XENOTECH

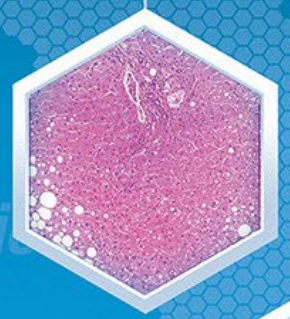
A BioIVT Company

25 Years

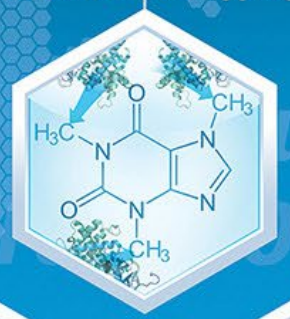
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

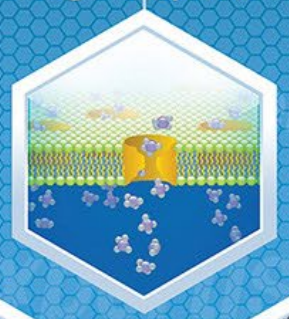


Reaction Phenotyping

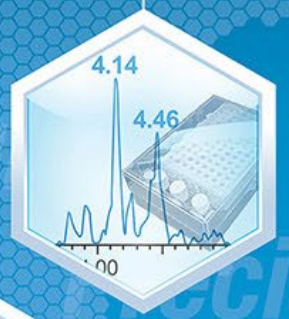


Enzyme Induction & Inhibition

Drug Transporters



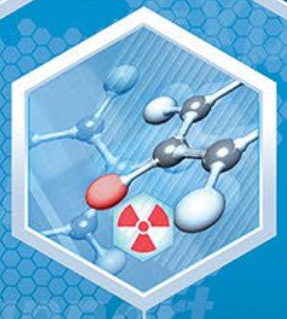
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

In Vitro ADME and Drug-Drug Interaction Considerations for Toxicologists

Pallavi Limaye, Ph.D., DABT

Director of Consulting

XenoTech, LLC

Outline

- Why run these studies?
- Types of *in vitro ADME &* Drug-Drug Interaction (DDI) studies
- Areas of concern: Proper design & interpretation
- When to conduct?
- Important highlights

Why conduct these studies? Is this just box checking?

No! The information is important for multiple aspects:

1. Provide deeper understanding of the molecule
   - Metabolism, enzymes involved in metabolism etc.
   - The information generated from DDI studies goes on the drug label.
   - From the pharma company's perspective these studies help decide on a different candidate early on
2. Prepare for clinical studies
   - Prediction of FIH dose and DDI risk
3. Comply with regulatory guidance

Regulatory Guidance

FDA: Final January 2020

In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

January 2020
Clinical Pharmacology

EMA: Final 2013

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 June 2012
CPMP/EWP/560/95/Rev. 1 Corr. 2**
Committee for Human Medicinal Products (CHMP)

Guideline on the investigation of drug interactions

Discussion in the Efficacy Working Party (EWP)	June/October 1996 February 1997
Transmission to the CPMP	March 1997
Transmission to interested parties	March 1997
Deadline for comments	September 1997
Re-submission to the EWP	December 1997
Approval by the CPMP	December 1997
Date for coming into operation	June 1998
Draft Rev. 1 Agreed by the EWP	April 2010
Adoption Rev. 1 by CHMP for release for consultation	22 April 2010
End of consultation Rev. 1 (deadline for comments)	31 October 2010
Agreed by Pharmacokinetics Working Party	February 2012
Adopted by CHMP	21 June 2012
Date for coming into effect	1 January 2013

This guideline replaces guideline CPMP/EWP/560/95.

Keywords	***Interaction, guideline, metabolism, inhibition, induction, transport, enzyme, transport protein, transporter, absorption, food, distribution, PBPK, herbal, SmPC***

* The correction concerns section 5.3.4.1 (p 26) and the corresponding decision tree no. 6 (p 61) to read "if the observed Ki value is lower or equal to /.../"; Appendix VII, Table 5 to read "See section 5.4.2".* Decision tree 4.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

PMDA: Final 2019

事　務　連　絡
平成 31 年 2 月 8 日

各都道府県衛生主管部（局）薬務主管課　御中

厚生労働省医薬・生活衛生局医薬品審査管理課

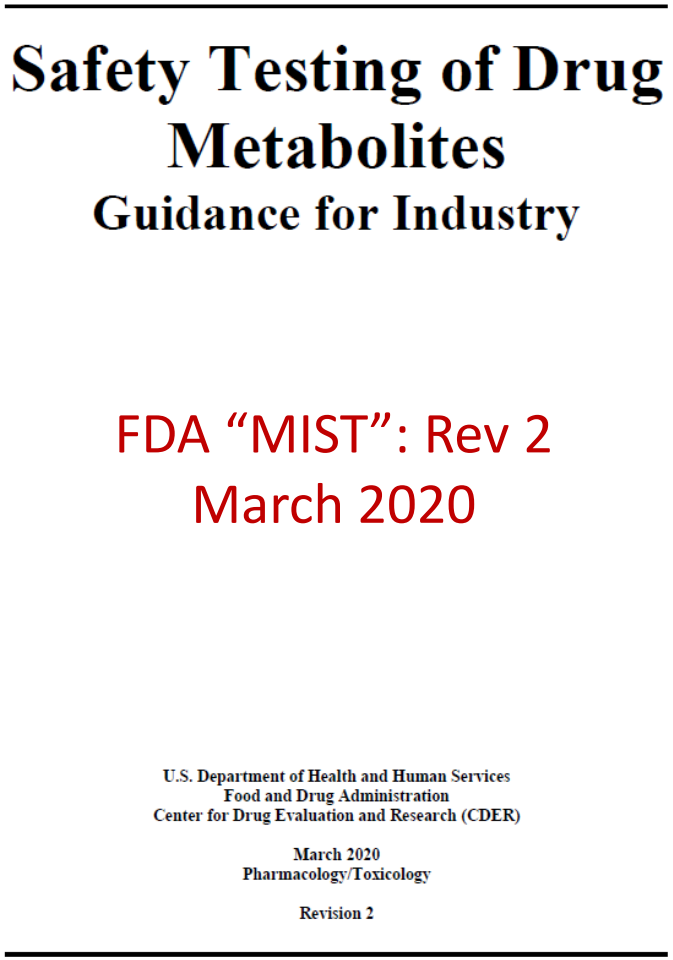
「医薬品開発と適正な情報提供のための薬物相互作用ガイドライン」等の英文版の送付について

標記について、別添１及び２のとおり取りまとめましたので、貴管下関係業者に対して周知方お願いします。

別添１　Guideline on drug interaction for drug development and appropriate provision of information

別添２　Question and Answer for the "Guideline on drug interaction for drug development and appropriate provision of information"

Additional Guidance



Safety Testing of Drug Metabolites
Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

March 2020
Pharmacology/Toxicology

Revision 2

FDA "MIST": Rev 2
March 2020

Guidance for Industry

M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2010
ICH

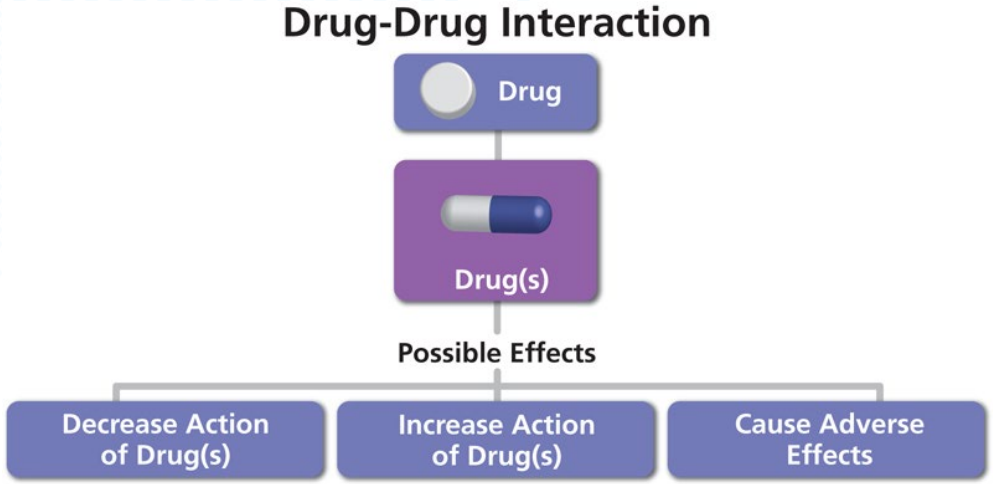
Revision 1

FDA / ICH: Final 2010

ADME and DDI

Compounds are evaluated for ADME properties

- **A**bsorption – Drug Transporters, passive diffusion
- **D**istribution – Drug Transporters, passive diffusion
- **M**etabolism – Drug Metabolizing Enzymes (CYP450s, UGTs, etc.)
- **E**xcretion – Drug Metabolizing Enzymes and Drug Transporters



Retrieved from https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/213/drug-drug-interaction

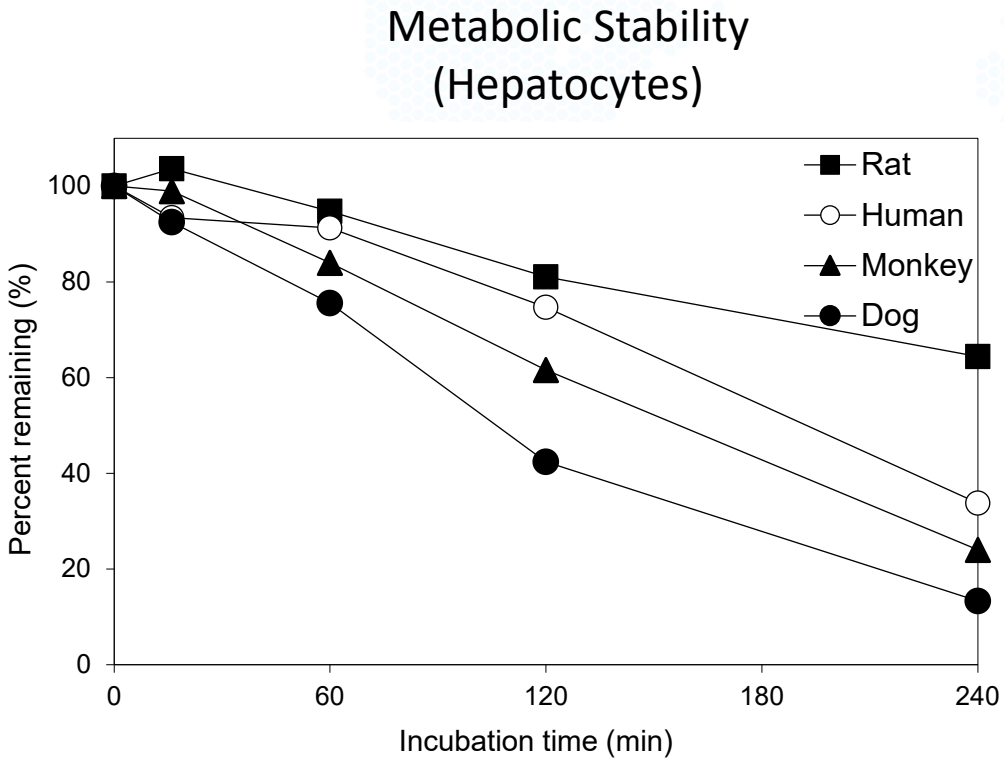
In vitro ADME & DDI study types

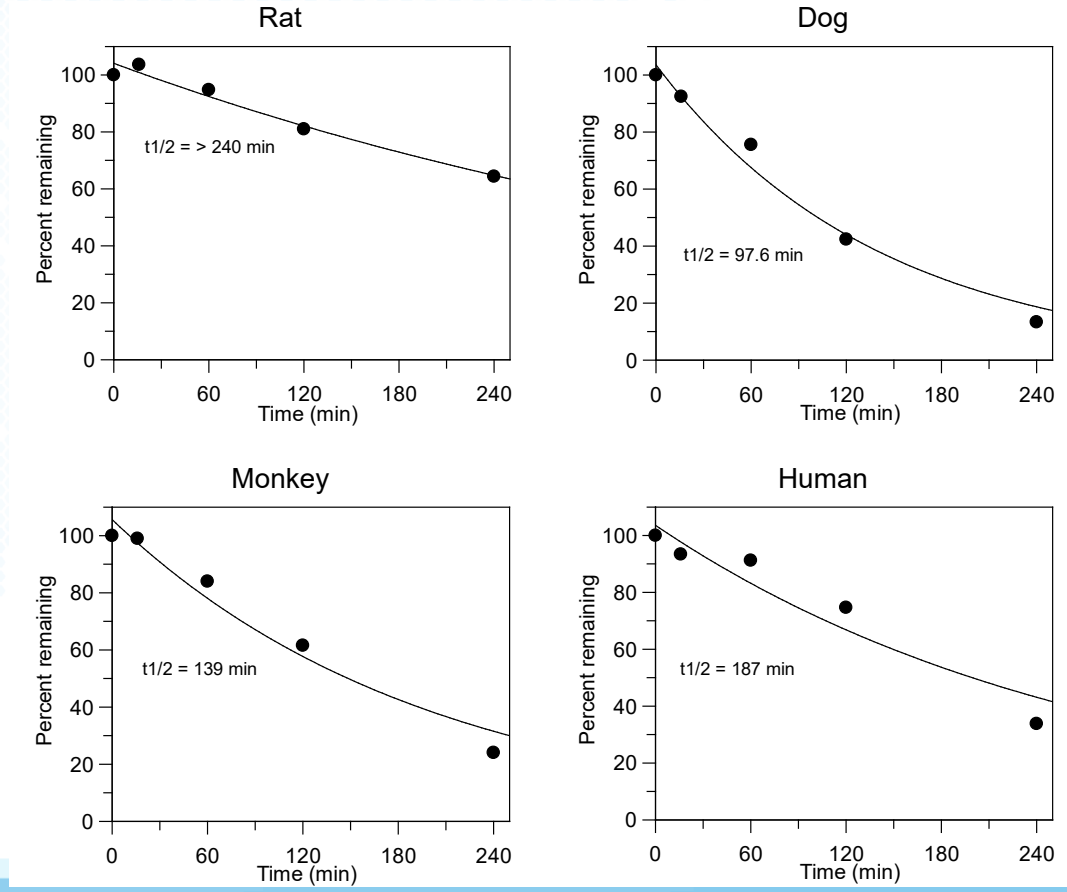
ADME component	Type of in vitro study
Drug Metabolism (M, E)	1. Inter-species comparative metabolism
	2. Metabolite ID – Qualitative analysis of metabolite profile
	3. Reaction phenotyping – Determine which CYPs are metabolizing
Drug Metabolizing Enzymes (M, E)	1. CYP Inhibition – Profile specific CYP inhibitions
	2. CYP induction – Induction potential for specific CYPs
Drug Transporters (A, D, E)	1. Transporter substrate – Determine Transporter substrate profile
	2. Transporter inhibition – Profile specific inhibition of major Transporters

Drug Metabolism Studies

Drug Metabolism: 1. Inter-Species Comparative Metabolism

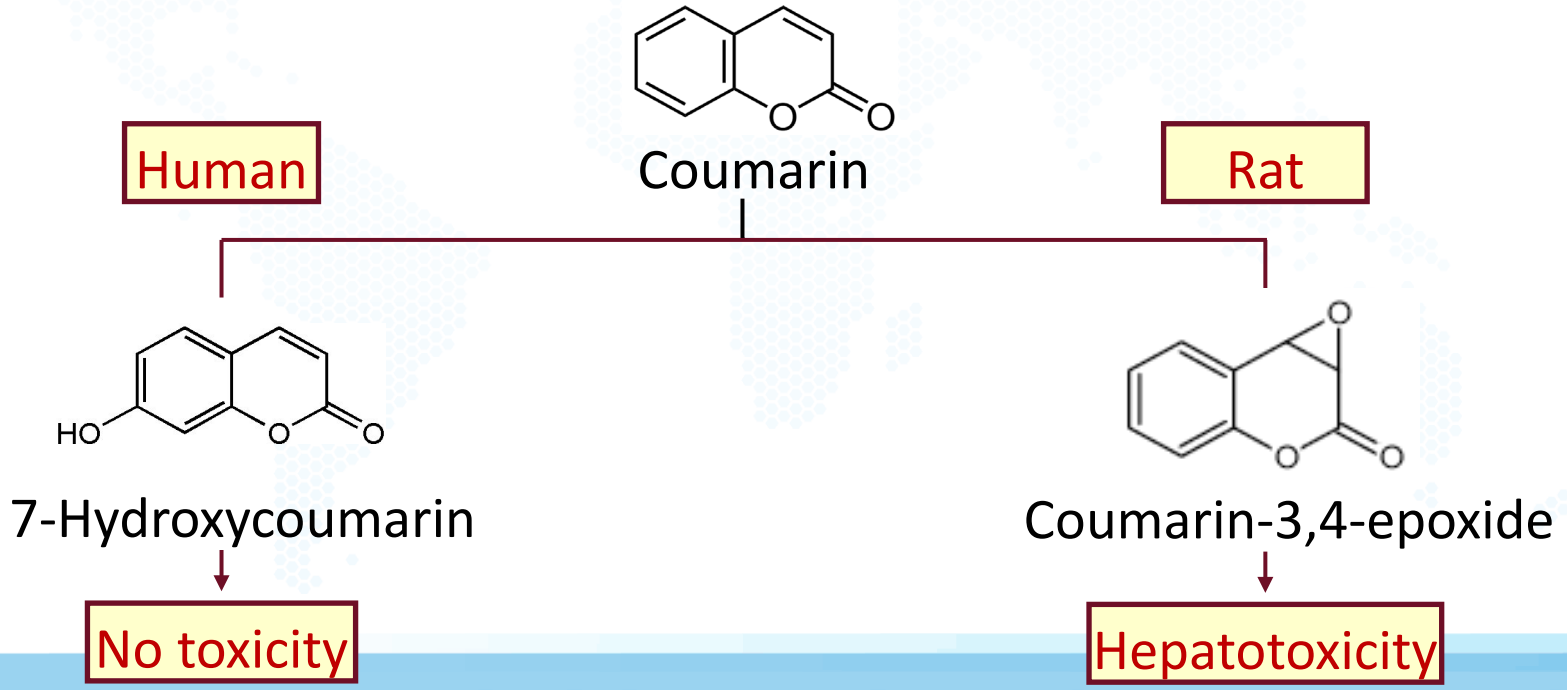
- Design: Drug incubations with hepatocytes or subcellular fractions from various species
- Typical species: Human, Rat, Mouse, Dog, Rabbit, Monkey, Pig





Drug Metabolism: 2. Inter-Species Comparative Metabolite ID

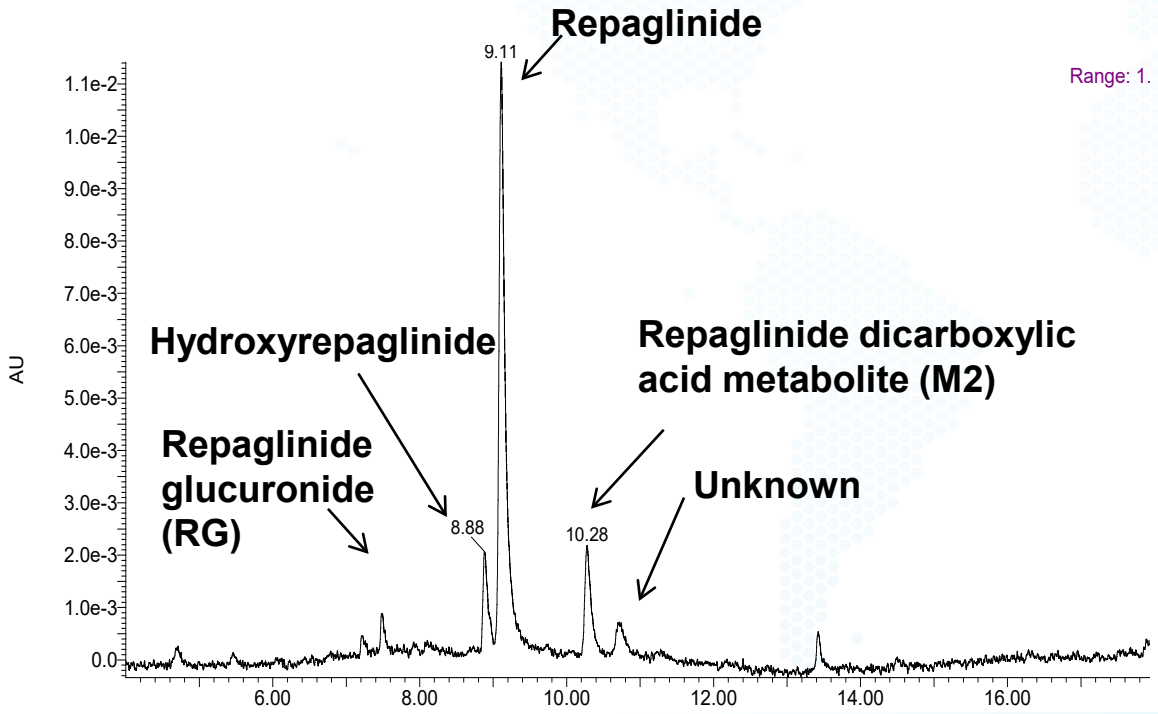
- **Goals:**
- Complete profile of metabolites
- Are there human specific metabolites?
- Which other species have a similar metabolic profile?



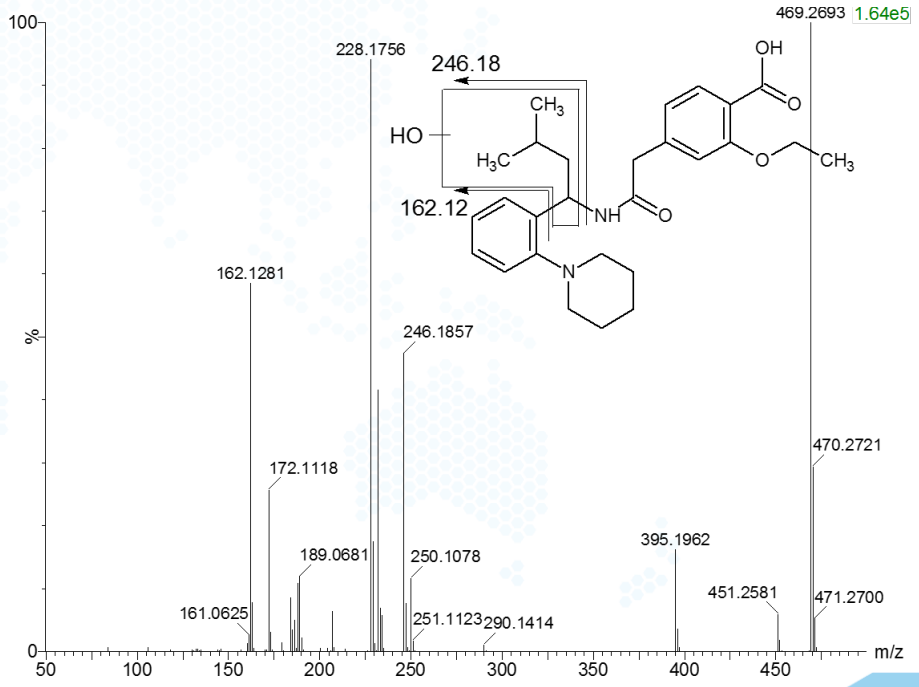
Metabolite ID

LC-MS/MS analysis – Qualitative identification of the metabolites

50 μM Repaglinide; Human hepatocytes; 60 minutes; 37°C



Hydroxyrepaglinide LC-MS/MS

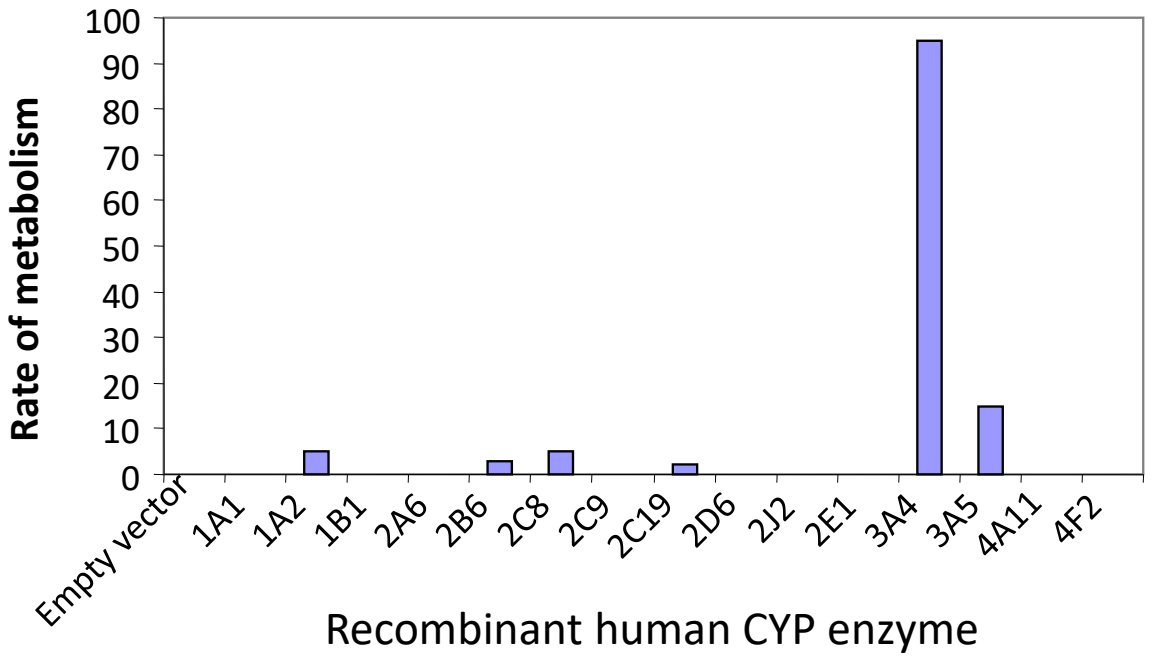


Cross-species Met ID

Component	Retention time (min)	Mass shift	Proposed biotransformation	Mouse	Rat	Dog	Pig	Human
C1	3.43	255.9889	Sulfation + glucuronidation	+	+	+	+	+
C2	3.63	354.0783	Di-glucuronidation + hydrogenation	+	+	+	+	+
C3	3.78	159.9135	Di-sulfation	+	+	+	+	+
C4	4.00	258.0045	Sulfation + glucuronidation + hydrogenation	+	+	+	+	+
C5	4.41	161.9298	Di-sulfation + hydrogenation	+	+	+	+	+
C6	4.44	194.0428	Glucuronidation + oxygenation + hydrogenation	ND	ND	ND	+	+

Drug Metabolism: 3. CYP Reaction Phenotyping (Victim potential)

- Design: Incubate drug + recombinant human CYPs or human liver microsomes or hepatocytes ± selective inhibitors
- Goal: Determine which CYPs drive the metabolism of the drug
- Unique CYP metabolism is of concern



High DDI potential: few enzymes involved

Follow-up studies:

- Confirm with selective inhibitors
- Evaluate non-CYP pathways in hepatocytes

Terfenadine
(Seldane® - withdrawn)

Fexofenadine
(Allegra®)

Drug Metabolizing Enzymes (Perpetrator potential)

Drug Metabolizing Enzymes: 1. CYP Inhibition

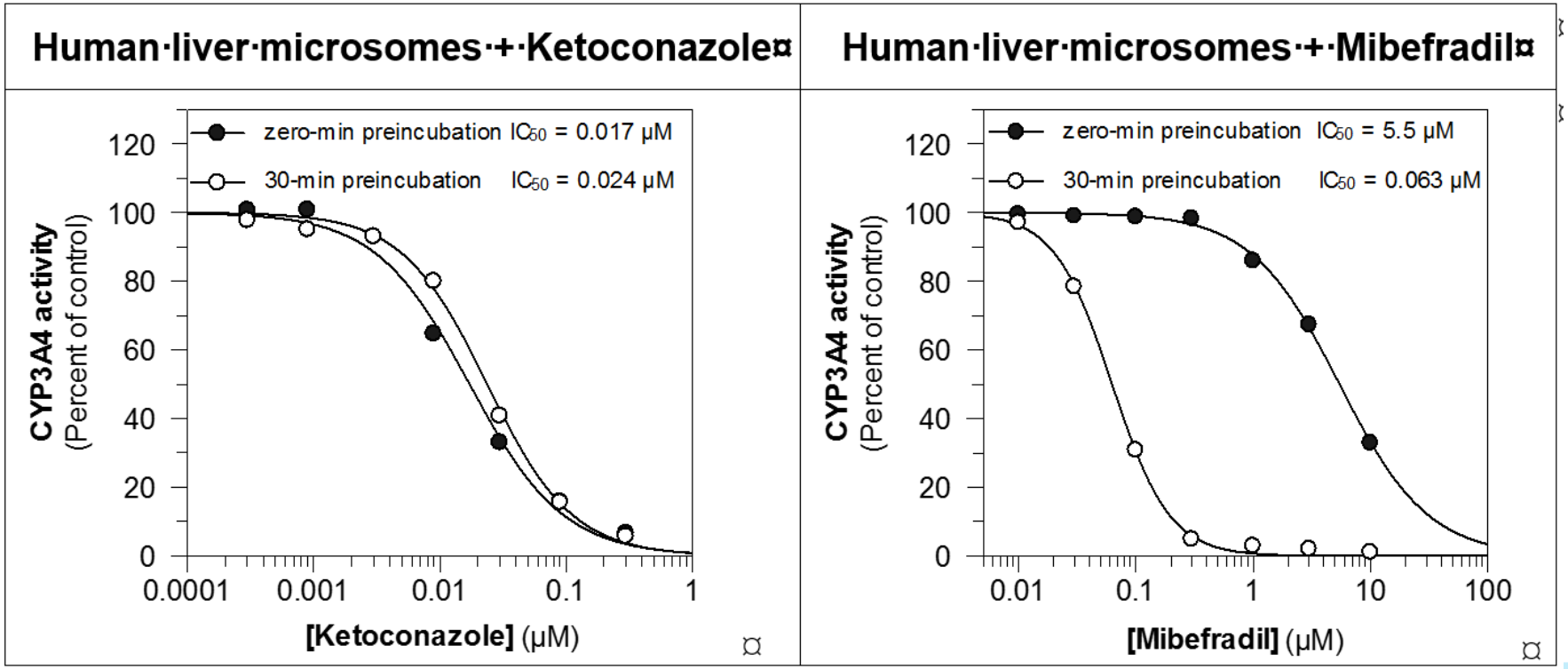
- Design: Drug incubations with HLM + marker substrate ± pre-incubation
- Goal: Predict clinically relevant inhibition of CYP enzymes

CYP	Activity Assay
CYP1A2	Phenacetin *O*-dealkylation
CYP2B6	Bupropion hydroxylation
CYP2C8	Amodiaquine *N*-dealkylation
CYP2C9	Diclofenac 4´-hydroxylation
CYP2C19	*S*-Mephenytoin 4´-hydroxylation
CYP2D6	Dextromethorphan *O*-dealkylation
CYP3A4	Testosterone 6β-hydroxylation
CYP3A4	Midazolam 1´-hydroxylation

Drug Metabolizing Enzymes: CYP Inhibition (Direct vs. Time dependent)

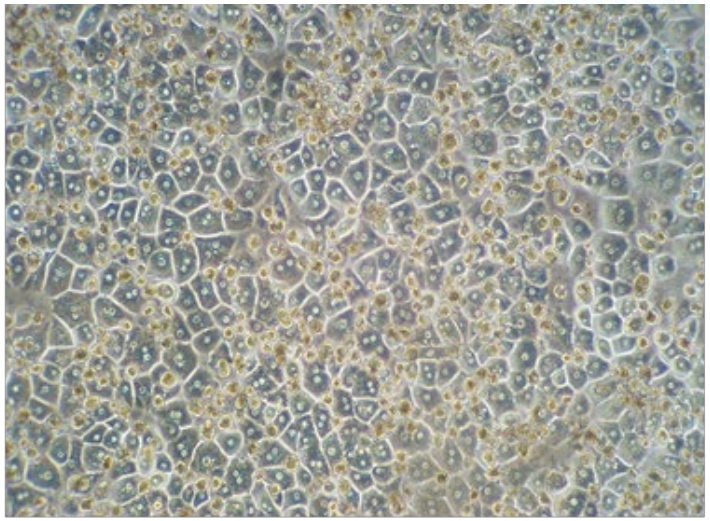
Ketoconazole: Potent inhibitor of CYP3A4 precludes coadmin of other drugs

Mibefradil: Removed from market in 1998 due to potential for fatal DDIs

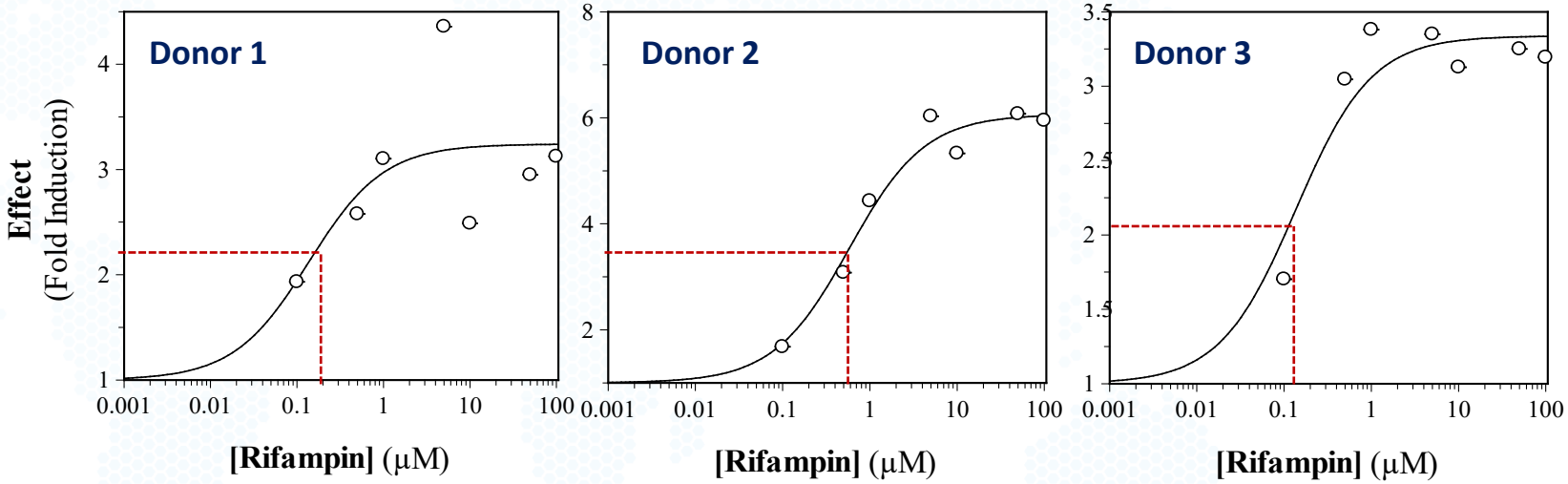


Drug Metabolizing Enzymes: 2. CYP Induction

- Design: Drug incubations in cultured human hepatocytes, Measure mRNA of various CYPs
- Goal: Predict clinically relevant induction of CYP enzymes



Cultured Human Hepatocytes



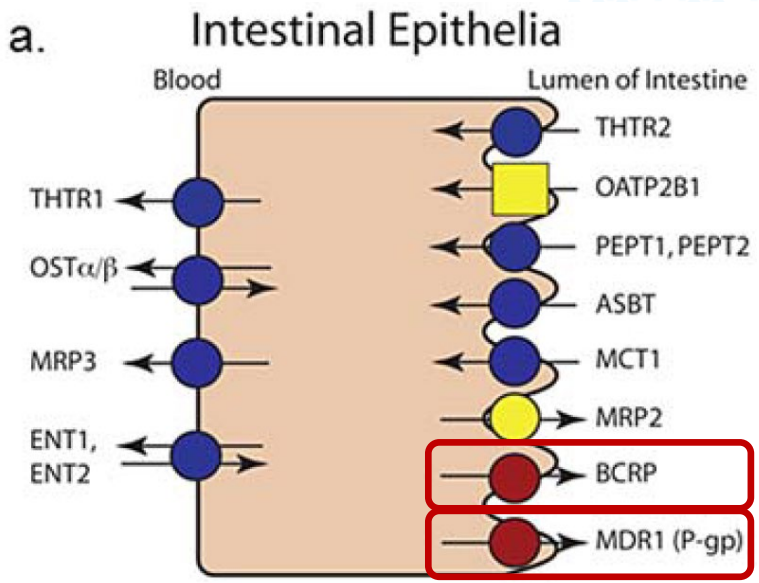
Parameter	Value	Std. Error
Emax	3.2417	0.3163
EC50	0.1371	0.1427

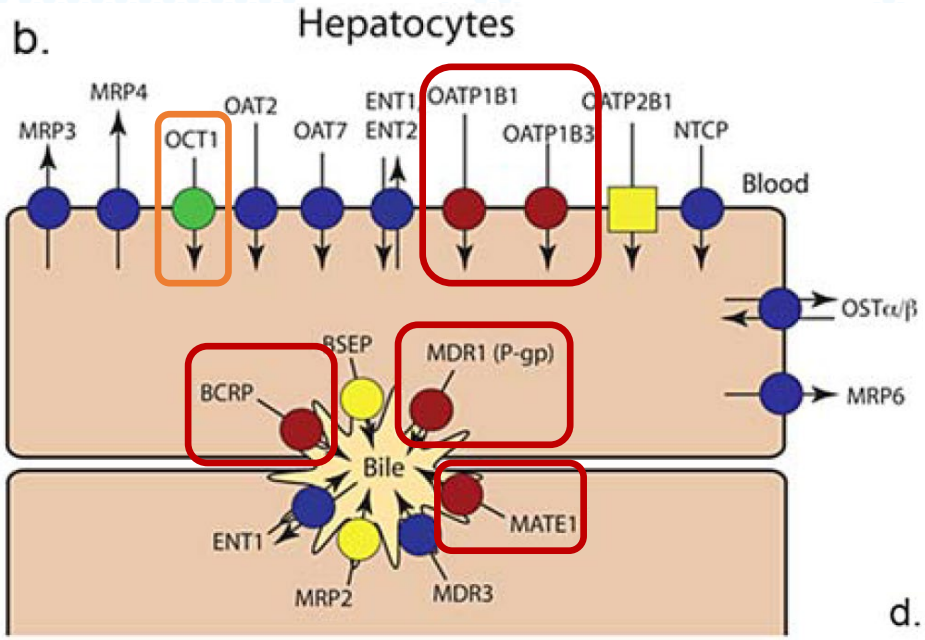
Parameter	Value	Std. Error
Emax	6.0649	0.1981
EC50	0.5795	0.1244

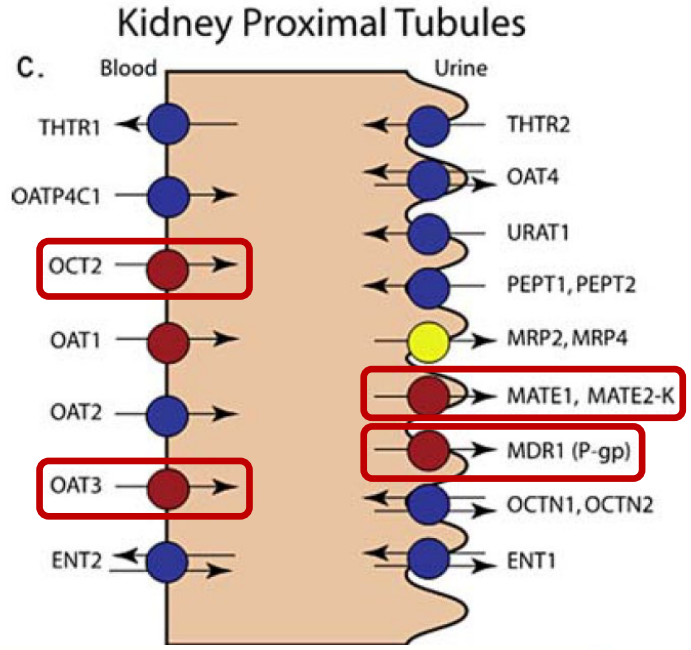
Parameter	Value	Std. Error
Emax	3.3378	0.1200
EC50	0.1362	0.0528

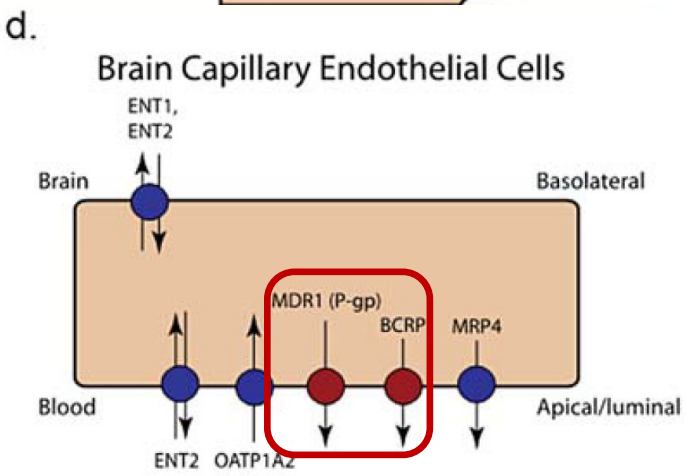
Drug Transporters
(Victim and Perpetrator potential)

Transporters









Inhibition for all in red (FDA & PMDA); **orange (EMA)**

Substrate potential:

P-gp and BCRP (all orally administered drugs)

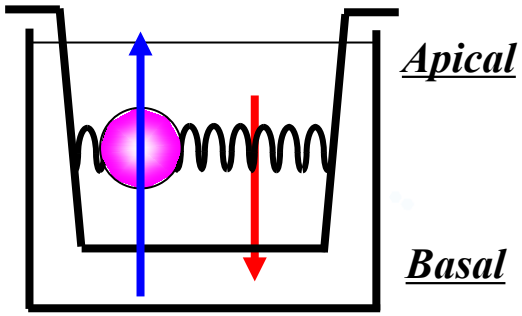
Hepatic uptake: If hepatic metabolism or biliary secretion ≥25%

Renal: If active renal secretion ≥25% of total clearance

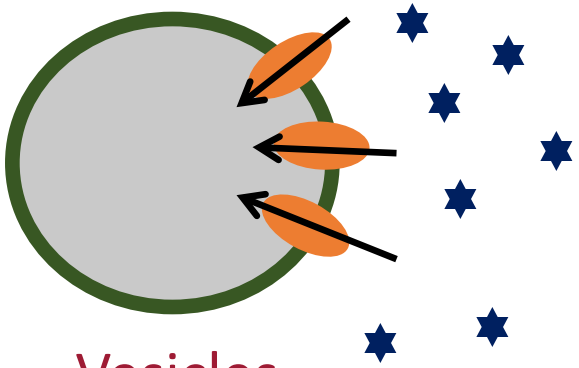
Figures from Zamek-Gliszczynski et al. ITC3 (2018) *CPT* 104:890-899

1. Transporter Substrate

- Design: a) Drug incubations with transporter-expressing cells
  b) Confirmation of specificity with positive control inhibitors
- Goal: Predict a drug's ability to be transported by specific transporters

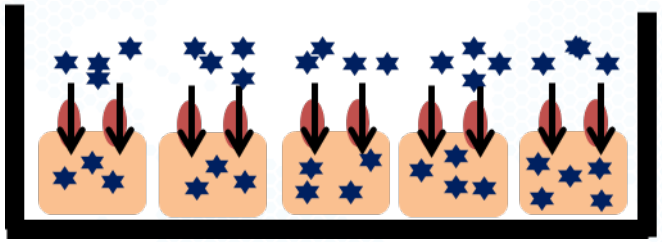


Trans-well



Vesicles

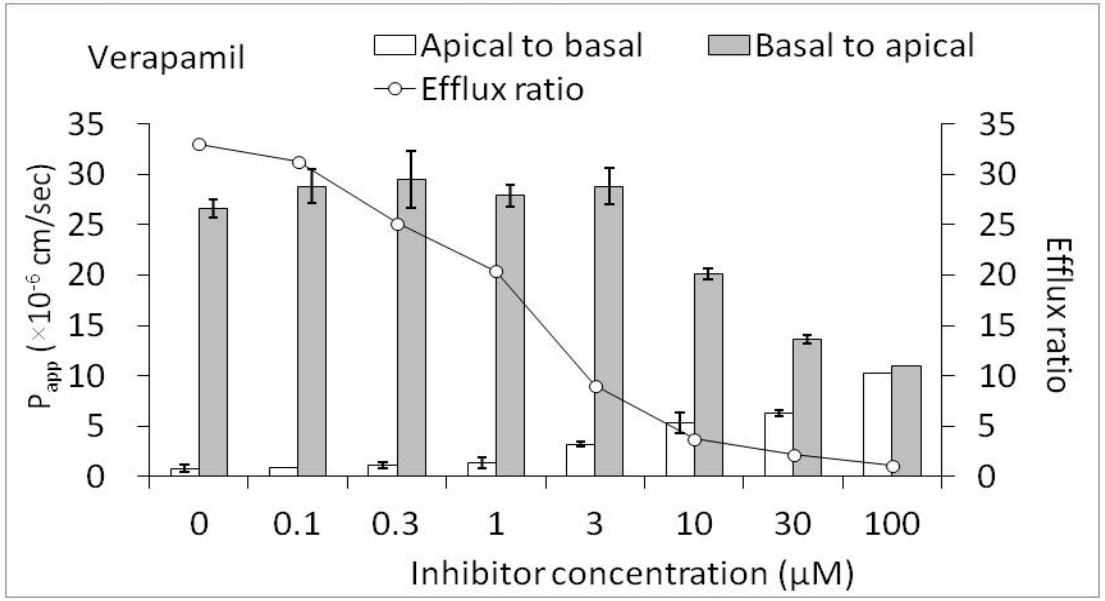
Transporter-expressing cells



Control cells

2. Transporter Inhibition

- Design: Drug incubations with transporter-expressing cells or vesicles and marker substrate
- Goal: Predict clinically relevant inhibition of major transporters



Areas of Concern

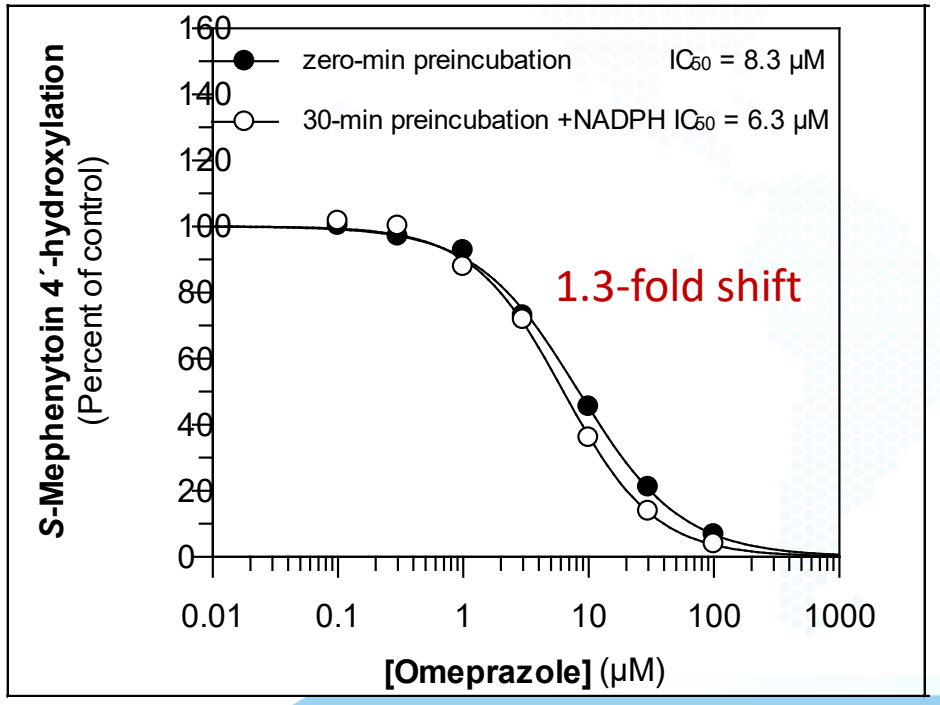
Areas of concern: CYP Inhibition study design

False negative results arise from poorly designed studies

- Example: Clinically relevant time-dependent inhibition of CYP2C19 by omeprazole missed with high [protein] and long marker substrate incubation

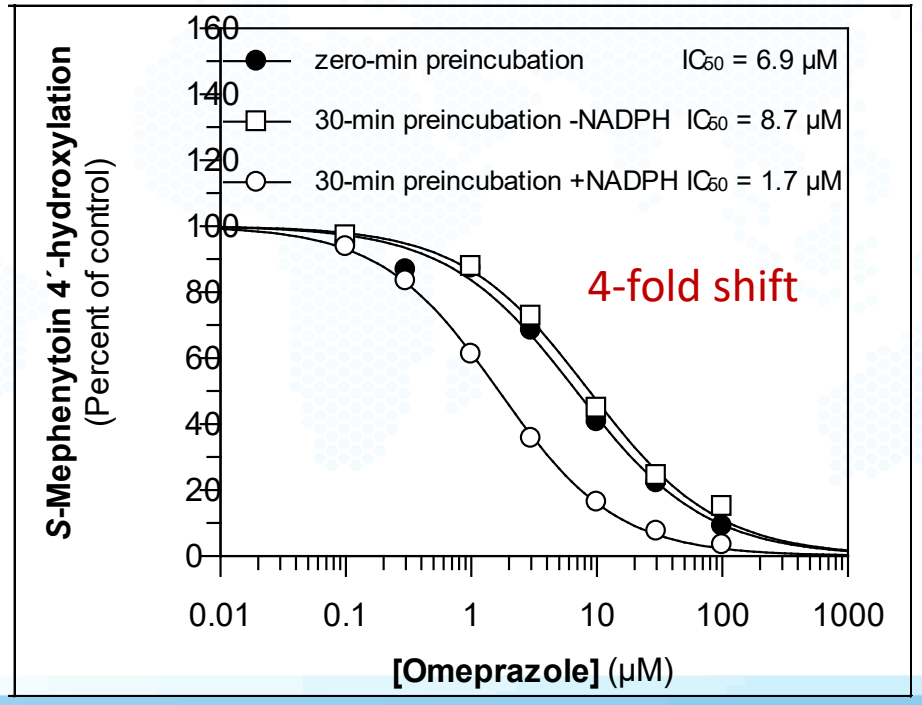
Missed

1 mg/mL protein, 30 min substrate incubation



Detected

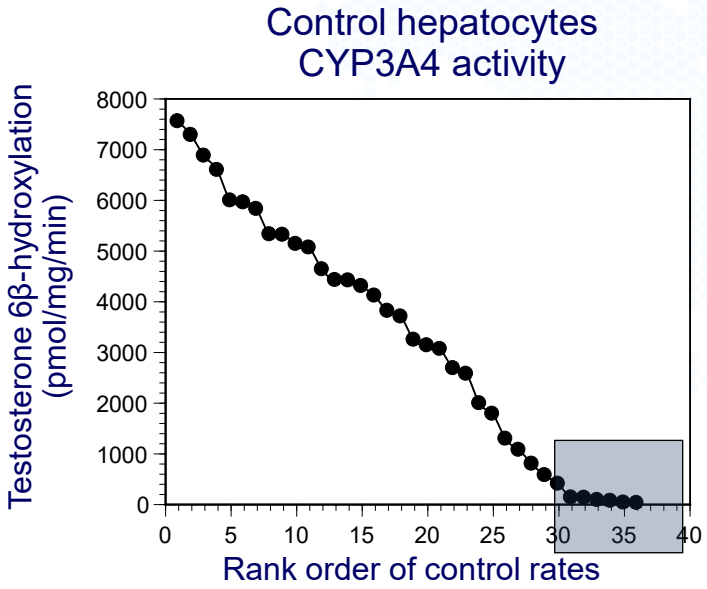
0.1 mg/mL protein, 5 min substrate incubation

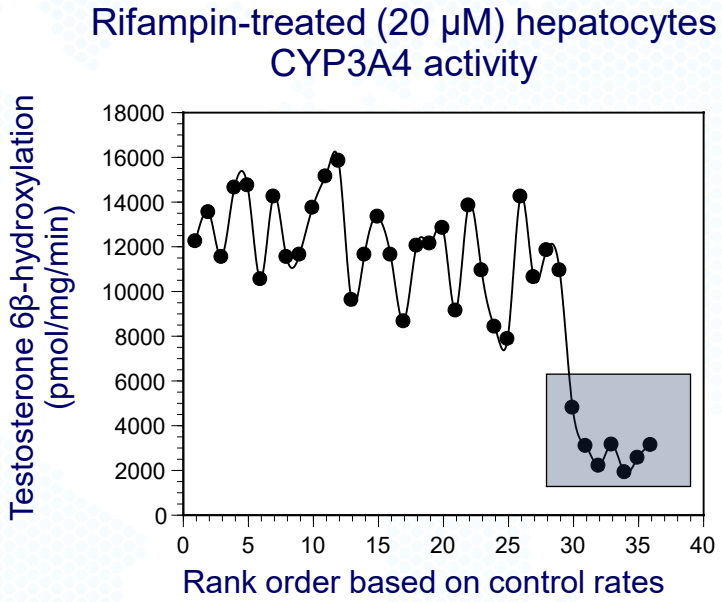


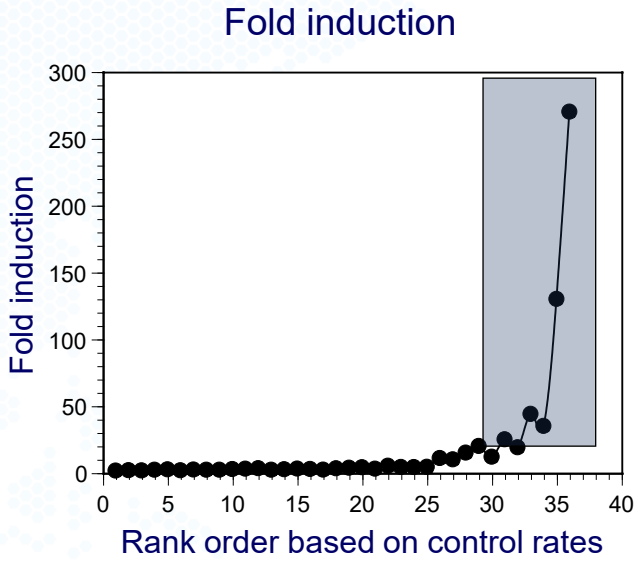
Time-dependent inhibition of CYP2C19 by omeprazole is readily detectable with HLM at 0.1 mg/mL with a 5-min substrate incubation period (right) but not at 1.0 mg/mL with a 30-min incubation period (left)

Areas of concern: CYP Induction study design

- CYP induction studies: positive controls with very large induction





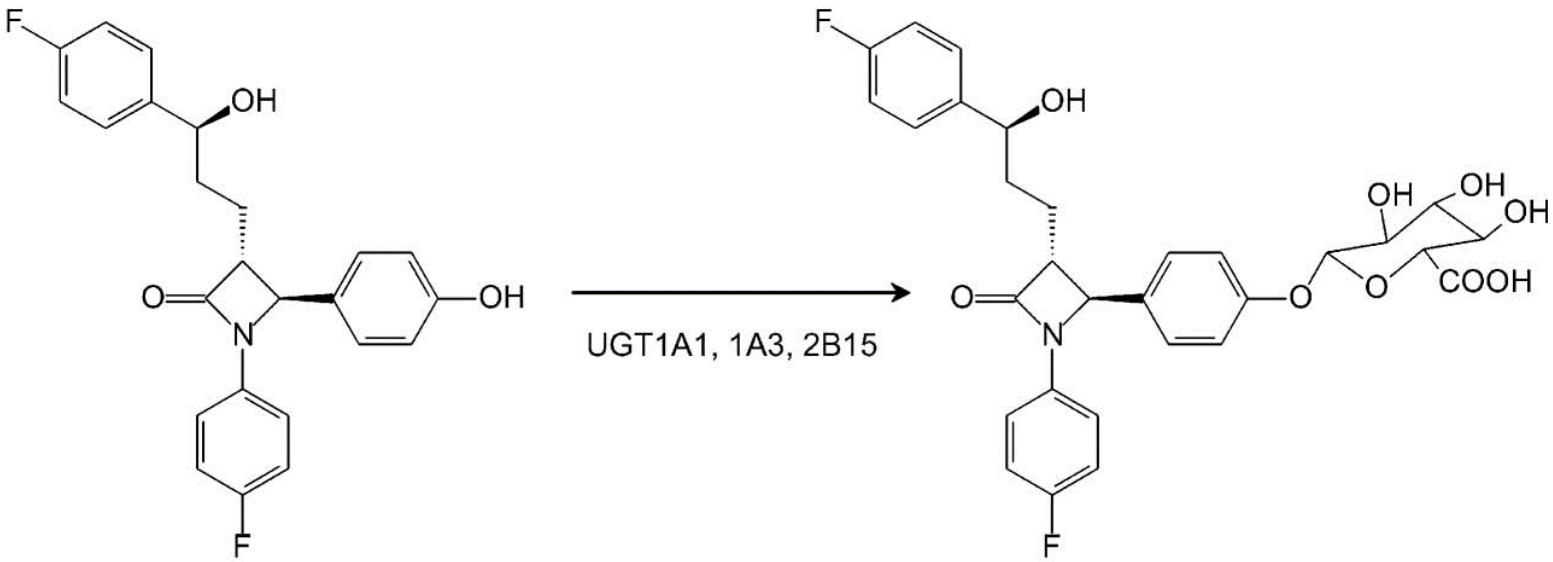


- When it comes to induction, more is not always better
- A high fold-induction (>20 fold) of CYP3A4 activity by rifampin is a sign of hepatocellular dedifferentiation of the cultured human hepatocytes

Areas of concern: Reaction phenotyping study design

Metabolism studies: Choose the right test system based on the structure

- Ezetimibe is oxidized by CYP3A4 however results with HLM & NADPH alone can be misleading.



Ezetimibe

Ezetimibe glucuronide

Oxidation does not occur clinically due to rapid phenolic glucuronidation. Recombinant human UGTs or human hepatocytes would be a better test system.
CYPs are not the only enzyme system.

Timing of ADME studies

Drug Development Pipeline – Timeline of *in vitro* DDI studies

Type of drug	Lead optimization	Pre-IND	Phase I to NDA
Typical small molecule	1. Comparative metabolism 2. Metabolite ID 3. Screening for others	1. CYP inhibition/Induction 2. Transporter inhibition 3. Limited transporter substrate	1. Reaction phenotyping 2. Additional transporter substrate (dependent on routes of elimination)
Small molecule with orphan, breakthrough status, etc.	1. Comparative metabolism	May be able to defer	1. Metabolite ID 2. CYP inhibition 3. Transporter inhibition 4. Reaction phenotyping 5. CYP induction
Peptides, oligos, ADCs, other biologics	May be able to defer	May be able to defer	1. Metabolite ID 2. CYP inhibition 3. Transporter inhibition 4. Reaction phenotyping 5. CYP induction

Priority depends on strategy for each drug & need for de-risking at each stage
FDA: "Collect enough DDI information to prevent patients from being unnecessarily excluded"

Conclusions: *In vitro* ADME & DDI studies

- Provide understanding of drug characteristics and insight concerning future performance in *in vivo* systems; notably concerning predictive toxicology, dose/species selection for IND enabling studies, and FIH trial considerations.
- Satisfaction of regulatory interests is critical for prevention of delays
- Prioritization varies based on drug class and program de-risking needs
- Conduct and interpretation can be deceptively simple; they both benefit expert design and understanding
- Provide as much information of the drug as possible for appropriate guidance

Thank you

Questions or Comments?