The 2012 FDA Draft Guidance on Drug-drug Interactions: Enzyme Induction and Beyond

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The “Guidance for Industry”

Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

Reaction phenotyping
DDI (inhibition & induction)
In vivo clinical studies
Labeling

Transcription

Promoter (CYP3A4) → Gene (CYP3A4) → CYP3A4 mRNA → CYP3A4

Conc. of Drug
Time (hr)

Drug → Drug-OH

loss of efficacy
General Scheme of Model-based Prediction: Investigational Drug Interacting with CYP Enzymes

CYP inhibition
(reversible and time-dependent)

- Measure enzyme activity in human liver microsomes
- Estimate DDI parameters

CYP induction

- Measure mRNA change by investigational drug in cultured human hepatocytes from 3 or more donors
- Estimate DDI parameters

Basic Models

Is increase in mRNA > a predefined threshold?
Or
Is the calculated R value < 0.9?

\[ R_3 = \frac{1}{1 + d \times E_{\text{max}} \times [I] / (EC_{50} + [I])} \]

Mechanistic Static Model

\[ \text{AUCR} = \frac{1}{(A_g \times B_g \times C_g) \times (1-F_g) + F_g} \times \frac{1}{(A_h \times B_h \times C_h) \times F_m + (1-F_m)} \]

OR,

Dynamic Model (PBPK)

Mechanistic Models

Is AUCR > 1.25 (inhibition) or < 0.8 (induction)

Conduct a clinical study using an appropriate probe substrate

Yes

Conduct a clinical study using an appropriate probe substrate
Basic Induction Model

\[ R_3 = \frac{1}{1 + \left( d \times E_{\text{max}} \times [I] \right) \div (\text{EC}_{50} + [I])} \]

Is increase in mRNA > a predefined threshold?

or

Is the calculated R < 0.9

\( d = 1 \)

\([I]\) = maximum total (free+unbound) systemic concentration

\( E_{\text{max}} \) is the maximum induction response

\( \text{EC}_{50} \) is the concentration causing half maximal effect

What if due to solubility or cytotoxicity issues the \( E_{\text{max}} \) and \( \text{EC}_{50} \) can not be determined?

Options?

AUC/F2 Method?

Basic equation is designed to eliminate false negatives, but unfortunately leads to increased false positives, hence the need to move to the mechanistic models
Mechanistic Static Induction Model

\[ \text{AUCR} = \frac{1}{(A_g x B_g x C_g) x (1-F_g) + F_g} \times \frac{1}{(A_h x B_h x C_h) x F_m + (1-F_m)} \]

- **Gut**
  - \( C = 1 + \frac{d \times E_{\text{max}} \times [I]}{[I] + EC_{50}} \)
  - \([I]_g = F_a \times K_a \times \text{Dose/Q}_{\text{en}} \)
  - \([I]_h = f_u \times (C_{\text{max}} + (F_a \times K_a \times \text{Dose/Q}_h)) \)

- **Liver**

**Interpretation:** Is AUCR > 1.25 (inhibition) or < 0.8 (induction)
Basic to Mechanistic Model

**Basic Model**

- \( d = 1 \)
- \([I] = 2.3 \text{ uM}\)
- \( E_{\text{max}} = 25 \)
- \( EC_{50} = 5.3 \text{ uM}\)

\[ R_3 = 0.12 \]  
\((R_3 < 0.9 \text{ implies induction})\)

**Mechanistic Static Model**

- \( d = 0.5 \)
- \( F_a = 1 \)
- \( K_a = 0.03/\text{min}\)
- Dose = 81 uM
- \([I] = 2.3 \text{ uM}\)
- \( F_u = 0.012 \)
- \( E_{\text{max}} = 25 \)
- \( EC_{50} = 5.3 \text{ uM}\)
- \( K_{\text{deg},g} = 0.00032/\text{min}\)
- \( K_{\text{deg},h} = 0.00048/\text{min}\)
- \( F_{g,h} \) (midazolam)

\[ \text{AUCR} = 0.21 \]  
\((\text{AUCR} < 0.8 \text{ implies induction})\)

Induction Expected

‘Moderate Inducer’
Model System for Evaluating Induction

- Primary cultures of human hepatocytes (fresh or cryopreserved)
  - What about immortalized hepatocytes or NHR assays?
    “At present, data generated from other in vitro systems are considered complementary and may be reviewed along with data generated with cultured hepatocyte systems.”
  - EMA, “very well justified” immortalized cell lines acceptable

- Evaluate 3+ donors to account for interindividual variability
  - If one donor is positive in Basic model, the drug is considered an inducer and “follow-up evaluation is needed” (ie. Mechanistic)

- Need to determine performance of hepatocytes in identifying enzyme induction potential with ‘sufficient’ number of clinical inducers (how many?)
Model System for Evaluating Induction

• “The changes in the mRNA level of the target gene should be used as an endpoint”
  – Not consistent with EMA guidance (activity & RNA)
  – Activity should be an option in cases when the test compound is not an inhibitor

• Inclusion of negative controls
  – Could provide value in distinguishing real induction from the ‘noise’ of the assay, but no guidance given on what to use or at what concentration.

• Evaluate CYP1A2, 2B6, and 3A
  – If CYP3A is positive, then evaluate CYP2Cs (2C8, 2C9, and 2C19)
    – Generally, CYP2C induction is less than CYP3A and mediated by factors beyond PXR and CAR (Fahmi et al, DMD 2010 & Chen/Goldstein, CDM 2009)
## In Vitro CYP Inducers (Table 2)

<table>
<thead>
<tr>
<th>CYP</th>
<th>In vitro inducer as positive controls</th>
<th>Recommended concentration (uM) of positive controls</th>
<th>Reported fold induction in enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Omeprazole</td>
<td>25-100</td>
<td>14-24</td>
</tr>
<tr>
<td></td>
<td>Lansoprazole</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2B6</td>
<td>Phenobarbital (CITCO-EMA)</td>
<td>500-1000 (&lt;100 nM)</td>
<td>5-10</td>
</tr>
<tr>
<td>2C8</td>
<td>Rifampin</td>
<td>10</td>
<td>2-4</td>
</tr>
<tr>
<td>2C9</td>
<td>Rifampin</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>2C19</td>
<td>Rifampin</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>2D6</td>
<td>None identified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>Rifampin</td>
<td>10-50</td>
<td>4-31</td>
</tr>
</tbody>
</table>

No information on ‘reported fold induction in mRNA response’
<table>
<thead>
<tr>
<th>CYP</th>
<th>Strong Inducers &gt;80% decrease in AUC</th>
<th>Moderate Inducers 50-80% decrease in AUC</th>
<th>Weak Inducers 20-50% decrease in AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Montelukast, phenytoin, smokers</td>
<td>Moricizine, omeprazole, phenobarbital</td>
<td></td>
</tr>
<tr>
<td>2B6</td>
<td>Efavirenz, rifampin</td>
<td>Nevirapine</td>
<td></td>
</tr>
<tr>
<td>2C8</td>
<td>Rifampin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C9</td>
<td>Carbamazepine, rifampin</td>
<td>Aprepitant, bosentan, phenobarbital, St. John’s wort</td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td>Rifampin</td>
<td></td>
<td>Artemisinin</td>
</tr>
<tr>
<td>3A4</td>
<td>Avasimibe, carbamazepine, phenytoin, rifampin, St. John’s wort</td>
<td>Bosentan, efavirenz, etravirine, modafinil, nafcillin</td>
<td>Amprenavir, aprepitant, armodafinil, clobazamechinacea, pioglitazone, prednisone, rufinamide, vemurafenib</td>
</tr>
<tr>
<td>2D6</td>
<td>None known</td>
<td>None known</td>
<td>None known</td>
</tr>
</tbody>
</table>
“… it is critical to evaluate the time it takes for the enzyme activities to return to normal when induction or TDI is involved so that a third crossover period in which the interacting drug is removed”
A Novel Mechanism of Enzyme Induction: Non Classical PXR or CAR-Mediated Induction
Both PXR and CAR can cause CYP3A4 gene activation and enzyme induction leading to significant drug interactions.
Comparative PXR Binding and Transactivation Results

**PXR Binding Assay**

- **BMS-536924** binds to PXR: \( IC_{50} = 1.0 \text{ uM} \)
- **BMS-665351** does not bind to PXR: \( IC_{50} = 30 \text{ uM} \)

**SAR**

**PXR Transactivation Assay**

- **BMS-536924**
  - \( EC_{50} = 5 \text{ uM} \)
- **BMS-665351**
PXR and Hepatocyte Data on Both Sets of Compounds

**BMS-536924**
- Binds to PXR
- Activates PXR
- Induces CYP3A4 in Primary Human Hepatocytes

**BMS-665351**
- Do Not Bind to PXR
- Do Not Activate PXR
- Induces CYP3A4 in Primary Human Hepatocytes

Rifampicin-positive control for PXR
Two Mechanisms of CAR-Mediated Enzyme Induction

Constitutive Androstan Receptor Mechanism 1

Mechanism 2

Transit of CAR from the cytoplasm to the nucleus by any mechanism is termed Translocation.

PB-phenobarbital
Activation of CAR and PXR in HepG2 Cells

**CAR3 Expression Assay**

- **HepG2**
  - **hCAR3/CYP3A4 (PXRE/XREM)**

**PXR Expression Assay**

- **HepG2**
  - **hPXR/CYP3A4 (PXRE/XREM)**

**BMS-665351 does not activate human CAR or PXR**

**CITCO-positive control for CAR**
**CAR Translocation Assay: BMS-665351**

Human hepatocytes infected with adenovirus fluorescently tagged with CAR (AD/EYFP-hCAR)

**hCAR1 Localization in HL-#37**

<table>
<thead>
<tr>
<th>CTL (0.1% DMSO)</th>
<th>PB 1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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</table>

<table>
<thead>
<tr>
<th>BMS-665351 (1 µM)</th>
<th>BMS-665351 (5 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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</table>

### Relative Count (%)

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>PB 1 mM</th>
<th>1 µM</th>
<th>5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C=N</td>
<td>0</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>N</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

**BMS-665351 does not translocate CAR**

C - cytoplasm
N - nucleus
Does the Induction Have Anything to do with CAR?

CAR3 Over Expression System (HepG2)

CAR is involved in the CYP3A4 induction response

PK11195 – a selective CAR deactivator

BMS-665351: 10 uM
CITCO: 1 uM
PK11195: 10 uM
Does BMS-665351 Induce the Expression of CAR?

BMS-665351 induces the expression of CAR in cell lines and human hepatocytes. Does not induce the expression of PXR.
Summary

Further studies necessary to link the increase in CAR expression to the increased expression of CYP3A4
- CAR promoter-reporter assay and siRNA CAR knock-down
- Does this mechanism of CYP3A4 induction translate to an in vivo DDI?
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