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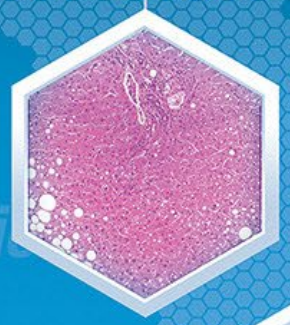
25 Years

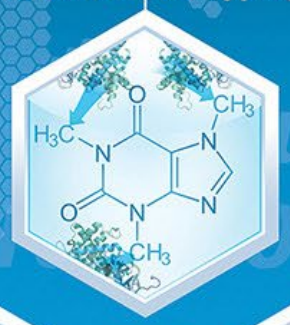
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

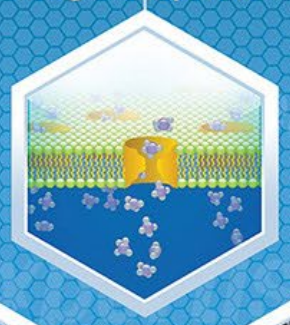


Reaction Phenotyping

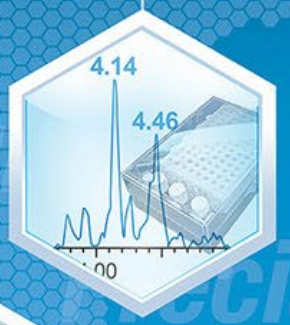


Enzyme Induction & Inhibition

Drug Transporters



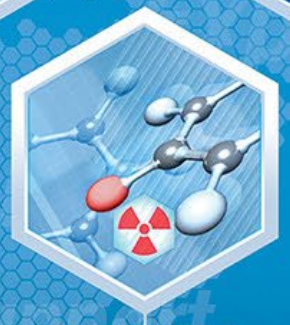
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

Welcome to the webinar...

We will begin shortly

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Design Intricacies and Decision-Making Strategy for Drug-Drug Interaction Studies

Andrew G. Taylor, Ph.D.
Manager of Technical Support for Services

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Design Intricacies and Decision-Making Strategy for DDI Studies

6 December 2022

Andrew G. Taylor, Ph.D.

Outline

- Why run these studies?
- Predominant types of *in vitro ADME* & Drug-Drug Interaction (DDI) studies & test systems
- Importance of proper study design & data interpretation
- When to conduct in vitro DDI ADME studies?
- Additional highlights and information

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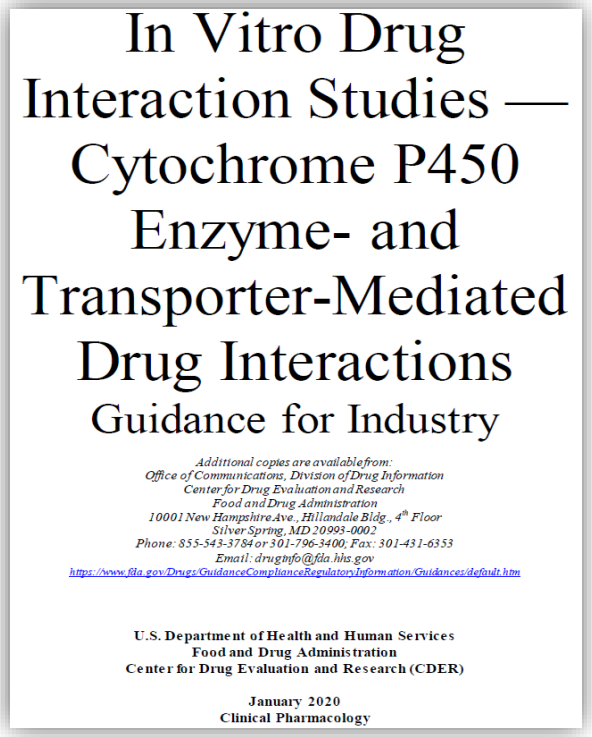
Regulatory Guidance

FDA: Final January 2020



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

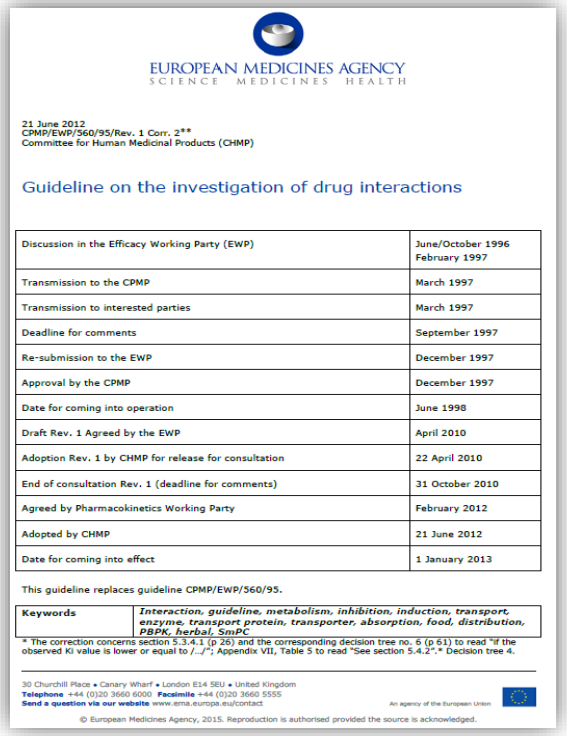
Guidance for Industry

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

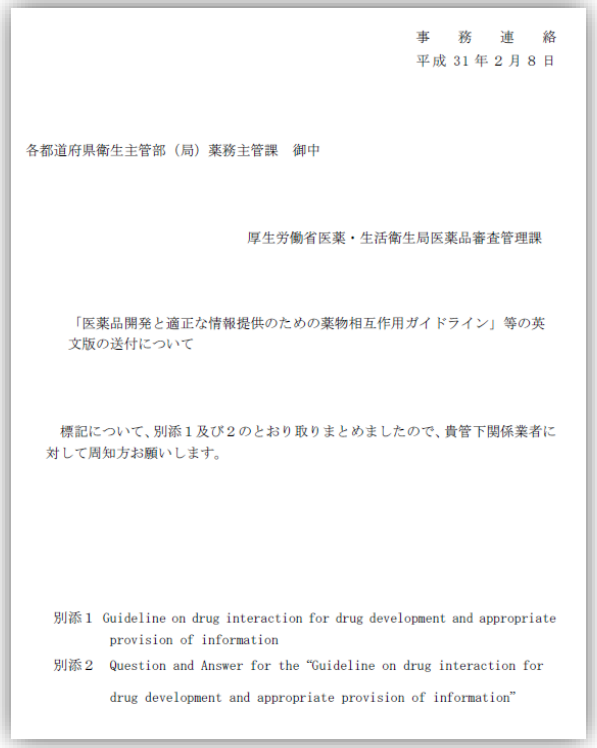
This guideline replaces guideline CPMP/EWP/560/95.

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
An agency of the European Union

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

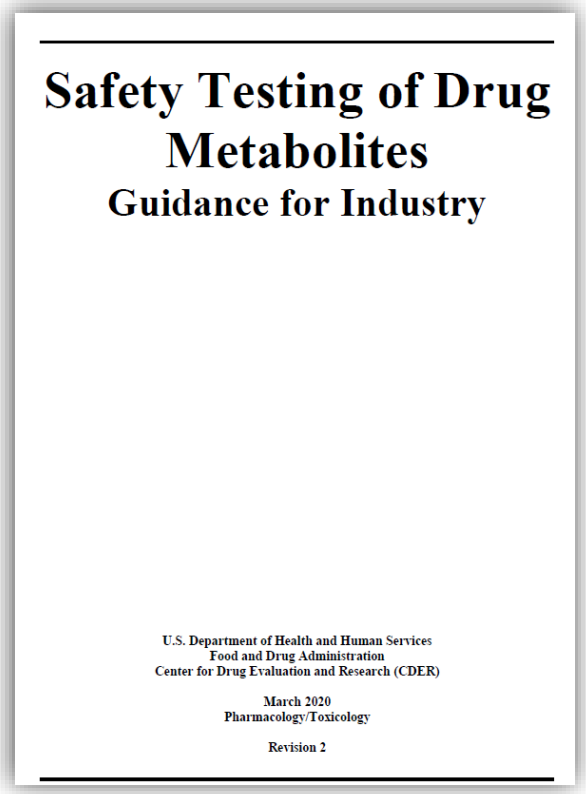
「医薬品開発と適正な情報提供のための薬物相互作用ガイドライン」等の英文版の送付について

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

FDA "MIST": Rev 2 March 2020



Safety Testing of Drug Metabolites

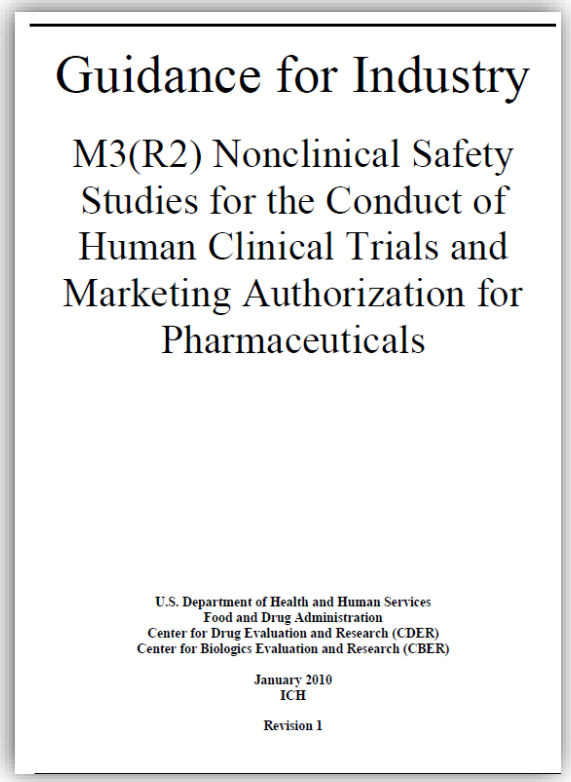
Guidance for Industry

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 / ICH M3: Final 2010



Guidance for Industry

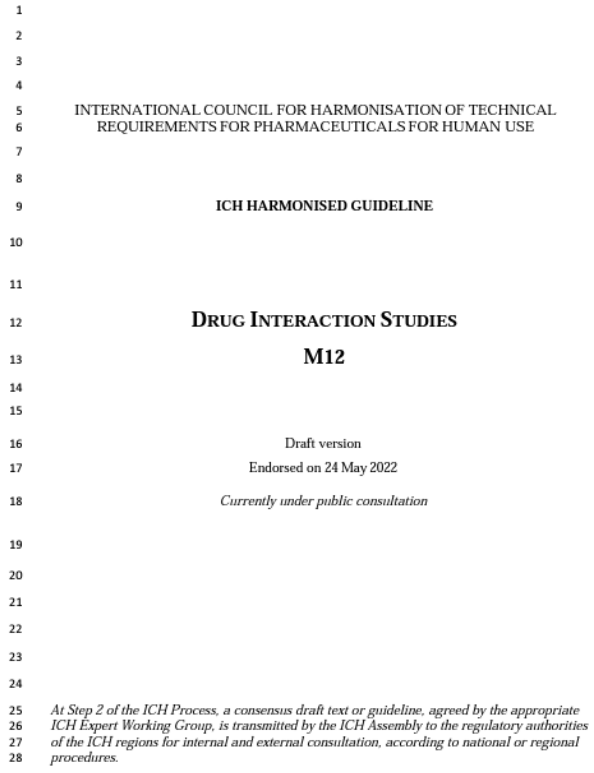
M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

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

ICH M12: Draft



INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DRUG INTERACTION STUDIES

M12

Draft version

Endorsed on 24 May 2022

Currently under public consultation

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

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In vitro ADME & DDI study types

ADME component	Type of in vitro study
Drug Metabolism (M, E)	1. Inter-species comparative metabolism – C_{lint} & $t_{1/2}$
	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

Test Systems for in vitro DDI Experiments

- **Subcellular fractions** – non-living, cell/tissue lysates that have been fractionated to enrich for certain enzyme activities.
  - **S9**
  - **Microsomes**
  - **Cytosol**
  - These subcellular fractions are derived from many relevant drug metabolizing organs/tissues. May require co-factors.
- **Cryopreserved primary hepatocytes** – living, isolated directly from living liver tissue and frozen for use at a later time. From multiple small animal models and human livers.

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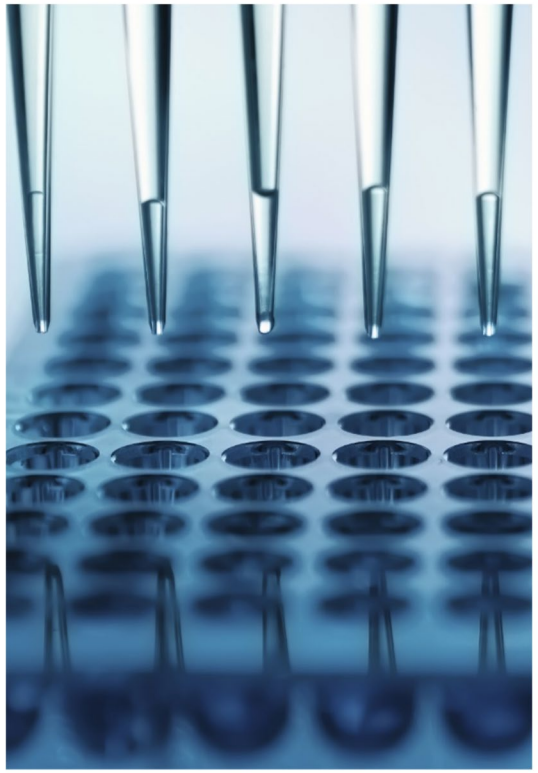
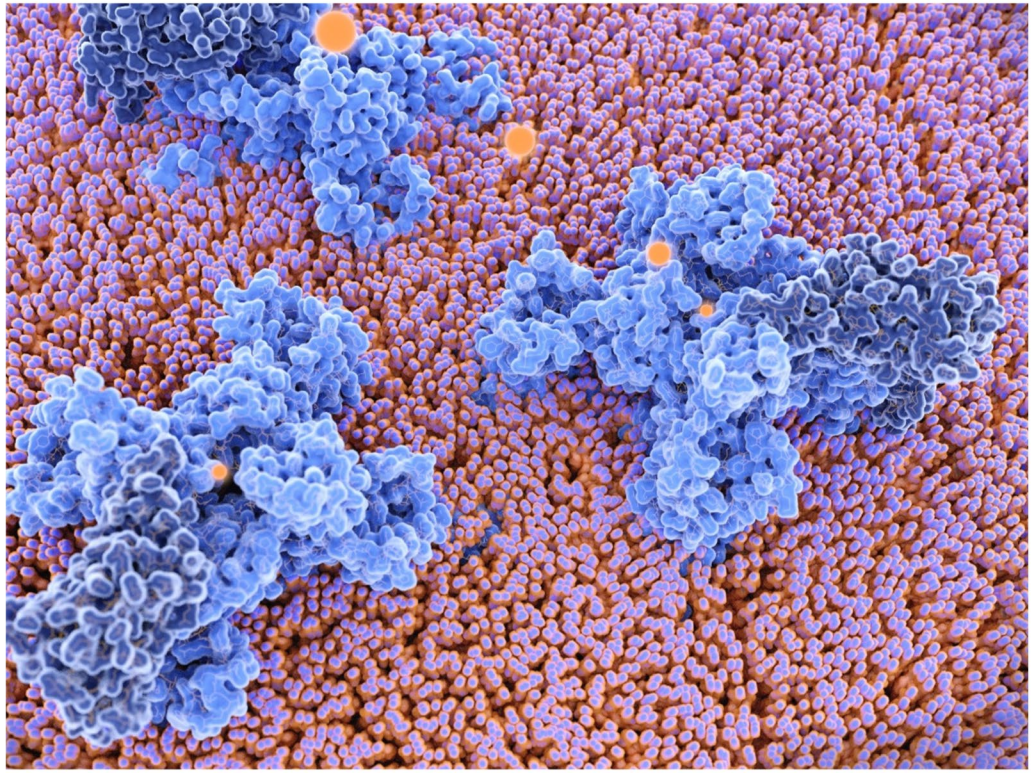
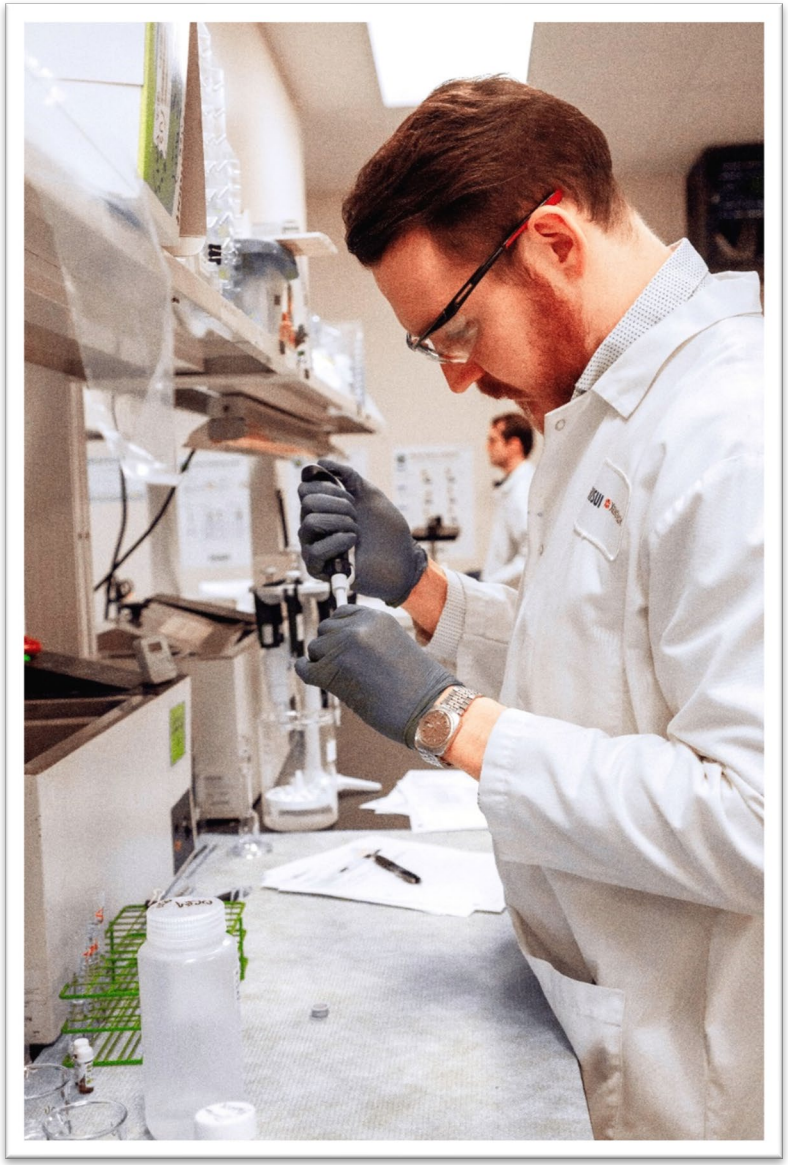
Drug Metabolism Studies

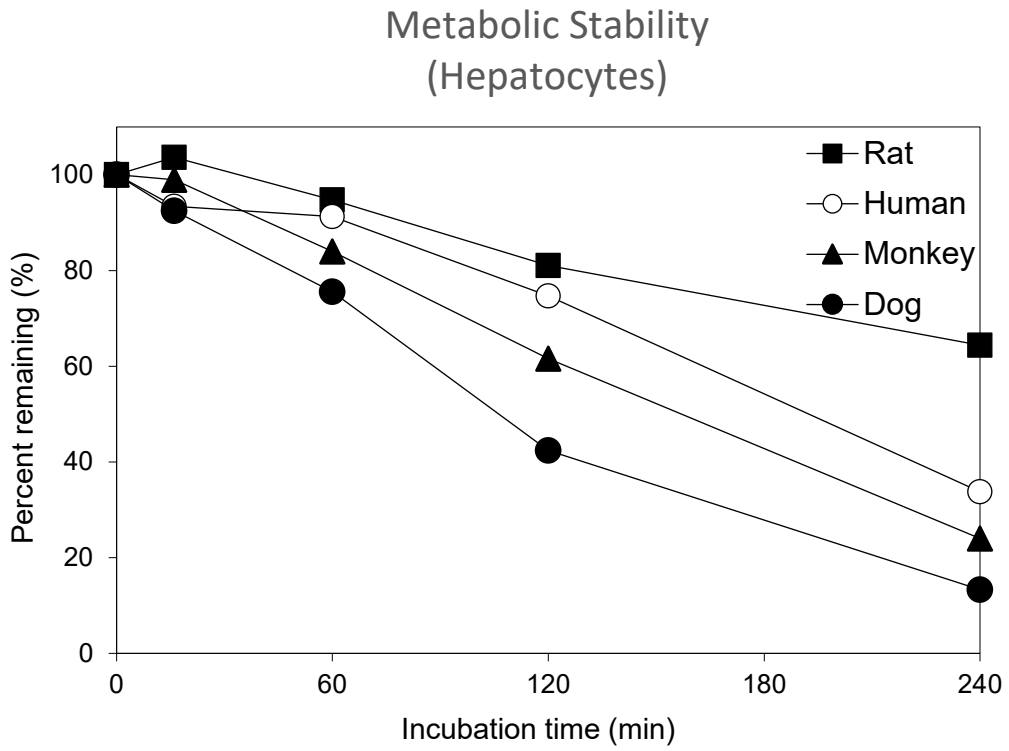


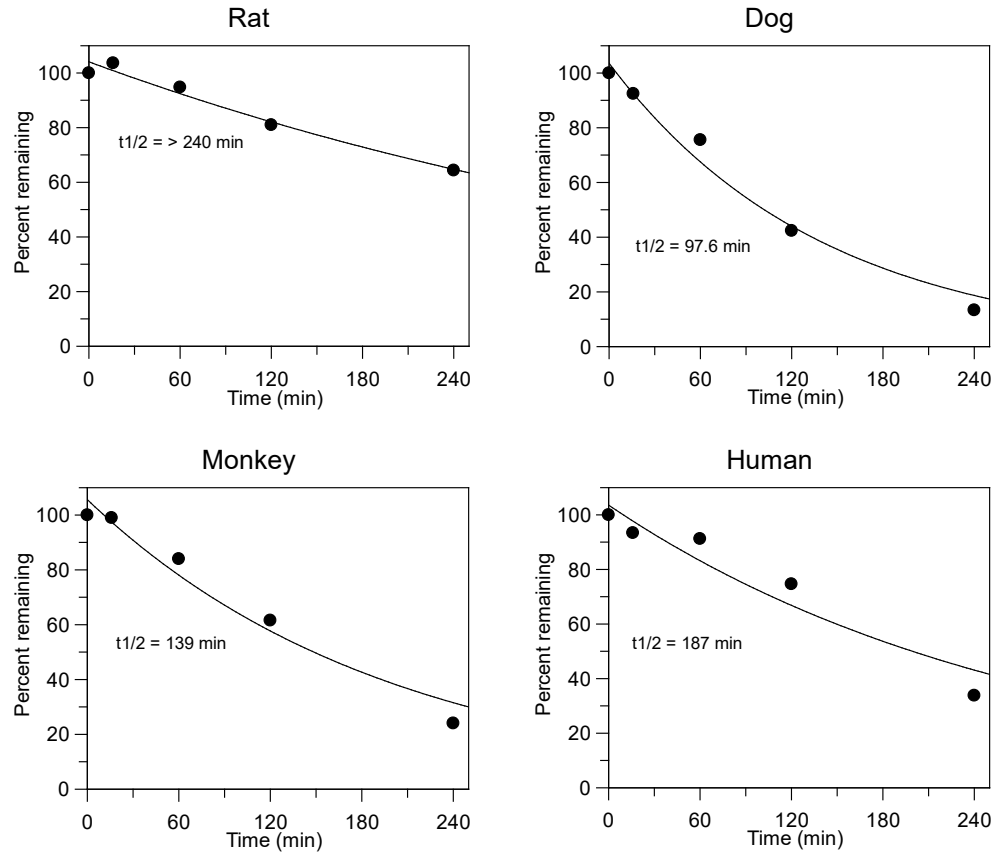




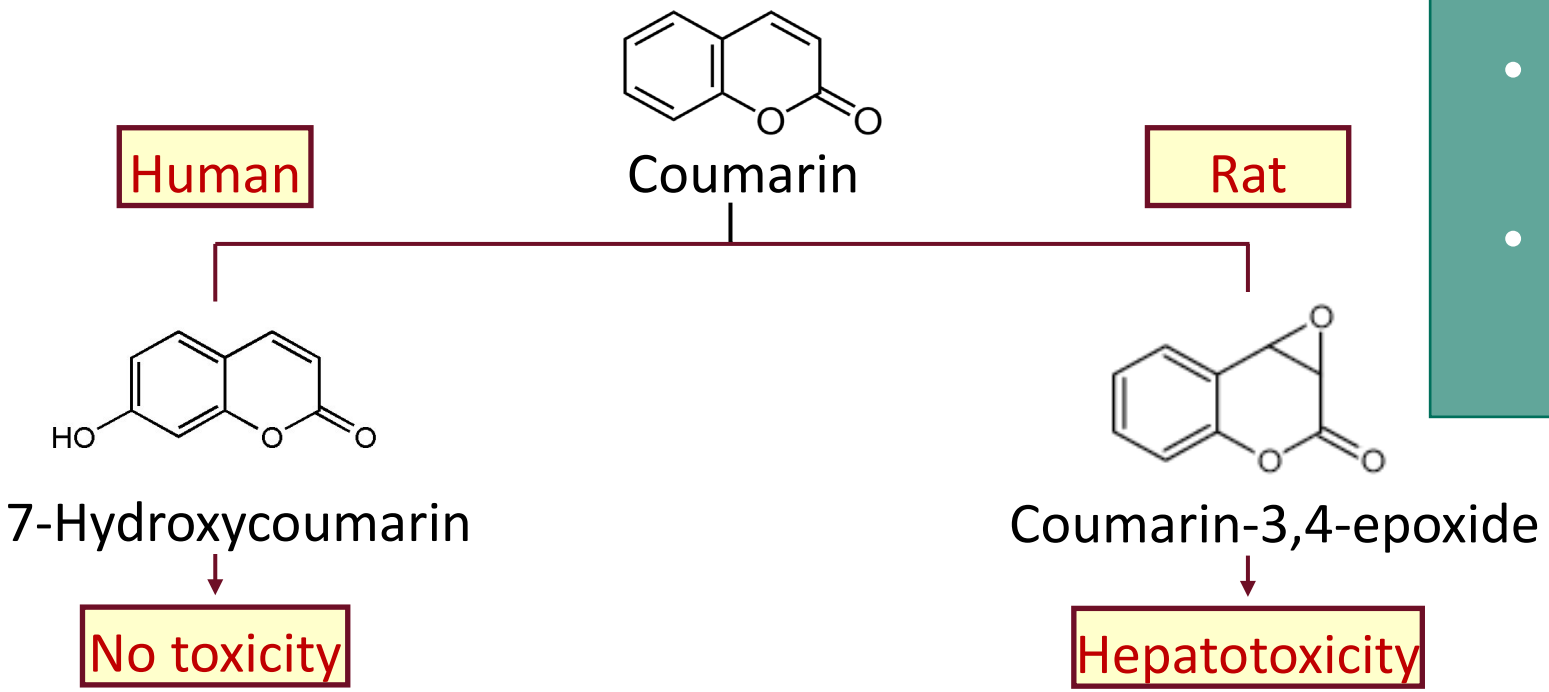
Drug Metabolism: Inter-Species Comparative Metabolism

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





Drug Metabolism: Inter-Species Comparative MetID



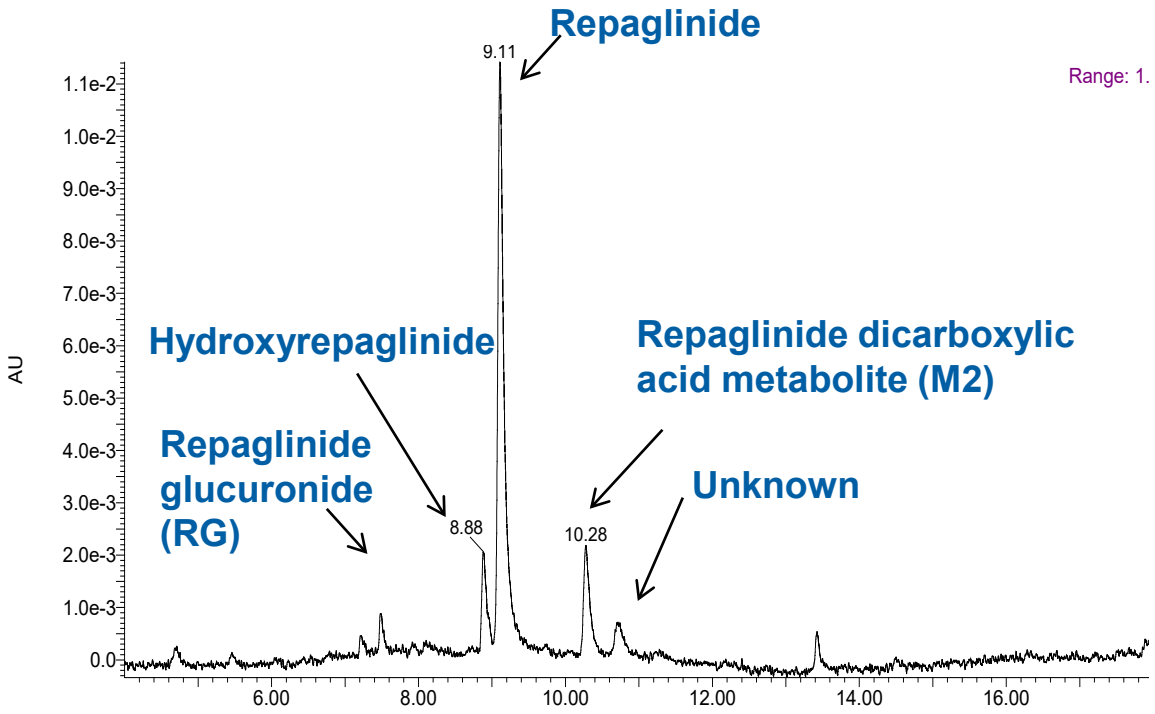
Goals:

- Complete profile of metabolites
- Are there human specific or disproportionate metabolites?
- Which other species have a similar metabolic profile?

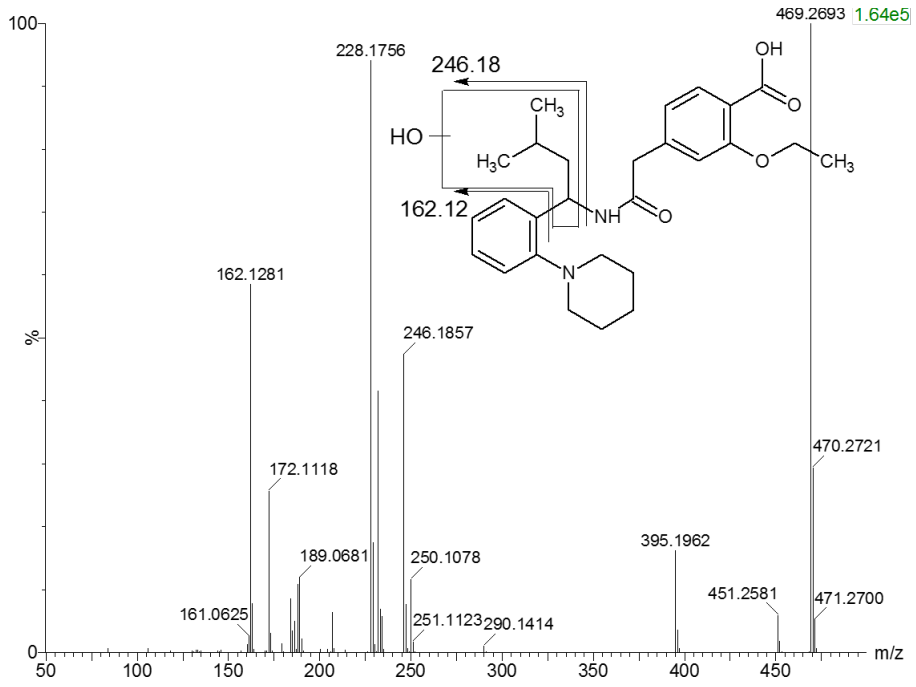
Metabolite Characterization & ID

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

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



Hydroxyrepaglinide LC-MS/MS



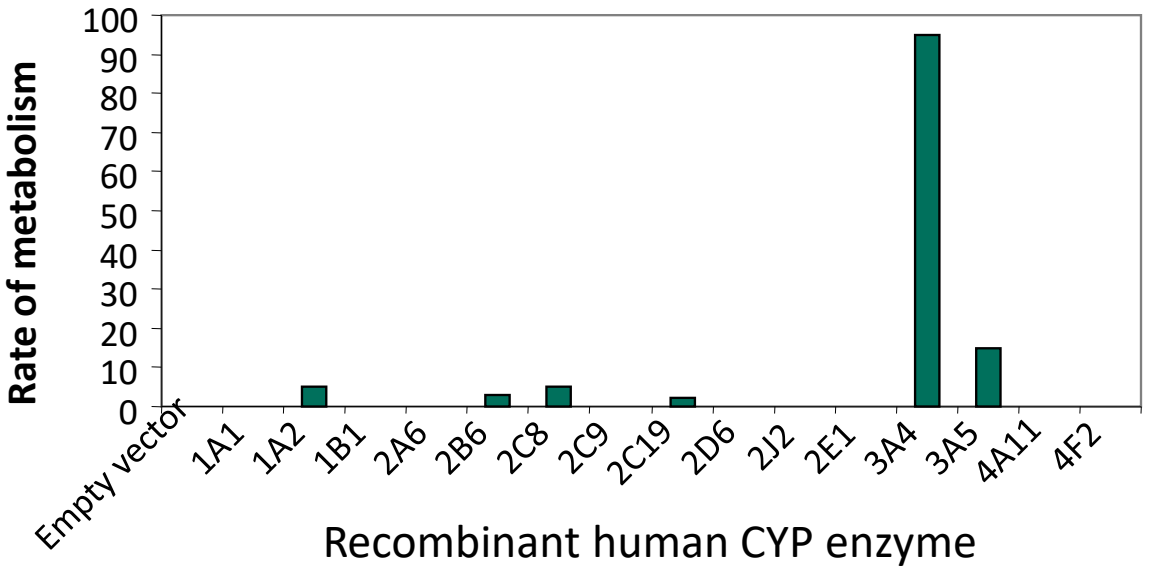
Cross-Species Met ID

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: CYP Reaction Phenotyping (Victim potential)

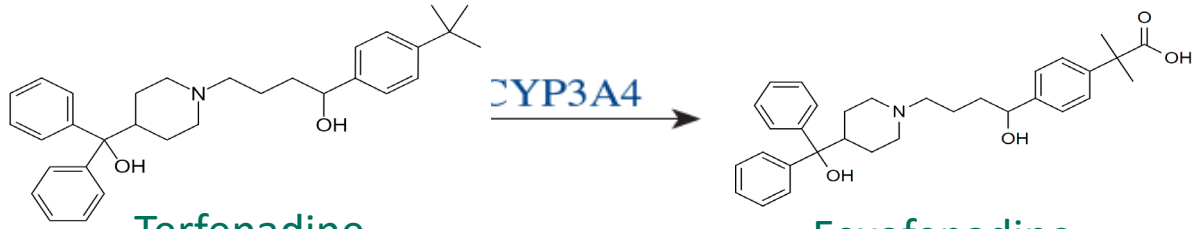
- Design: Incubate drug + recombinant human CYPs or human liver microsomes or hepatocytes ± selective inhibitors
- Goal: Determine which CYPs drive the metabolism of the drug
- Unique CYP metabolism is of concern

High DDI potential: few enzymes involved; Avoiding DDI, multiple pathways are preferred



Follow-up studies:

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



Terfenadine
(Seldane® - withdrawn)

Fexofenadine
(Allegra®)

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Drug Metabolizing Enzymes (Perpetrator Potential)

Enzyme Induction

Enzyme Inhibition

Drug Metabolizing Enzymes: CYP Inhibition

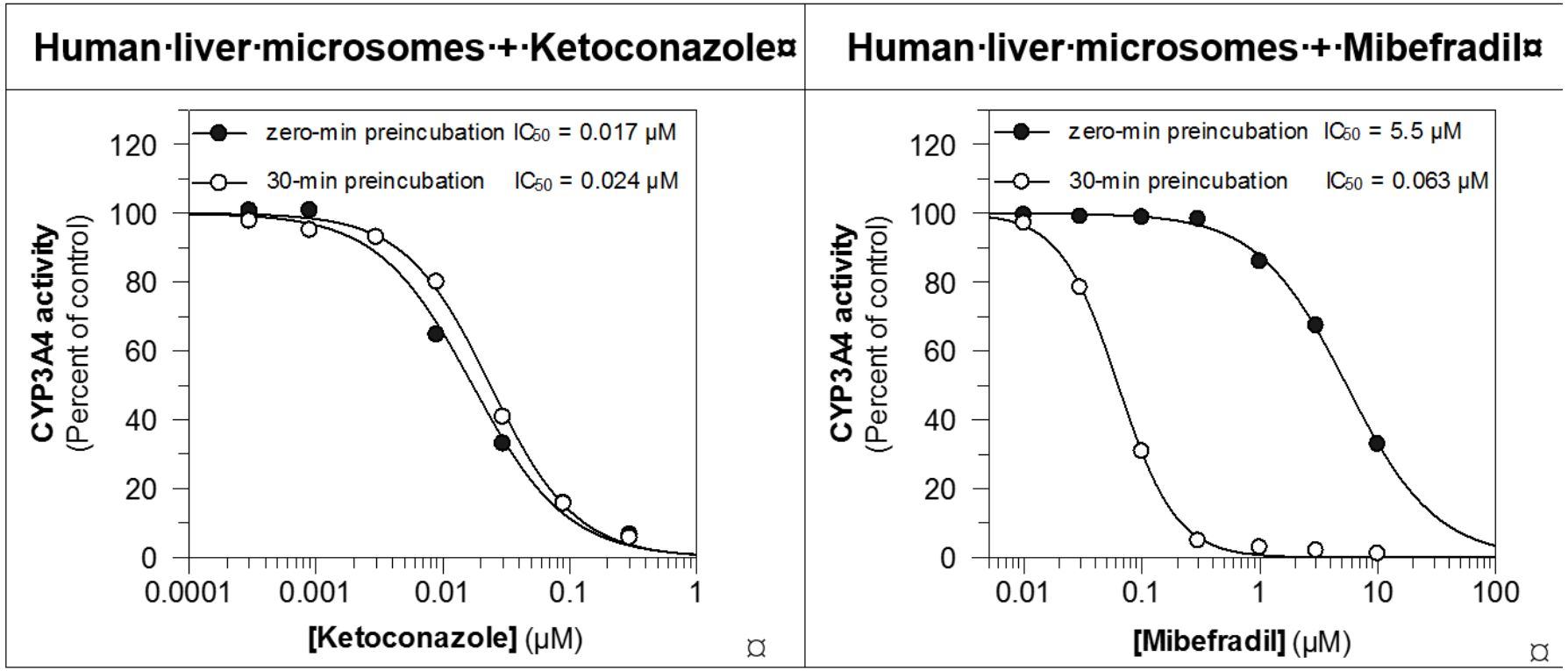
Design:
Drug incubations with HLM + marker substrate ± pre-incubation

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

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



Ketoconazole:

Potent inhibitor of 3A4 precludes coadministration of other drugs

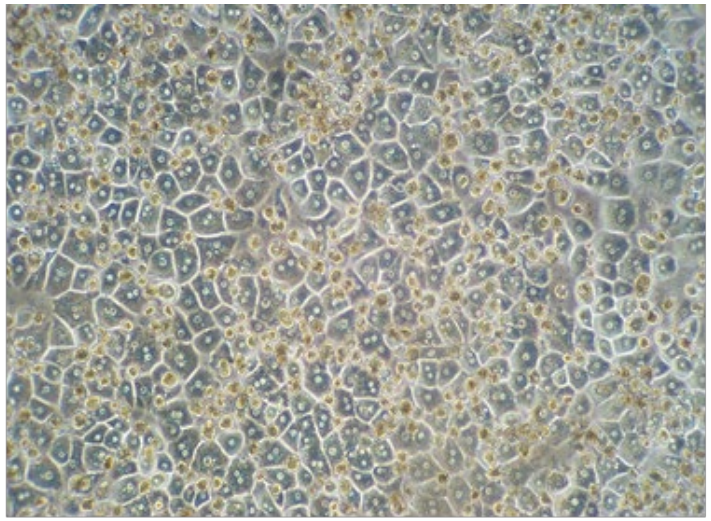
Mibefradil

Removed from market in 1998 due to potential for fatal DDIs

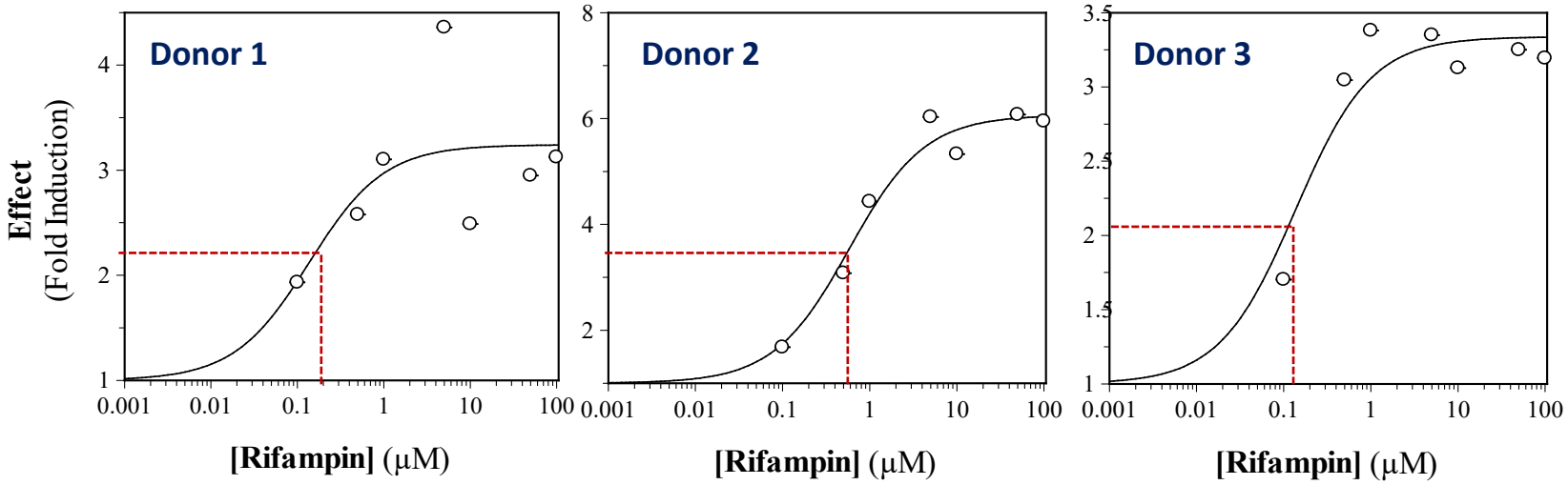
TDI has greater DDI risk

Drug Metabolizing Enzymes: CYP Induction

- Design: Drug incubations in cultured human hepatocytes, measure mRNA (or activity) of various CYPs
- Goal: Assess induction of CYP enzymes
- For both inhibition and induction studies, basic or mechanistic models are further utilized to determine the clinical DDI potential of a drug and requirement of a clinical DDI study.



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

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Drug Transporters

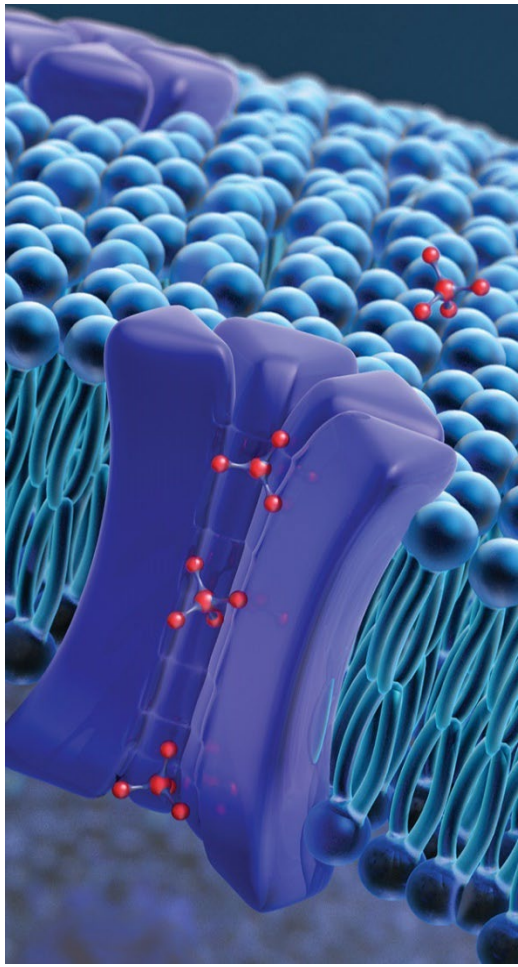
Substrate Potential (Victim)

Transporter Inhibition (Perpetrator)

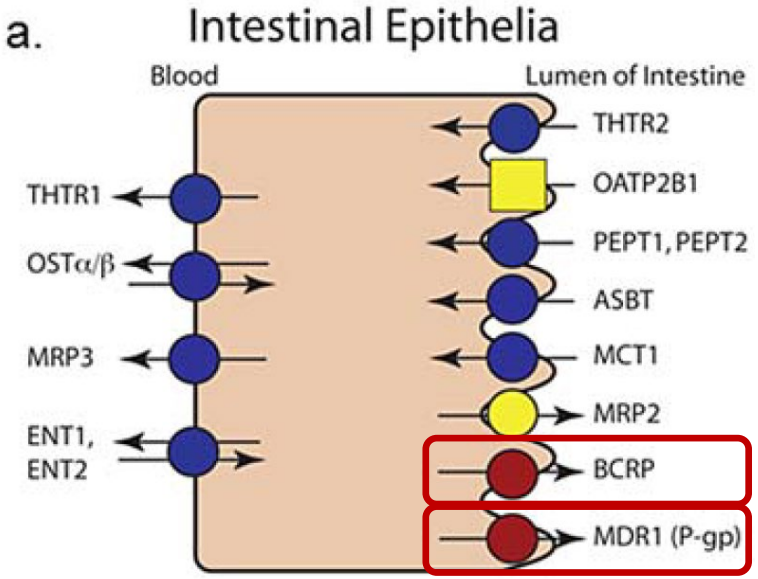
"Why conduct drug transporter DDI studies?"

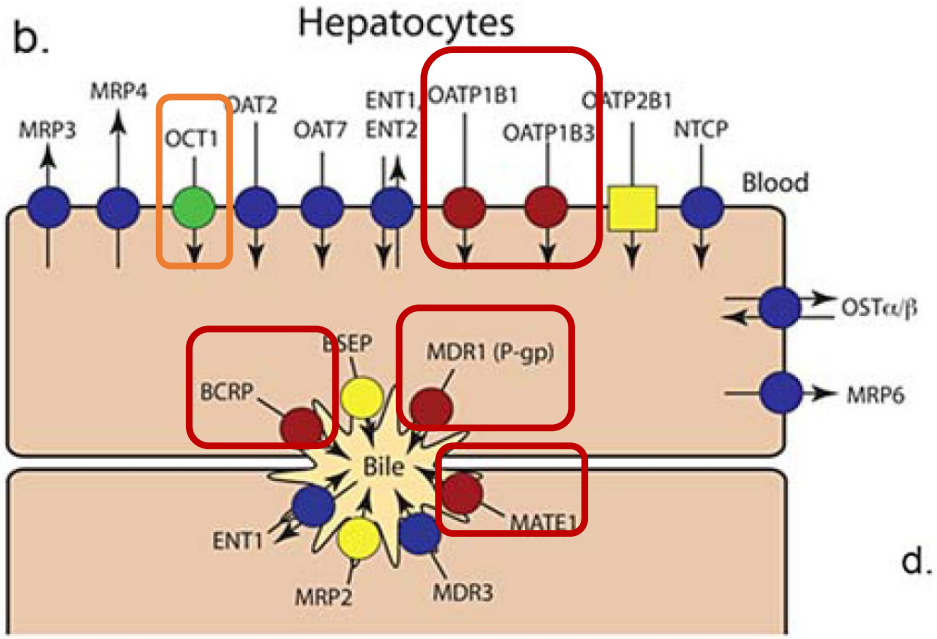
ADME

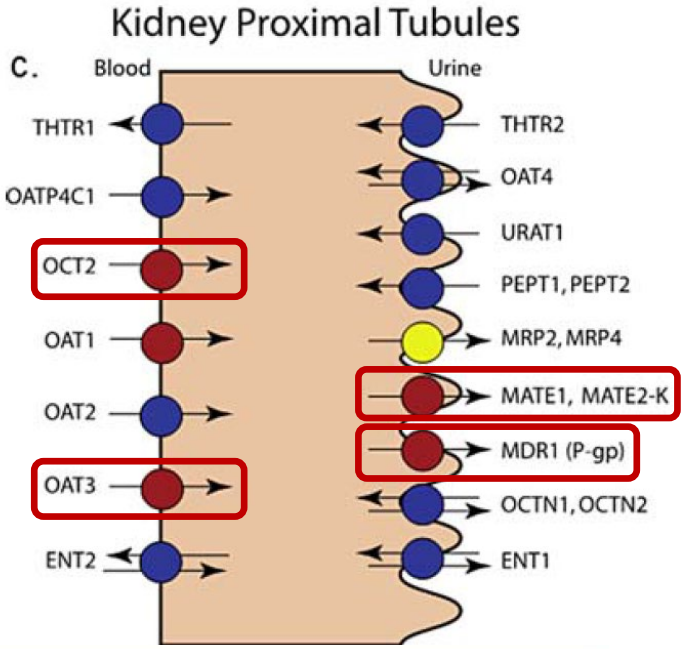
- Drug absorption, distribution, tissue-specific drug targeting, and elimination
- Drug-drug interactions
  - Clearance of transporter substrates (Victims) can be impacted by transporter inhibitors or inducers (Perpetrators)
  - Toxicity or loss of efficacy
- Real world example - Statins
  - Hepatic uptake transporter (OATPs) substrates: taken up in the liver, reduce cholesterol
  - Cyclosporine inhibits OATPs: up to 10-fold increase in statin exposure
  - Toxic side effect: rhabdomyolysis (skeletal muscles break down, cells released into bloodstream, can lead to kidney failure and possibly death)

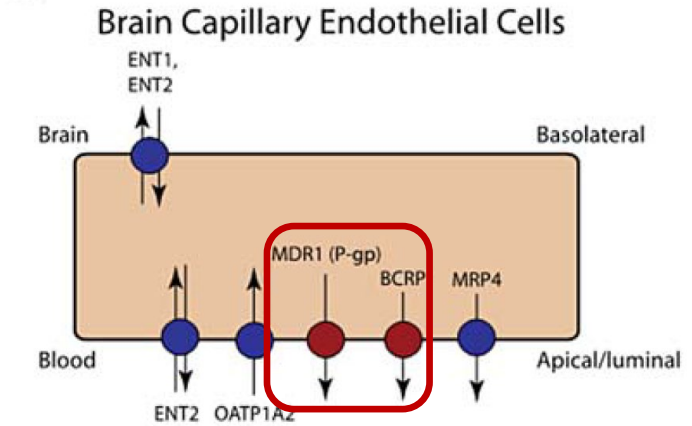


Transporter studies









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

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Importance of proper study design & data interpretation for in vitro DDI studies

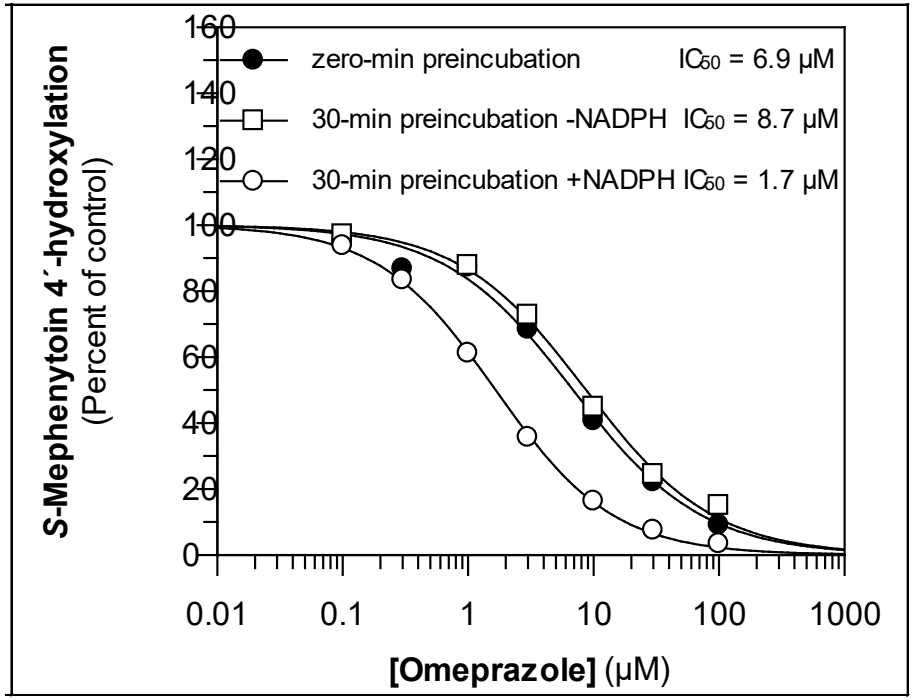
Importance of study design: CYP inhibition study

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

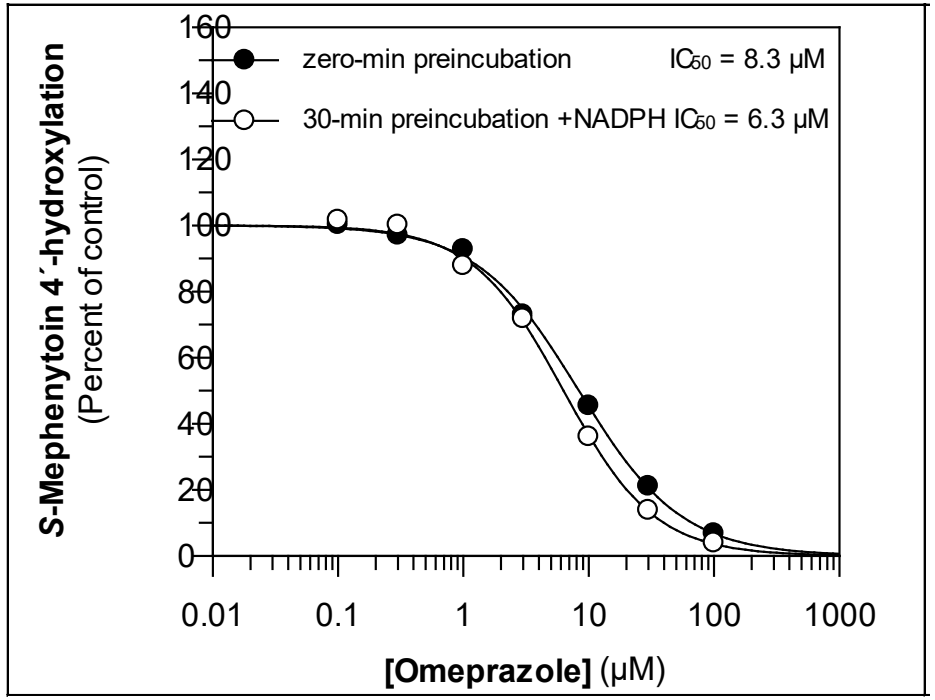
Detected

0.1 mg/mL protein, 5 min substrate incubation



Missed

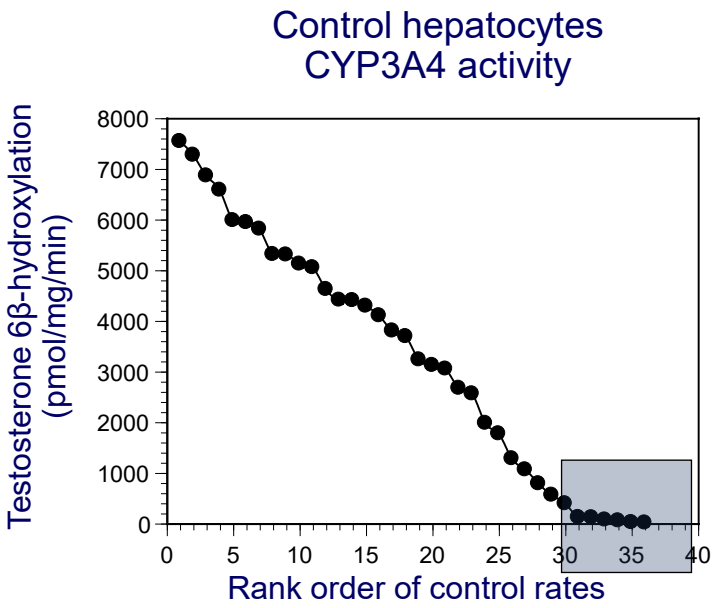
1 mg/mL protein, 30 min substrate incubation

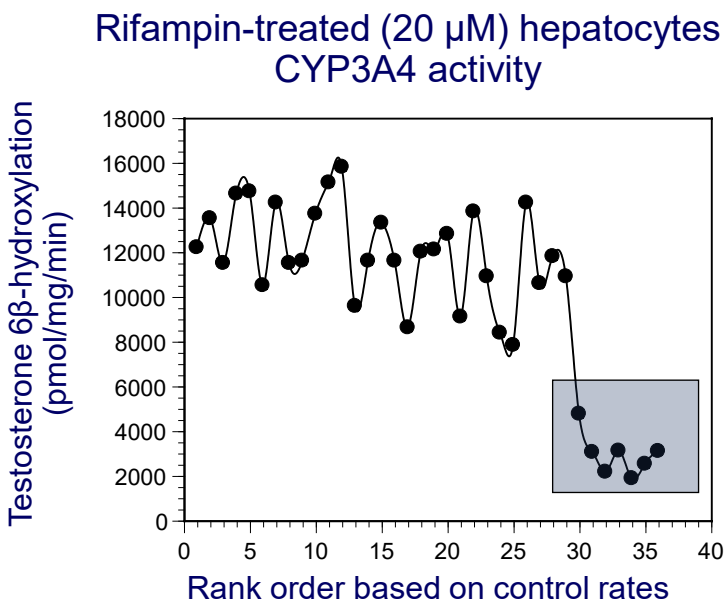


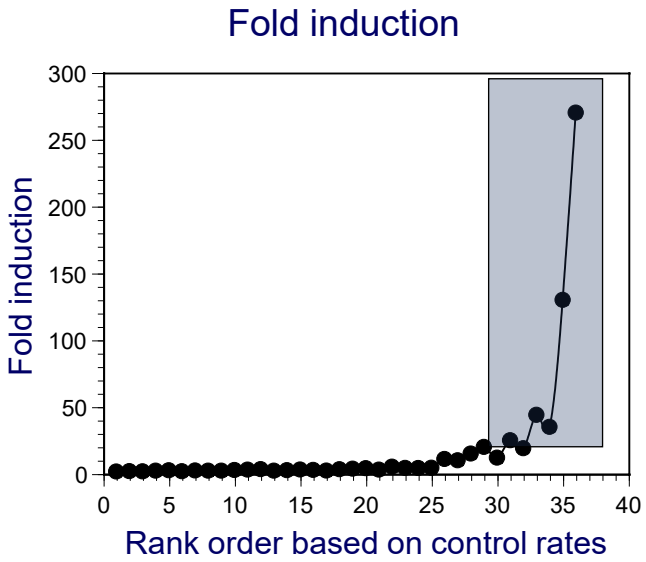
Time-dependent inhibition of CYP2C19 by omeprazole is readily detectable with HLM at 0.1 mg/mL with a 5-min substrate incubation period **(left)** but not at 1.0 mg/mL with a 30-min incubation period **(right)**

Proper test system selection: CYP Induction study

CYP induction studies: positive controls with very large induction





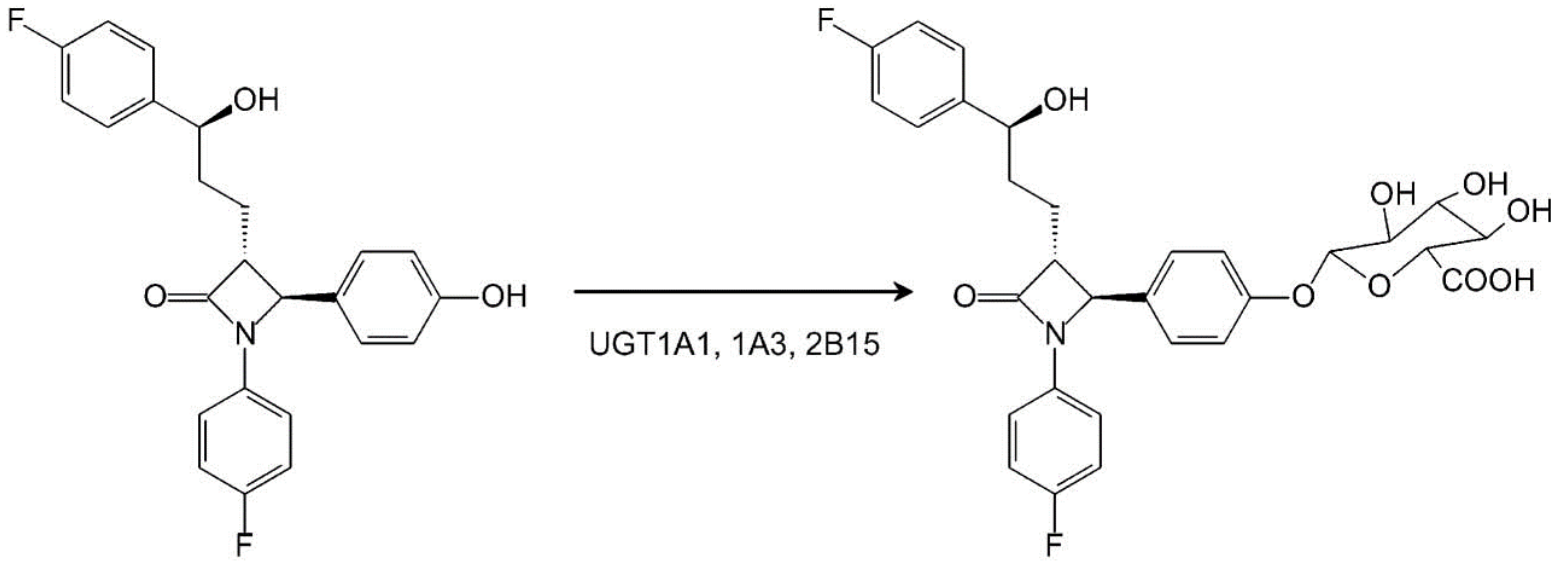


- When it comes to induction, more is not always better
- A high fold-induction (>20 fold) of CYP3A4 activity by rifampin is a sign of incomplete hepatocellular differentiation of the cultured human hepatocytes

Proper test system selection : Reaction phenotyping

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.

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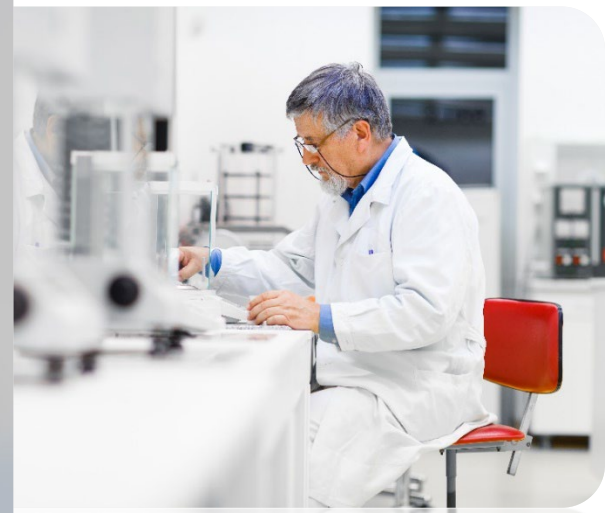
Timing of ADME studies



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Drug Development Pipeline: Timing *in vitro* DDI studies

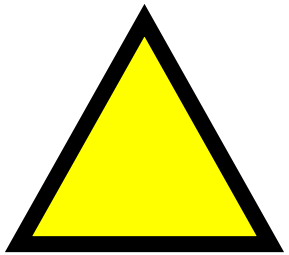
Priority depends on strategy for each drug & need for de-risking at each stage

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 2. CYP Induction 3. Transporter inhibition 4. 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

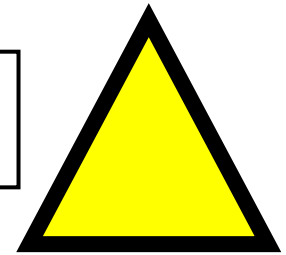
“Why conduct these studies? Is this just box checking?”

No. The information in aggregate has real utility:

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 can inform go/no-go decisions for a drug candidate
   - Predictive toxicology and dose selection for certain non-clinical *in vivo* studies
2. Prepare for clinical studies
   - Prediction of FIH dose and DDI risk
3. Satisfy regulatory expectations and comply with regulatory guidance



These studies may appear deceptively simple – maximizing insight can be complex



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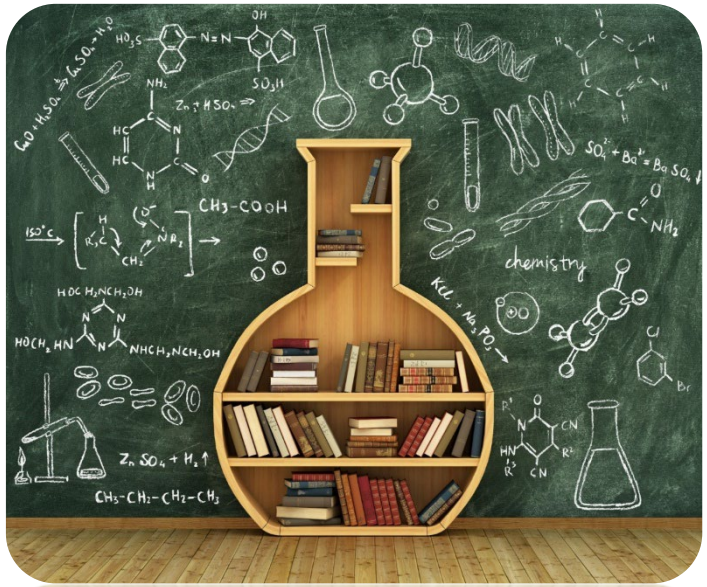
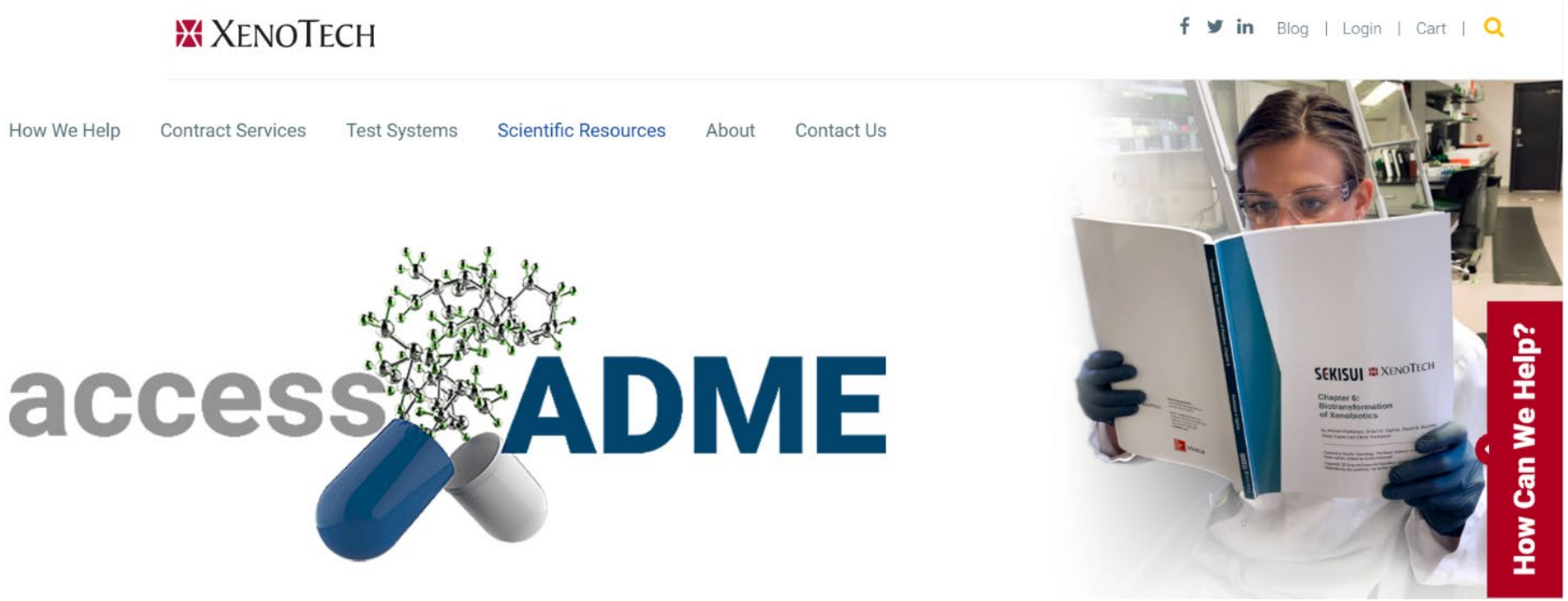
For questions or further resources:

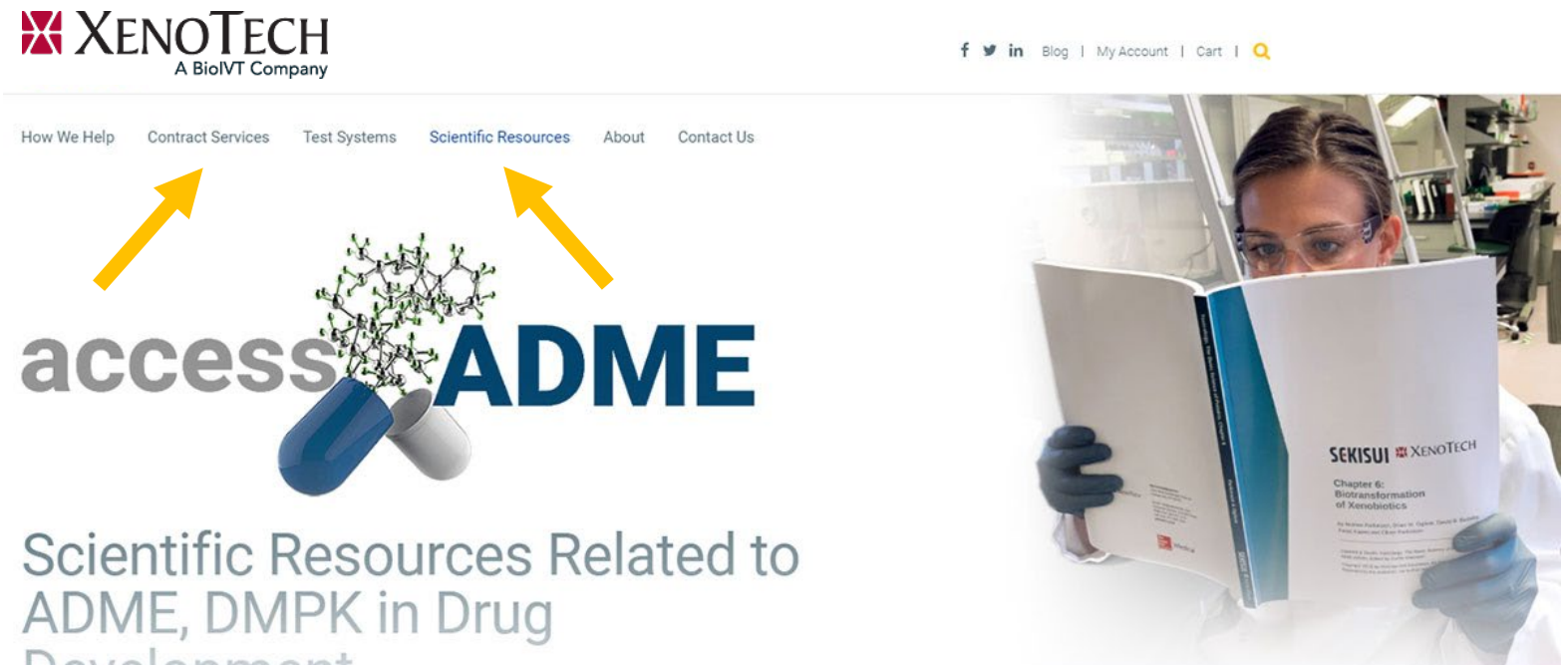




Further Resources

https://www.xenotech.com/access-adme-research-resources/

- Videos
- Webinars
- Blogs
- And more!



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Webinar Topic Request Form:
www.xenotech.com/scientific-resources/upcoming-webinars

XenoTech's Services & Products:

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- Metabolite Identification
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In Vivo ADME/PK & Distribution
- QWBA
- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

Bioanalytical Pharmacology
- In Vitro Ligand Binding & Radioreceptor Assays
- Immunoassays

Chemical Synthesis
- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

Consulting...

Cellular Products
- Hepatocytes (Cryo/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions
- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

Custom Products
- Various Species, Tissues & Preparations

Research Biobank
- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

JCRB Cell Lines...