Metabolomic Biomarkers for Drug-Induced Renal Damage

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Outline of Presentation

1. Treatment-related adverse events
2. Toxicity in clinical care
3. Drug development attrition
4. Biomarkers and metabolomics
5. Cisplatin nephrotoxicity study
6. Precision medicine
7. PBPK models
Key Messages

- Kidney damage following progression of primary diseases increased by 330% from 1990 to 2011
- Incidence rates for drug-induced kidney damage range from 1.3 to 10%
- There are no qualified renal safety biomarkers that outperform Scr and BUN for assessing kidney damage
- Metabolomics can render new biomarkers which can identify renal toxicity before histological changes occur

Interindividual Variation in Drug Response

“Medicine is a science of uncertainty and an art of probability”

Any medicine can produce beneficial or harmful effects in any specific patient at any given time

William Osler (1896)
Simplified View of Underlying Cause of Heterogeneity in Drug Response

Adapted from David Jackson, Molecular Health
More Realistic Depiction of the Complexity of Biological Pathways

INCOMPLETE UNDERSTANDING OF DISEASE BIOLOGY
There is a hidden epidemic......

It costs tens of thousand of lives each year – more than from auto accidents, falls, fires and drownings combined.

It costs $300B annually

IT’S A DRUG PROBLEM – BUT NOT THE KIND YOU THINK

It’s what happens when people take prescription drugs and get no benefit, or worse, experience serious adverse events
Dramatic Rise in AE Reporting to FDA

Fig. 1. The FDA Adverse Event Reporting System (FAERS) recorded nearly 800,000 AEs in 2011 and, based on the count though September 2012, Pharmaceutical Commerce projects nearly 900,000 for 2012. “Expedited” AEs are more serious ones, reported to FDA by manufacturers; while “direct” AEs come from healthcare providers or patients.
Characteristics of Treatment-Emergent Adverse Events (TEAE)

- Undesirable toxicity resulting from off-target effects or extension of on-target pharmacology (Type A)

- Unanticipated or unexplained toxicity sometimes called idiosyncratic (Type B)

- Risks occurring in subpopulations related to intrinsic (e.g., disease, age) or extrinsic factors (e.g., DDIs)

- Distinguish severe from mild on the basis of [probability x severity]
Type B TEAE Are Especially Problematic

- Not dependent on dose or systemic exposure and are less predictable than Type A TEAEs
- Do not occur in most patients but rather unpredictably and rarely in a few patients either pre- or post-marketing
- Undiscovered during drug development or regulatory review because of small clinical trial populations
- Clinical signs for Type B TEAEs are different from the desired pharmacological effects of a drug ("off-target/pathways")
- Proposed mechanism of many Type B TEAEs is immune–mediated toxicity or unrecognized pharmacology
Miley suffered a sinus infection and was prescribed cephalexin. She experienced flu-like symptoms and an “extreme allergic reaction” and has a rash over her entire body. She will be hospitalized for up to 27 days.

Hypersensitivity — Allergic reactions in the form of rash, urticaria, angioedema, and, rarely, erythema multiforme, Stevens-Johnson syndrome, or toxic epidermal necrolysis have been observed. These reactions usually subsided upon discontinuation of the drug. In some of these reactions, supportive therapy may be necessary. Anaphylaxis has also been reported.
Status Quo vs Innovation in Drug Safety

- Safety assessments have not really changed over the past 40 years
- International regulatory guidances make it easy to for companies to assess toxicity
- Regulatory agencies are conservative by nature and resistance to change
- “We need innovations in drug development and regulatory science with regard to drug safety”
Polypharmacy: Why do CV events occur much more with rosiglitazone than with pioglitazone in diabetics when taken with other drugs?
Case Study I: Patient With Ovarian Cancer

A 59 yo patient was admitted to the hospital cancer ward and started on 100 mg/m² of IV cisplatin once every 4 weeks. Serum creatinine and BUN was monitored daily.

The $S_{cr}$ began to climb 24 hours after dosing and increased 48% after 28 days. BUN increased from 10 to 28 mg/dl during the same time period. If creatinine levels exceed 3.5 mg/dl, then dialysis will be necessary. The patient probably has permanent kidney damage and will not cope with another cisplatin session.
Cisplatin Facts

- Approved in 1989 for ovarian cancer
- Most commonly used chemotherapy drug
- Increases survival rate by one year
- Renal tubular damage is dose-related
- Disposition is key to mechanisms of toxicity

- 50% of dose excreted unchanged into kidneys within 24 hours
- Concentrated in renal cortex and proximal renal tubule
- Must enter target cells to cause renal damage
- Basolateral transporters (OCT2) in cisplatin uptake
A Bean-Shaped Organ Equal in Weight to a McDonald’s Quarter Pounder

- Renal clearance is one of the major mechanisms of drug elimination (high blood flow rate)
- Drugs and their metabolites are concentrated in the tubules (active transporters)
- Renal cells exposed to high levels of drugs and metabolites cause epithelial and interior cell damage
Microscopic Look at Kidney Damage 5 Days After Cisplatin

Problem: increases in Scr lag behind histological changes risking progressive and possible irreversible changes in renal function before becoming aware of toxicity

Choudhury et al, Nat Nephrology (2006), 2, 80-91
Serum Creatinine: Strengths and Limitations

Most commonly used biomarker of renal function but diagnosis of acute renal damage is challenging

① Measures GFR
② Influenced by multiple non-renal factors
③ Delayed rise to new steady-state
④ Rises only after 50% of kidney function is lost
⑤ Increases lag behind structural damage
⑥ Is not unique to drug-induced toxicity
“Look at that poor lady on dialysis. She is so depressed and in pain with muscle cramps. She is always tired and can’t sleep at night. And, now, she has been diagnosed with ovarian cancer.”
Renal Toxicity in Cancer Patients

As many as 30-50% of ICU patients will develop renal insufficiency at some point because of treatment-related toxicity.

Rapid intervention is critical because renal insufficiency compromises treatment benefit, reduces QOL and decreases overall survival.

Current renal safety biomarkers – Scr and BUN – are inadequate for addressing this problem.
Serious Unmet Medical Need

Metabolomics may render new biomarkers for earlier and more accurate identification of acute kidney injury from medications that would allow for prompt intervention to avert permanent renal damage.
Drug Development Scenario
You are a member of a PhRMA project team and your modeling group recommends using a low (10 mg) and high dose (40 mg) of a new statin in a pivotal phase 3 clinical trial. You speak with confidence that the 40 mg dose will not cause any renal toxicity based on animal tox data and an earlier 6 week clinical trial with 2,400 patients.

In the phase III clinical trial with 17,000 patients, 33% exposed to 40 mg for up to 60 months had an increase in $S_{CR}$ of $>30\%$ above baseline. Three patients developed acute renal failure. The company discontinued the clinical trial because potential progression to renal failure in the remaining patients represented an unacceptable risk that FDA would never accept.
Root-Cause Analysis of Suspended Programs

Data: 359 Phase III Clinical Trials and 95 NDAs

- Efficacy
- Safety
- Business
- Other

Hay et al, Nat Biotech (2014), 32 (1), 40-51
Toxicity of Major Organ Systems in Preclinical or Clinical Trials

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>FUNCTIONAL CHANGE</th>
<th>INCIDENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>QTc prolongation</td>
<td>35%</td>
</tr>
<tr>
<td>Liver</td>
<td>LFT elevation</td>
<td>29%</td>
</tr>
<tr>
<td>Brain</td>
<td>Seizures</td>
<td>11%</td>
</tr>
<tr>
<td>Kidney</td>
<td>Renal insufficiency</td>
<td>7%</td>
</tr>
<tr>
<td>Blood</td>
<td>Neutropenia</td>
<td>5%</td>
</tr>
<tr>
<td>Immune System</td>
<td>Allergic reaction</td>
<td>4%</td>
</tr>
</tbody>
</table>

Late Phase Trial Failures Are Extremely Costly

Phase III clinical trial costs exceed $26,000 per patient

How do you explain when things don’t go as you assumed with drug development?
If Google can predict flu trends – if Amazon can predict what we’ll buy – if PredPol can predict where crime will happen – if Skyhook can predict where the best nightlife is............darn it, why can’t you predict if a patient will experience drug toxicity?
Metabolomic biomarkers: their role in the critical path

Laura K. Schnackenberg, Richard D. Beger*
FDA Critical Path Initiative

**Advancing Regulatory Science**

*Moving Regulatory Science into the 21st Century*

Regulatory Science is the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of all FDA-regulated products.

On February 24, 2010, FDA launched its **Advancing Regulatory Science Initiative (ARS)**, building on the achievements of existing Agency programs, like the Critical Path Initiative’s groundbreaking efforts to transform the way medical products are developed, evaluated, and manufactured.

**Guidance for Industry**

**Qualification Process for Drug Development Tools**
Biomarker Definition

A characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention

Categories of Biomarkers

30,508 biomarkers across 17 therapeutic areas and 1063 indications and 47% were in oncology

Source: GVK Bio Online Biomarker Database (Sreeni Devidas)
Clinical Use of Biomarkers

30,508 biomarkers across 17 therapeutic areas and 1063 indications and 17% were for safety

Source: GVK Bio Online Biomarker Database (Sreeni Devidas)
Use of Biomarkers in Clinical Trials

- Over the past 10 years the number of studies using biomarkers increased 6-fold
- Biomarker studies are relatively small (< 20%)
- 70% of biomarker studies were in phase 1-2
- Biomarkers used in 37% of oncology clinical studies
- Most common use was for patient selection

Hayashi, Drug Disco Ther, 2012
Newer Urinary Biomarkers of Acute Renal Injury: Site and Mechanism

<table>
<thead>
<tr>
<th>BIOMARKER</th>
<th>TYPE OF INJURY AND LOCATION</th>
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<tbody>
<tr>
<td>Cystatin C</td>
<td>Functional marker (glomerular filtration)</td>
</tr>
<tr>
<td>Albumin</td>
<td>Functional marker (glomerular filtration)</td>
</tr>
<tr>
<td>Total protein</td>
<td>Up-regulated proteins (glomerular filtration)</td>
</tr>
<tr>
<td>Kidney injury molecule (KIM-1)</td>
<td>Up-regulated protein (proximal tubule)</td>
</tr>
<tr>
<td>Beta-2-microglobulin</td>
<td>Functional marker (proximal tubule reabsorption)</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Functional marker (distal tubule epithelium)</td>
</tr>
<tr>
<td>Trefoil factor-3</td>
<td>Functional marker (tubular reabsorption)</td>
</tr>
</tbody>
</table>

“The more novel the biomarker, the more difficult validation (linking biomarker change to histological change) can become”

Major toxic effects of cisplatin are nephrotoxicity and ototoxicity.
Metabolomics: Alternative Approach to Renal Drug Safety Biomarkers

- Adverse outcomes in animals
- NOEL doses, in vitro assays, interspecies differences
- Lack of biomarkers
- Unanticipated toxicity
- Unexplained toxicity
- Failure to predict

ANIMAL MODEL

METABOLOMERIC SIGNATURE

CLINICAL INTERPRETATION

J-M Sauer, PSTC, Critical Path Institute
Why Metabolomics?

• Faster and more reproducible “omics” technology -- directly reflects biological events and uses accessible fluids urine and plasma

• Shows changes in levels of endogenous or exogenous metabolites in biological fluids due to physiological stimuli from drugs vs baseline or control

• **Metabolomic signatures** represent patterns of useful biomarkers that increase or decrease in relationship to drug-induced renal toxicity
Our Three Step Approach to Research

- Rodent
  - Metabolomic signature of drug-induced kidney damage
- Human
  - Verify signature in individuals who develop nephrotoxicity
- Rodent
  - Explore potential neuroprotective drugs
Schematic of Research Workflow

- **Integrative Systems Biology: Mechanistic and Complimentary**
  - Systems pharmacology
  - PK/PD and PBPK models of E/R
  - *In silico* target and pathway analysis

**Exploratory or Learning**

- Demonstration of drug-induced renal damage in rodents

**BIOMARKERS**

**Confirming or Applying**

- Prediction of drug-induced damage in humans
Focus on Major Biomolecule Classes

Targeted (known) metabolites: biomarkers associated with aminoaciduria and glycosuria – phenotypes of renal damage

1. Dramatic 1-18-fold increase in urinary excretion of amino acids and dipeptides; concomitant 5-fold decrease in tissue levels after 28 days of cisplatin dosing in rodents beginning with changes at days 1 and 5 with no histopathological changes

2. Marked 4.5-fold rise in polyamines beginning as early as days 1 and 5

3. Continuous decrease in renal tissue purine and pyrimidine nucleosides beginning at days 1 and 5, decrease as much as 5-fold at 28 days

4. Other including lipid metabolites, carbohydrates, energy-related metabolites and vitamins

Study #1: Exploratory Rodent Study

- **Saline**
  - Plasma samples predose (-5 minutes) and at 1, 5, 15, 50 and 75 minutes post-dose

- **0.5 mg/kg**
  - Urine samples predose and between 0-10, 20-30, 40-50, 60-70, minutes post-dose

- **3.5 mg/kg**
  - Kidney tissue predose and at day 1, 7, 14 and 28 fat each dose level along with other tissues for future investigation

- **7.5 mg/kg**
  - Battery of clinical chemistry tests, histology exam, miRNA and possibly 7 previously validated rodent safety biomarkers
Sample Preparation, Instrumentation and Data Analysis

Agilent 6460 Triple Quad with Agilent 1260 Infinity LC

For Research Use Only. Not for use in diagnostic procedures.
Metabolite Identification Challenge

1. Prior studies of nephrotoxicity yielded as many as 657 metabolites in urine and 547 in kidney tissue

2. Published studies of cisplatin rendered as many as 232 metabolites in kidney tissue and 192 in urine

3. 35% of metabolites were identifiable and 65% had unnamed structural identities

4. Boil down the number of metabolites to ~ 50 – those that progressively increased or decreased from days 1-28, rank-ordered by magnitude of change

Automated comparison to reference library using Agilent proprietary software
Target Identification and Pathway Analysis: \textit{MASE}^R

- \(~2M\) ADE cases with detailed molecular views
- Any drug:AE pairs, high frequency molecular targets, enzymes and transporters
- Risk comparisons of drug:AE pair safety profiles
- Risk evaluation by drug usage or ATC categories
- Statistical signal detection metrics (e.g., PRR)
- Risk comparisons of drug:AE pair safety profiles
- Molecular dissection of ADEs at level of targets, pathways, PK DDIs
- Alternative target therapy drugs
Data Analysis and Modeling Process

Evaluate the dose- and time-dependent course of renal damage using changes in metabolomic biomarkers in plasma urine and tissue; correlate with histological changes

Measure the time course of cisplatin concentration in plasma, urine and kidney tissue; correlate with time course of histological changes

Classify metabolomic biomarkers in plasma and urine into those showing early (rapid) and late (delayed) rank-order increases or decreases compared to saline
Model Building: Built “Fit for Purpose”

“The essence of mathematics is not to make simple things complicated, but to make complicated things simple”

Stan Gudder
Professor of Mathematics
University of Denver
Mathematical Physiologically-Based PK Model

Systems Pharmacology

Mechanistic -- Mechanism of off-target drug effects, metabolomics and integrated “omics”

PBPK
Semi-mechanistic -- Bridging: animals-to-humans, explore scenarios not studied before

PK/PD
Empirical – Model PK/PD using renal safety biomarkers

Multiple Omics -- Multiple Models
Study #2: Observational Human Study

- Be able to propose metabolomic biomarkers that reflect the site and mechanisms of cisplatin toxicity

- Obtain plasma and urine samples from subjects with acute renal toxicity (ART) who received drugs known to potentially cause nephrotoxicity

- Compare the rodent metabolomic signature to that of humans with ART to propose a tool for detection and diagnosis of drug-induced renal damage
Study #3: Nephroprotective Rodent Study

- Identification of mechanism-based metabolomic biomarkers of drug-induced renal toxicity
  - Explore basolateral transporters that play a role is cisplatin uptake in renal cortex
  - Select a known nephroprotective, OCT-2 inhibitor (cimetidine) and repeat rodent cisplatin studies
  - Major focus on proximal tubule organic cation transporter-2 or OCT-2 which accumulates cisplatin in proximal tubule
Precision Medicine

An emerging approach to diseases and treatments that takes into account an individual's unique variation in genes, environment and lifestyle.

Omic technologies – particularly genomics – have enabled sub-classification of diseases based on pathway mutations and identified target therapies for greater efficacy.
Precision Safety Medicine

MALES
Prostate, colon, melanoma, urinary bladder and non-Hodgkin lymphoma account for 75% of cancers

FEMALES
Breast, uterine, colon, melanoma, thyroid and non-Hodgkin lymphoma account for 75% of cancers

How can we identify the 1 in 7 cancer patients that get renal toxicity?

Metabolomics Can Redefine Our Future Thinking About Diseases and Treatments

Adenocarcinoma (1999): classified by anatomic and histologic criteria

Adenocarcinoma (2019): augmented by metabolomic pathway biomarkers
Summary

- **Using metabolomics:** we can identify very early candidate biomarkers of kidney damage that can detect nephrotoxicity much earlier than histopathology, BUN and Scr.

- **Future efforts:** look at other drugs, validate biomarkers in humans inadvertently made toxic with cisplatin. Look at mechanisms of and site of toxicity. Correlate with 7-member panel from the FDA as further validation.
Thank You For Your Time and Attention

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Questions?