In Vitro ADME & Drug-Drug Interaction Considerations for Toxicologists

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SEKISUI XenoTech Director of Scientific Consulting
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

**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., H心中 Bldg., 4 Floor
Silver Spring, MD 20990-0002
Phone: 855-543-7884 or 1-800-796-3400, Fax: 1-811-6353
Email: druginfo@dhs.mil.gov


**Guideline on the investigation of drug interactions**

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discussion in the EWP</td>
<td>June/July 96</td>
</tr>
<tr>
<td>Transmission to the CHMP</td>
<td>March 1997</td>
</tr>
<tr>
<td>Transmission to interested parties</td>
<td>March 1997</td>
</tr>
<tr>
<td>Deadline for comments</td>
<td>September 97</td>
</tr>
<tr>
<td>Re-submission to the EWP</td>
<td>December 97</td>
</tr>
<tr>
<td>Approval by the CHMP</td>
<td>December 97</td>
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<tr>
<td>Data for coming into operation</td>
<td>June 1998</td>
</tr>
<tr>
<td>Draft Rev. 1 agreed by the EWP</td>
<td>April 2010</td>
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<tr>
<td>Adoption Rev. 1 by CHMP for release for consultation</td>
<td>24 April 2010</td>
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<tr>
<td>End of consultation Rev. 1 (deadline for comments)</td>
<td>31 October 2010</td>
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<tr>
<td>Agreed by Pharmacokinetics Working Party</td>
<td>February 2012</td>
</tr>
<tr>
<td>Adopted by CHMP</td>
<td>21 June 2012</td>
</tr>
<tr>
<td>Date for coming into effect</td>
<td>1 January 2012</td>
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</table>

This guideline replaces guideline CPMP(E/EW/540).585.

Keywords: interactions, guidelines, metabolism, inhibition, interaction, transport, impact, pharmacokinetics, transport, absorption, distribution, food, administration.

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**FDA: Final January 2020**

**EMA: Final 2013**

**PMDA: Final 2019**

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**Clinical Pharmacology**

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**IN VITRO – IN VIVO CONTRACT RESEARCH & TEST SYSTEMS**
Additional Guidance

Safety Testing of Drug Metabolites
Guidance for Industry

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

FDA / ICH: Final 2010
Compounds are evaluated for ADME properties

- Absorption – Drug Transporters, passive diffusion
- Distribution – Drug Transporters, passive diffusion
- Metabolism – Drug Metabolizing Enzymes (CYP450s, UGTs, etc.)
- Excretion – Drug Metabolizing Enzymes and Drug Transporters

Drug-Drug Interaction

# In vitro ADME & DDI study types

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

**Metabolic Stability (Hepatocytes)**

**Rat**
- $t_{1/2} = > 240$ min

**Dog**
- $t_{1/2} = 97.6$ min

**Monkey**
- $t_{1/2} = 139$ min

**Human**
- $t_{1/2} = 187$ min
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?

**Diagram:**
- **Human**
  - 7-Hydroxy coumarin
  - No toxicity
- **Rat**
  - Coumarin-3,4-epoxide
  - Hepatotoxicity
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

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention time (min)</th>
<th>Mass shift</th>
<th>Proposed biotransformation</th>
<th>Mouse</th>
<th>Rat</th>
<th>Dog</th>
<th>Pig</th>
<th>Human</th>
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<tbody>
<tr>
<td>C1</td>
<td>3.43</td>
<td>255.9889</td>
<td>Sulfation + glucuronidation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C2</td>
<td>3.63</td>
<td>354.0783</td>
<td>Di-glucuronidation + hydrogenation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C3</td>
<td>3.78</td>
<td>159.9135</td>
<td>Di-sulfation</td>
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<td>+</td>
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<td>4.00</td>
<td>258.0045</td>
<td>Sulfation + glucuronidation + hydrogenation</td>
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<td>C5</td>
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<td>194.0428</td>
<td>Glucuronidation + oxygenation + hydrogenation</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
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</tr>
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</table>

*ND* indicates not determined.
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

<table>
<thead>
<tr>
<th>CYP</th>
<th>Activity Assay</th>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin O-dealkylation</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion hydroxylation</td>
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<tr>
<td>CYP2C8</td>
<td>Amodiaquine N-dealkylation</td>
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<tr>
<td>CYP2C9</td>
<td>Diclofenac 4′-hydroxylation</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4′-hydroxylation</td>
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<tr>
<td>CYP2D6</td>
<td>Dextromethorphan O-dealkylation</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Testosterone 6β-hydroxylation</td>
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<tr>
<td>CYP3A4</td>
<td>Midazolam 1′-hydroxylation</td>
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</table>
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](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
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<tr>
<td>Emax</td>
<td>3.2417</td>
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</tr>
<tr>
<td>EC50</td>
<td>0.1371</td>
<td>0.1427</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
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<td>Emax</td>
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<td>EC50</td>
<td>0.5795</td>
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<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
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<tr>
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<tr>
<td>EC50</td>
<td>0.1362</td>
<td>0.0528</td>
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</table>
Drug Transporters
(Victim and Perpetrator potential)
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

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

**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)

**Missed**
- 1 mg/mL protein, 30 min substrate incubation
  - 1.3-fold shift
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.

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

<table>
<thead>
<tr>
<th>Type of drug</th>
<th>Lead optimization</th>
<th>Pre-IND</th>
<th>Phase I to NDA</th>
</tr>
</thead>
</table>
| **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
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- Drug Transport
- Drug Metabolism
- Enzyme Inhibition & Induction
- Protein Binding
- Metabolite Identification
- ADME Screening
- Toxicology

**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 (CryolFresh, 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**

**Metabolite Production Kits**

**JCRB Cell Lines...**