Assessing modeling and simulation tools and methods for predicting metabolic-based DDI
Update from the IQ Work Group

Presented by Scott Obach on behalf of the Induction Working Group
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October 6, 2011
North Jersey Drug Metabolism Discussion Group
Outline

- IQ Consortium DDI Work Groups
- Sub-objectives of the Induction Working Group – General overview
- Models/approaches for the prediction of clinical CYP3A induction
- A look at the new FDA draft decision tree for induction (R3 value)
- Summary/Conclusions
IQ Consortium Drug Interaction Work Groups

General overview

- 2009: Meeting between individuals from PhRMA Drug Metabolism Technical Group and FDA Office of Clinical Pharmacology
  - Agreement that the area of modelling and simulation of DDI from in vitro data is a valuable endeavor
  - Established Industry-FDA-Academia work groups
  - Main Objective: To test various models and approaches for predicting DDI using agreed upon input parameters
  - Two teams formed
    - Inhibition+Inactivation – Discussion for another time
    - Induction – We’ll share findings now

To identify method(s) that can reliably use in vitro data to provide quantitative forecasts of clinical DDI across a broad range of drugs and provide a recommendation as to what approach to use to inform the need for clinical DDI studies.

Scope: Development, not early research
Sub-objectives – Induction Working Group

General overview

• Define acceptable approaches for the prediction of the clinical induction of CYP3A (models used, input parameters needed, what type of data is needed)

• Standardize input parameters that can be standardized (e.g. $fm_{CYP}$, $k_{deg}$)

• Define criteria for quality of input parameters

• Define the extent to which we need to qualify the models

• Work together to qualify the models identified on the ability to predict clinical DDI

These sub-objectives are in support of the main objective
Predictions of Induction DDI – General Principle

- Most cytochrome P450 (CYP) induction prediction models use a relationship which include the parameters:
  - EC$_{50}$
  - E$_{max}$
  - [Inducer]

- These prediction models are generally based upon the same principal, that being the law of mass action for receptor binding (and activation since induction is an agonist property)

\[
\text{Effect} = \frac{E_{max} \times [\text{Ind}]}{EC_{50} + [\text{Ind}]}
\]

- EC$_{50} = 15 \mu\text{M}$
- E$_{max} = 20\text{-fold}$
Approaches for prediction of clinical CYP3A induction

What models/approaches are being used now?

**general methods and examples of approach(es):**

- **Empirical Approaches**
  - % of positive control

  *FDA Draft Guidance – Drug Interaction Studies 2006; Bjornsson et al DMD 2003 31:815*

- **Correlation Methods**
  - Relative Induction Score
  - $C_{\text{max}}/EC_{50}$


- **Mathematical Equations (Mechanistic Static Models)**
  - Net effect model

  *Fahmi et al. 2008 DMD 36:1698*

- **Physiologically-Based Pharmacokinetic (Mechanistic Dynamic Models)**
  - Simcyp

*Almond et al 2009 Curr Drug Metab 10:420*
Qualification of the different models/approaches

Identification of trials and available in vitro induction data

- Initially identified trials to model from the literature (U. Washington Drug Interaction Database) or from internal sources
  - Literature searches were focused on DDI studies involving common CYP3A substrates (e.g. midazolam, triazolam, alprazolam, simvastatin, buspirone, etc)
  - Compiled a list of perpetrators that caused clinical induction or no induction/inhibition of these CYP3A substrates

- Using the initial search, four victim drugs were chosen to further investigate based upon the number of trials that were available and differences in \( f_{m_{\text{CYP}}} \) and \( F_G \) (midazolam, alprazolam, nifedipine, and simvastatin)

- Identified the \textit{in vitro} (internal) mRNA induction data (and activity) we had with respect to the inducers/non-inducers in the identified trials (EC\(_{50}\) and \( E_{\text{max}} \) data) and consolidated it for use in modeling (median values)
# Trials chosen for the model comparison

<table>
<thead>
<tr>
<th>perpetrator</th>
<th>victim</th>
<th># of trials</th>
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<tbody>
<tr>
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<td>midazolam</td>
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<td>rosiglitazone</td>
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<tr>
<td>Merck MK-1</td>
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<td>Merck MK-2</td>
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<td>pioglitazone</td>
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<tr>
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<tr>
<td>ranitidine</td>
<td>nifedipine</td>
<td>2</td>
</tr>
</tbody>
</table>

- 28 trials (17 victim/perpetrator pairs)
Empirical Approaches

- e.g. % of positive control

3. Endpoints for Subsequent Prediction of Enzyme Induction

When analyzing the results of experiments to determine whether a drug induces an enzyme activity, the following issues are relevant.

(a) A drug that produces a change that is equal to or greater than 40% of the positive control can be considered as an enzyme inducer in vitro and in vivo evaluation is warranted.

\[ \% \text{ positive control} = \frac{\text{activity of test drug treated cells} - \text{activity of negative control}}{\text{activity of positive control} - \text{activity of negative control}} \times 100 \]

Intended to indicate an *in vitro* induction response
Empirical Approach- Pros and Cons

e.g. % of positive control

Pros

- Can identify non-inducers

Cons

- Not proven to be predictive of DDI – may lead to false negatives/false positives
- Does not account for EC_{50} value
- What \textit{in vitro} [I] would be relevant to decision making for the clinics?
- What are appropriate positive controls?

\textit{Too simple to be reliably predictive}
Correlation Methods

- Sigmoidal relationships observed between RIS and the percent decrease in the AUC values of a CYP3A substrate (e.g. midazolam) after co-administration of enzyme inducers

- Relationships used to predict *in vivo* induction from RIS for test compounds

Relative Induction Score (RIS) = \( \frac{E_{\text{max}} \times [\text{Ind}_u]}{EC_{50} + [\text{Ind}_u]} \)

*What value to use for [I]?*
The value of [I]

Inducer concentration to use in correlation methods or in static models

All are surrogates... however, some have been preferred e.g. free $C_{\text{max}}$ for inactivation and induction and free portal $C_{\text{max}}$ for reversible inhibition*

$$\text{Systemic}$$

- Total $C_{\text{max}}$
- Free $C_{\text{max}}$ ($C_{\text{max}} \cdot \text{fu}$)

$$\text{Hepatic}$$

- Total Portal $C_{\text{max}}$
- Free Portal $C_{\text{max}}$ (Portal $C_{\text{max}} \cdot \text{fu}$)

$$\text{Gut, } [I]_G = \frac{k_a \cdot D \cdot F_a}{Q_{\text{ent}}}$$

Portal $C_{\text{max}} = C_{\text{max}} + \frac{k_a \cdot D \cdot F_a}{Q_H}$

$k_a =$ absorption rate constant
$D =$ dose
$F_a =$ fraction of dose absorbed
$Q_H =$ hepatic blood flow
$Q_{\text{ent}} =$ enterocytic blood flow

Correlation Methods – Pros and Cons

Pros

- Published reports of good correlation with clinical outcome for CYP3A inducers
- Can use these models with relatively simple assumptions about human PK (e.g. $C_{\text{max}}$). No need to simulate the entire conc.-time profile

Cons

- A calibration curve is needed for each laboratory/lot of hepatocytes for calibrator compounds (~6-8+)
- Does not account for CYP3A inducers that are also inhibitors/inactivators
- Limited to CYP enzymes in which there is sufficient clinical data available to set up a calibration curve (e.g. CYP3A)
- Unlikely universal cut-off criteria (e.g. RIS value) can be established
Universal cut-off values of RIS are not recommended as they can be very different between investigators.

Similar conclusions were made for $C_{\text{max,}u}/EC_{50}$. 
Correlation methods- Results

Relative Induction Score (RIS) Model

Predictions of clinical DDI were made by use of the RIS calibration curve (RIS vs. observed DDI) established for all the trials (n=28, 17 victim/perpetrator pairs)

The RIS Model worked very well in the prediction of the induction/non-induction effect within the 0.8-fold (-20%) boundary

* True negatives or false positives with respect to induction
Correlation methods - Results

$C_{max}/EC_{50}$ Model

Predictions of clinical DDI were made by use of the linear regression line of $C_{max}/EC_{50}$ vs. observed DDI established for all the trials ($n=28$, 17 victim/perpetrator pairs).

The $C_{max}/EC_{50}$ Model also worked very well in the prediction of the induction/non-induction effect within the 0.8-fold (-20%) boundary.

* True negatives or false positives with respect to induction
Mathematical Equations (Mechanistic Static Model)

\[ \frac{\text{AUC'}_{po}}{\text{AUC}_{po}} = \left( \frac{1}{[A_H \times B_H \times C_H] \times f_m + (1 - f_m)} \right) \times \left( \frac{1}{[A_G \times B_G \times C_G] \times (1 - F_G) + F_G} \right) \]

\[ A = \frac{k_{\text{deg}}}{k_{\text{deg}} + \frac{[I] \times k_{\text{inact}}}{[I] + K_i}} \]

\[ B = 1 + \frac{d \times E_{\text{max}} \times [I]}{[I] + EC_{50}} \]

\[ C = \frac{1}{1 + \frac{[I]}{K_i}} \]

**e.g. Net Effect Model**

- **inactivation (TDI)**
  - Incorporates:
    - \( f_m \) (fraction of victim drug metabolized by the affected enzyme)
    - \( F_G \) for CYP3A (fraction of the victim drug escaping first pass metabolism in the gut)
    - Inactivation, induction, and reversible inhibition equations

- **induction**

- **reversible inhibition**

H = hepatic
G = gut
Mathematical Equations – Pros and Cons

Pros

- No calibration curves needed – savings in time, effort, cost
- Can be used to predict DDI for compounds that are both inducers and inhibitors/inactivators of CYPs in gut and liver
- As long as $f_{m_{CYP3A}}$ is estimated properly, can be used for different probe (victim) substrates

Cons

- Requires more input parameters compared to Correlation Methods (e.g. $f_{m_{CYP3A}}$ and $F_G$ for victim)
- It requires an empirical calibrator/scalar factor to bridge in vitro data to in vivo (e.g. $d$)
Mathematical Equations – Results

Net Effect Model

\[ [I] = \text{Total C}_{\text{max}} \]

\[ [I] = \text{Free C}_{\text{max}} \]

\[ [I] = \text{Free Portal C}_{\text{max}} \]

where:

\[ [I] = \text{free C}_{\text{max}} \]

for induction and TDI

\[ [I] = \text{free Portal C}_{\text{max}} \]

for reversible inhibition

The Net Effect Model also proved to be a good model to predict the induction/non-induction effect within the 0.8-fold (-20%) boundary.

One trial (MK-2) is off-scale in the true negative quadrant (predicted DDI of 1.6-3-fold, depending on [I])

* True negatives or false positives with respect to induction
**PBPK-based (Mechanistic Dynamic Model)**

- **e.g. Simcyp**

Incorporates:
- Various algorithms incorporating both physiological and test compound properties
- Simulates time-concentration profiles of both perpetrator and victim
- Inactivation, induction, and reversible inhibition

**Graphical Representation**

- Systemic concentration of carbamazepine (ng/mL)
- Systemic concentration of alprazolam (ng/mL)

**Data**

100 mg tid carbamazepine 10 days + 0.8 mg alprazolam on day 8

- **Actual** = 58% increase in clearance
- **Predicted** = 52% increase in clearance

PBPK-based Model – Pros and Cons

Pros

- No calibration curves needed – savings in time, effort, cost
- Can be used to predict DDI for compounds that are both inducers and inhibitors/inactivators of CYPs in the gut and liver
- Physiologically-based model, incorporates drug and system dynamics and population aspects

Cons

- Accurate predictions require extensive knowledge of drug (victim and perpetrator) parameters
- Requires normalization of *in vitro* induction parameters with rifampin positive control (for IVIVE)
- Requires that concentration-time profile of the perpetrator to be accurately predicted
PBPK-based – Results

Simcyp Model

Time-based model more predictive of actual DDI (AUC) than ‘Steady-state’ model. Also proven to be a good model to predict the induction/non-induction effect within the 0.8-fold (-20%) boundary.

* True negatives or false positives with respect to induction
Relative accuracy of the various models

GMFE = geometric mean fold-error
GmedFE = geometric median fold-error

<table>
<thead>
<tr>
<th>Correlation Methods</th>
<th>Mathematical Equations</th>
<th>PBPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIS</td>
<td>C_{\text{max}}/EC_{50}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Free</td>
</tr>
<tr>
<td>GMFE</td>
<td>1.99</td>
<td>1.67</td>
</tr>
<tr>
<td>GMedFE</td>
<td>1.56</td>
<td>1.29</td>
</tr>
<tr>
<td>Scaling Factor (d)**</td>
<td>0.35</td>
<td>0.51</td>
</tr>
</tbody>
</table>

In general, all the models predict the actual DDI with similar accuracy, particularly using free or free portal $C_{\text{max}}$ for $[I]$ in the non-PBPK models.

* Simcyp time-based model, AUC ratio (steady-state model for AUC ratio: GMFE = 2.27; GMedFE = 2.42)
**The scaling parameter for induction (i.e., $d$) in each of the four sets of predictions was estimated through linear regression to a value that minimized the GMFE of the prediction via linear weighted least-squares regression.
Predictions of DDI within 2-fold of actual

Although all models can properly predict DDI within the 0.8-fold (-20%) boundary, importantly, the least amount of under-predictions were observed with the Net Effect model.

[\[I\] = C_{max,u} for static models]
New FDA draft guidance decision tree

Possible Model for Decision-Making
- NME as a CYP inhibitor or inducer -

CYP inhibition (reversible and TDI)

- Measure enzyme activity in human liver microsomes
- Estimate DDI parameters (e.g., $K_i$, $K_{inact}$, $K_d$

CYP induction

- Measure mRNA in cryopreserved human hepatocytes
- Estimate DDI parameters (e.g., $E_{max}$, $EC_{50}$, $y$

How well does R3 work with our dataset?

Basic static model

Calculate “R”
1. Reversible inhibitor, $R_1 = 1 + [I]/K_i$
2. TDI, $R_2 = (K_{obs} + k_{deg})/k_{deg}$ and $K_{obs} = K_{inact} [I]/(K_i + [I])$

$R > 1.1^3$

Mechanistic model: static or dynamic (PBPK when human data are available)

NME likely a CYP inhibitor

Calculate “AUCR”

$AUCR > 1.25$ (inhibition)$^4$
$AUCR < 0.8$ (induction)$^4$

Conduct clinical study using appropriate probe substrates$^5$

NME likely a CYP inducer

$\hat{mRNA} >$ a predefined threshold (at $E_{max}$ concentration)$^2$

or

Calculate $R_3 = 1/(1 + E_{max} \times [I] \times (EC_{50} + [I]))$

$\hat{mRNA} >$ a predefined threshold$^2$ or $R_3 < 1/1.1$

1. TDI: time-dependent inhibition.
2. $E_{max}$, Drug Metab Dispo, 2010
3. $E_{max}$, Drug Metab Dispo, 2009
4. Proposed for discussion.

 Courtesy of Dr. Shiew-Mei Huang
Evaluation of the “R3” value to assess risk

R3 = 1/[1+(E_{max} \times [I]^\gamma)/(EC_{50}^\gamma +[I]^\gamma)], where \gamma = 1 in this case

R3 value, using [I] as free C_{max} or free portal C_{max}, appears to work well to categorize DDI risk within the R3 = 0.9 boundary. Using total C_{max} would result in many over-predictions and some false positives.
Summary/Conclusions

Highlights of the Working Group outcomes thus far...

- Defined and worked towards the qualification of approaches to predict clinical induction of CYP3A

  • Results of the predictions
    - All the models evaluated appeared to predict the DDI effect within the range of the 0.8-fold (-20%) boundary
    - Some differences in the models to predict within 2-fold of actual, with the Net Effect Model having the least amount of under-predictions in this dataset.

  • Interpretation across-Co.
    - It is not recommended to establish universal cut-off values (RIS, $C_{\text{max}}/EC_{50}$, and even fold-change), as these are likely only valid for a particular lot of hepatocytes and the laboratory

  • Ease of use
    - The lack of the need to set-up of calibration curves, such as for the Net Effect Model or PBPK models, is a major advantage to these models.
    - mRNA is the preferred data to use in these models (better dynamic range and differentiation of induction vs. inhibition/inactivation)

- Proposed some good starting estimates for those input parameters that we believe can be standardized (with the inhibition team) and defined quality of some important input parameters (e.g. $EC_{50}$ and $E_{\text{max}}$)
Induction Working Group
A global collaboration across Academia, Pharma, and the FDA

• Heidi Einolf (Novartis)
• Scott Obach (Pfizer)
• Lei Zhang (FDA)
• Ping Zhao (FDA) – ad hoc
• Odette Fahmi (Pfizer)
• Mohamad Shebley (Abbott)
• Chris Gibson (Merck)
• Liangfu Chen (GSK)
• Jash Unadkat (University of Washington)
• Mike Sinz (BMS)
• Jose Silva (J&J)
Back-up slides
Input parameters needed in the different models

A review

- **Correlation Methods**
  - Of test perpetrator (and calibrator compounds): EC$_{50}$, E$_{max}$, [I]
  - Known clinical DDI effect of calibrator compounds

- **Mathematical Equations (Mechanistic Static Models)**
  - Of test perpetrator: EC$_{50}$, E$_{max}$, [I]
  - Scaling factor “d”
  - If also a reversible and/or TDI: $K_i$ and/or $K_l$ and $k_{\text{inact}}$ (also $k_{\text{deg \, CYP3A4}}$), $f_{\text{mic}}$
  - Depending on value used for [I]: $k_a$, $f_a$, dose may be needed (e.g. hep$_{\text{intlet}}$ C$_{max}$), $f_u$
  - Of victim: $f_{m_{\text{CYP}}}$, $F_G$

- **Physiologically-Based Pharmacokinetic (Mechanistic Dynamic Models)**
  - Of test perpetrator (and rifampin control as a calibrator): EC$_{50}$, E$_{max}$
  - If also a reversible and/or TDI: $K_i$ and/or $K_l$ and $k_{\text{inact}}$, $f_{\text{mic}}$
  - Of both victim and perpetrator: physical chemical properties, protein binding and blood cell distribution, absorption parameters, $V_{ss}$, renal and hepatic clearances (correct $f_{m_{\text{CYP}}}$ being calculated for victim), etc.
Input parameters that can be standardized

- $f_{m_{\text{CYP}}}$ and $F_G$ for common CYP3A victim drugs (input values we chose to use for this evaluation)
  - **Midazolam**
    - $f_{m_{\text{CYP}}}$ range 0.86-0.94 (used 0.90)
    - $F_G$ point estimate 0.5
  - **Alprazolam**
    - $f_{m_{\text{CYP}}}$ 0.8
    - $F_G$ 0.94
  - **Nifedipine**
    - $f_{m_{\text{CYP}}}$ 0.71
    - $F_G$ 0.78
  - **Simvastatin**
    - $f_{m_{\text{CYP}}}$ 0.92
    - $F_G$ 0.58

- $k_{\text{deg}}$ CYP3A
  - **Hepatic**
    - $k_{\text{deg}}$ 0.02/h ($t_{1/2}$ 36h)
  - **Intestinal**
    - $k_{\text{deg}}$ 0.03/h
Details of the modeling using Simcyp

- Design of the trials were as described in the publication
  - Demographics (number of subjects, gender, age range)
  - Dose(s), dosing regimen, route of administration

- Victim drug input parameters were used as already provided in Simcyp (Version 10.10 SP1)

- Perpetrator drug input parameters were qualified (in most cases, the compound file had to be built)
  - Ensured in vitro induction parameters ($EC_{50}$ and $E_{\text{max}}$) were identical as those used in the other models/approaches (calibration/normalization with rifampin was included)
  - Other DDI parameters were included when available from the literature ($K_i$, $k_{\text{inact}}$ and $K_I$)
  - Qualified the perpetrator input parameters by comparing simulated time-concentration curves and PK parameters with actual clinical trials
Perpetrator compound model building
Qualification of perpetrator inputs for PBPK modeling

- Example: Rosiglitazone 8 mg single dose (SD)

![Graph showing systemic concentration of Rosiglitazone over time]

<table>
<thead>
<tr>
<th></th>
<th>AUC (ng/mL.h)</th>
<th>% change compared to actual</th>
<th>Tmax (h)</th>
<th>% change compared to actual</th>
<th>CMax (ng/mL)</th>
<th>% change compared to actual</th>
<th>Dose (mg)</th>
<th>CL (Dose/AUC) (L/h)</th>
<th>% change compared to actual</th>
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<tbody>
<tr>
<td>Mean</td>
<td>2984.80</td>
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<td>0.91</td>
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<td>515.61</td>
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<td>8.00</td>
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<td>Mean</td>
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<td>2.71</td>
<td>5.05</td>
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</table>

Simulated multiple dose (MD) PK is not substantially different than SD
Evaluations with and without rifampin calibration

**PBPK-Simcyp model**

- **AUC ratio**
- **$C_{\text{max}}$ ratio**

<table>
<thead>
<tr>
<th>Observed DDI</th>
<th>With RIF Calibration</th>
<th>Without RIF Calibration</th>
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<tbody>
<tr>
<td>GMFE</td>
<td>2.40</td>
<td>2.45</td>
</tr>
<tr>
<td>GMedFE</td>
<td>1.35</td>
<td>1.54</td>
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<table>
<thead>
<tr>
<th>“Steady-state” AUC ratio vs. Observed AUC ratio</th>
<th>With RIF Calibration</th>
<th>Without RIF Calibration</th>
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<tbody>
<tr>
<td>GMFE</td>
<td>3.48</td>
<td>3.70</td>
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<tr>
<td>GMedFE</td>
<td>1.79</td>
<td>2.21</td>
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<table>
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<th>With RIF Calibration</th>
<th>Without RIF Calibration</th>
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</thead>
<tbody>
<tr>
<td>GMFE</td>
<td>2.44</td>
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<tr>
<td>GMedFE</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Without RIF calibration, a consistently greater DDI (induction effect) is predicted. In general, the calibration with RIF resulted in lower GMFE or GMedFE values.
Why the “40%” rule may result in false neg/pos

Simulation Based Upon Fitted RIS Model

- Each curve represents a prediction of DDI from a RIS calibration curve for a test compound.
- Each compound (curve) has an EC$_{50}$ of 1 and a different E$_{max}$, represented as a certain % of RIF.
- As the C$_{max}$/EC$_{50}$ ratio is varied, the projected DDI varies.

Theoretically, even compounds with a low % of RIF (e.g. ≤25%), could have the potential for a DDI, if “[I]/EC$_{50}$” is high enough (>0.1)
Relative induction of CYP3A4 mRNA by several compounds in replicate experiments

Human Cryopreserved hepatocytes Lot Hu4165

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Conc (uM)</th>
<th>Run 1</th>
<th>Run 2</th>
<th>Run 3</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Run 6</th>
<th>Run 7</th>
<th>Run 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modafinil</td>
<td></td>
<td>10%</td>
<td>5%</td>
<td>7%</td>
<td>4%</td>
<td>4%</td>
<td>12%</td>
<td>6%</td>
<td>5%</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>100</td>
<td>48%</td>
<td>31%</td>
<td>25%</td>
<td>14%</td>
<td>23%</td>
<td>27%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>Topiramate</td>
<td>100</td>
<td>21%</td>
<td>41%</td>
<td>15%</td>
<td>18%</td>
<td>22%</td>
<td>31%</td>
<td>20%</td>
<td>14%</td>
</tr>
<tr>
<td>Nafcillin</td>
<td>100</td>
<td>79%</td>
<td>44%</td>
<td>37%</td>
<td>18%</td>
<td>27%</td>
<td>23%</td>
<td>26%</td>
<td>26%</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>100</td>
<td>81%</td>
<td>88%</td>
<td>54%</td>
<td>47%</td>
<td>31%</td>
<td>36%</td>
<td>30%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Evidence that some compounds may not follow rifampin CYP3A4 mRNA induction to the same extent in different experiments.
**EC$_{50}$ and $E_{\text{max}}$ values determined for Rifampin**

*Within Co. run-to-run variability using the same lot of hepatocytes*

### Company 1

<table>
<thead>
<tr>
<th>Run Name</th>
<th>Lot Hu4165</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C100614</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>C100607</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>C090810</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>C100628</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>C100628M</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>24</strong></td>
<td></td>
</tr>
<tr>
<td><strong>CV%</strong></td>
<td><strong>48%</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Company 2

<table>
<thead>
<tr>
<th>Run Name</th>
<th>Celsis Lot NPV</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>18</strong></td>
<td></td>
</tr>
<tr>
<td><strong>CV%</strong></td>
<td><strong>51%</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Company 3

<table>
<thead>
<tr>
<th>Run Name</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>25</strong></td>
</tr>
<tr>
<td><strong>CV%</strong></td>
<td><strong>43%</strong></td>
</tr>
</tbody>
</table>

### Company 4

<table>
<thead>
<tr>
<th>Run Name</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>43</strong></td>
</tr>
<tr>
<td><strong>CV%</strong></td>
<td><strong>24%</strong></td>
</tr>
</tbody>
</table>
Common fitting algorithms for *in vitro* induction data

**Calculation of $E_{\text{max}}$ and EC$_{50}$ values**

- **Simple $E_{\text{max}}$ model:**
  \[
  \text{Effect} = \frac{E_{\text{max}} \times [I]}{\text{EC}_{50} + [I]}
  \]

- **Sigmoidal $E_{\text{max}}$ model:**
  \[
  \text{Effect} = \frac{E_{\text{max}} \times [I]^\gamma}{\text{EC}_{50}^\gamma + [I]^\gamma}
  \]

- **Sigmoid 3-parameter:**
  \[
  \text{Effect} = E_{\text{max}} / (1 + \exp(-(I - \text{EC}_{50})/\gamma))
  \]
EC_{50} and E_{max} values

Collection of data across Co.

- *In vitro* induction data compiled across several Pharma Co. (Pfizer, Novartis, Abbott, Merck, BMS, J&J)
  - rifampin (n=20), carbamazepine (n=7), nifedipine (n=7), phenobarbital (n=8), phenytoin (n=8), pioglitazone (n=7), rosiglitazone (n=5), troglitazone (n=4)
  - Additional data provided by Pfizer (nafcillin, pleconaril, omeprazole, ranitidine) and Merck (MK-1, MK-2)

- Several Co. evaluated each of the 3 fitting algorithms to determine if one algorithm should be favored over the others
  - Akaike Information Criterion (AIC)
    
    \[
    \text{AIC} = N \times \ln \left( \text{sum of squared residuals} \right) + 2P, \text{ where } N = \# \text{ of observations and } P = \# \text{ of parameters fit in the model}
    \]

    *A minimum AIC would be regarded as the best representation of the data. Used for rank ordering models by goodness of fit (with penalty for increasing number of estimated parameters).*
Calculation of EC₅₀ and Eₘₐₓ values

Use of different fitting algorithms - Example: Nifedipine

mRNA data

**Simple Eₘₐₓ model**

\[ \text{Effect} = \frac{E_{\text{max}} \times [I]}{EC_{50} + [I]} \]

**Sigmoidal Eₘₐₓ model**

\[ \text{Effect} = \frac{E_{\text{max}} \times [I]^{-\gamma}}{EC_{50}^{-\gamma} + [I]^{-\gamma}} \]

**Sigmoid 3-parameter**

\[ \text{Effect} = \frac{E_{\text{max}}}{1 + exp\left(-\left([I] - EC_{50}\right)/\gamma\right)} \]

---

<table>
<thead>
<tr>
<th>[Nifedipine], µM</th>
<th>Fold change in CYP3A4 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

**Eₘₐₓ (SE) = 23.5 (4.2)**  
**EC₅₀ (SE) = 19.9 (10)**  
**R² = 0.9130**  
**AIC = 29**

**Eₘₐₓ (SE) = 18.5 (1.4)**  
**EC₅₀ (SE) = 13.3 (2.2)**  
**R² = 0.9699**  
**AIC = 23**

**Eₘₐₓ (SE) = 18.2 (0.59)**  
**EC₅₀ (SE) = 14.6 (1.2)**  
**R² = 0.9927**  
**AIC = 13**

Standard error (SE) lowest
Calculation of EC$_{50}$ and E$_{\text{max}}$ values

Comparisons of different algorithms

Overall, the team felt that there was no consistent trend and substantial difference to give universal preference of one fitting algorithm over another.

The recommendation would be to monitor the standard error given and if comparing data, use the same model.
Compiled Co. EC$_{50}$ and E$_{\text{max}}$ data

*Consistently used the Sigmoid 3-parameter model*

<table>
<thead>
<tr>
<th></th>
<th>RIF</th>
<th>CBM</th>
<th>NIF</th>
<th>PB</th>
<th>PHT</th>
<th>PIO</th>
<th>RSG</th>
<th>TRO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E$_{\text{max}}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (CV%)</td>
<td>36.8 ± 42 (115%)</td>
<td>18.3 ± 11 (59%)</td>
<td>37.5 ± 34 (91%)</td>
<td>36.6 ± 23 (62%)</td>
<td>19.4 ± 11 (59%)</td>
<td>26.6 ± 17 (64%)</td>
<td>26.1 ± 9.2 (35%)</td>
<td>52.6 ± 40 (76%)</td>
</tr>
<tr>
<td>Median</td>
<td>21.6</td>
<td>14.9</td>
<td>28.9</td>
<td>33.6</td>
<td>17.6</td>
<td>21.1</td>
<td>23.9</td>
<td>37.6</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>EC$_{50}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (CV%)</td>
<td>1.46 ± 1.5 (101%)</td>
<td>47.0 ± 31 (65%)</td>
<td>15.0 ± 12 (80%)</td>
<td>331 ± 229 (69%)</td>
<td>42.0 ± 38 (90%)</td>
<td>20.6 ± 18 (85%)</td>
<td>34.3 ± 18 (52%)</td>
<td>8.35 ± 4.8 (58%)</td>
</tr>
<tr>
<td>Median</td>
<td>0.849</td>
<td>39.1</td>
<td>14.5</td>
<td>264</td>
<td>28.0</td>
<td>24.0</td>
<td>34.9</td>
<td>9.07</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

The combined data set showed variability in the *in vitro* induction parameters in the range of 35-127%. Based on the results of the predictions, these median values appeared to work relatively well in the models.

RIF = rifampin; CBM = carbamazepine; NIF = nifedipine; PB = phenobarbital; PHT = phenytoin; PIO = pioglitazone; RSG = rosiglitazone; TRO = troglitazone