Biomarkers & Liver Injury

Is Serious DILI Predictable?

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The views being presented are my own and not an official position of the FDA
Overview of Presentation

• Development of DILI biomarkers: What is the challenge?

• Drugs that cause serious DILI
  – pathways of toxicity & injury

• Patient susceptibility profiles
  – pharmacogenomic factors
  – epigenetics
  – non-genetic host characteristics

• Predicting serious liver injury
  – promise of metabonomics, proteomics & small RNAs
  – multivariate approaches in the modeling of DILI

• Summary
Development of DILI biomarkers

What is the challenge?

• Need for biomarkers that reliably predict which drugs will cause idiosyncratic drug AEs, portend serious injury before it has occurred, or indicate who are susceptible patients

• Different drugs incite serious DILI through different poorly elucidated mechanisms

• Serious/life-threatening events are rare and typically not be seen in clinical trials

• How a weak signal of mild injury in a small test population will ‘play out’ after marketing is difficult to predict
Development of DILI biomarkers

What is the challenge?

• For each drug rare combinations of poorly elucidated exposure conditions, host factors & genetic defects are required for serious DILI

• Validation of biomarker(s) depends on comprehensive assessment of adequately designed & performed clinical studies

• A key objective is to predict serious DILI in an individual early after beginning drug treatment, either before any injury or at a phase when injury is mild & easily reversible
Pathways of toxicity & injury
Opportunities for DILI biomarker development

Susceptibility Factors
- Genetic & Non-genetic factors

Xenobiotic Processing
- Absorption
- Distribution
- Metabolism
- Excretion

Injury
- Altered Cell functions
  - Necrosis
  - Apoptosis

Injury Amplification
- Necrosis
- Apoptosis

Cytoprotection & Adaptation
- Cell preservation

Recovery & Regeneration
- Cell proliferation & replacement

P-G variants
Epigenetic markers
Nutritional states
Other Drugs/EtOH

Drug intermediates
Metabonomic markers
Proteomic profiles
Transcriptome
Circulating RNAs
Enzyme activities

Cytokines
Growth factors
Apoptotic markers
Injury markers

ALT/bilirubin
Liver function tests
Hepatocellular mass
The Promise of DILI Predictive Biomarkers

Yogi’s Principles at Play

‘It’s tough to make predictions….. especially about the future.’

Quid Pro Quo

‘You can observe a lot by watching.’
# MHC alleles & DILI Susceptibility

<table>
<thead>
<tr>
<th>Drug</th>
<th>HLA</th>
<th>Odds Ratio* (Case/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amox/Clavulanate¹</td>
<td>DRB1*1501</td>
<td>10X</td>
</tr>
<tr>
<td>Ximelegatran²</td>
<td>DRB1*07</td>
<td>4X</td>
</tr>
<tr>
<td>Lumaricoxib³</td>
<td>DRB1*1501</td>
<td>6X</td>
</tr>
<tr>
<td>Flucloxacillin⁴</td>
<td>B*5701</td>
<td>80X</td>
</tr>
<tr>
<td>Ticlodipine⁵</td>
<td>A*3303</td>
<td>13X</td>
</tr>
</tbody>
</table>

2. Kindmark et al., *Pharmacogenomics J.*, 2007
3. [www.aasld.org/conferences/educationtraining/Documents/Hepatoxicit/Wright](http://www.aasld.org/conferences/educationtraining/Documents/Hepatoxicit/Wright)
4. Daly et al., *Nature Genetics*, 2009
5. Hirata et al., *Pharmacogenomics J.*, 2008

* Odds Ratio does not reflect predictive value or absolute risk measures
Valproic Acid & DILI

Pharmacogenomic susceptibility

• Alpers-Huttenglocher Syndrome (heritable neurometabolic disorder with high risk for VPA-induced ALF)
  – Extreme phenotype caused by homozygous or compound heterozygous mutations of POLG (mitochondrial γ DNA polymerase)

• Idiosyncratic VPA hepatotoxicity
  – 7/14 adult & pediatric cases linked to simple heterozygous missense mutations in Polymerase domain of POLG*
  – Odds ratio relative to ethnically matched controls ~ 24
  – Point mutations associated with extended mutability of mtDNA in yeast; nonetheless in humans DILI phenotype only unmasked with VPA
  – Alleles with these POLG mutations as common as <8.6% in some populations
  – P-G screening of POLG prior to VPA treatment may emerge as strategy to identify some patients with increased risk for VPA-induced ALF

* JD Stewart et al., Hepatology, 2010
Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio)

TA Manolio et al. Nature 2009
’Classical’ DILI Susceptibility Factors

- Pre-existing conditions or diseases
- Age & Gender
- Nutritional status
- Alcohol (chronic vs acute)
- Concomitant drugs
- Genetic variants
  - drug clearance, metabolism & secretion into blood or bile
- Multiple DILI phenotypes
  - different individual responses to certain drugs
Epigenetic Mechanisms & DILI Susceptibility

• To date not systematically interrogated

• Mechanisms of interest to study
  • Epigenetic changes caused by drug treatment
    • Genomic sites of C-G methylation & demethylation
    • Targeted effects on histone acetylation & deacetylation
    • Associated changes in patterns of gene expression
  • Pretreatment epigenetic signatures linked to DILI susceptibility
DILI Susceptibility

Epigenetic Mechanisms for Investigation

M. Szyf, Tox Sci., 2007
Drug Processing

Absorption, distribution, metabolism & elimination (ADME)

• ADME analysis of NMEs useful for
  – assessments of PK & PD (‘on-target’ effects)
  – modifications of dosing driven by drug-drug & disease-drug interactions
  – predictions of dose-related toxicity (e.g. APAP hepatotoxicity)

• ADME not typically useful in predicting rare idiosyncratic DILI

• Emerging biological measures of xenobiotic processing that may provide informative biomarkers include metabonomic, proteomic and transcriptonomic changes in liver cells, serum and urine

• miRNA and mRNA profiles in serum may also prove useful as predictors of DILI

• Whether predictors of serious DILI appear at an early phase of drug exposure before liver injury develops remains to be shown
Circulating RNAs

- Liver-specific mRNAs and miRNAs released by liver cells into serum in microvesicles & exosomes after hepatotoxic doses of DGAL & APAP\textsuperscript{1,2}
- mRNA microarrays show drug-specific & organ-injury specific signatures
  - Clustering of transcripts reflect altered expression of canonical pathways\textsuperscript{2}
    - APAP: oxidative stress, apoptosis, necrosis, immune responses
    - DGAL: lipid metabolism, complement, coagulation, acute phase reactions
  - Liver specific mRNAs (haptoglobin, fibrinogen, albumin) increase at hepatotoxin doses that do not increase ALT/AST; also may occur earlier
- Analysis of serum and liver miRNAs by microarrays or quantitative PCR\textsuperscript{1} demonstrates increases of tissue specific species (e.g. mir-122 & mir-192)
  - Prior to ALT increases and histopath changes

\textsuperscript{1}K Wang et al. PNAS, 2009; \quad \textsuperscript{2}BA Wetmore et al., Hepatology, 2010
MicroRNA Production
Early Prediction of DILI with APAP

*Rodent metabonomic biomarkers*

- Rat model of pre-treatment metabonomic profiles in urine as predictors of DILI\(^1\)
  - Predictive power of liver injury responses to toxic doses of D-galactosamine and APAP assessed using NMR profiles of pre-treatment urine samples
  - Correlations between pre-treatment NMR principle components & post-treatment histological changes are promising

- Each component is an independently regulated set of compounds

- Based on goodness-of-fit multivariate analysis, compounds whose levels are most predictive include taurine, TMAO, betaine

\(^1\)TA Clayton et al., Nature, 2006
Early Prediction of DILI with APAP

*Human metabonomic biomarkers*

- 33% volunteers treated with APAP 4 gm/day develop increased serum ALT after 5 days of dosing

- Study of early post-treatment metabonomic profiles in urine as predictors of mild DILI\(^1\)
  - Not designed to predict progression from mild to serious liver injury

- Principle Component Analysis of NMR signatures after 1 day of dosing predicted increased ALT levels with 74% accuracy
  - Based on goodness-of-fit analysis, most metabolites whose levels early on were predictive of ALT increases are not related to APAP

\(^1\)JH Winnike et al., Clin Pharm & Ther., 2010
Early Prediction of DILI with APAP

Study Subjects: ALT increases vs no increases

Dosing days 6-7 2-3

1Adapted from JH Winnike et al., Clin Pharm & Ther., 2010

24 March 2011 FDA-AASLD-PhRMA HepTox Steering Committee
Pathways of toxicity & injury

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Changes in biomarker specificity and clinical severity measures at different phases of DILI

Clinical Severity (B)

Specificity for DILI (A)

Time from Initiation (C)

DILI Progression Step

Profile A^1

Profile A^0

Profile B^1

Profile B^0

Profile C^1

Profile C^0

High

Low
Serious DILI Predictive Modeling

Complexity

• Human liver functions are homeostatic with multiple layers of function that are relevant to predictions of DILI risk

• Pathways for xenobiotic processing, adaptation and regeneration are overlapping and redundant

• Knowledge of the biosystem is still limited

• Perturbations of individual biosystem elements can have a range of effects on DILI risk

• Prediction of serious DILI is based on conditional probability

• A ‘perfect storm’ of DILI risk factors may still generate a positive predictive value for serious DILI <<100%
Biomarkers to Predict Serious DILI

**Bio-systems Perspective**

- **DILI Course**
  - Resolution vs Progression
- **Liver Organ Response**
  - Adaptation vs Accelerated Damage
- **Cell Functions**
  - Hepatocyte drug uptake, metabolism, transport, & clearance
  - Organelle dysfunction, necrosis, apoptosis, recovery
  - Immune & inflammatory cell activation
- **Molecular Effects**
  - Reactive metabolites, neoantigens, signal transduction

Transcriptonomics

Proteomics

Metabonomics

24 March 2011

FDA-AASLD-PhRMA HepTox Steering Committee
Challenges

‘If you don't know *where* you're going, you might not get there.’  (Berra)

A fortiori

‘If you don’t know *how* to get there, you also may not get there’  (Avigan)
Attributes of an Ideal Predictive Model in Physics¹

- Contains few arbitrary elements
- Explains all existing observations
- Is elegant (few fudge factors; simple as possible, but not simpler)
- Makes detailed predictions

¹S Hawking & L Mlodinow, The Grand Design, 2010
Predicting Serious DILI

Challenges for bio-system modeling

• Drug-induced liver effects occur in complex hepatic system with many interconnected components
  • hepatic metabolism, xenobiotic clearance, cytoprotection, adaptation, innate & adaptive immunity, hepatocellular regeneration, etc.

• Multiple steps between xenobiotic initiation and acceleration of liver injury
  – inevitable uncertainty (‘chaos effect’) in accurately predicting serious DILI
  – Rechallenge after DILI may not always lead to DILI
  – positive predictive value for single DILI biomarkers <<100%

• Drugs with rare DILI events difficult to study; dose-related drugs can be studied & modeled more easily (e.g. APAP)

• Individual susceptibility factors should be incorporated into model
Predicting Serious DILI
Is it like forecasting the weather?
Search for Predictive DILI Biomarkers

• A key objective is to identify markers that predict serious DILI early after beginning drug treatment, either before injury or at a phase when injury is mild & easily reversible

• Evaluation depends on stratified comparisons between estimated & measured outcomes in prospective studies (goodness-of-fit statistical analysis)

• Matched drug-treated and non-treated controls are essential

• Animal models are a necessary step but do not supplant the need for human studies
Summary

- Although we look forward to remarkable advances in the prediction of serious DILI, absolute predictors are unlikely.

- Predictive modeling of serious DILI will likely be a composite of biomarker measures that include circulating mRNA/miRNA, serum/urinary metabolites & P-G /epigenetic markers.

- Biomarker measures would reflect early and intermediate steps leading to DILI progression or adaptation/recovery:
  - drug processing
  - patient susceptibility factors
  - cytoprotective responses
  - immune responses
  - regenerative responses
  - injury or apoptotic pathways
FDA DILI website: www.fda.gov/Drugs/ScienceResearch/ResearchAreas