Are in vitro metabolism and DDI studies critical for an IND?

AAPS 2019 Pharmsci 360, San Antonio, Texas
04 November 2019
Booth 1206
Greg Loewen, Sekisui-XenoTech
gloewen@xenotechllc.com
Road Map

• IND – Short discussion
• Guidance documents – IND and DDI
• In Vitro Assays
  – Permeability assays
  – Protein binding
  – Metabolic stability
  – Cross species metabolite identification
• Drug-drug interaction assessment
  – Reaction phenotyping
  – Inhibition
  – Induction
  – Drug Transporters
What is an IND?

- IND = Investigational New Drug
- 21CFR Part 312 (Investigational New Drug Application)
- Scope: “This part contains procedures and requirements governing the use of investigational new drugs, including procedures and requirements for the submission to, and review by, the Food and Drug Administration of investigational new drug applications (IND's). An investigational new drug for which an IND is in effect in accordance with this part is exempt from the premarketing approval requirements that are otherwise applicable and may be shipped lawfully for the purpose of conducting clinical investigations of that drug.”

Link to eCFR 21Part312
IND Content

- Chemistry, Manufacturing, and Controls (CMC)
  - Information on the drug substance and drug product pertaining to the composition, manufacturer, stability, and controls

- Clinical Protocols Investigator Information
  - Detailed protocols for clinical studies, qualifications of the clinical investigators, commitments to obtain informed consent. Assess the potential to expose subjects to unnecessary risks.

- Pharmacology and Toxicology Information (Pharm/Tox)
  - Summary of toxicology findings (in vitro or animal studies) to support use in humans
  - Description of the pharmacologic effects and mechanisms of action, secondary pharmacology, safety pharmacology

  - Pharmacokinetics: Information on the absorption, distribution, metabolism and excretion (ADME)

Guidance for Industry

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

In Vitro Metabolism- and Transporter-Mediated Drug-Drug Interaction Studies Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology, Guidance and Policy Team at CDER_OCP_GPT@fda.hhs.gov.

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

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

October 2017
Clinical Pharmacology
Section III. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

- In vitro **metabolic and plasma** protein binding data for animals and humans should be evaluated before initiating human clinical trials.

- ...and in vitro biochemical information relevant to **potential drug interactions** should be available before exposing large numbers of human subjects or treating for long duration.

- Compare human and animal **metabolites**...

- **Nonclinical characterization of a human metabolite(s)** is only warranted when ... greater than **10 percent** of total drug-related exposure and **at significantly greater levels in humans** than the maximum exposure seen in the toxicity studies.
Permeability (Absorption and Excretion)

In vitro permeability to predict absorption, bioavailability, tissue distribution and routes of excretion

- **Test system**: polarized cells grown on transwell plates
- **Permeability** measured in the A to B and B to A directions. Efflux Ratio > 2 indicates a transporter interaction
- **Oral bioavailability**: Determined by absorption and metabolism
  - Absorption includes disintegration, dissolution and permeation
  - Metabolism: first pass in the intestine and liver
- **Bio Waiver**: Waiver of in vivo bioavailability and bioequivalence... for class I compounds. Based on high solubility and high permeability

<table>
<thead>
<tr>
<th>Test Systems</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco-2</td>
<td>Human colorectal carcinoma derived cell line – intestinal permeability</td>
</tr>
<tr>
<td>MDCKII and LLC-PK1</td>
<td>Canine or porcine derived cell line - Passive permeability</td>
</tr>
<tr>
<td>MDCKII-MDR1, LLC-PK1-MDR1</td>
<td>Transfected with human transporters – transporter interaction or tissue specific permeability</td>
</tr>
<tr>
<td>PAMPA</td>
<td>Artificial membrane – passive permeability</td>
</tr>
</tbody>
</table>

Diagram:
- **Apical**
- **Basal**
Permeability, more than absorption
BCS, BDDCS and ECCS

Class 1
High solubility
Metabolism

Class 2
Low solubility
Metabolism

Class 3
High Solubility
Elimination of Unchanged Drug

Class 4
Low Solubility
Elimination of Unchanged Drug

Class 1A
Clearance: metabolism (≥70%). Eliminated as metabolites (≥70%). Is absorption permeability limited: NO

Class 1B
Clearance: hepatic uptake (≥70%). Eliminated as metabolites (≥70%). Is absorption permeability limited: NO

Class 2
Clearance: metabolism (≥70%). Eliminated as metabolites (≥70%). Is absorption permeability limited: NO

Class 3A
Clearance: renal (≥70%). Eliminated as parent in urine (≥70%). Is absorption permeability limited: YES

Class 3B
Clearance: hepatic uptake or renal (≥70%). Eliminated as parent in bile or urine (≥70%). Is absorption permeability limited: YES

Class 4
Clearance: renal (≥70%). Eliminated as parent in urine (≥70%). Is absorption permeability limited: YES

Adapted from:
BCS: Amidon, 1995
BDDCS: Wu and Benet, 2005
ECCS: Varma et. Al., 2015
Plasma Protein Binding (Distribution)

Used to predict the free fraction (unbound) of the drug in circulation in pre-clinical species and humans

- **Free Drug**: In most cases, only free drug interacts with a target (or off target)
- **Fraction unbound** can be used to predict the human dose from in vitro and pre-clinical models
- **Toxicity**: Exposure in the toxicological species can be related to the human exposure
- **DDI**: Free fraction is used in most of the in vitro DDI predictions to determine if a clinical DDI experiment is needed (Di et. al., Jphamsci, 2017)
- **Red Blood Cell Partitioning**: Important if the ratio is not 1
- **α-1-acid glycoprotein** and **Albumin** binding: important in certain disease states
Plasma Protein Binding (Methods)

**Equilibrium Dialysis**

- Compound Equilibrates across membrane
- RED device
- 96 well plate with 48 samples
- Dialysis membrane (MWCO 8K, 12K)
- Multiple sampling

**Ultrafiltration**

- Centrifugation to apply 2000g
  - Fast process (good for low stability compounds)

**UltraCentrifugation**

- Spike test article in plasma
  - Centrifuge ~ 500,000 g for 5-6 hours at 37C
  - Long process
  - Low NSB
Metabolism (Metabolism and Excretion)

• Routes of clearance
  – Metabolic stability comparison of human and pre-clinical species
  – Predication of human clearance

• Metabolic Stability: Are major routes of metabolism non-CYP mediated (e.g., does the clearance in microsomes match in vivo)?
  – AO? Use pooled human liver cytosol, hepatocytes OR S9 ± Hydralazine and ± 1-ABT
  – FMO? Use pooled HLM ± heat inactivation of FMO
  – UGTs? Use HLM with and without UDPGA
  – SULTs? Use S9 with PAPS

• May need to consider transporters
Metabolite characterization

Timing of *in vitro* studies –

- **ICH M3(R2)**, “In vitro metabolic ... data for animals and humans...”

- In Vitro DDI guidance: FDA suggests metabolite characterization studies precede reaction phenotyping

- “Metabolic pathway identification experiments identify the number and structures of metabolites produced by a drug . . . Data obtained from metabolic pathway identification experiments help to determine whether and how to conduct a reaction phenotyping study”

- Also consider the FDA’s 2016 [MIST guidance](#)
### Timing of metabolite characterization studies –

<table>
<thead>
<tr>
<th>Stage</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>• Soft spot analysis from in-vitro samples</td>
</tr>
<tr>
<td>Pre-clinical</td>
<td>• In Vitro hepatocyte incubation, cross species metabolism</td>
</tr>
<tr>
<td></td>
<td>• In vivo samples from pre-clinical species</td>
</tr>
<tr>
<td>Phase I</td>
<td>• Clinical samples (non radio-labeled)</td>
</tr>
<tr>
<td></td>
<td>• Pre-clinical ADME study with radio-labeled</td>
</tr>
<tr>
<td>Phase II</td>
<td>• Human AME study – quantitative assessment</td>
</tr>
<tr>
<td></td>
<td>• Synthesize metabolites for quantification by LC/MS (earlier?)</td>
</tr>
<tr>
<td>Phase III</td>
<td>• Monitor metabolites in clinical studies</td>
</tr>
</tbody>
</table>
In vitro metabolite profiling experiments

• Incubate parent drug with appropriate test system
• Separate metabolites and matrix components by extraction and/or chromatography
• Detect metabolites by various techniques
  – Optical spectrophotometric detection (UV/vis, fluorescence)
  – Radiometric detection ($^{14}$C, $^3$H, $^{35}$S)
  – Mass spectrometric detection
• Perform structural elucidation of detected components with mass spectral data
Does MS signal equal abundance?

![Graph showing MS signal and abundance](image)

- **Propranolol**
- **Glucuronides**
- **OH + Sulfate**
- **OH + Glucuronide**
- **OH + Glucuronide**
- **N-dealkylation**
- **Hydroxylation**
Does MS signal equal abundance? NO
Example data output, Repaglinide high-resolution LC UV chromatogram (254)

RD117007_MSE_05Apr12_014 Sb (3,40.00); Sm (SG, 40x1)

50 μM Repaglinide
2.0 mg/mL HS9
30 minutes; 35°C; pH 7.4
NADPH-generating system

Unlabeled peaks are not related to repaglinide

Barbara, internal data, 2013
### Example data output, Repaglinide metabolite profile by mass spectrometry

<table>
<thead>
<tr>
<th>Component</th>
<th>( m/z ) value</th>
<th>Mass shift</th>
<th>Proposed biotransformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 (M0-OH)</td>
<td>469</td>
<td>+16</td>
<td>Hydroxylation</td>
</tr>
<tr>
<td>C2</td>
<td>451</td>
<td>-2</td>
<td>Dehydrogenation</td>
</tr>
<tr>
<td>C3</td>
<td>469</td>
<td>+16</td>
<td>Oxygenation</td>
</tr>
<tr>
<td>C4 (M4)</td>
<td>469</td>
<td>+16</td>
<td>Oxygenation</td>
</tr>
<tr>
<td>C5 (M1)</td>
<td>385</td>
<td>-68</td>
<td>N,N-didealkylation</td>
</tr>
<tr>
<td>C6</td>
<td>441</td>
<td>-12</td>
<td>O-deethylation + oxygenation</td>
</tr>
<tr>
<td>C7 (M5)</td>
<td>425</td>
<td>-28</td>
<td>O-deethylation</td>
</tr>
<tr>
<td>C8</td>
<td>469</td>
<td>+16</td>
<td>Oxygenation</td>
</tr>
<tr>
<td>C9</td>
<td>469</td>
<td>+16</td>
<td>Oxygenation</td>
</tr>
<tr>
<td>C10</td>
<td>471</td>
<td>+18</td>
<td>Oxygenation + reduction</td>
</tr>
<tr>
<td>C11 (M2)</td>
<td>485</td>
<td>+32</td>
<td>N-dealkylation + oxidation to the acid</td>
</tr>
<tr>
<td>C12</td>
<td>451</td>
<td>-2</td>
<td>Dehydrogenation</td>
</tr>
<tr>
<td>C13</td>
<td>451</td>
<td>-2</td>
<td>Dehydrogenation</td>
</tr>
<tr>
<td>C14</td>
<td>451</td>
<td>-2</td>
<td>Dehydrogenation</td>
</tr>
</tbody>
</table>

Barbara, internal data, 2013
## Accurate mass for biotransformation assignment

<table>
<thead>
<tr>
<th>Nominal mass shift (amu)</th>
<th>Biotransformation(s)</th>
<th>Elemental composition change</th>
<th>Accurate mass shift (amu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+14</td>
<td>Oxidation + dehydrogenation</td>
<td>+O -2H</td>
<td>+13.9792</td>
</tr>
<tr>
<td></td>
<td>Methylation</td>
<td>+CH₂</td>
<td>+14.0157</td>
</tr>
<tr>
<td>+16</td>
<td>Oxidation</td>
<td>+O</td>
<td>+15.9949</td>
</tr>
<tr>
<td></td>
<td>Heteroatom dealkylation within a heterocycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+18</td>
<td>Oxidation + hydrogenation</td>
<td>+O +2H +H₂O</td>
<td>+18.0106</td>
</tr>
<tr>
<td></td>
<td>Hydrolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+28</td>
<td>2x (Oxidation + dehydrogenation)</td>
<td>+2O -4H</td>
<td>+27.9584</td>
</tr>
<tr>
<td></td>
<td>Oxidation + dehydrogenation + methylation</td>
<td>+O -2H +CH₂</td>
<td>+27.9949</td>
</tr>
<tr>
<td></td>
<td>Di-methylation or ethylation</td>
<td>+2(CH₂)</td>
<td>+28.0313</td>
</tr>
<tr>
<td>+32</td>
<td>Di-oxidation</td>
<td>+2O</td>
<td>+31.9898</td>
</tr>
<tr>
<td></td>
<td>Oxidation + hydrogenation + methylation</td>
<td>+O +2H +CH₂</td>
<td>+32.0263</td>
</tr>
</tbody>
</table>
Example MS/MS fragmentation data, Lapatanib metabolite suspected of mechanism based inhibition

Barbara et. al., DMD, 2013
Timing of In vitro Pharmacokinetic DDIs

- 2017 FDA guidance on In Vitro DDI
- Section III. Evaluating Metabolism-Mediated Drug Interactions:
  - Reaction Phenotyping, CYP inhibition and CYP induction
  - “sponsors should initiate in vitro metabolic studies before” first-in-human studies
- Section IV. Evaluating Transporter-Mediated Drug Interactions
  - “The timing of the in vitro evaluation of each transporter may vary depending on the therapeutic indications of the investigational drug.”
- Note: Also covered in EMA 2013 (final), and PMDA 2018 (final)
Reaction Phenotyping

• “The sponsor should routinely evaluate CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 using in vitro phenotyping experiments...”
• “…undergoes significant in vivo metabolism that is not mediated by these major CYP enzymes.”
  – CYP2A6, CYP2J2, CYP4F2, and CYP2E1, Phase I (MAO, FMO, XO and AO) Phase II (UGTs)
• In vitro phenotyping experiments, Monitor:
  – metabolite formation (if available)
  – OR loss of substrate (assuming sufficient turnover)
• Time and protein to determine initial rate conditions
• Inhibition (chemical or antibody)
• Recombinant CYP enzymes
• Optional: $K_m V_{max}$ (for metabolite formation), $f_m$ and correlation analysis

The sponsor should develop validated and reproducible analytical methods to measure levels of the parent drug and each metabolite” (line 750)
Reaction Phenotyping (continued)

Recombinant CYP enzymes

Metabolism of <TA> (0.1 and 1 μM) in 30-min incubation with a panel of recombinant human CYP enzymes (10 pmol CYP per incubation)

Chemical or Antibody Inhibitors

Effect of monoclonal antibodies on the disappearance of <TA> (0.1 and 1 μM) with human liver microsomes (0.25 mg protein per incubation)
Inhibition of metabolizing enzymes

• “The sponsor should evaluate an investigational drug’s potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 in both a reversible manner (i.e., time-dependent inhibition (TDI)).”

• Follow up with $K_i$ (direct inhibition) or $K_i \cdot K_{\text{inact}}$ for TDI. Can skip the $K_i$ with a well designed experiment (Haupt et. al., 2015)

• Other enzymes? UGT when the compound is metabolized by UGT Microsomes, hepatocytes or recombinant enzymes

• Selective probe substrates (validated), short incubation times and low protein concentrations
Induction of metabolizing enzymes

• “The sponsor should evaluate the potential of an investigational drug to induce” CYP1A2, CYP2B6, and CYP3A4/5 and follow-up with CYP2C8, CYP2C9 and CYP2C19 IF CYP3A/5 is induced

• End pointes include mRNA and/or CYP enzyme activity (EMA requires mRNA).
  – But! For the CYP2Cs, mRNA does not work well, especially CYP2C19

• Hepatocytes from 3 donors (fresh or cryopreserved)

• Test article concentration in the culture media (spent media analysis)
  – Adjust dosing?

• Toxicity of the test article on the test system

![Cultured Human Hepatocytes]

![Graph: EC$_{50}$ = 0.58 µM, E$_{max}$ = 6.1 fold]
Drug Transporters

Transporters are membrane bound proteins that govern the transport of compounds in and out of cells.

In the intestine and at the BBB efflux (ABC) transporters predominate (particularly P-gp and BCRP) regulating absorption or entry into the brain.

In the liver (hepatocytes) and kidney (proximal tubules) uptake transporters (SLC) remove compounds from the blood and efflux (ABC) or SLC transporters pump them into the bile or urine.

If your compound is a substrate or inhibitor of transporters, it can be involved in a PK based DDI.

Figures adapted from Zamek-Gliszczynski et al., Clin Pharm Ther 92:5 2012.
Drug Transporters (Section IV of DDI guidance)

Transporters involved in DDI

- ABC (Efflux): P-gp, BCRP
- SLC (Uptake): OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K
- Others: BSEP, MRP2, OCT1 (and others as needed)

Transporter substrate (Victim)

- P-gp and BCRP are typically tested early (pre IND)
- SLC transporters: depends on route of elimination (post IND)
- Study designs and test systems can vary

Transporter inhibition (Perpetrator)

- Screens of all transporters done early (pre IND)
- Follow up with IC_{50}s as needed
In Vitro DDI comments

• Equations in the guidance document(s) to determine if a clinical DDI is needed
  – Some variation between the FDA, EMA and PMDA
  – Poster co-authored by Dr. Brian Ogilvie and Dr. Andrew Parkinson
• Generally for inhibition and induction (perpetrator potential), if the relevant clinical concentrations are not known, tested concentrations should be limited by solubility and toxicity.
• For substrate studies (victim potential), tested concentrations should be philologically relevant taking into consideration bioanalytical limitations and saturation of the enzyme in the test system.
• Metabolites may need to be tested for potential DDI in vitro.
Final Thoughts

• So, Are in vitro metabolism and DDI studies critical for an IND?
  – Metabolism: Yes
  – DDI studies, according to DDI guidance – YES
    • DDI risk assessment is important when dosing patients with poor safety profile
    • Useful for modeling PK and dose prediction for clinical study design
  – Depending on the program, lack of these data may or may not result in a clinical hold

• What if the DDI studies were conducted prior to the release of the latest guidance document?
  – May or may not be accepted by the regulatory agencies

• What do our clients say?
Thank You