Recent Advances in Reaction Phenotyping to Access Victim Drug-Drug Interaction

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Importance of Reaction Phenotyping

• Assess victim DDI
• Set inclusion / exclusion criteria
• Understand PGx, individual variability, co-med
• Impact confidence in CL prediction (non-CYP), IVIVE, dose, $t_{1/2}$
• Guide structural modification to reduce $f_m$
• If incorrect ....
  • Safety concerns, irrelevant DDI studies
  • Require much more work to figure out why
    – Experimentally and modeling and simulation
Balancing Various Clearance Pathways

Multiple Clearance Pathways to Minimize DDI Victim Potential
Structural Modification to Reduce CYP2D6 $f_m$

$f_m$ (CYP2D6) $> 0.95$

Major pathway: N-demethylation

Multiple CYPs

In Vitro/In Vivo Assays for Reaction Phenotyping

**In Vivo**
- $^{14}$C ADME
- DDI
- PGx

**In Vitro**
- HHEP
- HLM
- rh-Enzyme
- Parent Depletion
- Metabolite Formation
- Radio-labelled Material

- Reaction Phenotyping
  - Structure Modification
  - DDI Study Design
  - PK Variability
    - Individual Variability
    - Genetic Polymorphism
    - Disease State
    - Gender
    - Race
    - Age

*Li Di, Expert Opinion on Drug Discovery, 2017, 12:11, 1105-1115*
Current Challenges for Reaction Phenotyping

• Low Clearance

• Non-CYP Enzymes
  – Lack of selective inhibitors/substrates
  – Extrahepatic contribution

• Concentration dependence in $F_m$

• Time dependence in $F_m$
Challenges of Reaction Phenotyping for Low CL Compounds

High CL

- Steep decline
- Large difference +/- inhibitor
- Can detect high $f_m$ (>0.9)

Low CL

- Shallow decline
- Small difference +/- inhibitor
- $f_m$ limit is low (e.g. > 0.3)
Challenges of Reaction Phenotyping for Low CL

In order to achieve 90% inhibition, the limit of $\text{CL}_{\text{int}}$ needs to be 10-fold lower than regular $\text{CL}_{\text{int}}$ measurement.

<table>
<thead>
<tr>
<th>Assays</th>
<th>$\text{CL}_{\text{int}}$ Limit (mL/min/kg)</th>
<th>90% Inhibition $\text{CL}_{\text{int}}$ (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM (0.75 mg/mL, 1 hr)</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>HHEP Relay (0.5 Mcells/mL, 20 h)</td>
<td>1.5</td>
<td>15</td>
</tr>
<tr>
<td>HHEP Relay (2 Mcells/mL, 20 h)</td>
<td>0.36</td>
<td>3.6</td>
</tr>
</tbody>
</table>

- Moderate-high CL might need relay assay to achieve 90% inhibition
- The $\text{CL}_{\text{int}}$ limit can be lower if multiple pathways are involved
Current Tools for Reaction Phenotyping of Low CL Compounds

• rCYP
  – low power, problematic ISEFs, substrate dependent, non-physiological system
  – specifically addresses CYP reaction phenotyping
  – often only picks up CYP3A for low CL compounds – misleading

• Metabolite standards
  – chemical- or bio-synthesized, best applied with 1-3 metabolites/pathways
  – “major” metabolites might not have high enough coverage for overall CL
  – needs lead time and more suited for late stage development compounds

• Radio-labeled material
  – need material, expensive, tedious & long process ~ 4 weeks
  – has fallen out of favor with project teams

• HHEP relay with chemical inhibitors following substrate depletion
  – No need to have metabolite standards or radio-labeled material
  – No assumptions of clearance pathways
Transfer Supernatant

Accelerated Communication

A Novel Relay Method for Determining Low-Clearance Values

Li Di, Patrick Trapa, R. Scott Obach, Karen Atkinson, Yi-An Bi, Angela C. Wolford, Beijing Tan, Thomas S. McDonald, Yurong Lai, and Larry M. Tremaine

Pharmacokinetics, Dynamics and Metabolism, Pfizer Inc., Groton, Connecticut

Received April 24, 2012; accepted May 22, 2012
# HHEP Relay IVIVE of Commercial Drugs

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Metabolizing Enzymes</th>
<th>Human In Vivo $\text{CL}_{\text{int}}$ (mL/min/Kg)</th>
<th>Relay Method $\text{CL}_{\text{int}}$ (mL/min/Kg)</th>
<th>Fold Difference between In Vitro and In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>CYP3A, 2C19</td>
<td>15</td>
<td>15 ± 1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>CYP3A</td>
<td>5.9</td>
<td>4.8 ± 1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Theophylline</td>
<td>CYP1A2</td>
<td>2.6</td>
<td>2.8 ± 0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Timolol</td>
<td>CYP2D6</td>
<td>36-49</td>
<td>14 ± 5.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>CYP2C9</td>
<td>4.9</td>
<td>7.4 ± 0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>S-Warfarin</td>
<td>CYP3A, 2C9</td>
<td>4.5</td>
<td>4.2 ± 1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>CYP3A</td>
<td>2.1</td>
<td>3.3 ± 0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>CYP2D6, 1A2, 3A</td>
<td>4.4</td>
<td>3.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Zolmitriptan</td>
<td>CYP1A2, MAO</td>
<td>13</td>
<td>4.0 ± 1.9 *</td>
<td>3.3 *</td>
</tr>
</tbody>
</table>

*Underprediction of in vivo clearance due to extrahepatic contribution of MAO

**DMD, 2012, 57, 441-448**
## HHEP Relay IVIVE of Pfizer Compounds

<table>
<thead>
<tr>
<th>Therapeutic Area</th>
<th>Compounds</th>
<th>Human $\text{CL}_{\text{int},u}$ mL/min/Kg</th>
<th>HHEP Relay $\text{CL}_{\text{int},u}$ mL/min/Kg</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVMED</td>
<td>A</td>
<td>10.9</td>
<td>7.61</td>
<td>1.4</td>
</tr>
<tr>
<td>CVMED</td>
<td>B</td>
<td>4.93-7.23</td>
<td>4.43</td>
<td>1.1-1.6</td>
</tr>
<tr>
<td>Oncology</td>
<td>C</td>
<td>11.7</td>
<td>13.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Oncology</td>
<td>D</td>
<td>20.0</td>
<td>15.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Oncology</td>
<td>E</td>
<td>5.14</td>
<td>5.11</td>
<td>1.0</td>
</tr>
<tr>
<td>Oncology</td>
<td>F</td>
<td>40.8</td>
<td>11.5*</td>
<td>3.5* (AO)</td>
</tr>
<tr>
<td>Imflammation</td>
<td>G</td>
<td>4.2 (CL$_H$)</td>
<td>3.2 (CL$_H$)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Underprediction of in vivo clearance due to extrahepatic contribution of AO

### Prospective Prediction

- **Oncology:** 14.6 L/h (predicted), 11.7 (actual)
- **Neuroscience:** 0.9 mL/min/kg (predicted), 0.9 (actual)
- **CVMED:** 3.87 mL/min/kg (predicted), 3.95 (actual)
Novel HHEP Relay Reaction Phenotyping Assay

- Pre-incubation with Inhibitor
- Remove Inhibitor
- Incubate with Substrate
- Transfer Supernatant
- Next Relay

- MBIs to avoid the impact of inhibitor depletion during long incubation
- MBIs were removed from incubation after inactivation to increase selectivity by minimizing reversible inhibition (CYP, AO, CES, UGT, SULT, Transporters)

DMD, 44(3): 460-5, 2016
<table>
<thead>
<tr>
<th>CYP</th>
<th>Pre-Incub. Time (min)</th>
<th>Inactivators and Concentrations</th>
<th>Substrates</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>15</td>
<td>1 µM Furafylline</td>
<td>Phenacetin</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>2B6</td>
<td>15</td>
<td>10 µM Phencyclidine</td>
<td>Bupropion</td>
<td>OH-Bupropion</td>
</tr>
<tr>
<td>2D6</td>
<td>15</td>
<td>1.8 µM Paroxetine</td>
<td>Dextromethorphan</td>
<td>Dextrophan</td>
</tr>
<tr>
<td>2C8</td>
<td>30</td>
<td>100 µM Gemfibrozil Glucuronide</td>
<td>Amodiaquine</td>
<td>N-Desethyllamodiaquine</td>
</tr>
<tr>
<td>2C9</td>
<td>30</td>
<td>15 µM Tienilic Acid</td>
<td>Diclofenac</td>
<td>4’-OH-Diclofenac</td>
</tr>
<tr>
<td>2C19</td>
<td>15</td>
<td>8 µM Esomeprazole</td>
<td>Mephenytoin</td>
<td>4’-OH-Mephenytoin</td>
</tr>
<tr>
<td>3A</td>
<td>15</td>
<td>25 µM Troleandomycin</td>
<td>Midazolam</td>
<td>1’-OH-Midazolam</td>
</tr>
<tr>
<td>3A4</td>
<td>15</td>
<td>2 µM CYP3cide</td>
<td>Midazolam</td>
<td>1’-OH-Midazolam</td>
</tr>
<tr>
<td>Pan-CYP</td>
<td>30</td>
<td>1 mM ABT &amp; 15 µM Tienilic Acid</td>
<td>All Above</td>
<td>All Above</td>
</tr>
</tbody>
</table>

*Pan-CYP* indicates a pan-CYP condition, implying the use of multiple CYP enzymes in the phenotyping process.
CYP Inactivator Selectivity

ABT/Tienilic Acid inhibits certain UGTs
HHEP Relay Reaction Phenotyping Assay Validation

Erythromycin (3A)

- Time (hour)
- % Remaining
- Reported: mostly 3A
- Relay $f_m$: 0.84 3A

Erythromycin + TAO

- Time (hour)
- % Remaining

Tizanidine (1A2)

- Time (hour)
- % Remaining
- Reported $f_m$: 0.85 1A2
- Relay $f_m$: 0.83 1A2

Tizanidine + Furafylline

- Time (hour)
- % Remaining

DMD, 44(3): 460-5, 2016
Reported: 2D6 major
2C9 minor
Relay $f_m$: 0.74 2D6

Reported $f_m$: 0.8 -1 2C9
Relay $f_m$: 0.90 2C9
HHEP Relay Reaction Phenotyping Assay Validation

Reported $f_m$ : 0.5 3A, 0.5 2C19

Relay $f_m$: 0.56 3A, 0.42 2C19
Relay Reaction Phenotyping Application

- Compound is low CL
- $\text{CL}_{\text{int}} = 11.8 \text{ mL/min/kg}$ in HHEP relay
- Reaction phenotyping using MBI in the HHEP relay $\text{CYP3A } f_m = 0.34$, total CYP 0.71, non-CYP 0.29 (SULT, UGT)
- Guide design for Clinical Development Plan
- Human itraconazole DDI study:
  - 87% increased in AUC
  - Estimated human CYP3A $f_m = 0.47$

HHEP relay phenotyping data is consistent with clinical DDI results
Relay Reaction Phenotyping Application

- Compound is low CL
- $\text{CL}_{\text{int}} = 8.6 \text{ mL/min/kg}$ in HHEP relay
- rCYP indicated high CYP3A $F_m > 0.99$
- Clinical DDI study with ketoconazole CYP3A $F_{CL} = 0.6$
- HHEP relay reaction phenotyping
  - CYP3A $F_m = 0.76$ ($F_{CL} = 0.68$, 10% renal CL)
  - CYP2C8 $F_m = 0.15$
  - Total CYP $> 0.91$ (minor UGT pathway in humans $\sim 5\%$)

HHEP relay phenotyping data is consistent with clinical DDI results
Reaction Phenotyping with HHEP Relay Assay

- Drug candidate is low CL
- $CL_{int} = 2.8 \text{ mL/min/kg}$ in HHEP relay
- Assume all CYP3A based on rCYP data and 1$^{st}$ compound in the clinic
- HHEP relay reaction phenotyping
  - CYP3A $F_m = 0.36$
  - Non-CYP $\sim 0.60$
- Follow up studies suggested AO involvement. Data is consistent with azaheterocyclic structure.

HHEP relay reaction phenotyping is critical in diagnosing non-CYP pathway in order to guide clinical DDI studies and to predict clearance and dose.
Aldehyde Oxidase

- Hydralazine - a reported selective AO inhibitor, but...

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Substrate</th>
<th>% Inhibition ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 µM</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>phenacetin</td>
<td>38</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>bupropion</td>
<td>15</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>paclitaxel</td>
<td>5</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>diclofenac</td>
<td>7</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-mephenytoin</td>
<td>2</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>dextromethorphan</td>
<td>14</td>
</tr>
<tr>
<td>CYP3A</td>
<td>midazolam</td>
<td>11</td>
</tr>
<tr>
<td>AO</td>
<td>zaleplon</td>
<td>71</td>
</tr>
</tbody>
</table>

At ≥ 50 µM, significant inhibition of CYP1A2, 2B6, 2D6 and 3A, but clean for 2C families.
Hydralazine: MBI of CYP1A2


J. Pharm. Sci., 2018, Online
Substrate Concentration Dependence of Inhibition

\[ \frac{IC_{50}}{K_i} = 1 + \frac{[S]}{K_m} \]

\[ \frac{IC_{50t}}{K_i} = (1 + \frac{[S]}{K_m}) \times 0.693 / (k_{\text{inact}} \times t) \]

As substrate concentration decreases, IC\textsubscript{50} decreases (%inhibition increases)

Reductases: CBR1 and 11β-HSD1

## Selective Inhibitors of Reductases

<table>
<thead>
<tr>
<th>Properties</th>
<th>CBR1</th>
<th>11β-HSD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Fraction</td>
<td>Cytosolic (minor in microsomes)</td>
<td>Microsomal</td>
</tr>
<tr>
<td>Cofactor</td>
<td>NADPH</td>
<td>NADPH</td>
</tr>
<tr>
<td>Tissue Distribution</td>
<td>intestine, liver, kidney</td>
<td>liver, adipose,</td>
</tr>
<tr>
<td>Individual Variability</td>
<td>4-fold</td>
<td>3-fold</td>
</tr>
<tr>
<td>Substrate Reaction</td>
<td>Doxorubicin to Doxorubicinol (non-selective)</td>
<td>Cortisone to Cortisol (selective)</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>Menadione, Hydroxyl-PP-Me, PF-06932034 (13h) (all non-selective)</td>
<td>PF-915275 (selective)</td>
</tr>
</tbody>
</table>

![Selectivity at 1 μM](image)

**PF-915275**

_DMD, 2018, 46(7):1023-1029_
# 11β-HSD1 Involved in Doxorubicin Metabolism

<table>
<thead>
<tr>
<th>Experiments</th>
<th>HHEP Cell Density (Million Cells/mL)</th>
<th>% Inhibition of Doxorubicinol Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Average and Standard Deviation</td>
<td>29 ± 7.9</td>
<td></td>
</tr>
</tbody>
</table>

- 11β-HSD1 is identified for the 1\textsuperscript{st} time to involve in Dox metabolism (a drug on the market for more than 40 yrs and is one of the most prescribed anti-cancer drugs). Doxorubicinol formation: ~1/3 11β-HSD1, ~1/3 CBR1, and ~ 1/3 AKRs.
- Development of pan inhibitors of CBR1, 11β-HSD1 and AKR might be more effective in reducing cardiotoxicity.
Concentration Dependence in $F_m$

- Multiple enzymes involved with different $K_m$
- Saturation of low $K_m$, $F_m$ changes
- Examples: dextromethorphan, diazepam, and RO5263397
- Basimglurant
  - CYP3A major (10 $\mu$M)
  - CYP1A2 major (1 $\mu$M)
- Reaction phenotyping at clinical relevant concentrations or multiple concentrations

Time Dependence in $F_m$

- Auto-inhibition (TDI)
- Auto-induction
- Changes in $F_m$ from single dose vs. multiple dose
- PBPK modeling
Shift of Property Space: Consequence of HT-HLM Screening?

- **High CL CYP**
- **High CL Non-CYP**
- **Low CL CYP**
- **Low CL Non-CYP**

**Challenging Space (Knowledge Gap)**
- IVIVE, PGx,
- Tissue distribution
- Species difference
- DDI, inducibility,
- Individual variability
- Disease state
Further Information

- Questions: Li.Di@Pfizer.com
- Books: Drug-like Properties and Blood Brain Barrier
References

- Sophia M. Shi, Li Di, “The Role of Carbonyl Reductase 1 in Drug Discovery and Development”, Expert Opinion in Drug Metabolism and Toxicology, 2017, 13(8), 859-870.
- Xin Yang, Nathaniel Johnson, Li Di, “Cytochrome P450 Selectivity of Hydralazine”, under review
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