PREDICTING DRUG INTERACTIONS FOR NON-CYP ENZYMES
OUTLINE

Nonstandard programs provide the opportunity to reevaluate our assumptions, paradigms and quantitative approaches

Introduction
• Big picture
• Reaction types
• Relevant data and the drug discovery/development process
• Regulatory guidance

PBPK approaches

Case studies
• UGT Inhibition
• NAT Polymorphism
• CES-1 Inhibition
• Xanthine Oxidase Inhibition
Assess NCE as a perpetrator and a victim
(Including auto-inhibition and auto-induction)

Develop a narrative to underwrite patient safety based on sufficient characterization of drug disposition

Therapeutic index of the substrate

Fraction metabolized ($f_m$) of the substrate

Inhibition potency and $[I]$ at the enzyme active site

$K_i = 23$ nM
WHAT REACTION TYPES ARE WE TALKING ABOUT?

Oxidation

Cytochrome P450

Reaction mechanisms mimic many other drug metabolizing enzymes (oxidation, reduction, hydrolysis)

Flavin Monooxygenase (FMO)

Heat sensitive
Add drug to initiate rxn, not NADPH

Aldehyde oxidase (AO)

Often exhibits opposite reactivity (nucleophilic) from P450
Test activity with positive control

Xanthine Oxidase (XO)

Similar to AO, fewer substrates?

Monoamine oxidase (MAO)

Alcohol Dehydrogenase (ADH)

Aldehyde Dehydrogenase (ALDH)

Mechanisms of Drug Metabolism
Foti, RS, Rock, DA, Wienkers, LC, Wahlstrom, JL.
Encyclopedia of Drug Metabolism, 2012, John Wiley and Sons, Hoboken
WHAT REACTION TYPES ARE WE TALKING ABOUT?

Reduction

- Aldo-keto reductases (AKR)
  - Example: naltrexone

- Carboxyl Reductase
  - Example: haloperidol

NAD(P)H Quinone Oxidoreductase

- Example: menadione

P450, AO and XO may also contribute to reductive pathways

Drug Metabolism Chemical and Enzymatic Aspects

Mechanisms of Drug Metabolism
WHAT REACTION TYPES ARE WE TALKING ABOUT?

Hydrolysis

- Acetylcholinesterase (ACHE)
- Carboxylesterase (CES) 1b, 1c, 2
- Arylacetamide deacetylase (AADAC)
- Sialate O-acetylesterase (SIAE)
- Butyryl cholinesterase (BCHE)
- Paraoxenase/arylesterase (PON) 1, 2, 3
- Amidases (various)

P450 may also contribute to hydrolysis pathways

- Soluble epoxide hydrolase
- Microsomal epoxide hydrolase

Hydrolysis in stomach or gut (e.g. β-glucuronidase)

May not be enzymatically mediated

Drug Metabolism Chemical and Enzymatic Aspects

Mechanisms of Drug Metabolism
WHAT REACTION TYPES ARE WE TALKING ABOUT?

Conjugation

- UDP Glucuronosyltransferase
  - Acid stabilization of acyl glucuronides

- Sulfotransferase

Methyltransferases

- N-Acetyltransferase

- Glutathione S-Transferase
  - May not be enzymatically mediated

- Glutathione transferases: substrates, inhibitors and pro-drugs in cancer and neurodegenerative diseases
  - Oncogenesis 2018;7:8

Drug Metabolism Chemical and Enzymatic Aspects

Mechanisms of Drug Metabolism
WHEN DO WE OBTAIN RELEVANT DATA IN THE DRUG DISCOVERY AND DEVELOPMENT PROCESS?

Screening paradigm may assume P450-mediated clearance

- **Discovery**
  - Eliminate poor compounds
  - Tier 1 (Single point)
    - Metabolic Stability (Mic stability)
    - DDI Screens (%inhib)
    - PXR (CYP induction)
    - Permeability
    - Plasma Protein Binding
    - Structural Assessment

- **Screen**
  - Establish in vitro-in vivo correlation
    - Tier 2 (Multi-point)
      - IVIVC (Mic, hep CLint or ?)
      - DDI Characterization (TDI, IC50, IC50 shift)
      - Induction (hepatocytes)
      - Transporter (uptake or efflux, substrate or inhibitor)
      - Circulating metabolites/ADME in preclinical species

- **EO**
  - Underwrite patient safety (exclusion)
    - IND-enabling
      - Rxn Phenotyping (rEnzyme, mics or heps)
      - DDI Prediction (Ki, Ks, k\textsubscript{max})
      - Induction Prediction (EC50, E\textsubscript{max})
      - Transporter Prediction (Efflux ratio, IC50)
      - Multi-conc PPB, RBC Partitioning
      - DDI (other)

- **LO**
  - Refine understanding
    - Early Clinical Information
      - Circulating metabolites (single and multiple dose)
    - Early DDI studies (open exclusion criteria)
    - Rat ADME/Dosimetry to support human ADME
  - Develop label
    - Definitive Disposition
    - Human ADME
    - Label DDI studies

Approach with the end in mind – what concomitant medications are likely based on therapeutic area?
REGULATORY GUIDANCE

NCE as a substrate

- Conduct in vivo studies with the most potent inhibitor of the enzyme.
- Presence of significant interaction?
  - Yes: Study other inhibitors based on potency and likely co-administration.
  - No: Conduct in vivo studies with the most potent inducer.

NCE as an inhibitor

- The drug has one or more pharmacologically active metabolites.
- An enzyme appears, based on in vitro and ADME data, to catalyze a main metabolic pathway, which based on in vivo ADME data contribute to overall elimination by ≥25%.

1st stage: Basic DDI Model

- Enzyme inhibition is observed.
- Inhibition enhanced by pre-incubation is observed (MMI indicated).
- Conduct in vitro studies to determine Ki and k_{on}.

2nd stage: Mechanistic Static or PBPK Models

- May in vivo inhibition be excluded based on basic model?
  - Yes: No further study needed.
  - No: Perform multiple-dose DDI study with probe drug (see section 4.4).

Regulatory Guidance provides an overview for recommended clinical studies and staging for quantitative DDI modeling.
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**QUANTITATIVE PREDICTIONS: PBPK**

Physiologically-based pharmacokinetic (PBPK) modeling integrates in vitro, in vivo and in silico data to simulate outcomes.

Incorporates physiological and physicochemical properties

Treats the body as compartments connected by a circulatory system

Integrates in vitro and clinical observations to support M&S

PBPK is suited for modeling applications where:
- Changes in physiology or populations may impact PK variability
- Changes in physicochemical properties or formulations may alter PK
- Dynamic simulations of drug interactions are desired

EMERGING KNOWLEDGE OF DME TISSUE EXPRESSION

Key ADME processes:
- Dissolve in gastric and intestinal fluids
- Stable at physiological pH ranges
- Bioavailable
- Avoid efflux transport
- Partition into target tissue
- Selectively bind to target
- Avoid DDIs

Desired characteristics for oral drugs:
- Dissolve in gastric and intestinal fluids
- Stable at physiological pH ranges
- Bioavailable
- Avoid efflux transport
- Partition into target tissue
- Selectively bind to target
- Avoid DDIs

Based on dose route and drug distribution, are there DMEs of particular concern?

Emerging understanding of DME expression

Figure courtesy of the National Institute of General Medical Sciences “Medicines By Design”
Polymorphic DMEs with marked PK effects
- Alcohol dehydrogenase (ADH)
- Aldehyde dehydrogenase 2 (ALDH2)
- Butyrylcholinesterase
- CYP2C9
- CYP2C19
- CYP2D6
- CYP3A4/3A5
- Dihydropyrimidine dehydrogenase
- FMO 3
- NAT 2
- Thiopurine S-methyl transferase
- UGT1A1
- UGT1A4
- UGT2B17

Pharmacokinetic consequences of genetic polymorphisms can serve as an effective basis for PBPK model qualification

Prototypical PK observations

Special populations of concern?
- The ontogeny of NAT2 is not well defined
- 100% of children under 60 days of age exhibit slow acetylator phenotype
WHY SHOULD WE CARE ABOUT PBPK?

Application of PBPK to FDA IND/NDA Submissions (2008-2013, 33 Submissions)

Key regulatory questions in clinical pharmacology reviews:
• What intrinsic factors (age, gender, disease, polymorphism, etc) influence exposure?
• What extrinsic factors (drugs, herbal products, diet, etc) influence exposure?
• Based on exposure-response, what dosage regimen adjustments, if any, are recommended?

Potential for improved decision making
• Study Timing (delay until proof of concept achieved)
• Improved/abbreviated study design
• Necessity of clinical studies

PBPK enables the rational translation of in vitro or pre-clinical data to the clinical situation

What is necessary to underwrite PBPK model development and implementation?

What to consider in the big picture

• What is the intent of submission (i.e. study waiver)?
  • Is the degree of confidence in PBPK capabilities consistent across Regulatory Agencies?
• What is the impact of the analysis?
  • Simulations that affect the drug label/SmPC are high impact
• How much and what type of in vitro/clinical data is available?
  • High variability across clinical datasets may be problematic
• How much and what type of literature DDI data is available for verification?

Notes from the EMA PBPK Guidelines relevant to non-CYP enzymes

• “In case there are a limited number of inhibitors of the specific pathway and in vivo data on inhibition is scarce, the qualification could also be made using data on the consequences of genetic polymorphisms in the enzyme in question”

• “Again, the qualification will only be valid for situations covered by the qualification dataset, e.g. only for the specific enzyme(s), site of inhibition and the type of background data on which the simulations were based”

• “If the number of known in vivo inhibitors of the enzyme in question is limited, an attempt should be made to include all known inhibitors”

PBPK report elements

• Executive Summary
• Introduction
• Objectives
• Materials and Methods
• Overview of modeling strategy
• Model assumptions
• System specific parameters
• Drug specific parameters
• Parameter estimation
• Drug model structure
• PK/Clinical data
• Simulation design
• Model evaluation criteria
• Sensitivity analysis
• Software tools
• Results
• Model evaluation/qualification
• Model application
• Discussion/conclusions
• Appendices
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CASE STUDY 1: UGT INHIBITION

Specific UGT contribution to the metabolism of marketed drugs

Qualification of AZT PBPK model

Typical UGT2B7 Substrates

NSAIDS
Morphine
3-OHbenzodiazepines
AZT

Fluconazole/AZT DDI Prediction

Clinical DDI observations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Putative inhibitor/inducer</th>
<th>ΔGlucuronide</th>
<th>ΔParent</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT</td>
<td>Atovaquin</td>
<td>30% ↓ in AUC&lt;sub&gt;0&lt;/sub&gt;/AUC&lt;sub&gt;parent&lt;/sub&gt;</td>
<td>30% ↑ AUC</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td>50% ↓ Oral formation clearance</td>
<td>74% ↑ AUC</td>
</tr>
<tr>
<td>Probenecid</td>
<td></td>
<td>2-fold ↑ AUC</td>
<td>2-fold ↑ AUC</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td></td>
<td>27% ↓ in urinary clearance</td>
<td>No change</td>
</tr>
<tr>
<td>Didexyinosine</td>
<td></td>
<td>22% ↑ AUC</td>
<td>35% ↑ AUC</td>
</tr>
<tr>
<td>Lersiverine</td>
<td></td>
<td>19% ↑ AUC</td>
<td>35% ↑ AUC</td>
</tr>
</tbody>
</table>

FDA/EMA review of UGT induction submission

Early UGT DDI predictions using PBPK look promising

Drug-Derug Interactions for UDP-Glucuronosyltransferase Substrates: A Pharmacokinetic Explanation for Typically Observed Low Exposure Ratios

Drug Metabolism. 2004;32(11):1201-1208

Physiologically Based Pharmacokinetic Model Qualification and Reporting Procedures for Regulatory Submissions: A Consortium Perspective

Clin Pharmacol Ther. 2018; epub ahead of print

Application of Physiologically Based Pharmacokinetic Modeling to Predict the Pharmacokinetics of Zidovudine and its interaction with Fluconazole Using Recombinant UGT2B7 CLint inputs and UGT Tissue Scalars

Certara Poster. 2013

UDP-Glucuronosyltransferases and clinical drug-drug interactions
Pharmacology and Therapeutics. 2005;197-132
Do relevant in vitro tools to characterize DDI exist?

**Substrate**
- Recombinant enzyme?
- Selective control substrates?
- Selective chemical inhibitors/antibodies?
- Correlation analysis?
- Which cell type/subcellular fraction(s)?
  - Hepatocyte
  - Enterocyte
  - Microsome
  - S9, cytosol
- From which organ(s)?
- With which co-factor(s)?
- Have enzyme kinetics been run?

**Inhibitor**
- Selective substrate?
- Selective control inhibitor?

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**UGT INHIBITION, IN VITRO DDI TOOLS**

**MK-7246 Example**

**Recombinant enzyme**

**Enzyme kinetics**

**Enzyme inhibition**

**Enzyme abundance**

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**Integration of knowledge is critical for extrapolation to the clinical situation**

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**Multiplexed Targeted Quantitative Proteomics Predicts Hepatic Glucuronidation Potential**

*Drug Metab Dispos*. 2015;43(9):1331-1335

**Optimized Assays for Human UDP-Glucuronosyltransferase (UGT) Activities: Altered Alamethicin Concentration and Utility to Screen for UGT Inhibitors**

*Drug Metab Dispos*. 2012;40(5):1061-1065

**UGT2B17 Genetic Polymorphisms Dramatically Affect the Pharmacokinetics of MK-7246 in Healthy Subjects in a First-in-Human Study**


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**Table 4** Dose-normalized pharmacokinetic variables of MK-7246 in 13 healthy subjects in relation to UGT2B17 genotype

<table>
<thead>
<tr>
<th>UGT2B17 genotype</th>
<th>Cmax (nmol/L)</th>
<th>t1/2 (h)</th>
<th>AUC0-t (nmol*h/L)</th>
<th>AUC0-Inf (nmol*h/L)</th>
<th>AUC0-Inf/Clr (M1/M2:MK-7246)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1 (n=6)</td>
<td>0.22 ± 0.08</td>
<td>6.9 ± 0.1</td>
<td>5.87 ± 0.84</td>
<td>10.7 ± 2.9</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>*1/*2 (n=5)</td>
<td>0.94 ± 0.96</td>
<td>4.0 ± 0.9</td>
<td>14.66 ± 13.65</td>
<td>14.66 ± 13.65</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>*2/*2 (n=4)</td>
<td>18.11 ± 5.85</td>
<td>2.0 ± 0.4</td>
<td>148.03 ± 25.21</td>
<td>148.03 ± 25.21</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>*1/*1 (n=6)</td>
<td>0.62 ± 0.78</td>
<td>6.9 ± 0.1</td>
<td>10.7 ± 10.7</td>
<td>10.7 ± 10.7</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>*2/*2 (n=2)</td>
<td>8.0 ± 2.1</td>
<td>2.0 ± 0.4</td>
<td>148.0 ± 25.21</td>
<td>148.0 ± 25.21</td>
<td>1.7 ± 0.8</td>
</tr>
</tbody>
</table>

---
UGT INHIBITION

Risk factors for large AUC/I/AUC ratios
- High $f_m$
- High $[I]/K_i$
- High hepatic extraction
- Low $K_m$ (relative to [liver])

Effect of $[I]/K_i$ and $f_m$

Effect of $K_m$ and [drug]

200 mg dose of MW 500 drug = 1600 μM
Potential intestinal UGT inhibition
(UGT 1A1/1A8/1A10)

Potential UGT intestinal inhibition Silybin A & B
UGT 1A8/1A10 Ki: 40 – 60 uM
Silibinin dose 480 mg t.i.d

Effect of Intestinal Glucuronidation in Limiting Hepatic Exposure and Bioactivation of Raloxifene in Humans and Rats

Quantitative prediction and clinical evaluation of an unexplored herb–drug interaction mechanism in healthy volunteers
CPT: Pharmacometrics and Systems Pharmacology. 2015;4(12):701-710

Drug-Drug Interactions for UDP-Glucuronosyltransferase Substrates: A Pharmacokinetic Explanation for Typically Observed Low Exposure Ratios
Drug Metab Dispos. 2004;32(11):1201-1208

Negative DDI results may be useful for qualifying PBPK models
Isoniazid is an anti-tuberculosis drug predominantly metabolized by NAT2

NAT2 is polymorphic, with slow, intermediate and fast PK phenotypes

Efficacy readouts may be linked with the PBPK model based on phenotype

Exposure leading to toxicity in off-target organs was modeled using PBPK

The link between exposure, phenotype, efficacy and toxicity may be effectively incorporated into PBPK modeling
Oseltamivir is an anti-viral flu medicine administered as a pro-drug

CES-1 is the primary enzyme converting Oseltamivir to its active metabolite

Ethanol inhibits CES-1 activity

Qualification of oseltamivir PBPK model (genotypes)

Qualification of ethanol PBPK model

Integration of PBPK models for CES-1 inhibition

PBPK model qualification across clinical scenarios builds confidence in model application
**CASE STUDY 4: INHIBITION OF XANTHINE OXIDASE**

6-Mercaptopurine (6-MP) is a thiopurine found to be effective for the treatment of leukemia

- Xanthine oxidase mediates 6-MP clearance, along with hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and thiopurine methyltransferase (TPMT)
- High first pass clearance is observed

Co-administration of allopurinol markedly increases exposure of 6-MP

Allopurinol is an XO inhibitor developed to treat gout

Oxypurinol is a transition state analog and potent inhibitor of XO

**PK Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6-MP alone*</th>
<th>6-MP with allopurinol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (µM)</td>
<td>0.74 ± 0.28</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>t_{1/2} (hr)</td>
<td>2.4 ± 0.4</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>CLR (mL/min/m²)</td>
<td>417 ± 73</td>
<td>715 ± 56</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>0.12 ± 0.03</td>
<td>0.99 ± 0.05</td>
</tr>
</tbody>
</table>

Use of PBPK modeling is expanding to include tissue distribution and special populations

Mechanism of Inhibition of Xanthine Oxidoreductase by Allopurinol: Crystal Structure of Reduced Bovine Milk Xanthine Oxidoreductase Bound with Oxypurinol

Physiologically based pharmacokinetic model for 6-mercaptopurine: exploring the role of genetic polymorphism in TPMT enzyme activity

Inhibition of first-pass metabolism in cancer chemotherapy: interaction of 6-mercaptopurine and allopurinol.

Cytchrome P450 and Non–Cytochrome P450 Oxidative Metabolism: Contributions to the Pharmacokinetics, Safety, and Efficacy of Xenobiotics

CONCLUSIONS

- Prediction of DDIs for non-CYP enzymes is an emerging area of understanding
- Careful planning is required to ensure that appropriate in vitro experiments are run
- Non-routine tools may need to be developed and integrated as a drug progresses through the pipeline
- Keep the therapeutic area and common co-medications in mind
- Integrating in vitro, in silico and clinical information in a platform such a PBPK provides a powerful approach to predicting DDI magnitude
- Qualification of PBPK models for non-CYP enzymes may be challenging when limited clinical data are available