Cytochrome P450 Mediated Drug-Drug Interactions in Drug R&D

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Outline

• Part I
  – Drug Interaction and Regulatory Guidance

• Part II
  – CYP Inhibition and Induction in Drug-Drug Interaction
Part I
Drug Interaction and Regulatory Guidance
Outline

• Introduction
  – DDI
  – Cytochrome P450

• FDA draft guidance
  – Data analysis
  – DDI evaluation
  – Decision making
Drug-Drug Interactions

**Definition**

- Pharmacokinetic drug-drug interaction:
  - Broadly defined as the effects of one drug on the metabolic clearance of another, which may increase or decrease plasma/tissue drug concentrations to which with significant toxic consequences or therapeutic incompetence.

**Consequences**

- Exaggerated pharmacology and adverse effects including toxicity
- Decreased therapeutic effect and lack of efficacy
- Major concern for pharmaceutical industry and regulatory authorities
  - Five of 12 drugs withdrawn from the US market from 1997 to 2002 were prone to metabolic drug-drug interactions (Huang 2004)
Pharmacokinetic Drug-Drug Interaction

Drug A at SS

Drug B

Drug Concentration

Time

DDI

Therapeutic Window

Lack of Therapeutic Effect

Toxicity
Impact of Drug-Drug interaction on Medical Community

- Patients – Adverse drug effect, such as morbidity and mortality or lack of efficacy
- Physicians – Medical-legal liability
- Healthcare systems – Increased cost due to increased incidents
- Pharmaceutical companies – Medical-legal liability, drug attrition or restriction, increased cost in drug R&D
Types of Drug-Drug Interactions

• Role
  – Victim: susceptible to effects of enzyme inhibitors or inducers
    • Substrate
  – Perpetrator: alter the elimination or bioavailability of existing drugs

• Mechanism
  – **CYP mediated**
  – Transporter mediated
  – Other enzyme mediated
Cytochrome P450 (CYP or P450)

- A diverse super family of hemoproteins found in a variety of animals, plants, and microorganisms
- In mammals, present predominantly in liver, also found in intestine, kidney, lung, brain, adrenal cortex and other tissues
- In hepatocytes, P450s mainly localize in the ER
Function of CYP

- Major enzymes in drug metabolism for biotransformation of xenobiotics and endogenous components
- Monooxyenase reaction

\[
\text{RH} + \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{ROH} + \text{H}_2\text{O}
\]

# Human P450s

<table>
<thead>
<tr>
<th>Sterols</th>
<th>Xanthobiotics</th>
<th>Fatty acids</th>
<th>Eicosanoids</th>
<th>Vitamins</th>
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<td>1B1</td>
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<td>2J2</td>
<td>4F2</td>
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<td>2A7</td>
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<td>7A1</td>
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<td>7B1</td>
<td>2A6</td>
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<td>4F8</td>
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<td>21A2</td>
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<td>4V2</td>
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<tr>
<td>27A1</td>
<td>2E1</td>
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<td>4X1</td>
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<tr>
<td>39</td>
<td>2F1</td>
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<td></td>
<td>4Z1</td>
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<td>46</td>
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<td>3A5</td>
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<td></td>
<td>3A7</td>
<td></td>
<td></td>
<td>27C1</td>
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</tr>
</tbody>
</table>

*This classification is somewhat arbitrary (e.g., P450s 1B1 and 27A1 could be grouped in two different categories).*

*Source: Adapted from Guengerich (2003a, 2004).*
Elimination of Top 200 Most Prescribed Drugs in 2002: Role of CYPs

Weinkers and Heath, Nat. Rev. Drug Disc., 2005
Example - Effects of CYP/Transporter Inhibition on PK of Oral Antidiabetic Drugs

<table>
<thead>
<tr>
<th>Victim drug</th>
<th>Perpetrator drug (daily dose, mg)</th>
<th>Changes in pharmacokinetic parameters</th>
<th>Enzyme or transporter involved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AUC</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
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<tr>
<td>Thiazolidinediones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaglitzonide</td>
<td>Gemfibrozil (1200)</td>
<td>+239%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil (1200)</td>
<td>+222%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil (1200) + Itraconazole (100)</td>
<td>+291%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim (320)</td>
<td>+42%</td>
<td>NS</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Fluvoxamine (100)</td>
<td>+21%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil (1200)</td>
<td>+129%</td>
<td>+22%</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (400)</td>
<td>+47%</td>
<td>+17%</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim (320)</td>
<td>+37%</td>
<td>+14%</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim (400)</td>
<td>+31%</td>
<td>NS</td>
</tr>
<tr>
<td>DPP-4 inhibitors</td>
<td>Linagliptin</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Ritonavir (400)</td>
<td>+101%</td>
<td>+196%</td>
</tr>
<tr>
<td>Saxagliptin</td>
<td>Diitiazem (360)</td>
<td>+109%</td>
<td>+63%</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole (400)</td>
<td>+145%</td>
<td>+62%</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>Cyclosporine (600 single dose)</td>
<td>+29%</td>
<td>+68%</td>
</tr>
</tbody>
</table>

Example – Clinically Beneficial DDI

- HIV protease inhibitors (PI) generally have poor systemic bioavailability
- PIs are mainly metabolized by CYP3A and are potent CYP3A/P-gp inhibitors
- Ritonavir increases saquinavir concentrations at steady state by up to 30-fold,
  - Inhibition of first-pass metabolism mediated by CYP3A
  - Reduction in intersubject variance
  - Allow reduction of saquinavir dose and dosing frequency

Drug Interaction Studies: Objective

- Objective of interaction studies for a new drug is to determine:
  - whether any interactions are sufficiently large to necessitate a dosage adjustment of the drug itself or of the drugs with which it might be used
  - whether any interactions calls for additional therapeutic monitoring, or
  - whether there should be a contraindication to concomitant use when lesser measures cannot mitigate risk
Drug Interaction Studies: Guidance for Industry

- Guidance for Industry: Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling
  - New draft Guidance issued in Feb 2012 by USFDA Center for Drug Evaluation and Research (CDER)
  - “being distributed for comment purpose only”
  - Previous issue in Sep 2006

- Improvements/difference
  - Transporter focus
  - Physiologically-based pharmacokinetic modeling (PBPK)
  - Therapeutic protein (TP)-drug interactions
  - Mechanistic approaches for conduct and interpretation of DDI studies
Drug Interaction Studies: Decision Tree

Figure 2. Metabolism-Based Drug-Drug Interaction Studies — Decision Tree

- Conduct In Vitro Metabolism and Drug-Drug Interaction Studies in Human Tissues
  - Phase I enzymes: CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A, others
  - Phase II enzymes: UGTs (see Figure 3)

Is investigational drug a substrate of an enzyme responsible for ≥25% of its systemic clearance?

- Yes or inconclusive
  - Is investigational drug a substrate of multiple metabolizing enzymes together responsible for ≥25% of its systemic clearance?
    - No
      - Label as such based on in vitro and in vivo disposition data
    - Yes
      - Evaluate potential of complex drug-drug interaction

Presence of significant interaction?

- Yes
  - Conduct in vivo studies with strong inhibitor(s)/inducer(s) or if appropriate, compare PK in different genotypes

Dosage adjustment needed?

- Yes
  - Yes
  - No

- No
  - No

Presence of significant interaction?

- Yes
  - Conduct in vivo studies with most sensitive/species substrate(s)

Dosage adjustment needed?

- Yes
  - Yes
  - No

- No
  - No

Conduct in vivo studies with other less strong inhibitors/inducers selected based on likely co-administration or if appropriate, apply mechanistic modeling (see Figure 4)

Conduct in vivo studies with other substrates selected based on likely co-administration and/or narrow therapeutic range or if appropriate, apply mechanistic modeling (see Figure 4)

Label as such based on in vitro data

DDI Evaluation

**Figure 4. General Scheme of Model-Based Prediction: The Investigational Drug (and Metabolite Present at ≥25% of Parent Drug AUC) as an Interacting Drug of CYP Enzymes**

**CYP inhibition** (reversible and time-dependent inhibition, TDI)
- Measure enzyme activity in human liver microsomes
- Estimate DDI parameters

**CYP induction**
- Measure mRNA change by investigational drug in cultured human hepatocytes from ≥3 donors
- Estimate DDI parameters

**Basic models**
- Is the calculated R value > 1.1 (also, for CYP3A inhibitors given orally, is alternate R value > 1.1?)
  - Reversible inhibitor, \( R_i = 1 + [I]/K_i \)
  - TDI, \( R_T = (K_{ext} + K_{ext})/K_{ext} \) and \( K_{ext} = k_{max} * [I]/(K_i + [I]) \)

**Mechanistic models**
- Is AUCR > 1.25 (inhibition) or AUCR < 0.8 (induction)?
  - Estimate AUCR of a sensitive probe substrate using
    - a mechanistic static model
      \[
      \text{AUCR} = \left( \frac{A_k \times B_k \times C_k + (1 - F_s)}{A_k \times B_k \times C_k + (1 - F_s)} \right) \times \left[ A_k \times B_k \times C_k \right] \times f_m + (1 - f_m)
      \]
    - or a dynamic model, including PBPK

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

• R value
  – Equation
    • reversible inhibition: \( R_1 = 1 + \frac{[I]}{K_i} \)
    • Time-dependent inhibition: \( R_2 = \frac{K_{obs} + K_{deg}}{K_{deg}} \) where
      \( K_{obs} = \frac{k_{inact}[I]}{(K_I + [I])} \)
    • Induction: \( R_3 = \frac{1}{1 + dxE_{max}[I]/(EC_{50} + [I])} \)
  – Threshold
    • Inhibition: > 1.1, where [I] is the max total systemic concentration of
      the inhibitor
    • Induction: < 0.9

• Conservative, simple and practical
  – Eliminate the need for in vivo DDI study when R value below the
    threshold
  – Rank order inhibition potential across different CYP enzymes
DDI Evaluation

Figure 4. General Scheme of Model-Based Prediction: The Investigational Drug (and Metabolite Present at ≥25% of Parent Drug AUC) as an Interacting Drug of CYP Enzymes

- **CYP inhibition** (reversible and time-dependent inhibition, TDI)
  - Measure enzyme activity in human liver microsomes
  - Estimate DDI parameters

- **CYP induction**
  - Measure mRNA change by investigational drug in cultured human hepatocytes from ≥3 donors
  - Estimate DDI parameters

**Basic models**

- Is the calculated R value >1.1 (also, for CYP3A inhibitors given orally, is alternate R value >11)?
  - Reversible inhibitor, \( R_I = 1 + \frac{[I]}{K_I} \)
  - TDI, \( R_I = \frac{(K_{I+}, K_{I-})}{K_{I+}} \) and \( K_{I+} = \frac{K_{max}}{\frac{[I]}{K_{I+} + [I]}} \)

**Mechanistic models**

- Is AUCR ≥1.25 (inhibition) or AUCR ≤0.8 (induction)?
  - Estimate AUCR of a sensitive probe substrate using a mechanistic static model
    \[ \text{AUCR} = \frac{A_R \times B_R \times C_R \times (1-F_R) + F_R}{[A_R \times B_R \times C_R \times f_m + (1-f_m)]} \]
  - or a dynamic model, including PBPK

- Investigational drug likely a CYP inhibitor
- Investigational drug likely a CYP inducer

- Conduct a clinical study using an appropriate probe substrate

No: Label as non-inhibitor or non-inducer based on in vitro data
Yes: Label as non-inhibitor or non-inducer
Mechanistic Static Model

- Integrate more parameters in drug disposition and drug interaction mechanisms
- Predicted AUC ratio <0.8 or >1.25

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

<table>
<thead>
<tr>
<th></th>
<th>Gut</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversible inhibition</td>
<td>[ A_g = \frac{1}{\frac{[I]_g}{1 + \frac{[I]_g}{K_i}}} ]</td>
<td>[ A_h = \frac{1}{1 + \frac{[I]_h}{K_i}} ]</td>
</tr>
<tr>
<td>Time-dependent inhibition</td>
<td>[ B_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]<em>g \times k</em>{inact}}{[I]_g + K_I}} ]</td>
<td>[ B_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]<em>h \times k</em>{inact}}{[I]_h + K_I}} ]</td>
</tr>
<tr>
<td>Induction</td>
<td>[ C_g = 1 + \frac{d \cdot E_{max} \cdot [I]_g}{[I]_g + EC_50} ]</td>
<td>[ C_h = 1 + \frac{d \cdot E_{max} \cdot [I]_h}{[I]_h + EC_50} ]</td>
</tr>
</tbody>
</table>
PBPK Model

• Alternative to dedicated clinical studies – still developing

• Integrate both system and drug dependent parameters
  – Reflect dynamic of DDI – PK profile
  – Evaluate concurrent mechanism of DDI, including inhibitory metabolites
  – Population-based model provide insight into variability and uncertainty

• Suggested cutoff
  – Predicted AUC ratio <0.8 or >1.25
Figure 5. Using a PBPK Model to Explore Drug-Drug Interaction Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao et al. 2011).

**In vitro and in silico human ADME data**
- Physicochemical: LogP, pKa
- Absorption: \( P_{int} \)
- Distribution: B/P, \( K_d, K_a, f_{up} \)
- Metabolism and transport: \( K_m, V_{max}, I_{max}, C_{int} \)
- DDI: \( K_c, K_{int}, K_i \), Induction (EC50, \( E_{max} \), and \( \gamma \))

**Parameter input to build initial PBPK models**

**In vivo human PK data** (compartmental or PopPK)
- Absorption and first pass metabolism:
  \( F=F_p, F_a, K_a \)
- Distribution: \( V_v \)
- Elimination: \( CL, CL_{int} \)
- PK of metabolite(s) after parent drug administration
- PK of metabolite(s) after metabolite administration, when available

**Final PBPK model**

**Link two models:**
- Include all mechanisms (e.g., reversible inhibition, time-dependent inhibition, and induction)
- Use operating inhibitor/inducer concentration (e.g., unbound target tissue concentrations)

**Simulate drug-drug interactions**

**Evaluate drug-drug interaction potential**
- Predict substrate exposure ratio (AUC and \( C_{max} \)) and their variability
- Consider physiological/biological plausibility and evaluate parameter uncertainty
PBPK DDI Simulation: Simcyp Approach

Input parameters

Distribution

Log P
pKa
Protein binding

Clearance

Intrinsic CL
Protein binding
Blood/Plasma ratio

Absorption

Fa x Fg x Fh = F

PBPK disposition

Solubility
Effective Permeability (Peff)
Caco2, Pampa, MDCK
First pass (Liver - gut)
Transporter

Output

Simulation

C plasma or tissue vs Time
Case Study – PBPK Simulation & DDI Prediction

- Candidate cpd X: small basic molecule, BCS Class I, almost exclusively cleared by metabolism

- Request from project team: evaluate DDI potential of cpd X as a victim drug given its non-linear kinetics in function of dose and time

![Graph showing Dose Normalized (to 100mg) Observed and Mean Cmax](image1)

![Graph showing Dose Normalized (to 100mg) Observed and Mean AUCinf](image2)
Case Study – In vitro Studies

- In vitro experiments were performed to mechanistically characterize the clearance of cpd X in human kidney, lung and liver
- Mainly CYPs and FMOs involved
- CYP1A2 was the major driver of clearance and non-linearity
Case Study: Actual and simulated data

50 mg SAD plasma PK

400 mg SAD plasma PK

75 mg BID plasma PK

400 mg SAD Hep. Intr. Clearance
Case Study: DDI with Fluvoxamine (100 mg QD)

- 25 mg cpd x
- 100 mg cpd x
- 75 mg BID cpd x

- Cmax 5.7 fold
- AUC∞ 17 fold
- Cmax 1.7 fold
- AUC∞ 4.8 fold
- Cmax 2.1 fold
- AUCτ 2.8 fold

**Observed:**
- Cmax 5.0 fold
- AUC∞ 21 fold
- Cmax 2.1 fold
- AUC∞ 5.1 fold

- Candidate compound dose and dosing regimen is a crucial determinant in DDI simulations
Case Study: Impact on the Project

- The simulations demonstrated that the extent of DDI was mainly CYP1A2-mediated and dose-dependent: i.e. 17-fold AUC increase at low dose vs 3-fold AUC increase at pharmacological relevant dose. It was concluded that at clinical relevant doses the extent of interaction was less significant.

- PBPK modeling allows to optimally design clinical drug-drug interaction studies at different doses with several perpetrators using the optimal dosing regimen

- PBPK modeling enables to simulate inter-individual variability in PK and DDI in healthy volunteers and special populations (smokers, hepatic impairment,...)
### Summary of Parameters for DDI Evaluation

**Table 1** Important parameters needed for evaluation of complex drug interactions (modified from ref. 5)

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Parameters estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em> ADME and interaction</td>
<td>Enzymes/transporters involved in elimination and interaction</td>
</tr>
<tr>
<td></td>
<td>Drug distribution (e.g., $f_{up}$ and B/P)</td>
</tr>
<tr>
<td></td>
<td>Interaction mechanisms and parameters (e.g., $K_i$)</td>
</tr>
<tr>
<td></td>
<td>Initial $f_m$ estimation</td>
</tr>
<tr>
<td>Phase I dose escalation (oral administration)</td>
<td>$CL/F_{oral}$</td>
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<tr>
<td></td>
<td>$V/F_{oral}$</td>
</tr>
<tr>
<td></td>
<td>Likely $f_m$, $CL_r$, and metabolite data</td>
</tr>
<tr>
<td>Absolute oral bioavailability</td>
<td>$CL$</td>
</tr>
<tr>
<td></td>
<td>$V$</td>
</tr>
<tr>
<td></td>
<td>Likely $CL_r$, and metabolite data</td>
</tr>
<tr>
<td></td>
<td>$F_{oral}$</td>
</tr>
<tr>
<td><em>In vivo</em> mass balance (e.g., studies in humans using radiolabeled material)</td>
<td>Confirm $f_m$</td>
</tr>
<tr>
<td></td>
<td>Confirm $f_a$</td>
</tr>
<tr>
<td></td>
<td>Confirm $f_{er} CL_r$</td>
</tr>
</tbody>
</table>

ADME, absorption, distribution, metabolism, and excretion; B/P, blood-to-plasma ratio; $CL_r$, clearance; $CL_r$, renal clearance; $f_{er}$, fraction absorbed; $f_{e}$, fraction of the dose excreted unchanged in the urine; $f_{er}$, fraction metabolized; $F_{oral}$, oral bioavailability; $f_{up}$, unbound fraction in plasma; $K_i$, reversible inhibition constant; $V$, volume of distribution.

Comparison with EMA guidance

• European Medicines Agency (EMA): Guidance on the investigation of drug interactions, draft issued in April 2010

• Inconsistency in FDA and EMA guidance
  – Differences in static equations used in decision making
  – Enzyme induction assessment
  – List of transporters
  – Decision trees

• Harmonization and coordination between FDA and EMA are needed
Thank You! & Questions?

Janssen Research & Development
Drug Safety Sciences, Asia Pacific
Part 2 - CYP Inhibition and Induction in Drug-Drug Interaction
Outline

• CYP inhibition
  – Mechanisms
  – Study design

• CYP induction
  – Mechanisms
  – Study design

• Therapeutic protein-drug interaction

• Food and herb drug interaction
CYP Inhibition - Reversible

- **Mechanisms**
  - Competitive: inhibitor and substrate compete for binding
  - Uncompetitive: inhibitor binds when probe substrate is already bound
  - Noncompetitive: both inhibitor and substrate can bind to P450
  - Mixed-type: hybrid combination of the above three mechanisms

\[
\begin{align*}
I_{1}E + S & \xrightleftharpoons[\alpha_{1}K_{d}]{K_{i,1}\quad I_{1}ES} \\
E + S & \xrightleftharpoons[K_{d}]{k_{\text{cat}}} ES \rightarrow E + P
\end{align*}
\]

- Responsible for the majority (62%) of strong in vivo drug drug interactions (DDIs)
  - 33% of irreversible inhibitors cause strong DDIs [Chem Res Toxicol, 2009, 22(2):294-8]
Reversible Inhibition Assay and Role in Drug R&D

Wienkers and Heath, Nat Rev Drug Disc, 2005, 4: 825-833
Mechanism-Based Inactivation (MBI)

- **Definition**
  - “Mechanism-based enzyme inactivator is a relatively unreactive compound, having a structural similarity to the substrate or product for a particular enzyme that, via its normal catalytic mechanism of action, converts the inactivator molecule into a species which without prior release from the active site, binds most often covalently to that enzyme.” [R. silverman]

\[
E + I \xrightleftharpoons[k^{-1}]{k^{+1}} EI \xrightarrow{k_2} EI' \xrightarrow{k_4} Einact
\]

\[
\downarrow k_3
\]

E + P

MBI Characteristics

- Time dependence of inactivation
  - Time-Dependent Inactivation (TDI)
- Saturation
- Substrate protection
- Irreversibility
- Inactivator stoichiometry
- Involvement of catalytic step
- Inactivation prior to release of active species
TDI Impact on Drug R&D

• Complicate PK profile if the inactivated enzyme is the primary enzyme of metabolism
  - Non-linear PK, unpredictable drug accumulation
  - More problematic for drugs having narrow safety margin

• Substantially increase DDI potency
  - Irreversibly decrease enzyme activity
  - Prolonged alternation of enzyme activity even after drug administration discontinued (unlike reversible enzyme inhibition)

• Potential of reactive metabolite formation

• Difficult to overcome and predict
  - Quantitative in vitro/in vivo correlation needs to be established
  - DDI simulations (e.g., Simcyp) can be used for MBI
TDI Assay

DDI of Multiple Inhibitor Systems

• Inhibitory metabolites
  – An IC50 method to explore metabolism-dependent inhibition

• Racemic mixture of stereoisomers

• Co-administration of inhibitors of the same enzyme
CYP Inhibition Assay – Points to Consider

- Representative pool of human liver microsomes
- Low protein concentration
- Short marker substrate incubation time
- Robust assay
  - FDA/Pharma-accepted probe substrates and concentrations
  - Qualified/validated LC/MS/MS method
  - Stable isotope labeled internal standards
  - Consistency (negative and positive controls)
Cytochrome P450 Induction

- **Mechanism**
  - Promotion of gene activation or messenger RNA (mRNA) synthesis or inhibition of degradation of protein or mRNA
  - Most prominent mechanisms for CYP induction are ligand-dependent transcription activation of nuclear receptors, such as pregnane X receptors (PXR), constitutive androstane receptors (CAR) or aryl hydrocarbon receptors (AhR)
  - A slow regulatory process in contrast to the immediate response of CYP inhibition
  - Species differences in CAR and PXR activation
Mechanisms – PXR and CAR

RXR: retinoid X receptor
CCRP: cytoplasmic CAR retention protein,
HSP90: heat shock protein 90,
PP2A: protein phosphatase 2A,
PBREM: phenobarbital-responsive enhancer module,
XREM: xenobiotic responsive enhancer module

Kakizaki et al, Frontiers Biosci, 2011, 16: 2988-3003
Mechanisms - AhR
Example of CYP Induction-Mediated DDI

Figure 2. Median steady-state saquinavir (a) and ritonavir (b) plasma concentration–time profiles with and without rifampicin/isoniazid (n = 15). Error bars indicate interquartile ranges (25th and 75th percentiles). Open circles indicate drug concentrations without rifampicin/isoniazid. Filled circles indicate drug concentrations with rifampicin/isoniazid.

CYP Induction Assays

• PXR (Pregnane X receptor) activation
  – CYP3A and 2C

• Primary culture of human hepatocytes
  – CYP1A2, 2B6, and 3A

• Ex vivo
  – Liver microsomes prepared from treated animals (usually from tox studies)
  – Enzyme activity against vehicle controls
  – Species difference makes the prediction of clinical induction difficult
CYP Induction Assay – Human Hepatocyte Incubations

- **Endpoints**
  - Gene expression
    - qRT-PCR analysis of mRNA levels
  - Enzyme activity
    - Determined in the hepatocytes (*in situ*) or in microsomes prepared from the treated hepatocytes

- **CYP enzymes tested:** CYP1A2, 2B6, and 3A (minimum)

- **Assay specifics**
  - Freshly isolated or cryopreserved hepatocytes
  - At least 3 donors
  - Contain vehicle, positive and negative controls
  - Cell morphology and LDH release are used to assess cell toxicity
Therapeutic Protein (TP)-Drug Interactions

- A survey showed that drug labels for 38 out of 68 new TPs approved by the FDA by the end of 2008 (56%) included some information about drug interactions [Huang et al, Clin Pharmacol Ther, 2010, 87(4):497-503]
  - Most are cytokine products (81%) and mAb including cytokine modulators (65%)

- Cytokines and cytokine modulators can influence expression and stability of CYPs and transporters
  - Therapeutic mAb that modulate cytokine activities can indirectly influence by affecting cytokine concentrations

- Therapeutic mAb exposures can be altered by drugs that affect the production of anti-drug (TP) antibodies

- So far the importance of these interactions is lesser perceived compared with small molecules and lack of clinically significant interactions
  - No evidence of dosing adjustments pertaining to TP–drug interactions
CYP Activity Decrease by TPs

<table>
<thead>
<tr>
<th>CYP enzyme</th>
<th>Cytokines/cytokine modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>IFN-α, IFNα-2b, IFN-β, IL-2, IL-6, hGH&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>IL-1</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>IL-2, IL-10</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Tocilizumab&lt;sup&gt;b&lt;/sup&gt;, IFNα-2b, FN-β, IL-2, TNF-α, IL-6, hGH</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>IFNα-2b</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>IL-2, IFNα-2b</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Basiliximab, muromonab-CD3, tocilizumab&lt;sup&gt;b&lt;/sup&gt;, IL-1, IL-2, IL-6, IL-10</td>
</tr>
</tbody>
</table>

<sup>a</sup>/<sup>b</sup>: increase in activity

TP-Drug Interaction: Decision Tree

Therapeutic Protein (TP)

- Cytokine or cytokine modulator (that has known effects on CYPs or transporters)
- TPs intended to be used in combination therapy with
- Cases where studies can be considered important because of known mechanisms or general concerns other than its possible effect on CYPs or transporters

In vitro study TP → D: effect on CYPs or transporters

In vivo study TP → D: effect on CYPs or transporters (cocktail or individual studies)

1. TP → D
2. D → TP

In vivo interaction studies, e.g., crossover study, population PK, or parallel study

- No known or suspected mechanisms
- Yes, known or suspected mechanism; or potential for mechanism unknown

D → TP

In vitro or in vivo interaction studies

Population PK as initial assessment; may follow up with a formal study

Label describes study results and any important clinical actions

Label may indicate the potential for interaction with CYP or transporter pathways with a particular focus on its potential effect on narrow therapeutic range drugs (e.g., warfarin)

TP-Drug Interaction Study

• At present in vitro or animal studies have limited value in qualitative and quantitative projection of clinical interactions

• Some interactions between drugs and TPs based on mechanisms other than CYP or transporter modulation

• An important consideration in most TP–drug interaction studies is the need to conduct the study in patient populations instead of in healthy volunteers
Case Study – In vitro Assessment

- Drug Y is a human IgG monoclonal antibody against several cytokines.

- FDA PMR recommended an in vitro study to assess effect of cytokine modulation on the expression of major CYP enzymes. If no marked modulation observed, further exploration unnecessary.

- If an interaction is observed in vitro, a clinical DDI study would need to be conducted.

- Rationale

Cytokine related changes may affect drug metabolizing enzymes through regulation of nuclear hormone receptors such as PXR, AhR and CAR.

Morgan et al, DMD, 2008
Case Study: Our Approach

- **Experimental**
  - Used validated *CYP induction* method using cryopreserved human hepatocytes
  - Monitored functional activity and gene level of major 5 CYPs as requested + CYP2B6 (included in normal induction panel)
  - Incubated with IL-6 and TNF-α (Positive controls), IL-2 (negative control), and compound related cytokines

- **Results**
  - Results for IL-6, TNF-α and IL-2 were consistent with previously published studies
  - The cytokines that Drug X targeted did not effect major CYP activities

<table>
<thead>
<tr>
<th></th>
<th>CYP1A2</th>
<th>CYP2B6</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Control</td>
<td>Activity</td>
<td>mRNA</td>
<td>Activity</td>
<td>mRNA</td>
<td>Activity</td>
<td>mRNA</td>
</tr>
<tr>
<td>DMSO</td>
<td>100</td>
<td>1.19</td>
<td>100</td>
<td>1.04</td>
<td>100</td>
<td>1.03</td>
</tr>
<tr>
<td>IL-2</td>
<td>100</td>
<td>1.38</td>
<td>79.3</td>
<td>0.96</td>
<td>92.7</td>
<td>1.10</td>
</tr>
<tr>
<td>IL-6</td>
<td>77.6</td>
<td>0.87</td>
<td>69.7</td>
<td>0.39</td>
<td>65.2</td>
<td>0.38</td>
</tr>
<tr>
<td>TNF-α</td>
<td>27.3</td>
<td>0.65</td>
<td>65.2</td>
<td>1.15</td>
<td>83.1</td>
<td>1.05</td>
</tr>
</tbody>
</table>
Case Study: Impact on the Project

• Conclusion
  – The cytokines that Drug Y targeted did not appear to affect CYP1A2, 2B6, 2C9, 2C19, 2D6 and 3A4
  – In vitro model is able to assess direct in vitro DDI potential of cytokines, however multiple limitations exist in using isolated hepatocyte preparations

• Team proposed to change DDI wording for the USPI label, based on the in vitro study results without an in vivo DDI study

Well planned, timely in vitro study mitigated the need for a costly clinical trial
Food-Drug DDI

- Tropical fruit juices
  - Grapefruit juice:
    - 6’,7’-dihydroxybergamottin
    - Recovery of 3A function through enzyme regeneration: ca. 3 days
    - Enteric more than hepatic
  - Papaw juice
  - Star fruit juice
  - Pomegranate juice
  - Other citrus fruits
## Grapefruit Juice and Calcium Channel Blocker Interactions

### Table 1. Grapefruit juice and calcium channel blocker interactions

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reference</th>
<th>Grapefruit Juice</th>
<th>Compound</th>
<th>Interaction</th>
<th>Severity/onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>39</td>
<td>250 mL</td>
<td>5 mg, single-dose</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 15%</td>
<td>Minor/delayed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC ↑ 16%</td>
<td></td>
</tr>
<tr>
<td>Diltiazem</td>
<td>50, 53</td>
<td>250 mL</td>
<td>120 mg IR*, single-dose</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 22% ± 37%</td>
<td>Minor/delayed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC ↑ 20% ± 25%</td>
<td></td>
</tr>
<tr>
<td>Felodipine</td>
<td>1, 2, 10, 14, 21, 37, 41</td>
<td>250 mL single-strength; peeled grapefruit segments; intact grapefruit</td>
<td>Several single and multiple dose studies</td>
<td>Bioavailability can ↑ by two to three fold with similar size hemodynamic changes</td>
<td>Moderate/rapid</td>
</tr>
<tr>
<td>Isradipine</td>
<td></td>
<td>300 mL concentrated grapefruit juice</td>
<td>40 mg, single-dose</td>
<td>Not studied</td>
<td>Unavailable</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>44</td>
<td></td>
<td></td>
<td>AUC ↑ 43% ± 3.4% for [+] nicardipine and ↑ 91% ± 6.4% for [-] nicardipine</td>
<td>Moderate/rapid</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>38, 48</td>
<td>200 g of grapefruit pulp</td>
<td>20 mg, single-dose</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 40%</td>
<td>Minor/delayed</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>45</td>
<td>250 mL (751 mg naringin/L)</td>
<td>30 mg, single-dose</td>
<td>AUC ↑ 30%</td>
<td>Moderate/rapid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 24%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC ↑ 51%</td>
<td></td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>42, 43</td>
<td>250 mL single-strength</td>
<td>20 mg, single-dose</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 406% ± 73%</td>
<td>Moderate/rapid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AUC ↑ 198% ± 46%</td>
<td></td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>46</td>
<td>150 mL at −15, 10, −1/4, +5, and +10 h</td>
<td>20-mg, single-dose</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ↑ 99%</td>
<td>Moderate/rapid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 mL, normal strength</td>
<td>60 to 240-mg immediate release from twice daily</td>
<td>AUC ↑ 106%</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>49, 51</td>
<td></td>
<td></td>
<td>No significant Δ in C&lt;sub&gt;max&lt;/sub&gt; or AUC</td>
<td>Minor/delayed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Some interindividually variability</td>
<td></td>
</tr>
</tbody>
</table>

* Immediate release.
Herb-Drug Interaction

- St. John’s Wort
- Ginkgo
  - Treatment of cerebral insufficiency or peripheral vascular disease, and enhancement of memory function
  - CYP2C19 inducer
- Garlic
  - Treatment of hypercholesterolaemia, prevention of arteriosclerosis, improve immune function
  - CYP2E1, 3A and Pgp inhibitor
- Goldenseal
  - Antimicrobial for digestive disorders
  - CYP3A inhibitor
- Kava
  - For anxiety, depression, insomnia and restlessness
  - CYP2E1 inhibitor
St. John’s Wort

- Popular medicinal herb used for the treatment of depression
- Induce CYP isozymes, such as CYP3A4, 2C19, and 2C9, and P-gp
  - Hyperforin is one of the main components, a potent ligand for pregnane X receptor (PXR)
    - 10.4-fold induction CYP3A4 at 5 μM vs. 5.3-fold by rifampicin at 10 μM [Rahimi and Abdollahi, Expert Opin. Drug Metab. Toxicol. (2012) 8(6):691-708]
- Induction of CYP3A and P-gp activity by SJW (300mg three times daily for 10 days) was comparable in healthy volunteers from six ethnic populations, namely, Caucasians, African Americans, Hispanics, Chinese, Indians and Malays
- Contraindication
  - Not recommended in people taking immunosuppressants or cardiovascular drugs
  - With other medications, only with a low hyperforin content and under careful monitoring
  - Women should use additional preventive methods to avoid unintended pregnancy
Traditional Chinese Medicine (TCM) and DDI

• “The lack of information on the ADME characteristics, especially the metabolic stability and interaction potential between CYPs and herbs, increases ADR occurrence due to TCMs.”

Interactions between Phytochemicals from Traditional Chinese Medicines and Human Cytochrome P450 Enzymes

Jing-Jing Wu\(^1,2\), Chun-Zhi Ai\(^1\), Yong Liu\(^1\), Yan-Yan Zhang\(^1\), Miao Jiang\(^3\), Xu-Ran Fan\(^1\), Ai-Ping Lv\(^3\) and Ling Yang\(^1,^\dagger\)

\(^1\)Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; \(^2\)Graduate School of Chinese Academy of Sciences, Beijing, China; \(^3\)Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Dongzhimen, Beijing 100700, China
Future Developments

• More predictive in vitro/in vivo models for human DDI prediction
  – 3D liver platforms/hepatocyte models
  – Stem-cell derived human hepatocytes
  – Transgenic/humanized mouse

• Modeling and Simulation
  – Simcyp
    • First workshop in China: Feb 2012, Shanghai
  – PBPK: interplay between transporters and metabolizing enzymes

• Application in biologics/therapeutic protein development
Thank You!
&
Questions?

Janssen Research & Development
Drug Safety Sciences, Asia Pacific