ENDOGENOUS PROBES FOR DRUG-METABOLIZING ENZYMES & TRANSPORTERS: WHERE ARE WE NOW?

NEDMDG Meeting
September 16th, 2015

David Rodrigues, PhD
Transporter Sciences Group,
Pfizer, Groton, CT
a.david.rodrigues@pfizer.com
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• **Global view of PK-ADME-DDI**
• **Translational PK-ADME-DDI Science**
• **Where are we now?**
  - Push for endogenous enzyme & transporter probes
  - Pros/ “up-side”
  - Challenges/opportunities
  - Considerations
  - Comparison to drug probes
  - Looking forward
Global view of PK-ADME-DDI

PHARMACOKINETICS ○ ABSORPTION ○ DISTRIBUTION ○ METABOLISM ○ EXCRETION ○ DRUG-DRUG INTERACTIONS

PK-ADME-DDI SCIENCE CONTINUES TO EVOLVE!!
Global view of PK-ADME-DDI

PHARMACOKINETICS • ABSORPTION • DISTRIBUTION • METABOLISM • EXCRETION • DRUG-DRUG INTERACTIONS
Global view of PK-ADME-DDI

**PHARMACOKINETICS** ○ **ABSORPTION** ○ **DISTRIBUTION** ○ **METABOLISM** ○ **EXCRETION** ○ **DRUG-DRUG INTERACTIONS**
Translational PK-ADME-DDI Science

- How structure and phys-chem properties translate to in vitro properties
- How in vitro properties translate to in vivo PK-ADME-DDI profile
- How in vivo PK-ADME-DDI profile in one species translates to others
- How animal PK-ADME-DDI in vivo profile translates to human
- How individual (cohorts of) human subjects translate to populations
How structure and phys-chem properties translate to in vitro properties
How in vitro properties translate to in vivo PK-ADME-DDI profile
How in vivo PK-ADME-DDI profile in one species translates to others
How animal PK-ADME-DDI in vivo profile translates to human
How individual (cohorts of) human subjects translate to populations

Translational PK-ADME-DDI Science
Translational PK-ADME-DDI Science

BIOMARKER
“Generalized biological indicator of normal function, disease progression, drug target interaction, drug response”

Associated with pharmacological target biology and patient/subject genetic profile

- PD (result of target engagement/occupancy)
- Treatment response
- Disease progression
- Patient/subject (target genotype)

Not every TT is a biomarker
Not every biomarker is a pharmacodiagnostic
Translational PK-ADME-DDI Science

**Biomarker**

“Generalized biological indicator of normal function, disease progression, drug target interaction, drug response”

**Translation Trait (TT)**

“Biologic characteristic that leads to increased biomedical understanding in order to improve treatment or prevent disease”

Associated with pharmacological target biology and patient/subject genetic profile

- PD (result of target engagement/occupancy)
- Treatment response
- Disease progression
- Patient/subject (target genotype)

Not every TT is a biomarker

Not every biomarker is a pharmacodiagnostic
Translational PK-ADME-DDI Science

“ADME BIOMARKER”
“ENDOGENOUS BIOMARKER”
“P450 BIOMARKER”
“TRANSPORTER BIOMARKER”

“ENDOGENOUS MARKER (PROBE)”
“ENDOGENIC MARKER (PROBE)”
“EXOGENIC MARKER (PROBE)”
“DRUG MARKER (PROBE)”
“TRAIT MEASURE”
“BIOMEASURE”

TRANSLATIONAL TRAIT (TT)
“Biologic characteristic that leads to increased biomedical understanding in order to improve treatment or prevent disease”

Associated with drug itself or enzymes and transporters that govern its PK-ADME-DDI profile

- Drug PK
- Drug ADME
- DDI (probes)
- Subject phenotype (probes)
- Subject genotype (genotyping)

Exogenic (Xenobiotic)
- Enzyme
- Transporter
- Receptor

Endogenic (Endobiotic)

THERE WILL BE EXCEPTIONS?

# Translational PK-ADME-DDI Science

<table>
<thead>
<tr>
<th>Step(s)</th>
<th>Rank order (in vitro potency)</th>
<th>Strategy</th>
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<tbody>
<tr>
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<td>Animal &amp; translational trait calibrated</td>
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<tr>
<td>In Vitro Data</td>
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<td>Modeling &amp; Simulation</td>
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<td>Clinical Study (formal DDI)</td>
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</table>
## Translational PK-ADME-DDI Science

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<th>Step(s)</th>
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<th>IVIVE Conventional</th>
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</table>

In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: Qualitative relationships, quantitative predictions, and the rank-order approach

R. Scott Obach, PhD, Robert L. Walsky, BSc, Karthik Venkatakrishnan, PhD, J. Brian Houston, PhD, and Larry M. Tremaine, PhD  - Groton, Conn, and Manchester, United Kingdom

Prioritization of Clinical Drug Interaction Studies Using *In Vitro* Cytochrome P450 Data: Proposed Refinement and Expansion of the “Rank Order” Approach

A. David Rodrigues*
### Translational PK-ADME-DDI Science

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</table>

**Strategy**

**IVIVE**


M. Jamei · F. Bajot · S. Neuhoff · Z. Barter · J. Yang · A. Rostami-Hodjegan · K. Rowland-Yeo  

Toward Prospective Prediction of Pharmacokinetics in OATP1B1 Genetic Variant Populations

R Li1, HA Barton2 and TS Maurer1  

A Combined Model for Predicting CYP3A4 Clinical Net Drug-Drug Interaction Based on CYP3A4 Inhibition, Inactivation, and Induction Determined in Vitro

Odette A. Fahmy, Tristan S. Maurer, Mary Kish, Edwin Cardenas, Sherm Boldt, and David Nettleton
# Translational PK-ADME-DDI Science

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**Model for the drug-drug interaction responsible for CYP3A enzyme inhibition. I: Evaluation of cynomolgus monkeys as surrogates for humans (calibration)**

Cynomolgus Monkey as a Potential Model to Assess Drug Interactions Involving Hepatic Organic Anion Transporting Polypeptides: In Vitro, In Vivo, and In Vitro-to-In Vivo Extrapolation

Hong Shen, Zheng Yang, Gabe Minter, Yong-Hae Han, Cliff Chen, Praveen Balimane, Mohammed Jerral, Weiping Zhao, Renjie Zhang, Sanjith Kallipatti, Sabariya Selvam, Sunil Sukruthara, Prasad Krishnamurthy, Purit Marathe, and A. David Rodrigues
# Translational PK-ADME-DDI Science

## Step(s)
- **In Vitro Data**
- **Modeling & Simulation**
- **Predicted Human DDI**
- **Animal DDI (calibration)**
- **Clinical Study (translational trait)**
- **Predicted Human (calibrated)**
- **Clinical Study (formal DDI)**

## Strategy
- **Rank order (in vitro potency)**
  - Conventional
  - Animal calibrated
  - IVIVE

## IVIVE
- Translational trait calibrated
- Animal & translational trait calibrated

---

### In Vitro Data


**Evaluation of 6β-hydroxycortisol, 6β-hydroxy cortisolone and their combination as endogenous probes for inhibition of CYP3A4 in vivo**

Chi-Chi Peng, Ian Templeton, Kenneth E Thummel, Connie Davis, Kent L Kunze, and Nina Isoherranen

---

### Predicted Human DDI


**DOI 10.1007/s40062-014-1778-2**

**PHARMACOKINETICS AND DISPOSITION**

N¹-methylnicotinamide as an endogenous probe for drug interactions by renal cation transporters: studies on the metformin–trimethoprim interaction

Fabian Müller, Constanza A. Pontones, Berntold Reinmer, Marcus Muth, Eva Hauer, Daniel Auge, Renke Maas, Oliver Zelik, Martin F. Frömter

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### Animal DDI (calibration)

*Drug Metab Dispos.* 38(4): 653–660, April 2010

**Special Section on Transporters in Toxicity and Disease**


Yuichiro Imamura, Yuri Tsuruya, Katja Dammle, Dominik Heer, Yuji Kumagai, Kazuya Maeda, Nobuyuki Murayama, Noriko Okudaira, Atsushi Kurihara, Takashi Izumi, Yuichi Sugiyama, and Hiroyuki Kusuhara

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# Translational PK-ADME-DDI Science

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### In Vitro Data

### Modeling & Simulation

### Predicted Human DDI

### Animal DDI (calibration)

### Clinical Study (translational trait)

### Predicted Human DDI (calibrated)

### Clinical Study (formal DDI)

## Short Communication

**4β-Hydroxycholesterol as an Endogenous Biomarker of CYP3A Activity in Cynomolgus Monkeys**

Dehydroepiandrosterone sulfate, a useful endogenous probe for evaluation of drug–drug interaction on hepatic organic anion transporting polypeptide (OATP) in cynomolgus monkeys

Masaki Watanabe, Takao Watanabe, Masashi Yabuki, Ikumi Tamai
Induction of CYP3A in monkey

Z. Yang, H. Sun, BMS
Unpublished

4βHC in Cyno Monkey
(15 mg/kg RIF)
Translational PK-ADME-DDI Science

“An Evolving Clinical Tool Kit”

Probes Type

Conventional
- Dose single probe drug
  - Therapeutic dose
- Analyze plasma
  - Parent AUC
  - M/P Ratio
- Analyze urine
  - M/P Ratio

Evolved
- Dose probe drug cocktail
  - Therapeutic dose
- Analyze plasma
  - Parent AUC
  - M/P Ratio
- Analyze urine
  - M/P Ratio
- Analyze saliva
  - M/P Ratio

Trending
- Dose probe drug cocktail
  - Therapeutic dose
- Analyze plasma
  - Parent AUC
  - M/P Ratio
- Analyze urine
  - M/P Ratio
- Analyze saliva
  - M/P Ratio

DDI Assessment & Subject Phenotyping Approaches

“Endogenic” probe
- Dose probe drug cocktail
  - Microdose
- Dose single probe drug
  - Microdose
Translational PK-ADME-DDI Science

“An Evolving Clinical Tool Kit”

Has included limited sampling (plasma), urine collection < 24 hr, and “breath tests”

Probe Type

Conventional
- Dose single probe drug
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  - Parent AUC
  - M/P Ratio
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  - M/P Ratio
- Analyze saliva
  - M/P Ratio

Trending
- Dose probe drug cocktail
  - Therapeutic dose
- Dose single probe drug
  - Therapeutic dose
- “Endogenic” probe
  - Microdose
- Dose single probe drug
  - Microdose
- Analyze plasma
  - Parent AUC
  - M/P Ratio
- Analyze urine
  - M/P Ratio
- Analyze saliva
  - M/P Ratio

DDI Assessment & Subject Phenotyping Approaches
Translational PK-ADME-DDI Science

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- Analyze urine
  - M/P Ratio
- Analyze saliva
  - M/P Ratio

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- Dose probe drug cocktail
  - Therapeutic dose
- Dose single probe drug
  - Therapeutic dose
- Analyze plasma
  - Parent AUC
  - M/P Ratio
- Analyze urine
  - M/P Ratio
- Analyze saliva
  - M/P Ratio

DDI Assessment & Subject Phenotyping Approaches
Translational PK-ADME-DDI Science

“An Evolving Clinical Tool Kit”

Has included limited sampling (plasma), urine collection < 24 hr, and “breath tests”

Dosing of probe drugs at “sub-therapeutic” or GRAS doses (GRASD)

Probe Type

• Dose single probe drug
  • Therapeutic dose

Conventional

• Analyze plasma
  • Parent AUC
  • M/P Ratio
• Analyze urine
  • M/P Ratio

Evolved

• Dose probe drug cocktail
  • Therapeutic dose

• Dose single probe drug
  • Therapeutic dose

• Analyze plasma
  • Parent AUC
  • M/P Ratio
• Analyze urine
  • M/P Ratio
• Analyze saliva
  • M/P Ratio

Trending

• Dose probe drug cocktail
  • Therapeutic dose

• Dose single probe drug
  • Therapeutic dose

• Dose single probe drug
  • Therapeutic dose

• Analyze plasma
  • Parent AUC
  • M/P Ratio
• Analyze urine
  • M/P Ratio
• Analyze saliva
  • M/P Ratio

DDI Assessment & Subject Phenotyping Approaches
Translational PK-ADME-DDI Science

“An Evolving Clinical Tool Kit”

A Nanogram Dose of the CYP3A Probe Substrate Midazolam to Evaluate Drug Interactions
B Halama1, N Hohman1, I Burhenne1, I Weiss1, G Mikus1 and W E Haegele4

Pharmacogenomic/pharmacokinetic assessment of a four-probe cocktail for CYPs and OATPs following oral microdosing
Ichiro Ieiri1, Masato Fukae1, Kazuya Maeda2, Yukie Ando1, Miyuki Kimura3, Takeshi Hirota1, Takeshi Nakamura4, Kazuhide Iwasaki4, Shunji Matsuki3, Kyoko Matsuoguma5, Eri Kanda5, Mariko Deguchi5, Shin Irie5 and Yuichi Sugiyama2

Evaluation of Endogenous Metabolic Markers of Hepatic CYP3A Activity Using Metabolic Profiling and Midazolam Clearance
K-H Shin1, MH Choi1, KS Lim1, K-S Yu1, I-J Jang1 and J-Y Cho1

N-MethylNicotinamide Is an Endogenous Probe for Evaluation of Drug–Drug Interactions Involving Multidrug and Toxin Extrusions (MATE1 and MATE2-K)
S Ito1, H Kusuhara1, Y Kamagai7, Y Moriyama3, K Inoue5, T Kondo7, H Nakayama5, S Horita5, K Tanabe5, H Yuasa8 and Y Sugiyama8

The Basel Cocktail for Simultaneous Phenotyping of Human Cytochrome P450 Isoforms in Plasma, Saliva and Dried Blood Spots

The Use of Transporter Probe Drug Cocktails for the Assessment of Transporter-Based Drug–Drug Interactions in a Clinical Setting—Proposal of a Four Component Transporter Cocktail
Thomas Eber1, Naoki Ishiguro2, Mitchell E. Taur1
Table 3  Effect of ketoconazole on midazolam $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ (point estimates (90% CIs) of the log-transformed ratios with and without inhibitor)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>$C_{\text{max}}$</th>
<th>$\text{AUC}_{0-\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>4.42 (2.49–7.85)</td>
<td>19.5 (10.3–36.8)</td>
</tr>
<tr>
<td>0.0003</td>
<td>3.69 (2.85–4.79)</td>
<td>10.9 (7.80–15.1)</td>
</tr>
<tr>
<td>1</td>
<td>4.11 (1.98–8.53)</td>
<td>18.8 (14.5–24.4)</td>
</tr>
<tr>
<td>3</td>
<td>4.15 (3.06–5.65)</td>
<td>13.6 (10.9–16.9)</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration–time curve; CI, confidence interval; $C_{\text{max}}$, peak concentration.

“Conventional” MDZ PO dose = 2 – 15 mg (Wash Univ DDI db)
Translational PK-ADME-DDI Science

“An Evolving Clinical Tool Kit”

<table>
<thead>
<tr>
<th>P450/Transporter</th>
<th>Drug Cocktail</th>
<th>Dose (ug)</th>
<th>Assay</th>
<th>Valid quant range</th>
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<tbody>
<tr>
<td>CYP2C9</td>
<td>Warfarin</td>
<td>10</td>
<td>Parent (plasma)</td>
<td>50-50,000 ng/mL</td>
</tr>
<tr>
<td>CYP2C19/OATP</td>
<td>Glibenclamide</td>
<td>10</td>
<td>Parent (plasma)</td>
<td>1-1000 pg/mL</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Lansoprazole</td>
<td>50-70</td>
<td>Parent + 5-OH (plasma)</td>
<td>10-10,000 pg/mL</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan</td>
<td>100</td>
<td>Parent + Dextrophan (12 hr urine)</td>
<td>10-10,000 pg/mL</td>
</tr>
</tbody>
</table>

- No DDI, but studied impact of genotype on probe PK
WHERE ARE WE NOW?
High Level View

- Global view of PK-ADME-DDI
- Translational PK-ADME-DDI Science
- Where are we now?
  - Push for endogenous enzyme & transporter probes
  - Pros/ “up-side”
  - Challenges/opportunities
  - Considerations
  - Comparison to drug probes
  - Looking forward
Push for Endogenous Enzyme & Transporter Probes

- **Subject phenotyping**
  - Relate to genotype

- **DDI assessment**
  - Induction
  - Inhibition
  - Mixed mechanisms

**Recognized utility of “non-invasive” or “less invasive” approaches**

- Minimize pill burden, number of blood draws, etc
**Push for Endogenous Enzyme & Transporter Probes**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Transporters</th>
<th>Probes</th>
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<tbody>
<tr>
<td>UGT1A1</td>
<td>MRP3</td>
<td>Profile serum/plasma</td>
</tr>
<tr>
<td>MRP3</td>
<td>MRP2</td>
<td>Profile urine?</td>
</tr>
<tr>
<td>OATP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td></td>
<td>4βHC/Cholesterol ratio (plasma)</td>
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<tr>
<td>OATP</td>
<td></td>
<td>Formation CL (plasma/urine)</td>
</tr>
<tr>
<td>CYP3A</td>
<td>OAT3</td>
<td>Renal CL (plasma/urine)</td>
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<tr>
<td>MRP2</td>
<td>OATP1B1/1B3</td>
<td>CP-I/CP-III ratio (urine/plasma)</td>
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<tr>
<td>BCRP, MRP3, MRP4</td>
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<tr>
<td>MRP3</td>
<td>MRP4</td>
<td></td>
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<tr>
<td>FMO3</td>
<td></td>
<td>TMA/TMA-oxide ratio (urine)</td>
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<tr>
<td>OCT2</td>
<td>MATE1, MATE2K</td>
<td>Renal CL</td>
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<tr>
<td>OAT2?</td>
<td></td>
<td>Plasma AUC</td>
</tr>
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</table>

**Recognized utility of “non-invasive” or “less invasive” approaches**

- Minimize pill burden, number of blood draws, etc
Push for Endogenous Enzyme & Transporter Probes

No probe reported to date

- Pgp
- OATP1B1
- OATP1B3
- OATP2B1
- OCT1
- NTCP
- OAT1
- BCRP
- BSEP
- UGTs (beyond 1A1)
- CYP3A4
- CYP3A5
- CYP2D6
- CYP2C9
- CYP2C8
- CYP2C19
- CYP2B6
- CYP1A2

Recognized utility of “non-invasive” or “less invasive” approaches

✓ Minimize pill burden, number of blood draws, etc
Recognized utility of “non-invasive” or “less invasive” approaches

- Minimize pill burden, number of blood draws, etc

### Candidate probes

- **Pgp**
- **OATP1B1**
- **OATP1B3**
- **OATP2B1**
- **OCT1**
- **NTCP**
- **OAT1**
- **BCRP**
- **BSEP**
- **UGTs (beyond 1A1)**

### Table: UGT enzyme and Endobiotics

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<tr>
<td>1A1</td>
<td>Bilirubin*, estradiol (3-OH), T4</td>
</tr>
<tr>
<td>1A3</td>
<td>Chenodeoxycholic acid (24-OH), estrone, T4</td>
</tr>
<tr>
<td>1A4</td>
<td>Androstanediol, pregnanediol (3-OH, 17-OH)</td>
</tr>
<tr>
<td>1A5</td>
<td>Serotonin*, 5-OH-tryptophol</td>
</tr>
<tr>
<td>1A6</td>
<td>Estrone</td>
</tr>
<tr>
<td>1A7 (GIT)</td>
<td>Estrogens, T4, 20-HETE</td>
</tr>
<tr>
<td>1A8 (GIT)</td>
<td>Estrone</td>
</tr>
<tr>
<td>1A9</td>
<td>Hydroxysteroids, dopamine</td>
</tr>
<tr>
<td>1A10 (GIT)</td>
<td>Testosterone</td>
</tr>
<tr>
<td>2A1 (Olf)</td>
<td>Testosterone</td>
</tr>
<tr>
<td>2A2 (Olf)</td>
<td>Hyodeoxycholic acid</td>
</tr>
<tr>
<td>2A3 (GIT)</td>
<td>Bile acids</td>
</tr>
<tr>
<td>2B4</td>
<td>Steroid hormones, all-trans-retinoic acid, 20-HETE</td>
</tr>
<tr>
<td>2B7</td>
<td>12-HETE, 15-HETE</td>
</tr>
<tr>
<td>2B10</td>
<td>Testosterone</td>
</tr>
<tr>
<td>2B11</td>
<td>Dihydrotestosterone, Testosterone</td>
</tr>
<tr>
<td>2B15</td>
<td>Estradiol, testosterone</td>
</tr>
</tbody>
</table>

K.W. Bock / Biochemical Pharmacology 96 (2015) 77–82
Push for Endogenous Enzyme & Transporter Probes

No probe reported to date

- Pgp
- OATP1B1
- OATP1B3
- OATP2B1
- OCT1
- NTCP
- OAT1
- BCRP
- BSEP
- UGTs (beyond 1A1)
- CYP3A4
- CYP3A5
- CYP2D6
- CYP2C9
- CYP2C8
- CYP2C19
- CYP2B6
- CYP1A2

Candidate probes?

Detection of an endogenous urinary biomarker associated with CYP2D6 activity using global metabolomics


Recognized utility of “non-invasive” or “less invasive” approaches

✓ Minimize pill burden, number of blood draws, etc
Push for Endogenous Enzyme & Transporter Probes

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- OATP1B1
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✓ Despite greater need for mechanistic insight, agency focus on safety of drug probes
  - Some have suggested dosing of drug probes at sub-therapeutic doses

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☑ Increased need to evaluate interactions in special populations (beyond NHV)
  - Elderly, pediatric, pregnant women
  - Patients (cancer, organ impaired, infected, obese, diabetic, NAFLD, etc)

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✓ Increased need to evaluate interactions in special populations (beyond NHV)
  - Elderly, pediatric, pregnant women
  - Patients (cancer, organ impaired, infected, obese, diabetic, NAFLD, etc)

✓ Increasing need to study dose-response and time-course in support of modeling & simulation exercises
  - Greater leveraging of SAD/MAD Phase I studies possible ("DDI on the Phly")
  - "Calibration" of model-based IVIVEs possible (static, PBPK)
  - Compliment agency DDI decision trees
  - Earlier assessment of NCE as “none”, “weak”, “moderate” or “strong” perpetrator
  - Possibly delay, avoid or prioritize “formal” DDI study

Recognized utility of “non-invasive” or “less invasive” approaches
✓ Minimize pill burden, number of blood draws, etc
Push for Endogenous Enzyme & Transporter Probes

- More complex DDI mechanisms/considerations
  - Enzymes & transporters
  - Impact of genotype/phenotype
  - Reversible inhibition, TDI, induction, repression, de-repression, combinations!
  - DDI onset vs “wash out”
  - Biologics and small molecules
  - Multi-organ (“hepato-renal axis” and “entero-hepatic axis”)

- “Historical” literature precedent; impact of various “syndromes”
  - Rotor’s: OATP1B1/1B3
  - Dubin-Johnson: MRP2
  - PFIC2: BSEP
  - Crigler-Najjar (CN-1, CN-2), Gilbert’s: UGT1A1
  - No reports describing CYP2D6, CYP2C19, CYP2C9 PMs, etc

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  - Global metabolomics
  - Targeted metabolomics

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Pharmacogenomics + Metabolomics = “Pharmacometabolics”
Push for Endogenous Enzyme & Transporter Probes

“Hepato-Renal Axis”

Complex enzyme-transporter interplay likely

- Enzyme marker readout (M/P ratio) impacted by transporter DDI, geno/phenotype?
- Many transporter markers are conjugates; impact of enzyme (UGT, SULT)?
- Events in liver often register in urine (in addition to plasma)
Push for Endogenous Enzyme & Transporter Probes

“Hepato-Renal Axis”

- Largely differential SLC protein expression profile in liver versus kidney
- Consistent with mRNA data
- Implies “coordinated” uptake function
Hepato-Renal Axis: Example 1

Cholestasis causes dramatic changes bile acid profile

<table>
<thead>
<tr>
<th>Plasma BA</th>
<th>Normal</th>
<th>Cholestasis</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BA (nM)</td>
<td>2</td>
<td>112</td>
<td>50</td>
</tr>
<tr>
<td>Sulfated BA (nM)</td>
<td>40</td>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>Taurine/Glycine BA Ratio</td>
<td>0.2</td>
<td>1.9</td>
<td>9.5</td>
</tr>
<tr>
<td>CA/CDCA Ratio</td>
<td>0.3</td>
<td>2.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Primary/Secondary BA Ratio</td>
<td>1.6</td>
<td>5.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Amidated/Non-Amidated BA Ratio</td>
<td>3</td>
<td>2000</td>
<td>666</td>
</tr>
</tbody>
</table>

Hepato-Renal Axis: Example 1


Fig. 3. Urinary taurodihydroxycholate-3-sulfate in patients with chronic liver disease or cholestasis patients measured by ESIMS.
### Hepato-Renal Axis: Example 1

**Urinary ratio as marker for SULT2A1?**

<table>
<thead>
<tr>
<th>BA</th>
<th>hSULT2A1 $V_{max}/K_m$ ($\mu$L/min per mg)</th>
<th>Sulfate/Parent Ratio (Urine)</th>
<th>Sulfate/Parent Ratio (Plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCA</td>
<td>23.3</td>
<td>33</td>
<td>0.6</td>
</tr>
<tr>
<td>G-LCA</td>
<td>22.5</td>
<td>&gt;1000</td>
<td>25</td>
</tr>
<tr>
<td>T-LCA</td>
<td>16.7</td>
<td>1000</td>
<td>50</td>
</tr>
<tr>
<td>G-UDCA</td>
<td>1.02</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>T-UDCA</td>
<td>0.96</td>
<td>1.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>UDCA</td>
<td>0.91</td>
<td>14</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>DCA</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-DCA</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CDCA</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-DCA</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDCA</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-CDCA</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CA</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-CA</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Xenobiotica (2010) 40:184
### Increase in urinary ratio of CP-I vs CP-III in Dubin-Johnson syndrome subjects

<table>
<thead>
<tr>
<th>MRP2 (ABCC2) Genotype</th>
<th>% isomer I</th>
<th>% Isomer III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference subjects (urine)</td>
<td>≤ 30</td>
<td>~70%</td>
</tr>
<tr>
<td>DJS-Hetero (urine)</td>
<td>60</td>
<td>~40%</td>
</tr>
<tr>
<td>DJS-Homo (urine)</td>
<td>80</td>
<td>~20%</td>
</tr>
<tr>
<td>Reference subjects (feces)</td>
<td>~70</td>
<td>~30</td>
</tr>
<tr>
<td>Reference subjects (bile)</td>
<td>~80</td>
<td>~20</td>
</tr>
</tbody>
</table>

Coproporphyrin (CP)

### Hepato-Renal Axis: Example 2

#### Increase in urinary ratio of CP-I vs CP-III in Dubin-Johnson syndrome subjects

<table>
<thead>
<tr>
<th>MRP2 (ABCC2) Genotype</th>
<th>% isomer I</th>
<th>% Isomer III</th>
</tr>
</thead>
<tbody>
<tr>
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<td>80</td>
<td>~20%</td>
</tr>
<tr>
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<td>~30</td>
</tr>
<tr>
<td>Reference subjects (bile)</td>
<td>~80</td>
<td>~20</td>
</tr>
</tbody>
</table>

- No in vitro transporter data for CP-I or CP-III
- Absence of renal MRP2 does not impact CP-I renal secretion
  - Implicates other renal transporters (which ones ??)
- Urinary ratio impacted in Rotor syndrome (OATP implicated)
- Reports that hepatic MRP3 levels are elevated in DJS subjects (IHC data), no effect on MRP4 expression
  - MRP3 implicated in CP-I disposition

### Hepato-Renal Axis: Example 3

**Profile of bilirubin and its glucuronides in liver disease**

<table>
<thead>
<tr>
<th>Serum % Total Bilirubin</th>
<th>Unconjugated</th>
<th>Mono Gluc</th>
<th>Di Gluc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of samples</strong></td>
<td>C-8 isomer</td>
<td>C-12 isomer</td>
<td></td>
</tr>
<tr>
<td>Healthy children</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gilbert syndrome</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Crigler-Najjar syndrome</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Dubin-Johnson syndrome</td>
<td>42</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Rotor syndrome</td>
<td>21</td>
<td>12</td>
<td>43</td>
</tr>
</tbody>
</table>

**UGT1A1 ↓**

**MRP2 ↓**

**OATP ↓**

**Bilirubin**

**Bilirubin-Gluc**

**Bilirubin-diGluc**

**Bilirubin (%) of total**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unconjugated</td>
<td>15±13†</td>
</tr>
<tr>
<td>Monoconjugates</td>
<td>53±3</td>
</tr>
<tr>
<td>Diconjugates</td>
<td>32±14</td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
</tr>
</tbody>
</table>

* Results of five paired serum and urine samples from four patients.
† Mean ± SD.
‡ p<0.01 compared with serum.

Endogenous Probes: Pros/ “up side”

- No dosing required
  - Minimize pill burden
  - Facile phenotyping
  - Simplifies DDI assessment
- Can study perpetrator dose response
  - Determination of in vivo IC$_{50}$, EC$_{50}$

Slide 49
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  - On set, wash out
  - Multiple mechanisms, “net effect” over time
- Study DDI beyond 2 wks
Endogenous Probes: Pros/ “up side”

- **No dosing required**
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  - Simplifies DDI assessment

- **Can study perpetrator dose response**
  - Determination of in vivo IC$_{50}$, EC$_{50}$

- **Can “track” DDI time course**
  - On set, wash out
  - Multiple mechanisms, “net effect” over time

- **Study DDI beyond 2 wks**

- **No blood draw**
  - If leverage urine, saliva

- **Ability to “multiplex” endpoints**
  - “Simultaneous” readout across targeted enzymes and transporters (urine, plasma, saliva ?)
Endogenous Probes: Challenges/opportunities

- **Extent of ADME characterization?**
  - Routes of formation vs clearance?
  - Gut first pass under-represented (vs drugs)?
  - Rate-determining step(s)?
  - “Drug like” level of characterization?

- **Specificity for target protein?**
  - Enzyme, transporter?

- **“Validated” vs “established” probe drugs?**

- **Confounding factors?**
  - “Analytical” (isomers, matrix, standards, etc)?
  - Diurnal variation, disease?
  - Target pharmacology?
  - Inter-subject variability?

- **Sensitivity/dynamic range?**
  - Differentiate none, weak, moderate, strong DDI?
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  - Inter-subject variability?

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Considerations

Metabolic ratios should be better than single concentrations, urinary ratios are easily confounded

\[
C_{ss,\text{Metabolite}} = \frac{CL_f,\text{Metabolite} C_{ss,\text{parent}}}{CL_{\text{Metabolite}}}
\]

Metabolite concentrations will increase if parent increases without any changes in clearance pathways

\[
\frac{C_{ss,\text{Metabolite}}}{C_{ss,\text{parent}}} = \frac{CL_f,\text{Metabolite}}{CL_{\text{Metabolite}}}
\]

Metabolic ratios are a function of both formation and elimination clearances

\[
A_{e,m}/A_{e,p} = \frac{CL_{r,\text{Metabolite}} CL_f,\text{Metabolite}}{CL_r CL_{\text{Metabolite}}}
\]

Urinary ratios depend on renal clearances and metabolite formation and elimination clearances

Nina Isoherranen
Department of Pharmaceutics
University of Washington
• Even for 90% inhibition (Fxn ~0.1) $K_{el}$ for 4βHC is too low to render a robust change in plasma 4βHC Conc.
• 4βHC t1/2 = 17 days ($K_{el} = 0.041$ day$^{-1}$)

\[
\frac{dC_{4\beta HC}}{dt} = k_f \cdot Fxn_{altered} \cdot C_{C_{170}} - k_{el} \cdot C_{4\beta HC}
\]

\[
Fxn_{altered} = \left( \frac{1}{1 + \frac{[I]}{K_i}} \right) \cdot \left( \frac{k_{f \cdot [I]}}{k_{d \cdot [I]} + \frac{k_{f \cdot [I]}}{K_i + [I]}} \right) \cdot \left( 1 + V_{ind} \cdot e^{-\frac{([0.093/1.2] \cdot C_{PP3A})}{t_{1/2}}} \right)
\]

Does the Long Plasma Half-Life of 4β-Hydroxycholesterol Impact Its Utility as a Cytochrome P450 3A (CYP3A) Metric?

J Clin Pharmacol 2010;50:1330-1338
• Even for 90% inhibition (Fxn ~0.1) $K_{el}$ for 4βHC is too low to render a robust change in plasma 4βHC Conc.

• 4βHC $t_{1/2} = 17$ days ($K_{el} = 0.041$ day$^{-1}$)
Two weeks treatment

- Rifampicin
  - 600 mg QD
- Ketoconazole
  - 400 mg QD
Considerations

Kinetics

Dynamic Range

Specificity

CYP3A Inducer Increases 4βHC

CYP3A Inhibitor decreases 4βHC

Graphs showing the changes in plasma concentration of 4β-Hydroxycholesterol under different treatments over 2, 1, and 3 months.

- 2-Week Treatment
- 1-Month Treatment
- 3-Month Treatment
Creatinine CL as marker for OCT2, OAT2 and MATE inhibition (~10-20% active secretion only)

<table>
<thead>
<tr>
<th>Precipitant</th>
<th>% ↓ CL</th>
<th>C&lt;sub&gt;max,u&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt; Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OCT2</td>
</tr>
<tr>
<td>Cobicistat</td>
<td>8-20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Quinidine*</td>
<td>10</td>
<td>0.05</td>
</tr>
<tr>
<td>Dolutegravir</td>
<td>10-14</td>
<td>1.99</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>16-22</td>
<td>0.08</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>27</td>
<td>0.06</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>22</td>
<td>0.16</td>
</tr>
</tbody>
</table>

C<sub>max,u</sub>/IC<sub>50</sub> ratio > 0.1 (FDA)

- *Wash U DDI db and Pfizer in-house data (Sumathy Mathialagan)
## Considerations

### Pyrimethamine and trimethoprim as perpetrators

<table>
<thead>
<tr>
<th>OCT2/MATE Probe</th>
<th>Perpetrator</th>
<th>% ↓ CL(_{\text{renal}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>Pyrimethamine</td>
<td>~34</td>
</tr>
<tr>
<td>Thiamine</td>
<td></td>
<td>70-84</td>
</tr>
<tr>
<td>(N^1)-Methylnicotinamide</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>Carnitine*</td>
<td></td>
<td>~90</td>
</tr>
<tr>
<td>Metformin</td>
<td>Trimethoprim</td>
<td>14-35</td>
</tr>
<tr>
<td>(N^1)-Methylnicotinamide</td>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

*Apical OCTN2 substrate

\[
\text{CL}_{\text{renal}} = \frac{A_{e,0-t}(\text{urine})}{AUC_{0-t}} = (1 - \text{FR}) \times (f_u \times \text{GFR} + \text{CL}_{\text{sec}})
\]

### Table: Precipitant vs. \(C_{\text{max,ur}}/IC_{50}\) Ratio

<table>
<thead>
<tr>
<th>Precipitant</th>
<th>OCT2</th>
<th>MATE1</th>
<th>MATE2K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrimethamine</td>
<td>0.06</td>
<td>6.4</td>
<td>10</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.16</td>
<td>1.1</td>
<td>4.8</td>
</tr>
</tbody>
</table>

- CPT (2012) 923: 635
Considerations

- 6βHC/Cortisol ratio (urine)
- 6βHC formation CL (CL_f)

\[
CL_f = \frac{6\beta HC \ A_{e,0-t} \ (urine)}{Cortisol \ AUC_{0-t}}
\]
Considerations

- **6βHC/Cortisol ratio (urine)**
- **6βHC formation CL (CL<sub>f</sub>)**

$$CL_f = \frac{6\beta HC A_{e,0-t} \text{ (urine)}}{Cortisol AUC_{0-t}}$$

- **6βHC CL<sub>renal</sub>**

$$6\beta HC CL_{renal} = \frac{6\beta HC A_{e,0-t} \text{ (urine)}}{6\beta HC AUC_{0-t}}$$

$$= (1 - FR) \times (f_u \times GFR + CL_{sec})$$

75-90% of CL<sub>renal</sub>

• When evaluating CYP3A inhibition, recommend assessment of 6βHC and cortisol CL<sub>renal</sub>
• When using 6βHC as OAT3 probe, confirm whether or not test compound is CYP3A inhibitor
### Considerations

#### Various CYP3A perpetrators as hOAT3 inhibitors in vitro
(Estrone 3-O-sulfate as substrate; [S] < $K_m$)

<table>
<thead>
<tr>
<th>Perpetrator</th>
<th>IC$_{50}$ (µM)</th>
<th>$C_{\text{max,u}}$/IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itraconazole</td>
<td>&gt;300</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Itraconazole (hydroxy)</td>
<td>11.6 ± 0.38</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Itraconazole (keto)</td>
<td>&gt;40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Itraconazole (N-dealkyl)</td>
<td>&gt; 40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rifampicin (inducer)</td>
<td>&gt;300</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>17α-Ethinylestradiol</td>
<td>5.7 ± 1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>17α-Ethinylestradiol (3-O-Sulfate)</td>
<td>80 ± 13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>&gt;300</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Probenecid</td>
<td>4.1 ± 0.93</td>
<td>5.4</td>
</tr>
</tbody>
</table>

$C_{\text{max,u}}$/IC$_{50}$ ratio > 0.1 (FDA)

- When evaluating CYP3A inhibition, recommend assessment of 6βHC and cortisol CL$_{\text{renal}}$
- When using 6βHC as OAT3 probe, confirm whether or not test compound is CYP3A inhibitor

---

Sumathy Mathialagan, Pfizer (unpublished)
Impact of single dose probenecid (750 mg) on 6βHC as OAT3 probe

<table>
<thead>
<tr>
<th>Study</th>
<th>% Change</th>
<th>AUC(_{6\beta HC})</th>
<th>6βHC (A_e)(urine)</th>
<th>6βHC CL(_{renal})</th>
<th>6βHC CL(_{renal, sec})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>57% ↑</td>
<td>&lt;10% ↓</td>
<td>42% ↓</td>
<td>46% ↓</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>76% ↑</td>
<td>&lt;10% ↓</td>
<td>37% ↓</td>
<td>41% ↓</td>
</tr>
</tbody>
</table>

- Probenecid does not inhibit CYP3A4 in vitro
- No impact on 6βHC CL\(_f\)

<table>
<thead>
<tr>
<th>Probenecid</th>
<th>4.1 ± 0.93</th>
<th>5.4</th>
</tr>
</thead>
</table>

\(C_{max,u}/IC_{50}\) ratio > 0.1 (FDA)

- Probenecid (1000 mg); 27% ↓ in fexofenadine CL\(_{renal}\) (tubular secretion = 58%)
- Probenecid (750 mg); 54% ↓ in benzylpenicillin CL\(_{renal}\) (tubular secretion = 98%)
Endogenous Probes: Comparison to drug probes (1)

Two weeks of treatment with potent CYP3A inhibitor and inducer

- **Ketoconazole**
  - 400 mg QD

- **Rifampicin**
  - 600 mg QD

**Graphs**

- **4βHC** = 4β-hydroxycholesterol (plasma)
- **6βHCL:CL** = 6β-hydroxycortisol: cortisol ratio (urine)
- **6βHCO** = Formation CL of 6β-hydroxycortisol + 6β-hydroxycortisone (urine/plasma)
### Table 1: Induction ratios of P450 activities in healthy volunteers treated with daily doses of 20, 100, or 500 mg rifampicin for 14 days

<table>
<thead>
<tr>
<th></th>
<th>Daily rifampicin dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>Paraxanthine: caffeine (CYP1A2)</td>
<td>1.1 (8)</td>
<td>1.4 (8)</td>
</tr>
<tr>
<td>Losartan: E-3174 (CYP2C9)</td>
<td>0.9 (7)</td>
<td>1.1 (8)</td>
</tr>
<tr>
<td>Omeprazole: 5′-hydroxyomeprazole (CYP2C19)</td>
<td>2.5 (7)</td>
<td>3.8a* (8)</td>
</tr>
<tr>
<td>Omeprazole: omeprazole sulfone (CYP3A4)</td>
<td>3.0 (7)</td>
<td>2.8b (8)</td>
</tr>
<tr>
<td>Quinine: 3′-hydroxyquinine (CYP3A4)</td>
<td>1.6** (7)</td>
<td>3.0*** (8)</td>
</tr>
<tr>
<td>4β-hydroxycholesterol (CYP3A4)</td>
<td>1.5** (8)</td>
<td>2.5*** (8)</td>
</tr>
</tbody>
</table>

Rifampicin dose response (two weeks)
### Itraconazole CYP3A inhibition dose response (single dose)

<table>
<thead>
<tr>
<th>Itraconazole Single Oral Dose (mg)</th>
<th>% Decrease $\text{CL}_f$ (CYP3A5*3/*3 Subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$6\beta$-Hydroxycortisol</td>
</tr>
<tr>
<td>50</td>
<td>27 (15)</td>
</tr>
<tr>
<td>200</td>
<td>42 (43*)</td>
</tr>
<tr>
<td>400</td>
<td>51* (49*)</td>
</tr>
</tbody>
</table>

* $p$-value $\leq 0.017$

(6β-Hydroxycortisol/cortisol urinary ratio)

$$\text{CL}_f = \frac{6\beta-\text{Hydroxysteriod} A_{e,0-t(\text{urine})}}{\text{Parent } \text{AUC}_{0-t}}$$

- For IV midazolam, itraconazole (200 mg X 4 days) causes a 75 ± 25% decrease ($p < 0.05$) in plasma $1'$-hydroxymidazolam/midazolam ratio (CYP3A5*3/*3 subjects)

- CPT (2004) 76: 104
- CPT (2011) 89: 888
Endogenous Probes: Comparison to drug probes (4)

Oral contraceptive as CYP3A inhibitor vs clarithromycin and itraconazole

<table>
<thead>
<tr>
<th>Precipitant</th>
<th>% Decrease</th>
<th>6β-Hydroxycortisol CL_f</th>
<th>1'-HydroxyMDZ/MDZ Plasma AUC Ratio</th>
<th>MDZ AUC Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>43 - 64</td>
<td></td>
<td>36</td>
<td>1.2</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>58</td>
<td></td>
<td>90</td>
<td>5.5</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>42 - 51</td>
<td></td>
<td>86</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Oral MDZ

- Steroids (2014) 87: 137

Authors not able to explain “apparent inhibitory potency” of OC
Endogenous Probes: Looking forward

- Further validation of endogenous probes
  - Reporting of their utility with genotyped subjects
  - Utility with none, weak, moderate and strong perpetrators

- Increased PK-ADME characterization
  - Formation and CL pathways
  - In vitro enzyme and transporter phenotyping data
  - In vivo labeled studies (radio, stable)
Endogenous Probes: Looking forward

- **Further validation of endogenous probes**
  - Reporting of their utility with genotyped subjects
  - Utility with none, weak, moderate and strong perpetrators

- **Increased PK-ADME characterization**
  - Formation and CL pathways
  - In vitro enzyme and transporter phenotyping data
  - In vivo labeled studies (radio, stable)

- **Expansion of tool kit**
  - Identification of probes for additional CYPs (beyond CYP3A)
  - Probes for individual UGTs, SULTs
  - Individual SLCs and ABC transporters

- **Improved PBPK modeling**
  - Inclusion of compound files to support M&S (e.g., SimCYP, DILIsym)
Endogenous Probes: Looking forward

- Flexible strategies (fit for purpose) employing combinations of endogenous and drug probes (leverage SAD + 2-week MAD studies)
  - Multiplex endogenous probe readouts
  - GRAS dose drug probes (individual or cocktails)
- Regard endogenous probes as markers for enzyme-transporter “axes”, in addition to transporter-transporter (vectorial) axes
Endogenous Probes: Looking forward

- Flexible strategies (fit for purpose) employing combinations of endogenous and drug probes (leverage SAD + 2-week MAD studies)
  - Multiplex endogenous probe readouts
  - GRAS dose drug probes (individual or cocktails)
- Regard endogenous probes as markers for enzyme-transporter “axes”, in addition to transporter-transporter (vectorial) axes
- **Gut** transporters (e.g., BCRP, Pgp) will pose a challenge?
  - Endogenous probes may not be an option
  - GRAS dose digoxin (Pgp), sulfasalazine (BCRP) ??
- Greater integration of in vitro enzyme and transporter panel data
### Literature example of an integrated enzyme-transporter data set applied to two perpetrators with 6βHC as OAT3 probe

<table>
<thead>
<tr>
<th>Transporter/enzyme</th>
<th>Substrate</th>
<th>$K_i$ (μM)</th>
<th>Reference</th>
<th>Substrate</th>
<th>$K_i$ or IC$_{50}$ (μM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAT3</td>
<td>6β-OHF</td>
<td>12.1 ± 3.8</td>
<td>Imamura et al., 2013</td>
<td>6β-OHF</td>
<td>32.0 ± 6.2$^a$</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Estrone-3-sulfate</td>
<td>4.9 ± 1.4</td>
<td>Khamdang et al., 2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fexofenadine</td>
<td>1.3 ± 0.3</td>
<td>Tahara et al., 2006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCT2</td>
<td>Cimetidine</td>
<td>&gt;1000</td>
<td>Tahara et al., 2005</td>
<td>TEA</td>
<td>10 ± 1</td>
<td>Ito et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Famotidine</td>
<td>&gt;1000</td>
<td>Tahara et al., 2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MATE1</td>
<td>6β-OHF</td>
<td>&gt;300</td>
<td>Present study</td>
<td>6β-OHF</td>
<td>0.281 ± 0.033</td>
<td>Imamura et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TEA</td>
<td>0.077 ± 0.013</td>
<td>Ito et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TEA</td>
<td>0.046 ± 0.006</td>
<td>Ito et al., 2010</td>
</tr>
<tr>
<td>MATE2-K</td>
<td></td>
<td></td>
<td></td>
<td>Midazolam</td>
<td>&gt;100$^a$</td>
<td>Present study</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam</td>
<td>&gt;100</td>
<td>Present study</td>
<td>Cortisol</td>
<td>&gt;300$^a$</td>
<td>Present study</td>
</tr>
<tr>
<td>11β-HSD2</td>
<td>Cortisol</td>
<td>&gt;300</td>
<td>Present study</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$I_m$: Maximum unbound plasma concentration.

$^a$: Representing IC$_{50}$ value.
THANKS FOR YOUR ATTENTION!
ENDOGENOUS PROBES FOR DRUG-METABOLIZING ENZYMES & TRANSPORTERS: WHERE ARE WE NOW?

BACK UPS
Global view of PK-ADME-DDI

Computer-, technology- and molecular biology-enabled platforms

- Informatics
- Databases
- Data visualization
- Modeling & simulation
- Network theory
- Imaging

- Nano-dispensing
- Miniaturization
- Automation
- Assay multiplexing
- Arrays
- Bioanalysis

- Human tissue
- Cell culture
- Transgenics
- Animal models
- Recombinant methods
- Structural biology
“Translational PK-ADME-DDI Science”

From a compound’s projected PK-ADME-DDI profile to its observed PK-ADME-DDI profile in individual human subjects.

“Drug product with target PK-ADME-DDI profile”

“Translational medicine”

From molecules (bench) to man (bedside) to enable the right drug, dose, and schedule, in the right (stratified) patient.

“Targeted (precision) medicine”
• Dose, route, frequency
• Solubility
• Permeability
• CL, CL/Q_h, V_d, t_1/2
• P/T ratio, C_max
• F_oral, f_a, f_g, f_h, k_a
• f_u, plasma,
• RBC partitioning
• K_p,uu tissue
• M/P ratio
• K_m,enz, V_max,enz, CL_int,enz
• K_m,trans, V_max,trans, CL_int,trans

• Victim AUC, C_max ratio
• f_m
• f_renal
• f_bile
• f_m,CYPn
• f_m,UGTn
• f_transporter,n

• Perpetrator AUC, C_max ratio
• K_i, IC_50
• K_l, k_inact
• EC_50, E_max
“Target PK-ADME-DDI Profile”

- Acceptable physico-chemical properties
  - Compatible with formulation options that enable marketable doses
- Good absorption and small first-pass effect (gut and liver)
- Acceptable (linear) PK for the intended route/frequency of dosing
  - Half-life, peak-trough ratio, clearance
- “Balanced” clearance (no one route dominates; < 50% to total CL):
  - Renal and biliary excretion of parent drug
  - Metabolism to limited number of (none active/circulating) metabolites
- Metabolism catalyzed by several enzymes (≥ 2)
- Metabolism NOT dependent on polymorphic enzymes
- No chemically reactive metabolites
- High passive permeability or balanced uptake/efflux (passive vs active)
  - No one transporter governs active uptake/efflux
- Minimal-manageable drug interactions (as perpetrator and victim)
  - Other drugs
  - Endogenic molecules; bilirubin (hyperbilirbinemia) and bile acids (cholestasis)
- Wide therapeutic index (good efficacy, minimal toxicity)
```
<table>
<thead>
<tr>
<th>Property</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction absorbed ($f_a$)</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Oral bioavailability ($F_{oral} = f_a * f_g * f_h$)</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>Intrinsic clearance ($f_u * CL_{int}$; mL/min/kg)</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Hepatic clearance ($CL_h$; mL/min/kg) ($CL_h/Q_h$ ratio)</td>
<td>&lt; 10 (&lt; 0.5)</td>
</tr>
<tr>
<td>Fraction metabolized ($f_m$)</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Fraction metabolized by individual enzyme ($f_{m, enz}$)</td>
<td>&lt; 0.4 (if $f_m$ ~1)</td>
</tr>
<tr>
<td>Fraction cleared renal ($f_{renal}$)</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Fraction active uptake/efflux by individual transporter ($f_{trans}$)</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Enzyme and transporter $K_m$</td>
<td>&gt; 10 – 100 µM</td>
</tr>
<tr>
<td>Enzyme/transporter reversible IC$_{50}$ ($K_i$)</td>
<td>&gt; 40 µM (&gt; 20 µM)</td>
</tr>
<tr>
<td>CYP time-dependent inhibition IC$<em>{50}$ @ 30’ ($IC</em>{50}$ ratio 0’ vs 30’)</td>
<td>&gt; 20 µM (&lt;1.5)</td>
</tr>
<tr>
<td>PXR transactivation; EC$_{50}$ (%)</td>
<td>&gt; 20 µM (&lt;10%)</td>
</tr>
<tr>
<td>If protein binding &gt; 99%, HSA and hAGP $k_{off}$</td>
<td>&gt; 0.1 sec$^{-1}$</td>
</tr>
</tbody>
</table>
```

"Target PK-ADME-DDI Profile"
Transporter Expression in Human Liver & Kidney

<table>
<thead>
<tr>
<th>SLC</th>
<th>Gene</th>
<th>mRNA</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1</td>
<td>21A6</td>
<td>0.25</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>OATP2B1</td>
<td>21A9</td>
<td>0.07</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>OAT1</td>
<td>22A6</td>
<td>0.001</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>OAT2</td>
<td>22A7</td>
<td>0.80</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>OAT3</td>
<td>22A8</td>
<td>0.001</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>OAT4</td>
<td>22A11</td>
<td>0.001</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>OAT7</td>
<td>22A9</td>
<td>0.03</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>OCT1</td>
<td>22A1</td>
<td>2.2</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>OCT2</td>
<td>22A2</td>
<td>0.006</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>OCT3</td>
<td>22A3</td>
<td>0.12</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>NTCP</td>
<td>10A1</td>
<td>0.486</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Drug Metab Pharmacokin (2005) 20: 452
Endogenous Probes: Considerations (kinetics)

Metabolic ratios should be better than single concentrations, urinary ratios are easily confounded

\[
C_{ss,\text{Metabolite}} = \frac{CL_{f,\text{Metabolite}} C_{ss,\text{parent}}}{CL_{\text{Metabolite}}}
\]

Metabolite concentrations will increase if parent increases without any changes in clearance pathways

\[
\frac{C_{ss,\text{Metabolite}}}{C_{ss,\text{parent}}} = \frac{CL_{f,\text{Metabolite}}}{CL_{\text{Metabolite}}}
\]

Metabolic ratios are a function of both formation and elimination clearances

\[
A_{e,m} = f_{e,m} f_m D = \frac{CL_{r,\text{Metabolite}}}{CL_{\text{Metabolite}}} \cdot \frac{CL_{f,\text{Metabolite}}}{CL_{\text{parent}}} \cdot D
\]

\[
A_{e,p} = f_e D = \frac{CL_r}{CL_{\text{parent}}} \cdot D
\]

\[
A_{e,m} / A_{e,p} = \frac{CL_{r,\text{Metabolite}} CL_{f,\text{Metabolite}}}{CL_r CL_{\text{Metabolite}}}
\]

Urinary ratios depend on renal clearances and metabolite formation and elimination clearances

Nina Isoherranen
Department of Pharmaceutics
University of Washington
### Table 1
Percentage composition of bilirubin, its mono- and diglucuronides present in bile of normal, Gilbert’s syndrome and Crigler-Najjar syndrome affected individuals

<table>
<thead>
<tr>
<th>Composition</th>
<th>Clinical state</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Gilbert</td>
<td>CN2</td>
<td>CN1</td>
</tr>
<tr>
<td>Unconjugated bilirubin</td>
<td>1–3%</td>
<td>2–12%</td>
<td>10–81%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bilirubin monoglucuronide</td>
<td>6–18%</td>
<td>25–32%</td>
<td>12–63%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0%</td>
</tr>
<tr>
<td>Bilirubin diglucuronide</td>
<td>76–90%</td>
<td>59–82%</td>
<td>1–56%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0%</td>
</tr>
</tbody>
</table>

Clinica Chimica Acta 266 (1997) 63–74

At least for Gilbert, it is known that MRP2 function is normal (<27% CP-I in urine) [Strassburg et al]
Bilirubin summary

- Bilirubin: >90% in Plasma, ≤3% in Bile
- Bilirubin Mono-Gluc: ~6% in MRP1/3
- Bilirubin Di-Gluc: <1% in MRP1/3
- UGT1A1: Bilirubin Mono-Gluc to Bilirubin Di-Gluc
- Pgp: Bilirubin ≤3% in Hepatocyte
Probing the links between *in vitro* potency, ADMET and physicochemical parameters

M. Paul Gleeson*, Anne Hersey*, Dino Montanari† and John Overton¹

Relating Molecular Properties and in Vitro Assay Results to in Vivo Drug Disposition and Toxicity Outcomes

Jeffrey J. Sutherland,* John W. Raymond,† James L. Stevens,‡ Thomas K. Baker,‡ and David E. Watson*†

Cynomolgus Monkey as a Potential Model to Assess Drug Interactions Involving Hepatic Organic Anion Transporting Polypeptides: *In Vitro, In Vivo, and In Vitro-to-In Vivo Extrapolation*⁴⁴

Hong Shen, Zheng Yang, Gabe Mintsir, Yong-Hae Han, Clifford Chen, Praveen Balimane, Mohammed Jemal, Weiping Zhao, Renjie Zhang, Sanjith Kallipatti, Sabariya Selvam, Sunil Sukrutharaj, Prasad Krishnamurthy, Punit Marathe, and A. David Rodrigues

Microdosing and drug development: past, present and future

Graham Lappin¹, Robbi Noreika & Ed Bart

Introduction: Microdosing is an approach to early drug development where exploratory pharmacokinetic data are acquired in humans using inherently safe sub-pharmacologic doses of drug. The first publication of microdose data was 10 years ago and this review comprehensively explores the microdose concept from conception over the past decade, up until the current date.
Cytochrome P450 2J2, a new key enzyme in cyclophosphamide bioactivation and a potential biomarker for hematological malignancies. El-Serafi et al. Pharmacogenomics J. 2015 Jan 20. doi: 10.1038/tpj.2014.82. [Epub ahead of print].

TRANSLATIONAL TRAIT (TT)
“Biologic characteristic that leads to increased biomedical understanding in order to improve treatment or prevent disease”

Not every TT is a biomarker

BIOMARKER
“Generalized biological indicator of normal function, disease progression, drug target interaction, drug response”

Not every biomarker is a pharmacodiagnostic

PHARMACODIAGNOSTIC
“Specific biological and predictive indicator of a patient’s response to drug”
Endogenous Probes: Looking forward
In contrast to parent AUC, “minor” metabolic pathways (fm <0.3) are most sensitive for metabolite (assume impact on CL_{f_m,metabolite only})