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
Enzyme Induction Studies:
Services Overview

Andrew G. Taylor, Ph.D.
Manager, Technical Support for Services
SEKISUI XenoTech
Overview

• What is enzyme induction?
• Why is measuring CYP (or other enzyme) induction important?
• Basic terminology
• Regulatory guidance & expectations
• Types of induction studies
• Typical study design (definitive vs. screening)
• Example results
• Considerations & questions we ask clients up front
• SEKISUI XenoTech products for induction
General mechanism of enzyme induction

A receptor-mediated response to xenobiotics (xenosensors)

Drug

Receptor

AhR
CAR
PXR
PPAR

Note the cross-talk between CAR and PXR

DR cytosol → DR nucleus → Gene transcription

CYP mRNA

CYP protein

CYP activity

CYP1A
CYP2B
CYP3A
CYP4A
Induction DDI General Mechanism

Drug-1

Drug-2, a CYP3A4 Inducer

CYP3A4

Increased 3A4 enzyme
Increased 3A4 activity (likely)
Increased metabolism drug 1
Reduced exposure Drug 1
Reduced efficacy Drug 1

Normal Drug-1 metabolism
Intended Drug-1 exposure
Intended Drug-1 clearance
Intended Drug-1 efficacy
Terminology for Enzyme Induction

- **EC$_{50}$**
  - Concentration of the drug that gives the half-maximal response
  - Conceptually similar to IC$_{50}$, but looking at response instead of inhibition
  - It is a concentration (e.g., µM or mg/mL)

- **E$_{\text{max}}$**
  - “E” is the effect at drug concentration C
  - E$_{\text{max}}$ is the maximal effect at high drug concentrations when all receptors are occupied by the drug
  - It is fold change (compared to vehicle control)

- **Prototypical Inducer**
  - Compound known to induce a particular enzyme, a positive control (E.g., rifampin or phenobarbital \(\Rightarrow\) CYP3A4)
# Meeting Regulatory Expectations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test system (number of donors)</strong></td>
<td>Cryopreserved or fresh human hepatocytes (other systems considered complimentary) ( n \geq 3 )</td>
<td>Cryopreserved human hepatocytes (pre-characterized with mild to strong clinical CYP3A4 inducers) or fresh human hepatocytes ( n \geq 3 )</td>
<td>Cryopreserved human hepatocytes (pre-characterized with mild to strong clinical CYP3A4 inducers) or fresh human hepatocytes ( n \geq 3 )</td>
</tr>
<tr>
<td><strong>TA concentrations</strong></td>
<td>Sufficient to reach Emax not based on Cmax-ss total unbound</td>
<td>50x Cmax-ss unbound or if orally dosed 1/10th dose in 250 mL for CYP3A4</td>
<td>1/10th dose in 250 mL or 50x Cmax-ss total unbound, limit of in vitro solubility or in toxicity, includes Cmax-ss (and ideally reaches Emax) (recommend 8 concentrations)</td>
</tr>
<tr>
<td><strong>CYP emphasis</strong></td>
<td>1A2, 2B6, 3A4</td>
<td></td>
<td>1A2, 2B6, 3A4</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>Negative: Not specified</td>
<td>Negative: Not required</td>
<td>Vehicle: Test article specific</td>
</tr>
<tr>
<td></td>
<td>CYP specific Positive: Omeprazole (25-100 µM)</td>
<td>CYP specific Positive: Omeprazole (50 µM)</td>
<td>Negative: Flumazenil (25 µM)</td>
</tr>
<tr>
<td></td>
<td>Phenobarbital (500-1000 µM)</td>
<td>CITCO (100 nM)</td>
<td>CYP specific Positive: Omeprazole (50 µM)</td>
</tr>
<tr>
<td></td>
<td>Rifampin (10-50 µM)</td>
<td>Rifampin (20 µM)</td>
<td>Phenobarbital (750 µM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rifampin (20 µM)</td>
</tr>
</tbody>
</table>
## Meeting Regulatory Expectations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>End-point measurement</td>
<td>mRNA</td>
<td>mRNA (activity if decreases observed)</td>
<td>mRNA and activity</td>
</tr>
<tr>
<td>Positive induction response</td>
<td>Unspecified</td>
<td>6-fold increase in mRNA</td>
<td>6-fold increase in mRNA (in pre-characterized cryopreserved hepatocytes)</td>
</tr>
<tr>
<td>Concentration of TA in medium</td>
<td>Yes, on last day of treatment to obtain $C_{avg}$ OR change medium at intervals to reduce TA loss</td>
<td></td>
<td>Spent media collection on last day of treatment over 4 time points (analyzed by test article specific LC/MS/MS method or stored for future analysis)</td>
</tr>
</tbody>
</table>
# Meeting Regulatory Expectations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Toxicity assays</strong></td>
<td>Not specified</td>
<td>Yes, at highest TA concentration before and after incubation period</td>
<td>Recommended</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lactate Dehydrogenase Release (a measure of membrane integrity) (daily evaluation)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reduction of Resazurin (a measure of mitochondrial respiration) (day of harvest)</td>
</tr>
<tr>
<td><strong>Individuals vs. Average</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Individual cultures, worst-case scenario</td>
<td></td>
</tr>
<tr>
<td><strong>Methods for data interpretation</strong></td>
<td>• Basic models</td>
<td>• Basic models</td>
<td>• Correlation methods (RIS, etc.)</td>
</tr>
<tr>
<td></td>
<td>• Mechanistic-static model (net effect)</td>
<td>• Correlation methods (RIS)</td>
<td>• Basic models</td>
</tr>
<tr>
<td></td>
<td>• Dynamic models (PBPK)</td>
<td>• Mechanistic-static model (net effect)</td>
<td>• Dynamic models</td>
</tr>
<tr>
<td><strong>GLP-compliance</strong></td>
<td>Not required (spirit of GLP)</td>
<td>Not required</td>
<td>Available on request (Requires DSA)</td>
</tr>
</tbody>
</table>
**Study Types**

- **Ex vivo**
  - Investigates induction in laboratory animals (rat, mouse, dog, monkey)
  - Typically done following toxicology studies by the Sponsor as GLP multi-site study
  - Animals usually dosed by Sponsor and liver samples sent to SEKISUI XenoTech
  - Animals dosed with large amounts of test article
  - Microsomes or RNA from individual animals isolated from liver tissue
  - Tecan assays for activity or qPCR for mRNA expression levels performed

- **In vitro**
  - Enzyme induction examined in cryopreserved, characterized hepatocytes
  - Cultured hepatocytes treated with test article over 3 days in 60 mm dishes only
  - Small amounts of test article required
  - Hepatocytes from rat, dog, mouse, monkey and human have been used
  - Microsomal activity (or In situ activity)
  - Microsomes or RNA from treated hepatocytes
  - Tecan assays for activity or qPCR for mRNA expression levels performed
Definitive vs MTS EI Study Design

Definitive EI Study
- mRNA fold change for CYP1A2, 2B6 and 3A4 using qRT-PCR
- 3 lot hepatocytes, 72 hr treatment period (n =3 biological replicates)
- 6-8 TA concentrations
- EC_{50}/E_{max} data
- Vehicle control, Negative control for induction, Multiple positive controls (1 concentration)
- Spent media analysis at multiple time points
- Full submission report

Additional options:
- 2C8, 2C9 and 2C19 follow up (activity and/or mRNA)
- GLP dose solution analysis
- Pre induction study toxicity assessment

Medium-Throughput Screening EI Study
- mRNA fold change for CYP1A2, 2B6 and 3A4 using qRT-PCR
- 1 lot hepatocytes, 24 hr treatment period
- 3 TA concentrations (n = 3, pooled measured)
- Positive control included
- TA shipped in solution or as pre-weighed aliquots
- LDH
- Tabular data summary
Induction Example Data

[Chart showing fold change for different concentrations of drug, with 'Positive response' and 'Negative response' highlighted.]
Induction EC$_{50}$ and E$_{max}$ Example Data

<table>
<thead>
<tr>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_{max}$</td>
<td>3.3247</td>
<td>0.3163</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>0.1371</td>
<td>0.1427</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_{max}$</td>
<td>6.0649</td>
<td>0.1981</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>0.5795</td>
<td>0.1244</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_{max}$</td>
<td>3.3378</td>
<td>0.1200</td>
</tr>
<tr>
<td>EC$_{50}$</td>
<td>0.1362</td>
<td>0.0528</td>
</tr>
</tbody>
</table>
Induction Data Interpretation

- Determine fold change compared to vehicle control
  - Did the test article cause a fold change in mRNA or activity levels of greater than 2-fold?
  - Did the test article cause a response in mRNA or activity levels greater than 20% of the positive control?
  - Were the increases observed concentration dependent?
- If increases are observed, determine EC\textsubscript{50} and E\textsubscript{max} values for R\textsubscript{3} calculations (FDA 2020) – (requires I\textsubscript{max-u})
- Alternatively, compare test article response to range of weak to strong inducers (Relative Induction Score – CYP3A4 only)

Additional Considerations:
- Cytotoxicity observed?
- Does the mRNA response match the activity response?
- Should the mechanistic prediction model be used (e.g., AUCR values)?
Considerations and Questions for the Sponsor: In Vitro Studies

- TA specific considerations (solubility, binding, stability, molecular weight, molecule type, etc.)

- What question are you trying to answer? Just checking FDA boxes (human study) or anything other than human always good to know.

- FDA, EMA or both?
  - What do you plan to do with the data, plan on additional modeling, going to consultant, depend on us for interpretation? If using additional modeling (SimCYP for EMA) may suggest additional PC for induction. I.e., some clients may also want EC$_{50}$ of prototypical inducers in experimental design.

- C$_{\text{max-u}}$, physiological relevant concentrations, Plasma Protein Binding
Considerations and Questions for the Sponsor: Ex Vivo Studies

- What species
- Number of animals per sex
  - Pooled or individual
- Number of treatment groups
- GLP vs Non-GLP
- Multi-site work plan or protocol
- CYPs/UGT to be analyzed
- What question are they trying to answer

- Endpoints
  - mRNA, activity, Western Immunoblotting, ELISA—for activity WI and ELISA need 4-g liver tissue, mRNA only need ~150 mg
  - CYP specific probe substrates preferred
  - Do we have a preferred method of preparing the livers we do have a protocol to share? Yes!
SEKISUI XenoTech Products (CYP/UGT Induction)

- **Cryopreserved Attaching Primary Hepatocytes**
  - Individual donors
    - >6-7 fold mRNA induction (CYP1A2, CYP2B6, and CYP3A4)
    - >2 fold activity induction
  - Pooled primary human donors (screening only)
  - Multiple small animal species
- **Support Reagents**
  - Hepatocyte media
    - Thaw, Plate, Incubate, and Culture

- **Immortalized Hepatocytes (Fa2N-4)**
  - CYP induction screening (not so popular anymore)
- **Support Reagents**
  - MFE Hepatocyte mediums
Treated Animal Liver Subcellular Fractions

**Male Sprague-Dawley Rat**
- β-Naphthoflavone CYP1A
- β-Naphthoflavone and Phenobarbital CYP1A & 2B
- Phenobarbital CYP2B
- Isoniazid CYP2E
- Dexamethasone CYP3A
- Clofibric acid CYP4A
- Saline Control
- Corn oil Control

**Male Beagle Dog**
- β-Naphthoflavone CYP1A
- Phenobarbital CYP2B
- Rifampin CYP3A
- Clofibric acid CYP4A
- Saline Control
- Corn oil Control

**Male and Female Cynomolgus**
- β-Naphthoflavone CYP1A
- Omeprazole CYP4A
- Phenobarbital CYP2A & 2B
- Pyrazole CYP2E
- Rifampin CYP3A
- Saline Control
Thank you for watching!

For questions get in touch through the Contact Us tab on our website or use our Products pages to find our currently available test systems and reagents.
www.xenotech.com
Featuring publications, posters, webinars and other useful resources.

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

Sekisui XenoTech’s Services & Products:

In Vitro ADME/PK & DDI
- Drug Transport
- Drug Metabolism
- Enzyme Inhibition & Induction
- Protein Binding
- Metabolite Identification
- ADME Screening

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

JCRB Cell Lines...