Presentation Overview

• Regulatory guidance
• Brief review on drug-drug (Disease) interactions
• Down-regulation by large molecules
  – IL-6 as an example
  – Examples from literature
  – Data generated in-house

• Down-regulation by small molecules
  – Examples from literature
Large Molecules Defined

• In this presentation, “large molecules” refer to therapeutic proteins (TPs) intended for pharmaceutical use.
  – Include monoclonal antibodies, cytokines, growth factors, enzymes and other protein products.
Regulatory Guidance For DDI


- EMA (April, 2010) DRAFT Guideline on the Investigation of Drug Interactions
FDA Guidance

• If an investigational TPs is a cytokine or cytokine modulator, studies should be conducted to determine the TP’s effects on CYP enzymes or transporters.

• *In vitro* or animal studies have limited value in the qualitative and quantitative projection of clinical interactions.

• The *in vivo* evaluations of TPs in targeted patient populations can be conducted.

• When there are known mechanisms or prior experience with certain PK or PD, interactions, appropriate *in vitro* or *in vivo* assessments for possible interactions should be conducted.

• Does not discuss *in vitro* model or experimental details for study on CYP suppression by TP or small molecules.
EMA Guidance

- Mentions in several places that an *in vitro* model (e.g. human hepatocytes) for induction testing can be used for testing down-regulation.
  - “*These studies (induction) will also detect enzyme down-regulation…*”
  - “*mRNA could also be included and is mandatory for the interpretation of study results….. if a down-regulation is suspected based on the activity assay.*”

- Does not discuss the interaction with TPs.
Drug-Drug and Drug-Disease Interactions

• Cytokines are often found to cause global suppression or down-regulation of CYPs and transporters.
  – Cytokines may be elevated as result of disease state or administered as a drug.
  – May increase AUC of co-administered small molecule drugs.

• Suppression may be reversed (“de-suppression”) if the down regulating pathway is disrupted.
  – The antiarthritic drug Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor.
  – Prevents IL-6 mediated down-regulation of CYPs and may restore “normal” CYP activity (induction-like), leading to decreases in AUC of small molecule drugs.
Example: Tocilizumab and Simvastatin

IL-6 in Regulation of CYP Enzymes in Human Hepatocytes *in vitro*

- Dickmann et al used human hepatocytes as a model for study on IL-6-mediated CYP suppression.
- IL-6 caused a concentration-dependent suppression of CYP1A2 and 3A4 activity as well as multiple CYP mRNA expression when human hepatocytes from 9 donors were treated for 3 days with IL-6.
- A monoclonal antibody directed against IL-6 abolished or partially blocked IL-6-mediated suppression of CYP1A2 and CYP3A4 enzyme activity.
- The study demonstrate that human hepatocyte primary culture can be used for quantitative measurement of CYP suppression and desuppression.
- We wanted to reproduce in part this data in our lab.

*Dickmann et al. DMD 39:1415, 2011*
Cryopreserved human hepatocytes were plated in 24-well plates over night.

3-day treatments with IL-6 with or without in the presence of positive control inducers.

Enzyme activity “in situ” (CYP1A2, 2B6 and 3A4).

mRNA Isolation

RT-PCR (CYP1A2, 2B6 and 3A4)
The three lots chosen exhibited a wide range of CYP1A2 and CYP3A4 basal activities.

This permitted examination of how differing levels of basal activity might affect the detection of CYP suppression by IL-6.
Effect of IL-6 on CYP3A4 Activity and mRNA

- IL-6 (1 pg/mL - 500 ng/mL) was incubated with hepatocytes in presence of positive control inducer rifampicin (10 µM) or vehicle for 3 days.

- Concentration-dependent suppression of CYP3A4 for both activity and mRNA expression was observed.

- The suppression pattern of basal and induced CYP3A4 was similar.
Effect of IL-6 on CYP3A4 Activity and mRNA (EC50)

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>IL-6 (EC50 ng/mL)</th>
<th>IL-6 + RIF (ng/mL)</th>
<th>Em (IL-6 % Control)</th>
<th>Emax (IL-6 % of Control)</th>
<th>Em (IL-6 + RIF % Control)</th>
<th>Emax (IL-6 + RIF % of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>285</td>
<td>0.46</td>
<td>2.4</td>
<td>124</td>
<td>94</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>295</td>
<td>0.087</td>
<td>1.9</td>
<td>75</td>
<td>85</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>311</td>
<td>0.087</td>
<td>1.3</td>
<td>92</td>
<td>101</td>
<td>3</td>
<td>22</td>
</tr>
</tbody>
</table>

- EC50s for both activity and mRNA were similar for all three donors.
- The EC50 was shifted right for induced CYP3A4 for all three donors.
Effect of IL-6 on CYP2B6 Activity and mRNA

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>311</td>
<td>0.20</td>
<td>1.6</td>
<td>80</td>
<td>107</td>
<td>16</td>
<td>33</td>
</tr>
</tbody>
</table>

EC50 (ng/mL)  
Emin (% Control)  
Emax (% of Control)

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
<th>IL-6</th>
<th>IL-6 + PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>311</td>
<td>1.1</td>
<td>0.69</td>
<td>100</td>
<td>99</td>
<td>44</td>
<td>24</td>
</tr>
</tbody>
</table>

- IL-6 (1 pg/mL- 500 ng/mL) was incubated with hepatocytes for 3 days with positive control inducer phenobarbital (1000 µM) or its vehicle.
- Concentration-dependent suppression of CYP2B6 for both activity and mRNA expression was observed.
- The suppression pattern for both basal and induced CYP2B6 was similar.
Effect of IL-6 on CYP1A2 Activity and mRNA

- IL-6 (1 pg/mL- 500 ng/mL) was incubated with hepatocytes for 3 days in the presence of positive control inducer omeprazole (50 µM) or its vehicle.

- Concentration-dependent suppression of CYP1A2 for both activity and mRNA expression was observed except for CYP1A2 mRNA, where no significant suppression of induced CYP1A2 mRNA was seen (not examined in Dickmann et al).
Effect of IL-6 on CYP1A2 Activity and mRNA (EC50)

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>IL-6</th>
<th>IL-6 + OME</th>
<th>IL-6</th>
<th>IL-6 + OME</th>
<th>IL-6</th>
<th>IL-6 + OME</th>
</tr>
</thead>
<tbody>
<tr>
<td>285</td>
<td>5.5</td>
<td>7.6</td>
<td>91</td>
<td>132</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>295</td>
<td>2.0</td>
<td>4.2</td>
<td>132</td>
<td>118</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>311</td>
<td>0.96</td>
<td>2.0</td>
<td>107</td>
<td>106</td>
<td>15</td>
<td>40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>EC50 (ng/mL)</th>
<th>Emin (% Control)</th>
<th>Emax (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>285</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>295</td>
<td>2.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>311</td>
<td>2.4</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Suppression effects for both activity and mRNA were relatively similar for all three donors. CYP1A2 EC50 >> CYP2B6 ~ CYP3A4.
Effect of BD Matrigel® Overlay on CYP1A2 and CYP3A4 Suppression by IL-6

Use of BD Matrigel® overlay did not affect the assay sensitivity and suppression response by IL-6.
Effect of Kupffer Cells on Suppression by IL-6

• Kupffer cells are specialized macrophages located in the liver. The activation of Kupffer cells is associated with production of cytokines and immune response in the liver.

• Summary of Sunman et al. DMD 32:359, 2004
  – Human Kupffer cell/hepatocytes were co-plated at a ratio of 0/10, 1/10 and 4/10. Treatment of IL-6 with culture was conducted for three days.
  – IL-6 caused a decrease in CYP3A4 activity in all plate formats and no significant difference was found among these different formats.
  – The study suggested that IL-6 mediated-CYP3A4 suppression is not altered by Kupffer cells co-culture.

• Similar findings by Nguyen et al (DDI – Seattle, 2011)
No description on the down-regulation caused by small molecule drug candidates and its drug-drug interactions from FDA recent guidance.

It has been reported that small molecule drugs cause down-regulation of CYP enzymes (examples).

No much information is available from literature for drug interactions caused by down-regulation with small molecules.
Methods to Test Down-Regulation

• Plated human hepatocytes are the gold-standard model for testing both induction and down-regulation (EMA guidance).

• Enzyme activity is a common endpoint for induction testing. However, enzyme activity may be inhibited by the NCE (“masking”).
  – Direct or time-dependent inhibition

• How to address?
  – Inhibition can be tested in standard liver microsomal inhibition assays.
  – Test for inhibition in positive control inducer-treated wells (in situ assay). Induced cells ensure high uninhibited activity.
  – Test induction of activity in microsomes prepared from the treated hepatocytes (caution – the preparation of microsomes does not always wash out the NCE).
  – Can be assessed by mRNA or western blotting.
Direct Enzyme Inhibition by NCE

Inhibition assay: test item (in this case TAO or ritonavir) added at end of 3d treatment for 30 minutes, followed by wash and addition of probe substrate.
Hepatocytes from two donor livers were treated with TAO (0.2, 2, and 20 µM) for 3 days. After treatment, CYP3A4 activity and mRNA expression were determined.
The test article decreased CYP3A4 activity in a concentration-dependent fashion. Consistent in three donors.

No inhibition of enzyme activity found in liver microsomes or in hepatocytes \textit{in situ} inhibition tests.

No evidence of hepatocyte toxicity

mRNA data showed a similar pattern as activity.

The data suggest that the test article causes down-regulation of CYP3A4.
Mechanisms of Down-regulation by Small Molecules *in vitro*

- **Nuclear receptor antagonists/inhibitors**
  - Dietary Isothiocyanate Sulforaphane (example forthcoming)
  - HIV protease inhibitor A-792611 (example)
  - Antineoplastic agent ET-743 (Synold et al. Nat Med 7:584, 2001)

- **Disruption of interaction between PXR and co-activators**
  - Anticancer agent Camptothecin (CPT) (example)

- **Disruption of cellular microtubule network**
  - (Dvorák et al. Current Drug Metabolism, 6:545, 2005)
Direct PXR Antagonism-Sulforaphane (SFN)

- SFN is a biologically active phytochemical in the human diet and it is present at high concentrations in some vegetables such as broccoli.
- Treatment of human hepatocytes with SFN for 24 hours decreased midazolam clearance.
- SFN also decreased both basal and induced CYP3A4 mRNA expression.
- SFN efficiently inhibited SXR/PXR activity but not other nuclear receptors such as mPXR, rPXR, CAR, VDR, PPAR and RXR.
- SFN was specially bound to the purified SXR ligand binding domain.
- The study suggests that direct human SXR/PXR antagonism is responsible for CYP3A4 down-regulation caused by SFN.

A-792611 is a HIV protease inhibitor.

Decreases in CYP3A4 and CYP2B6 mRNA expression in cultured human hepatocytes treated with the drug for 48 h.

Inhibition of RIF or RIT-mediated PXR transactivation in HepG2 cells

Lack of interaction with other nuclear receptors

The data indicate that A-792611 is a functional PXR inhibitor

Disruption of Interaction between PXR and Co-activators- Camptothecin (CPT)

- CTP is a topoisomerase I inhibitor and used for cancer treatment.
- It did not decrease basal CYP3A4 activity, mRNA and protein but dramatically reduced RIF-induced CYP3A4 activity, mRNA and protein in cultured human hepatocytes treated with both RIF and CPT for 72 h.
- It did not compete LBD of PXR with agonists but inhibited RIF-induced PXR transactivation.
- It inhibited the binding of cofactor SRC-1 to hPXR.
- Data suggest that CPT may disrupt the interaction between PXR and co-activators and caused CYP3A4 down-regulation.

Chen et al. JEPET 334:999, 2011
Conclusions and Perspective

• Plated cryopreserved human hepatocytes may be a useful model in assessment of large and small molecule-induced suppression/down-regulation. It may help explain responses observed *in vivo*.

• Treatment of hepatocytes with IL-6 caused a concentration-dependent suppression effect on CYP1A2, 2B6 and 3A4. CYP3A4 was most sensitive to suppression by IL-6, followed by CYP2B6 and CYP1A2.
Conclusions and Perspective (continued)

• The effect on CYPs by IL-6 is more complicated than it has been thought. The differential effects on activity and mRNA (e.g. CYP1A2) suggests examination of both endpoints have value.

• Although the suppression of both activity and mRNA by IL-6 can be determined in the lots with lower basal activity, selection of donors with higher basal activity may be prudent to facilitate the quantitation under conditions of high suppression.

• The often complex biology of suppression responses may preclude meaningful predictions of pharmacokinetic parameters from in vitro data at this time.
George Zhang
e-mail: george_zhang@bd.com
tel: 781-935-5115 x2250