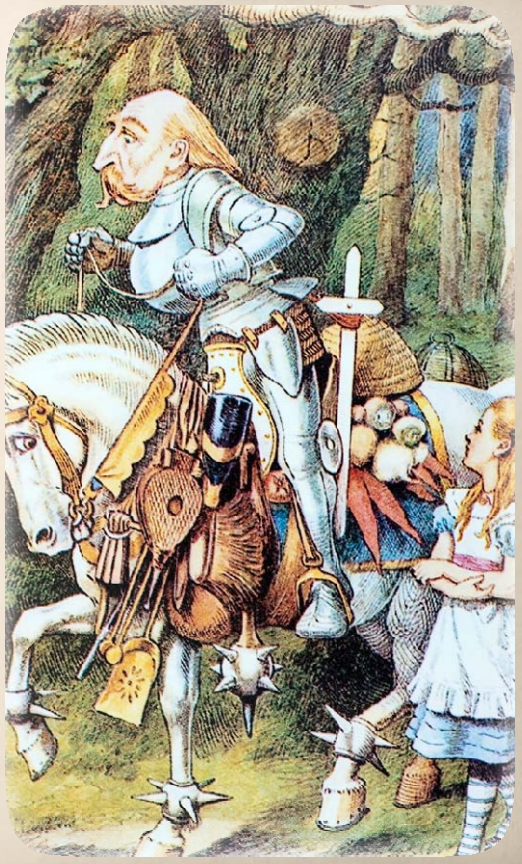
Test System Considerations

*`I see you're admiring my little box.' the Knight said in a friendly tone. `**It's my own invention** -- to keep clothes and sandwiches in. You see I carry it upside-down, so that the rain can't get in.'*

*`But the things can get out,' Alice gently remarked. `**Do you know the lid's open**?'*

-Through the Looking Glass, Lewis Carroll



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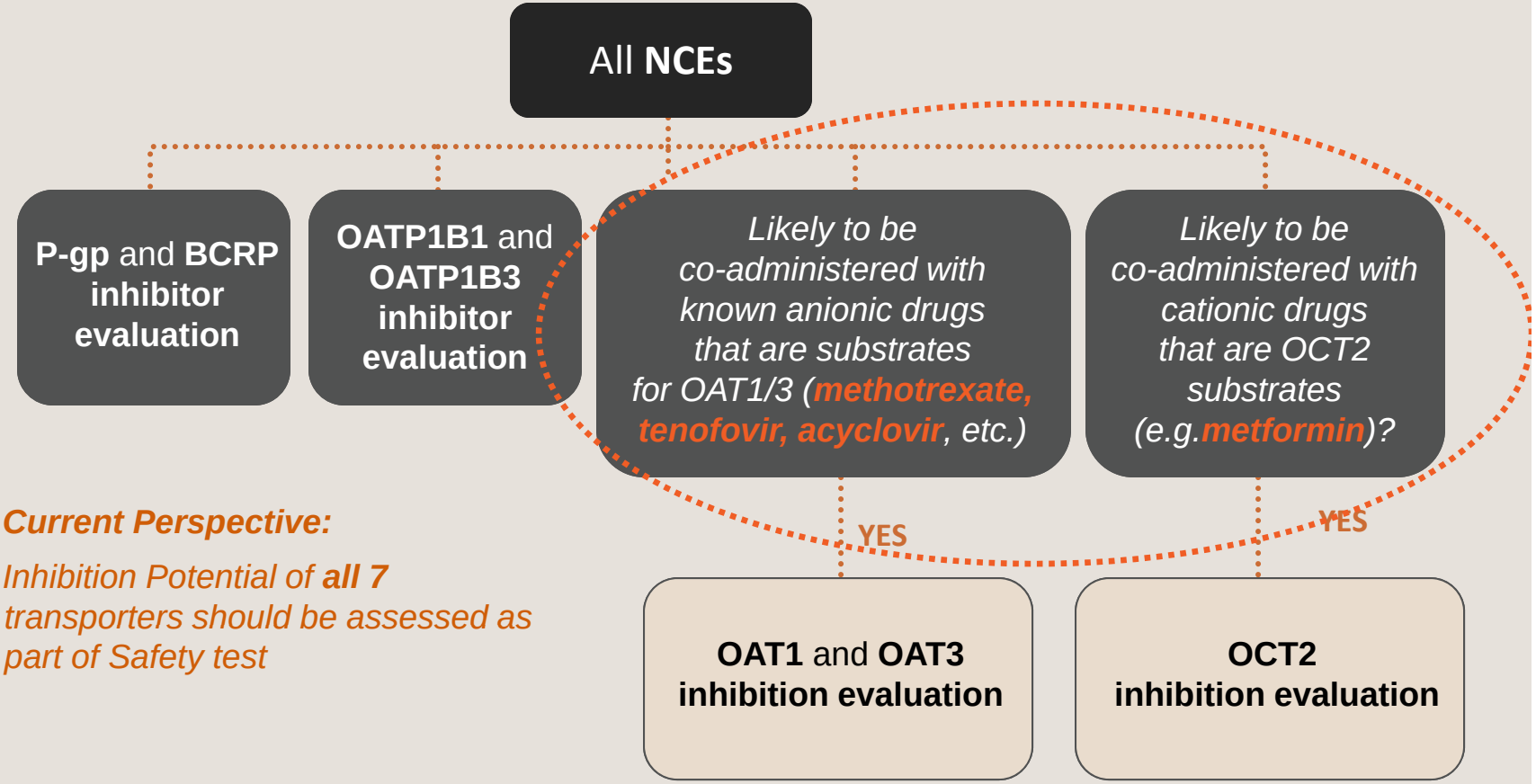
Evaluation of Inhibition Potential



Current Perspective:

*Inhibition Potential of **all 7** transporters should be assessed as part of Safety test*

- FDA Clinical Pharmacology Advisory Committee Meeting, March 17, 2010
- The International Transporter Consortium, *Nature Reviews*, March 2010
- FDA Comment on the Report from the ITC, Lei Zhang, AAPS Webinar, July 29, 2010

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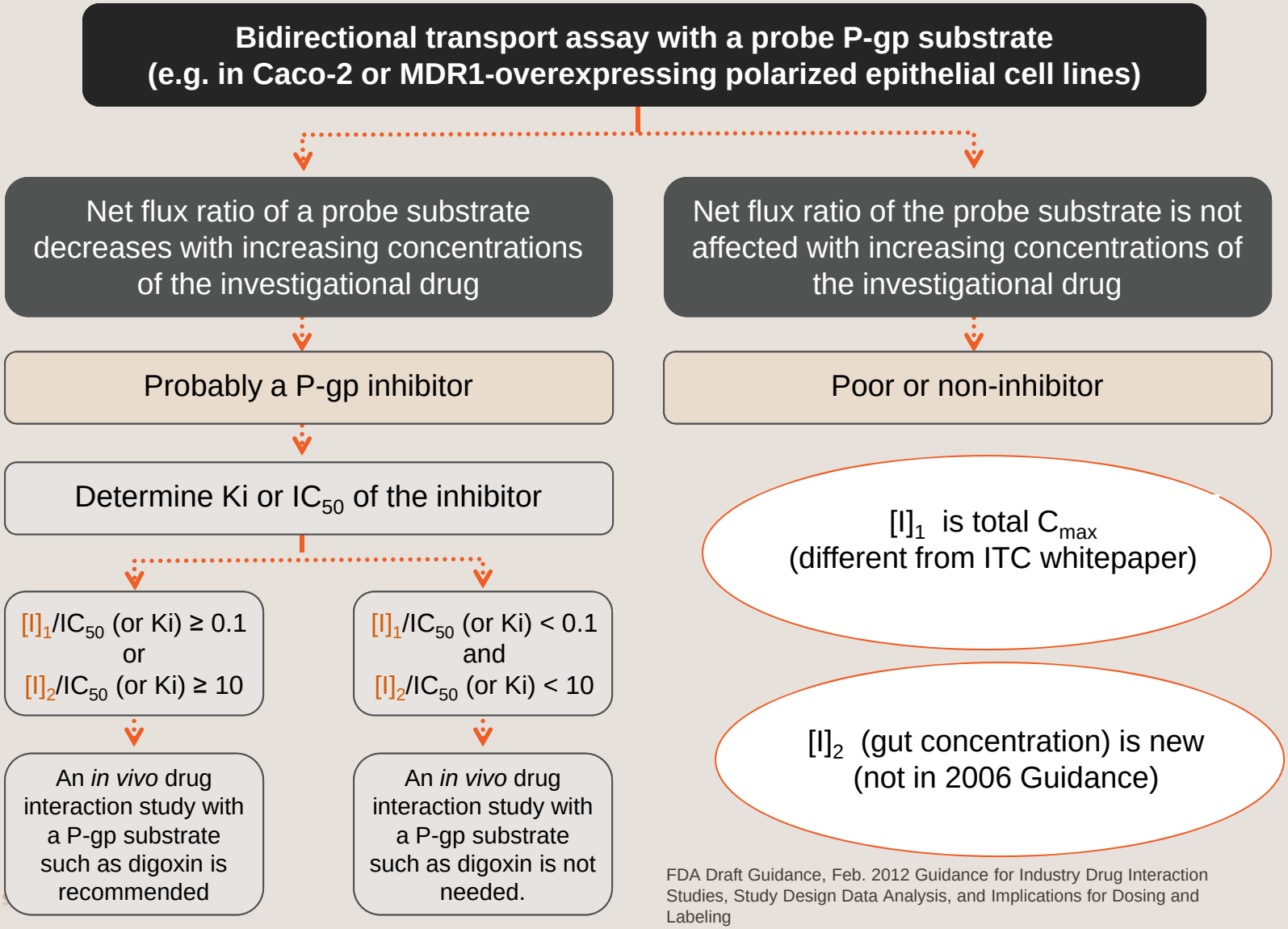
P-gp and BCRP Inhibition



FDA Draft Guidance, Feb. 2012 Guidance for Industry Drug Interaction Studies, Study Design Data Analysis, and Implications for Dosing and Labeling

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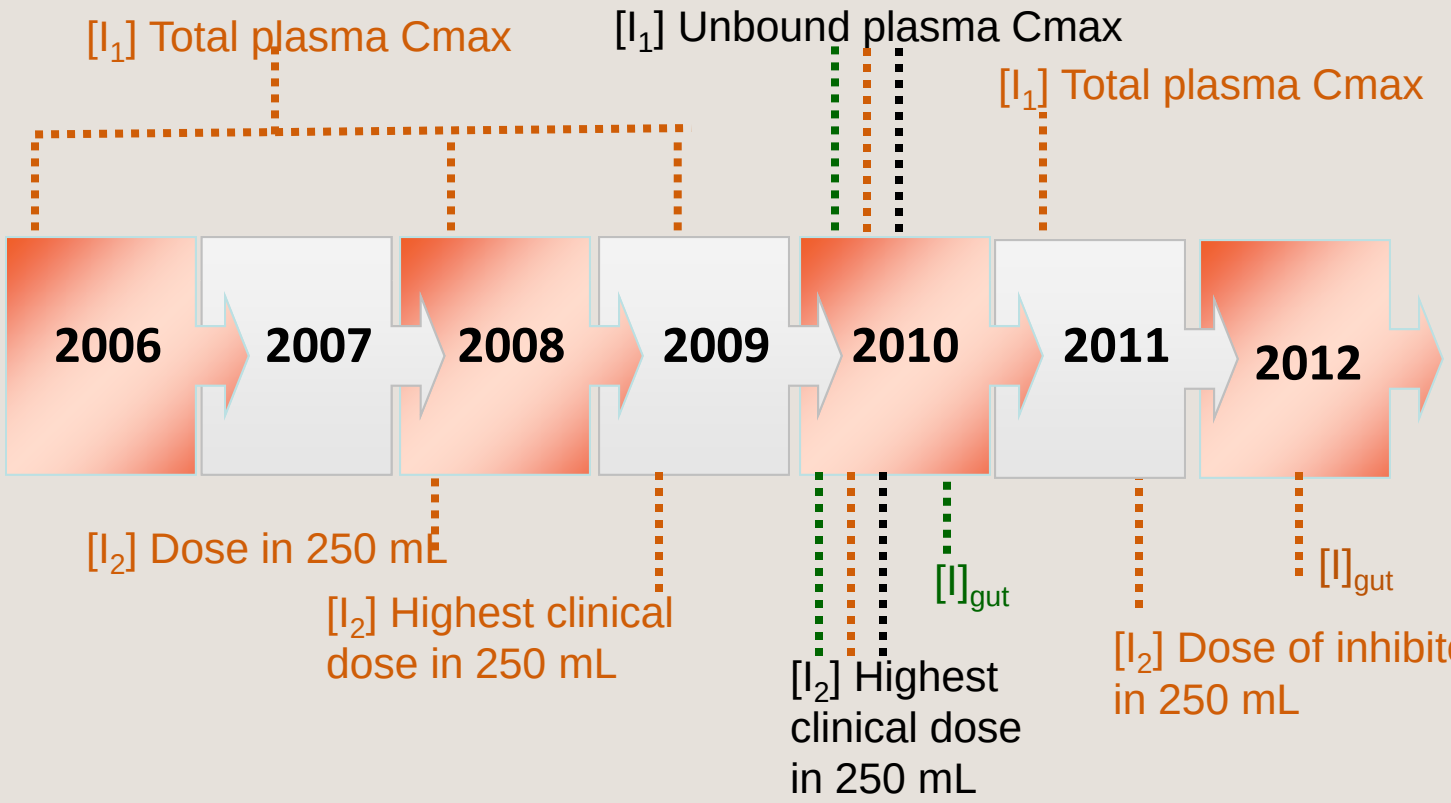
[I]dentity Crisis



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A regulatory viewpoint on transporter based drug interactions, Xenobiotica 2008
Predicting drug-drug interactions: An FDA Perspective, AAPS Journal 2009
EMA Guideline on the Investigation of Drug Interactions, 2010
Membrane Transporters in Drug Development, Nature Review 2010
Membrane Transporters in Drug Development: Comment on the Report from the ITC; Lei Zhang, AAPS Webinar July 2010

FDA
EMA
ITC

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[I] Matter: *In Vitro* and *In Vivo* Digoxin Data

	$[I_1]/IC_{50}$ (unbound)	$[I_1]/IC_{50}$ (total)	$[I_2]/IC_{50}$	Digoxin C_{max} (% change)	Digoxin AUC (% change)
Lapatinib	< 0.1	1	1355	N/A	180
Dronedarone	< 0.1	0.09	1349	N/A	150
Ranolazine	<0.1	0.04	2987	46	60
Darunavir	<0.1	0.33	146	15	58
Tolvaptan	<0.1	0.23	109	30	30
Etravirine	<0.1	0.04	76	19	18
Tetrabenzine	<0.1	0.01	6	13	2
Maraviroc	<0.1	0.01	13	4	0.5
Deferasirix	<0.1	0.003	4.3	-8.7	-8
Lacosamide	<0.1	0.01	1	4.8	2.4
Sitagliptin	<0.1	0.02	2	18	11

False Negatives **False Positives**

Agarwal, S, Zhang L, Huang S-M, Clin Pharmacol Ther 89(1): February 2011

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[I] Matter: *In Vitro* and *In Vivo* Digoxin Data

$[I_1]/IC_{50}$ (unbound)	6/11 (55%)	5 False Negatives
$[I_1]/IC_{50}$ (total)	9/11 (82%)	2 False Negatives
$[I_2]/IC_{50}$	9/11 (82%)	2 False Positives ← Solubility ← *In vitro/in vivo* probe substrate differences ← Possible P-gp induction ← Weak *in vitro* inhibitor

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IC_{50} Working Group: 2008-Present

Objective: To standardize IC_{50} evaluation

Considerations:

- Choice of test system
- Calculation techniques
- Intra- and inter-lab variability
- Calibration to enhance predictability

Findings:

- No “best” test system/equation
- Caco-2 and vesicles produce lower IC_{50} values than over-expressed systems.
- Use of AUC’ plots, accuracy plots, and ROC analysis enhances accuracy.

IC50 Working Group Recommendations, Oct. 2008-present. Caroline Lee. ITC2 Workshop, March 12, 2012.

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IC_{50} Working Group: ROC Analysis

	$[I]_1/IC_{50}$	$[I_2]/IC_{50}$	Accuracy	FN	FP
All Company	0.03	45	9/10	0/4	1/6 (17%)
FDA Guidance	0.1	10	8/10	0/4	2/6 33%

Application of "refined" *in vitro* cut-off:

- $[I_1]/IC_{50} = 0.03$
- $[I_2]/IC_{50} = 45$

IC50 Working Group Recommendations, Oct. 2008-present. Caroline Lee. ITC2 Workshop, March 12, 2012.

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Considerations: Solubility

Challenge

Low solubility

Recommended Excipients

PEG400
HP-B-CD
Tween 80
NMP
DMSO

Suitability Criteria

Integrity (lucifer yellow)
Rank order permeability
P-gp function
BCRP function

	Control	PS80
Integrity	✓	✓
Digoxin Efflux	16	11.4
E3S Efflux	33.6	16.7

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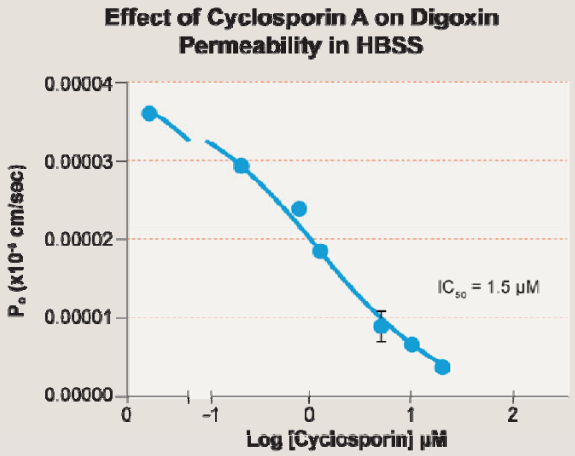
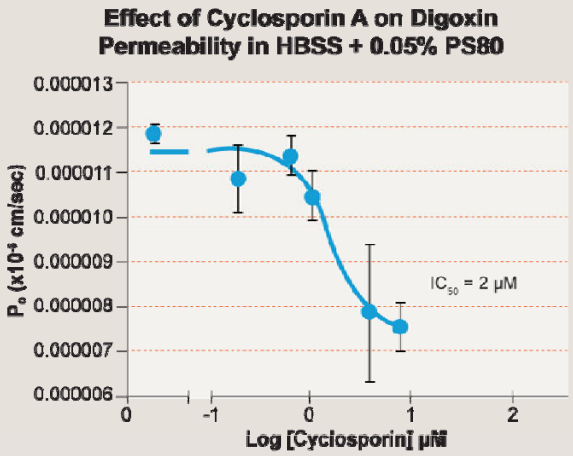
Considerations: Impact vs. Sensitivity

Comparison of HBSS to Polysorbate 80			
Solute	**Buffer**	$\frac{P_{B\text{-}A\ transcoil}}{P_{A\text{-}B\ transcoil}}$	J_{max}/K_M (10^{-5} cm/sec)
Loperamide	HBSS	35.5	313
	0.05% Polysorbate 80	5.0	40
Quinidine	HBSS	24.1	134
	0.05% Polysorbate 80	4.0	17
Talinolol	HBSS	43.7	6.8
	0.05% Polysorbate 80	3.6	0.8





Adapted from Burton, P.S. et al Strategy for Characterizing P-Glycoprotein Substrate and Inhibitor Potential for Sparingly Soluble Drugs, Poster, AAPS, 2008

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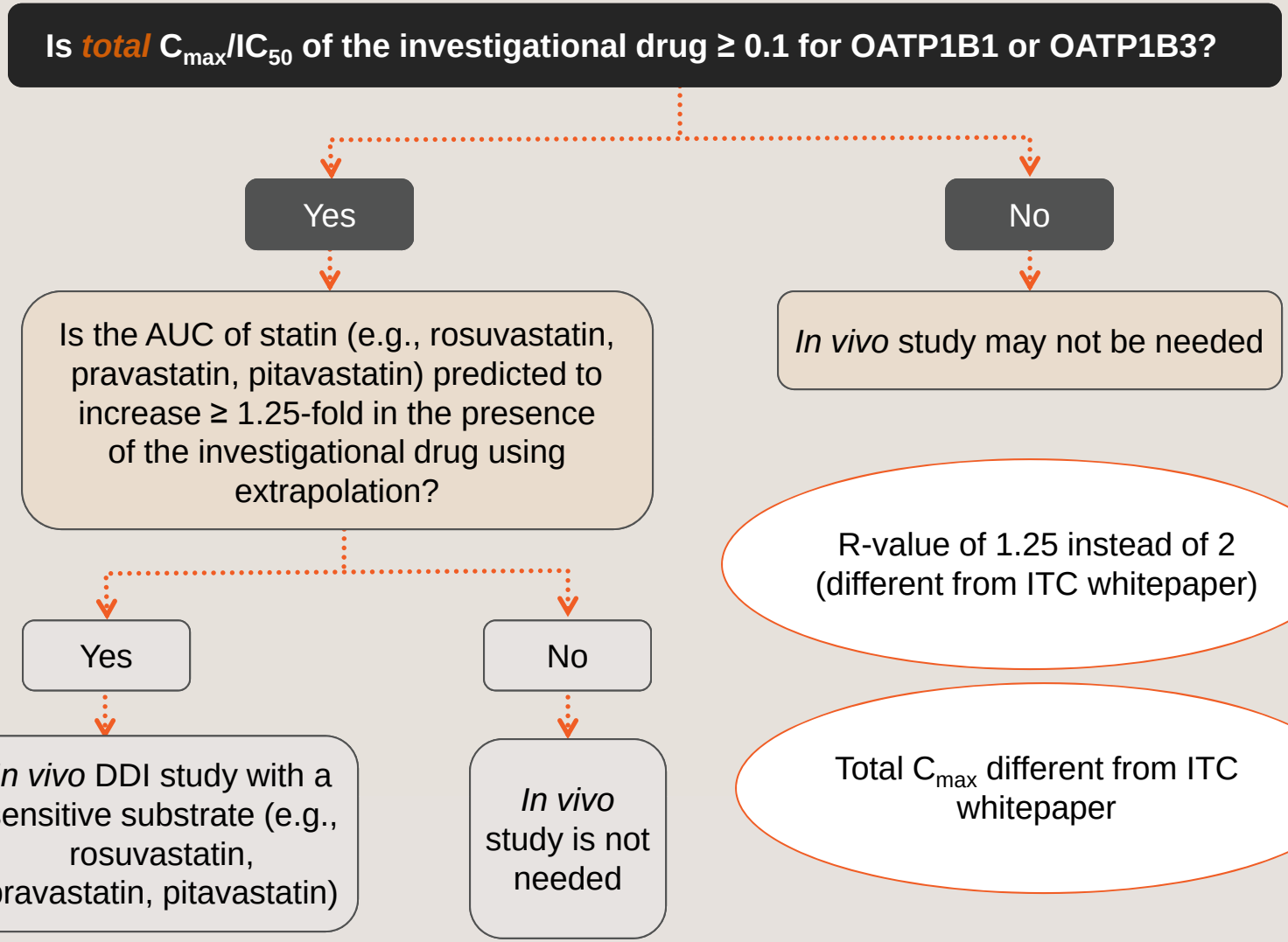
OATP1B1 and OATP1B3 Inhibition



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R-value Cutoff

ITC Whitepaper

$[I_1]_{unbound}/IC_{50} \geq 0.1$

FN **5 (21%)**	TP **19**
TN **2**	FP **2 (50%)**

$R \geq 2$

FN **6 (32%)**	TP **13**
TN **2**	FP **0 (0%)**

11 FN, 0 FP
61% accuracy

Using total C_{max} reduces false negatives

Including R introduces additional false negatives

FDA Guidance

$[I_1]_{total}/IC_{50} \geq 0.1$

FN **0 (0%)**	TP **24**
TN **2**	FP **2 (50%)**

$R \geq 1.25$

FN **5 (21%)**	TP **19**
TN **0**	FP **2 (100%)**

5 FN, 2 FP
75% accuracy

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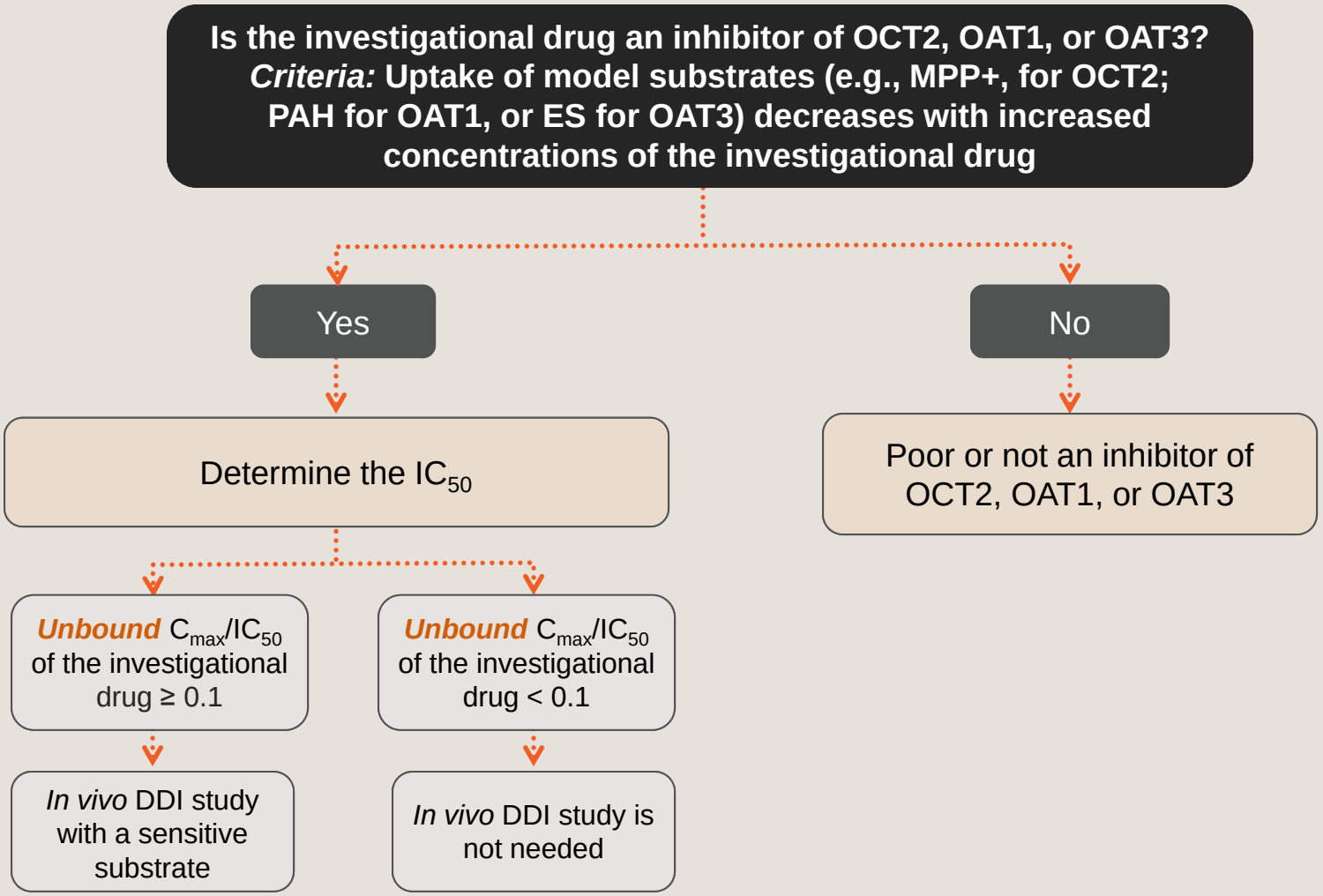
OAT1, OAT3, and OCT2 inhibition



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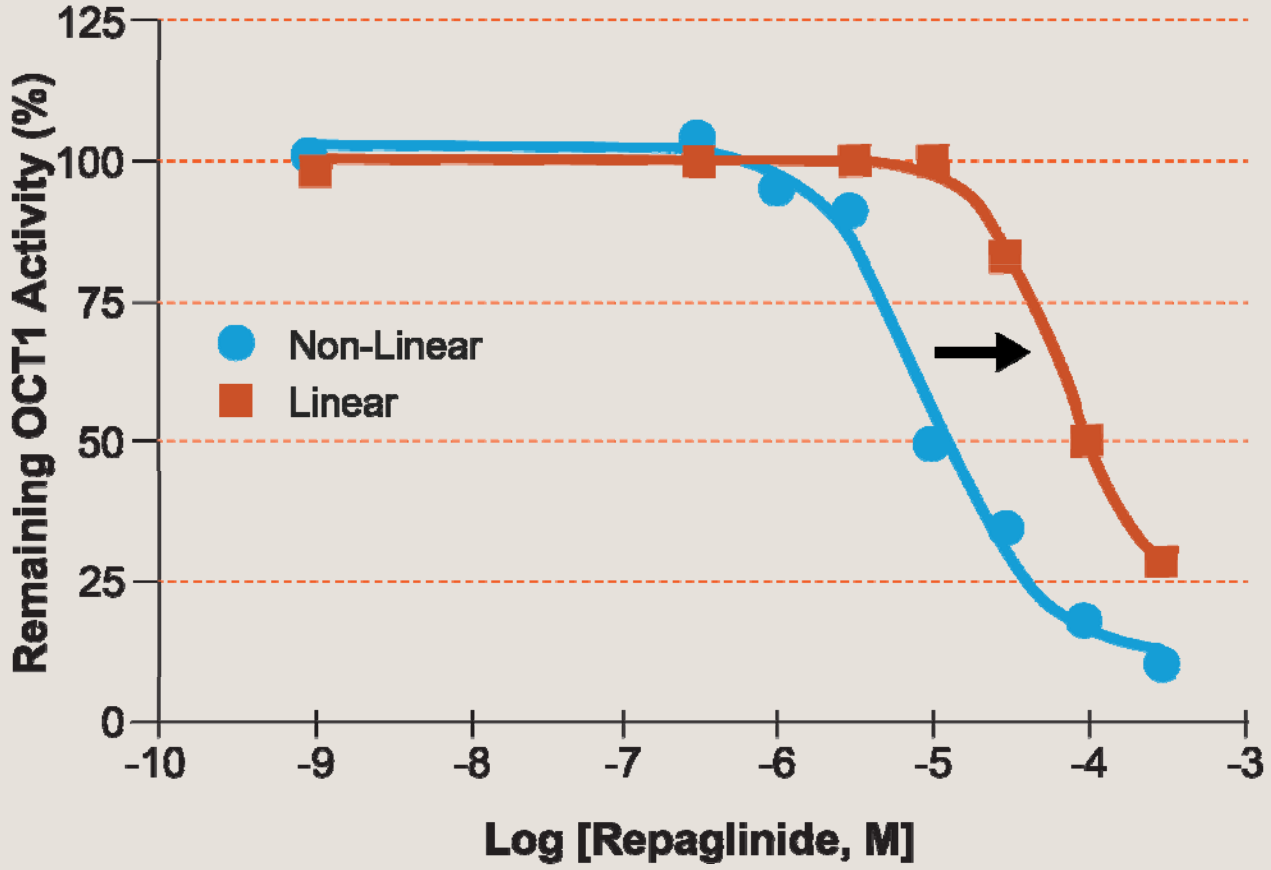
Considerations: Linearity and IC_{50}



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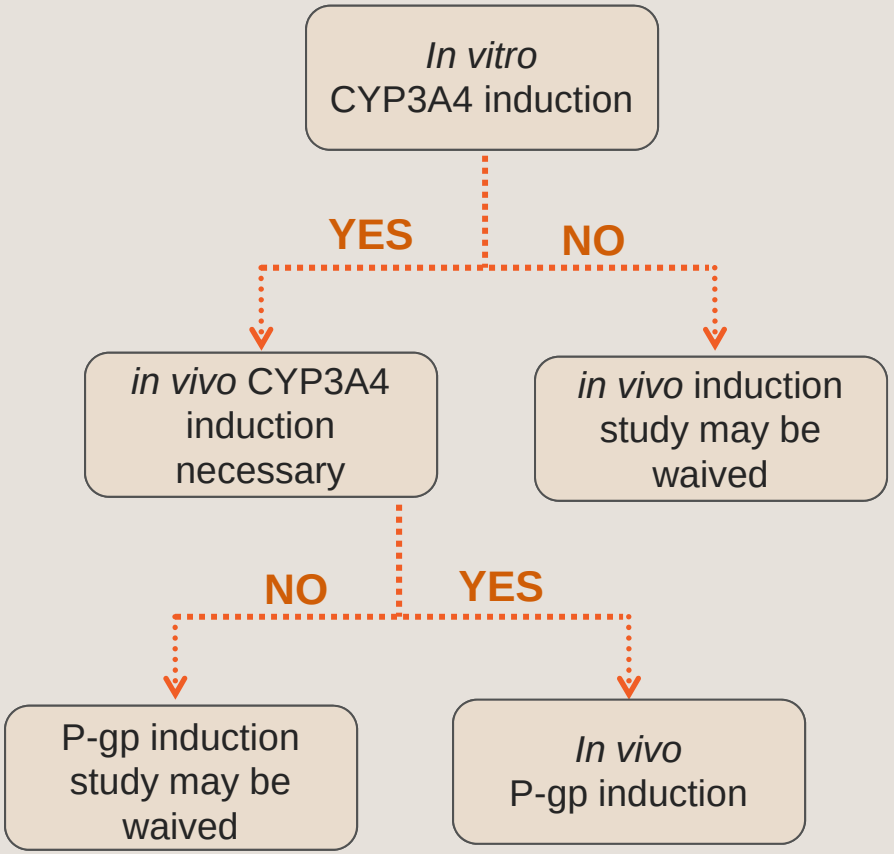
Induction

"Transporters can be induced by mechanisms similar to those for CYP enzymes…they share common nuclear factors…"

"…lack of a validated in vitro systems to study transporter induction, the definitive determination of induction potential…on transporters in based on in vivo induction studies…



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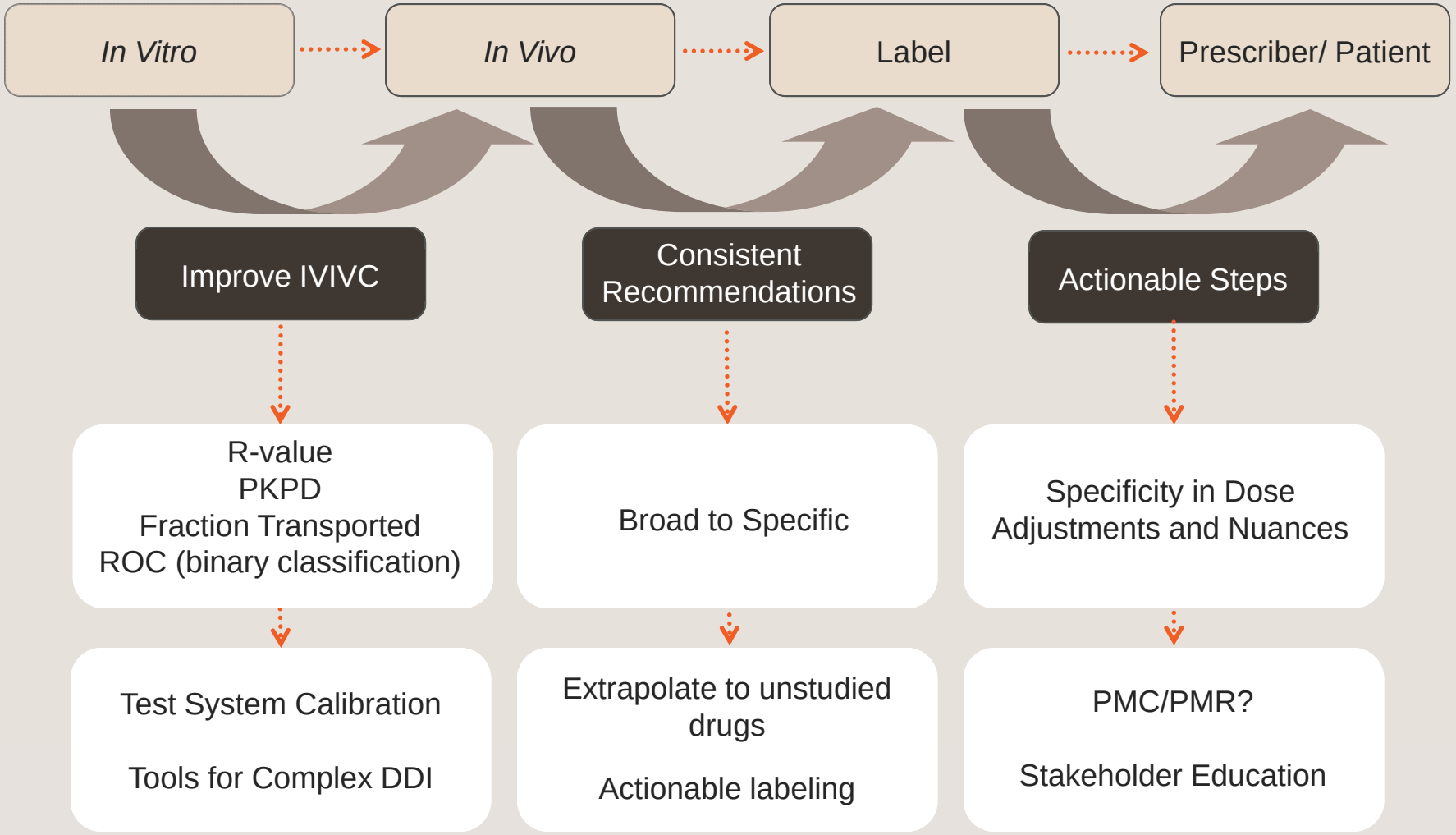
Improving Translatability: 2012 and Beyond



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Closing Thoughts

"…one can't believe impossible things."

-Alice, Through the Looking Glass, Lewis Carroll

*"Why, sometimes I've **believe**d as many as six **impossible** things before breakfast."*

-The White Queen, Through the Looking Glass, Lewis Carroll

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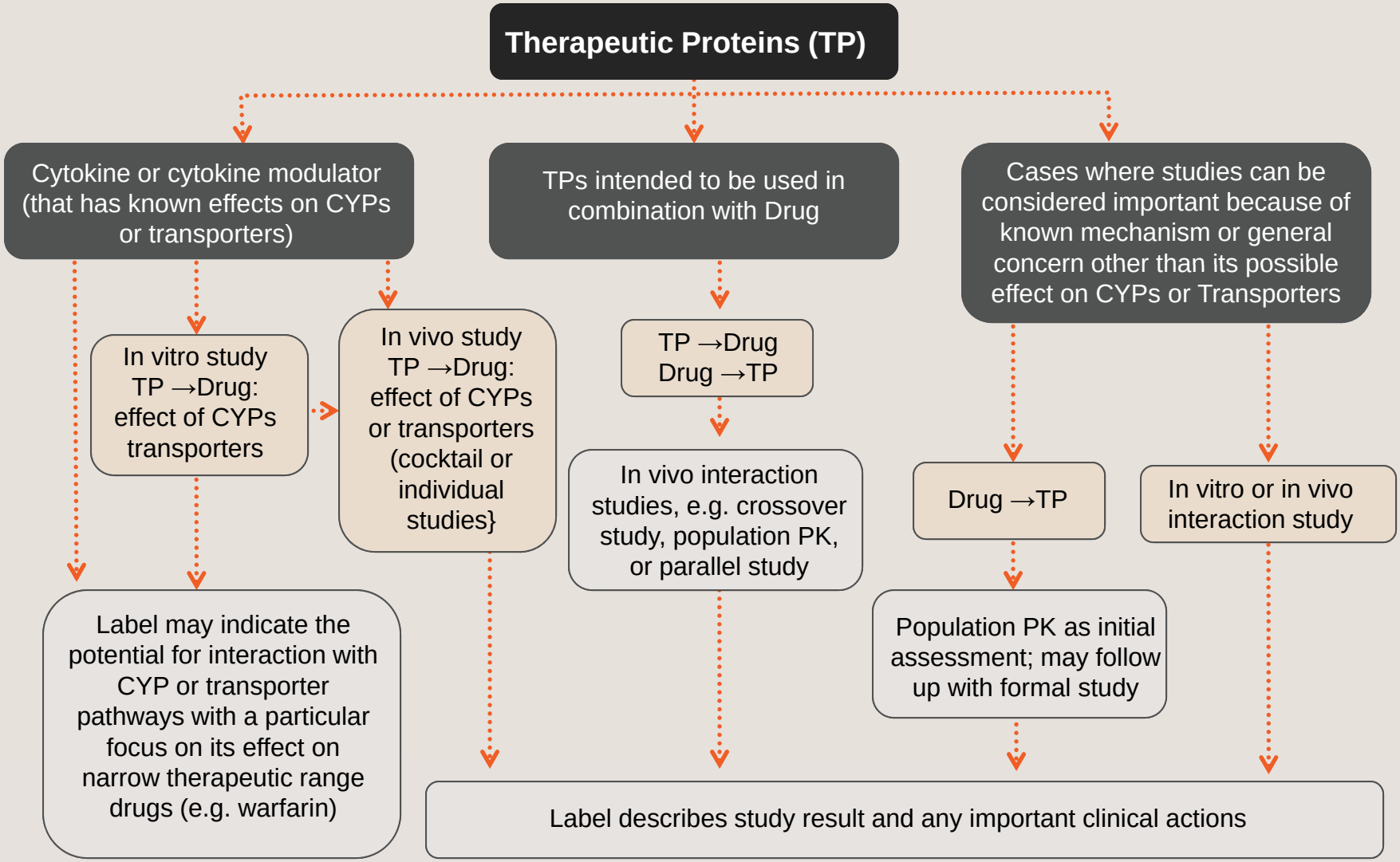
Therapeutic Proteins



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