

# Metabolomic Biomarkers for Drug-Induced Renal Damage

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# Acknowledgments



## University of Florida

- Naveed Shaik
- Mirjam Trame

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- Nigel Skinner
- Barbara Teets
- Adam Woodhouse

# 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

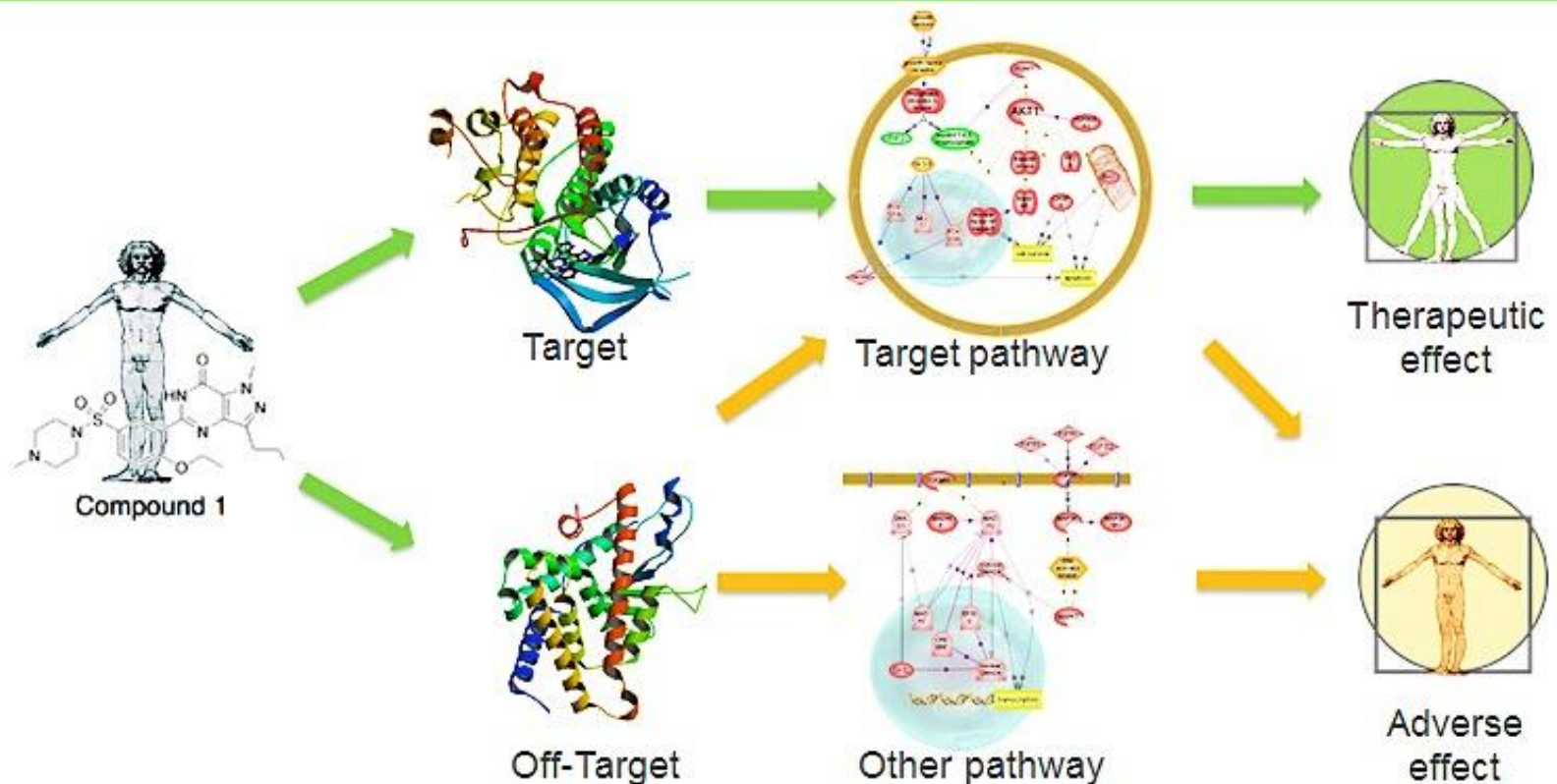


*William Osler (1896)*

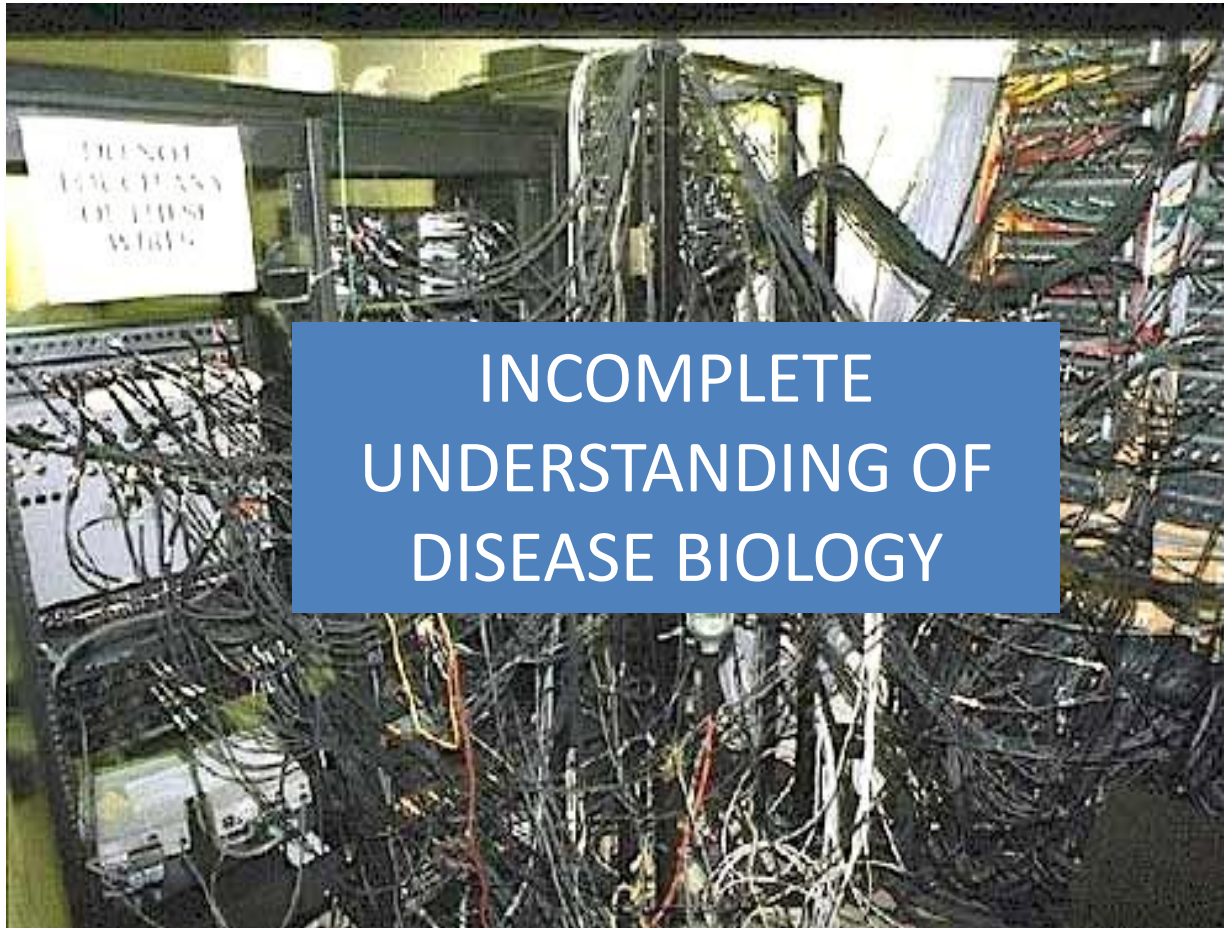
“Medicine is a science of uncertainty and an art of probability”

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

# Simplified View of Underlying Cause of Heterogeneity in Drug Response



# 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.

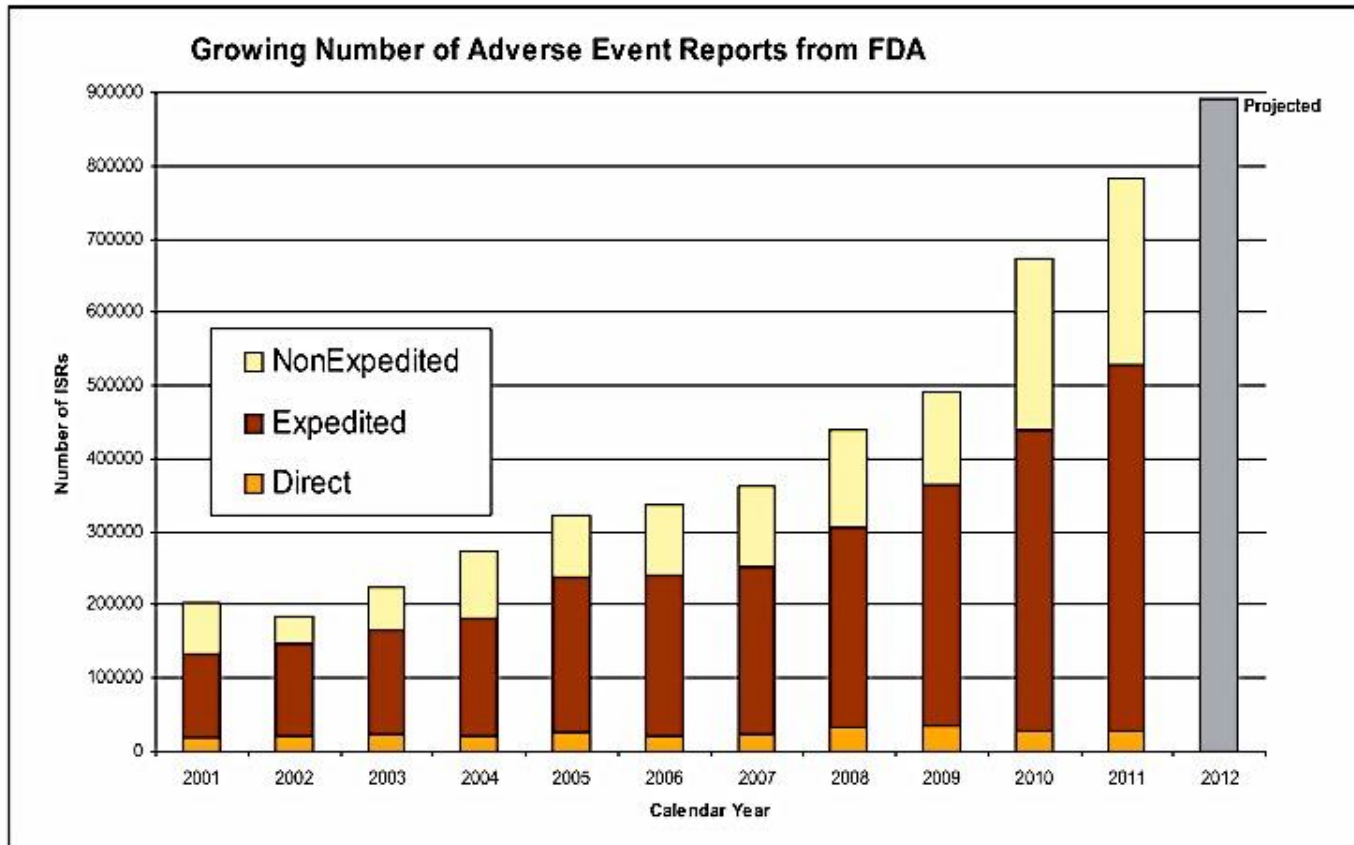
It costs \$300B annually

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 through 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

# SJS Is a Well-Known Type B Immunological TEAE



## **Miley Cyrus Drug Reaction May Have Triggered Deadly Syndrome**

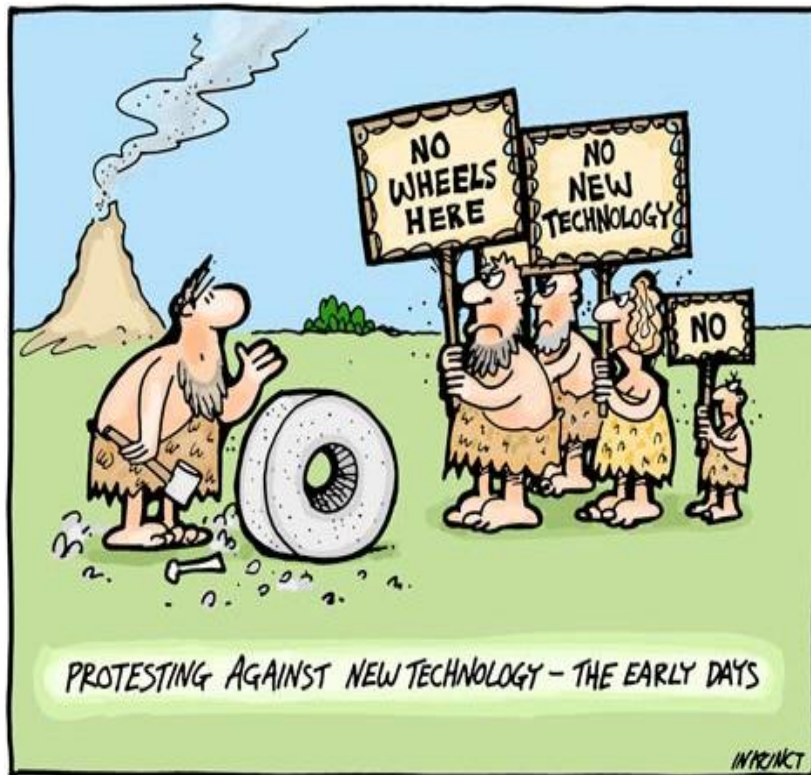
***Stevens-Johnson Syndrome Can Be Fatal***

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”

# Systems Pharmacology: Mechanistic Approach to Drug Safety Science

*Clinical Pharmacology & Therapeutics* (2011) **89** 6, 793–797. doi:10.1038/clpt.2011.55

## Pharmacological Mechanism-Based Drug Safety Assessment and Prediction

D R Abernethy<sup>1,2</sup>, J Woodcock<sup>1,2</sup> and L J Lesko<sup>1,2</sup>

### State of the Art

*Clinical Pharmacology & Therapeutics* (2013); **93** 5, 413–424. doi:10.1038/clpt.2013.29

### Systems Approaches in Risk Assessment

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Polypharmacy: Why do CV events occur much more with rosiglitazone than with pioglitazone in diabetics when taken with other drugs?

### SYSTEMS PHARMACOLOGY OF ADVERSE EVENT MITIGATION BY DRUG COMBINATIONS

Drugs are designed for therapy, but medication-related adverse events are common, and risk/benefit analysis is critical for determining clinical use. Rosiglitazone, an efficacious antidiabetic drug, is associated with increased myocardial infarctions (MIs), thus limiting its usage. Because diabetic patients are often prescribed multiple drugs, we searched for usage of a [More](#)

*Sci Transl Med* 9 October 2013:  
Vol. 5 no. 206 pp. 206ra140  
DOI:10.1126/scitranslmed.3006548



# Clinical Care Scenario



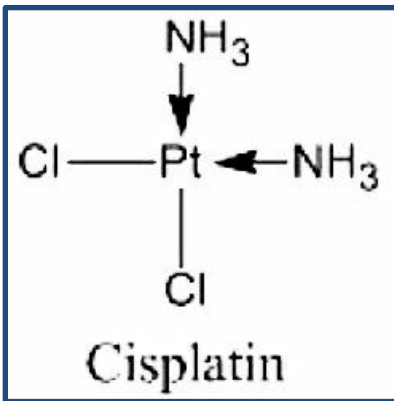
# Case Study I: Patient With Ovarian Cancer



A 59 yo patient was admitted to the hospital cancer ward and started on 100 mg/m<sup>2</sup> of IV **cisplatin** once every 4 weeks. Serum creatinine and BUN was monitored daily.

The S<sub>cr</sub> 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.**

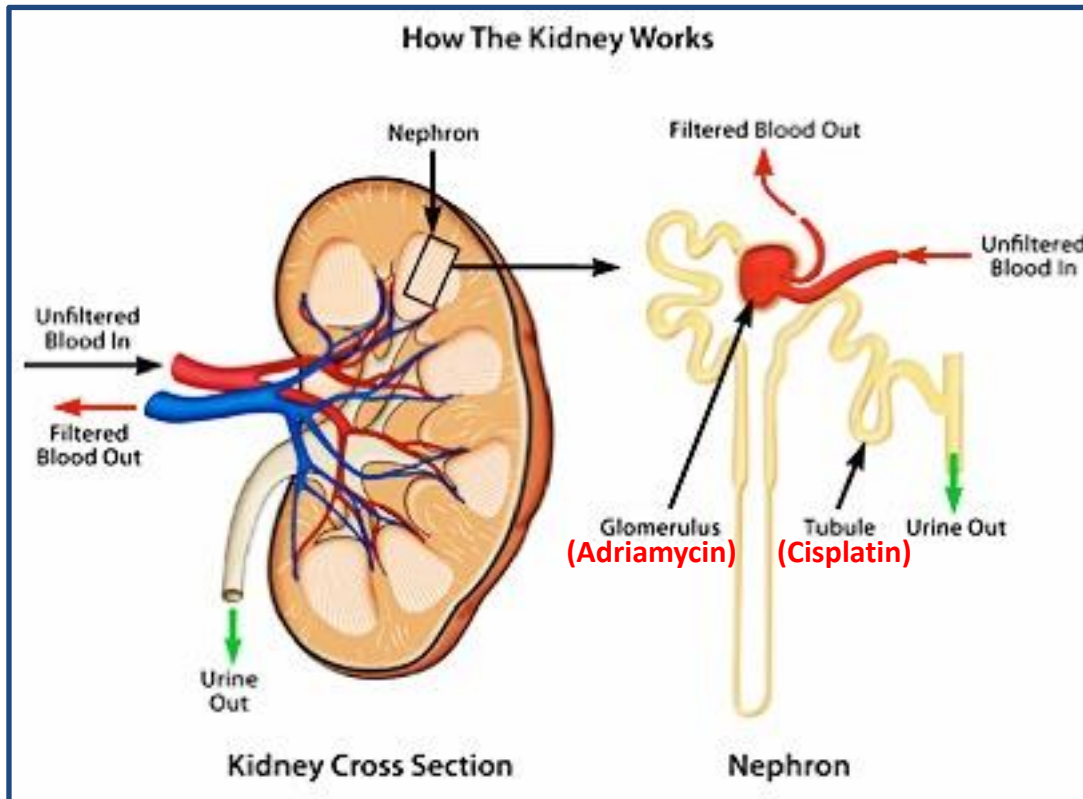
# Cisplatin Facts



- 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



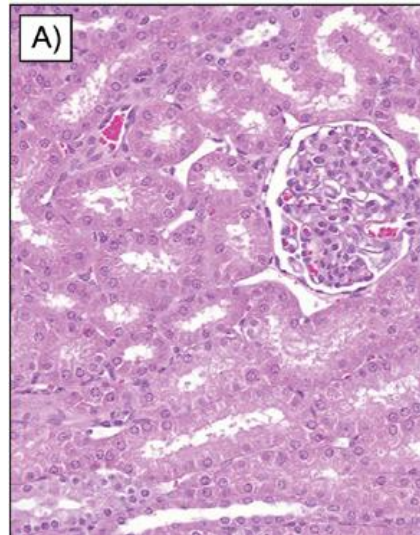
- Renal clearance is one of the major mechanisms of drug elimination (high blood flow rate)
- Drugs and their metabolites are concentrated in the tubules (active transporters)
- Renal cells exposed to high levels of drugs and metabolites cause epithelial and interior cell damage

# Microscopic Look at Kidney Damage 5 Days After Cisplatin

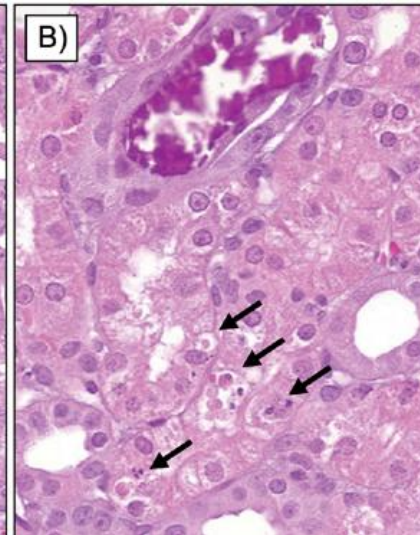


Scr  
BUN

NORMAL



DAMAGED



Patchy and denuded tubules

Necrosis and mineralization

Dilatation of renal tubules

Lymphocytic inflammation

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

# 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.*”

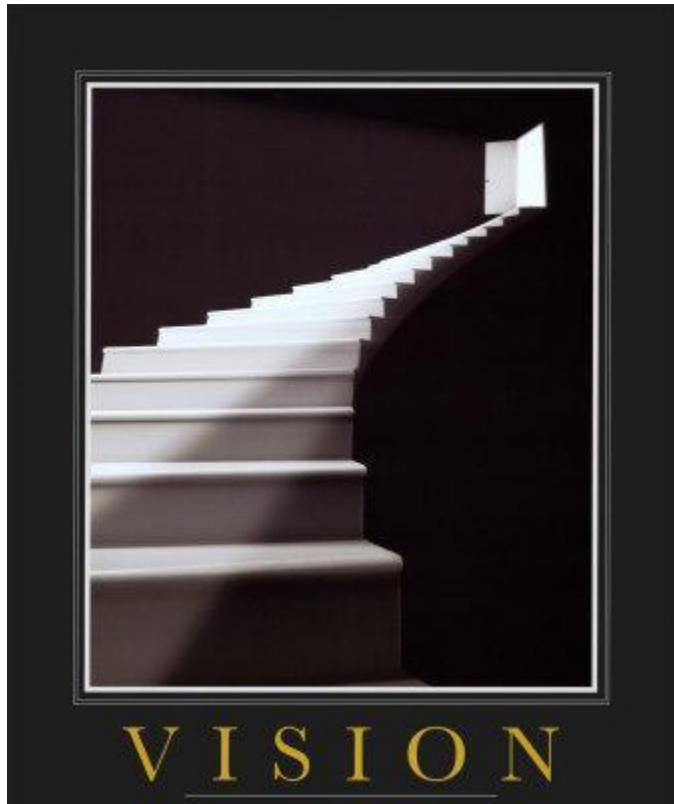
# Renal Toxicity in Cancer Patients

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



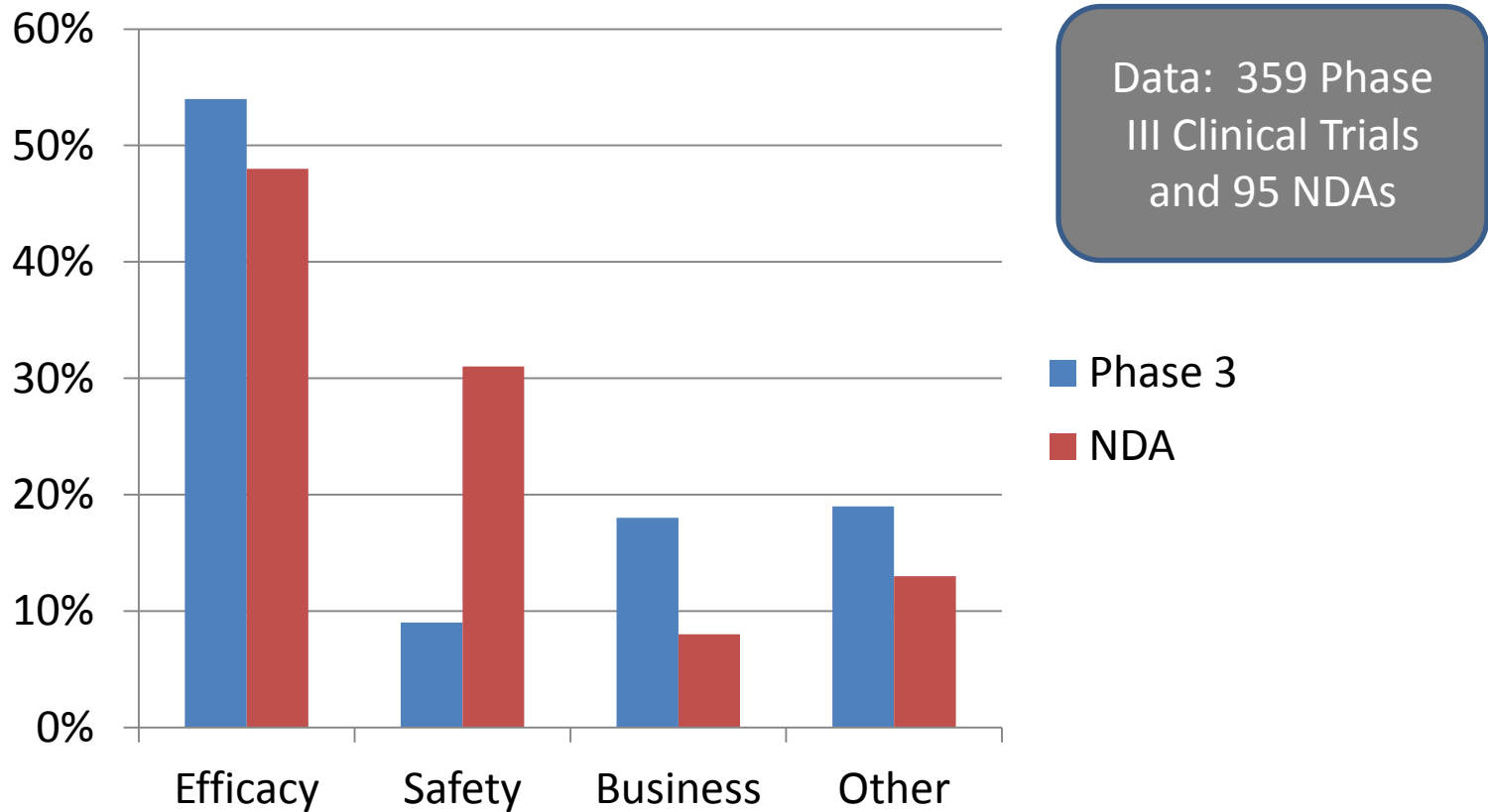
# Case Study II: Phase III Clinical Trial of a New Statin



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.

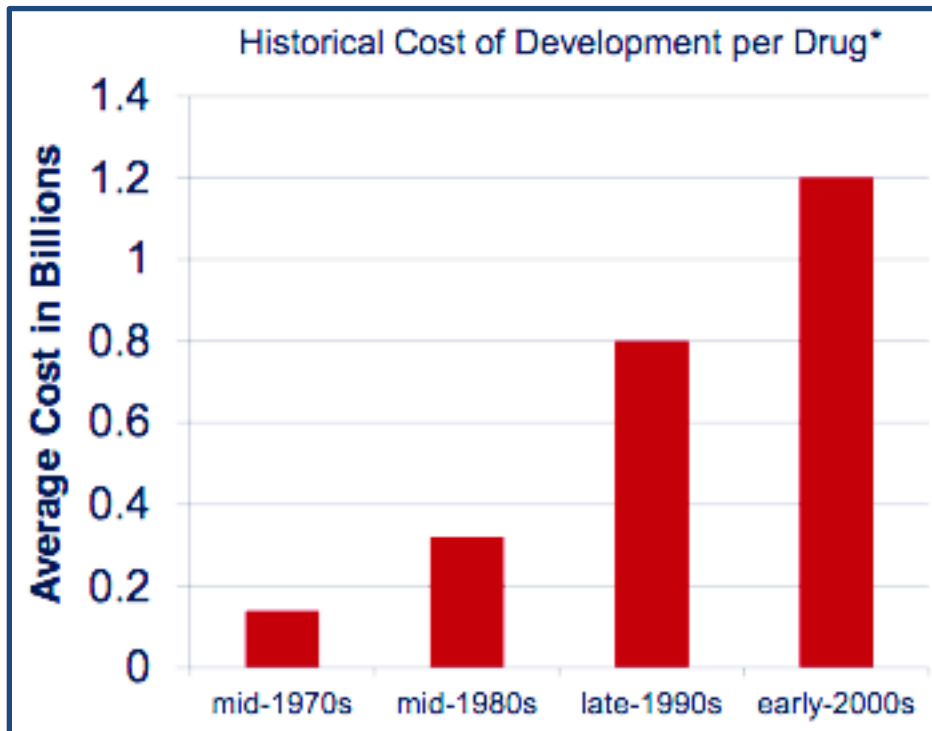
# Root-Cause Analysis of Suspended Programs



# Toxicity of Major Organ Systems in Preclinical or Clinical Trials

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

# Late Phase Trial Failures Are Extremely Costly



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



‘Biopharmaceutical Research Industry 2013 Profile.’ Pharmaceutical Research and Manufacturers of America. 2013.



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?**

# Biomarkers



ELSEVIER

DRUG DISCOVERY  
TODAY  
TECHNOLOGIES

Drug Discovery Today: Technologies

Vol. 4, No. 1 2007

*Editors-in-Chief*

**Kelvin Lam** – Harvard Stem Cell Institute, Harvard University, USA

**Henk Timmerman** – Vrije Universiteit, The Netherlands

Critical path

## Metabolomic biomarkers: their role in the critical path<sup>☆</sup>

Laura K. Schnackenberg, Richard D. Beger\*

Division of Systems Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, 72079-9502, United States

# 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

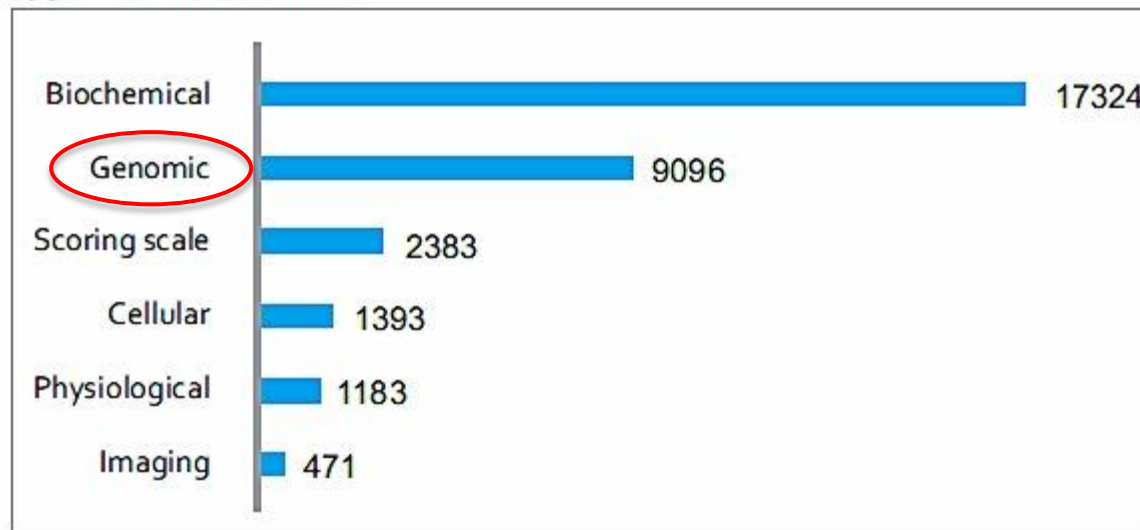
Qualification Process for  
Drug Development Tools

# 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

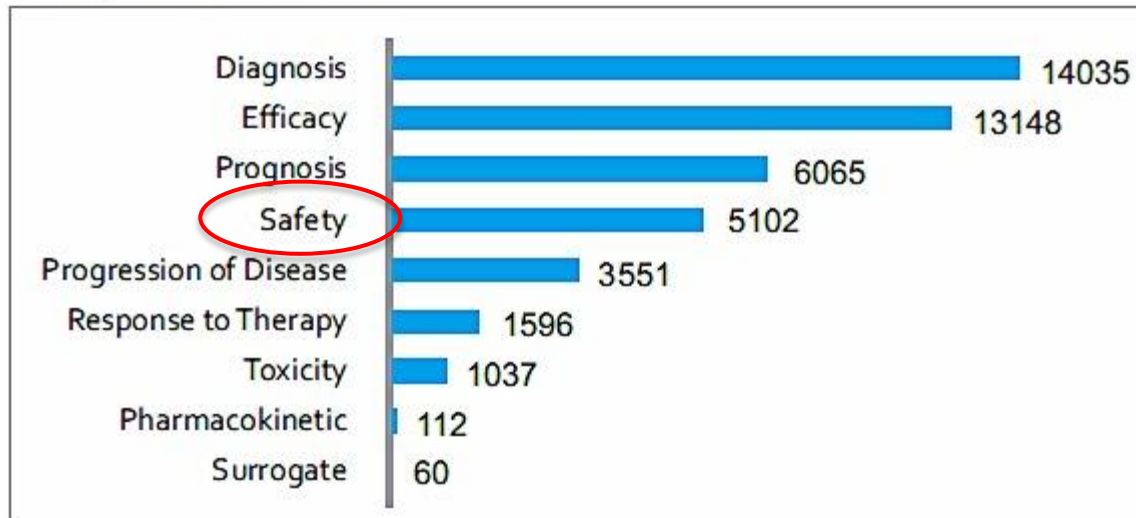
Types of Biomarkers



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

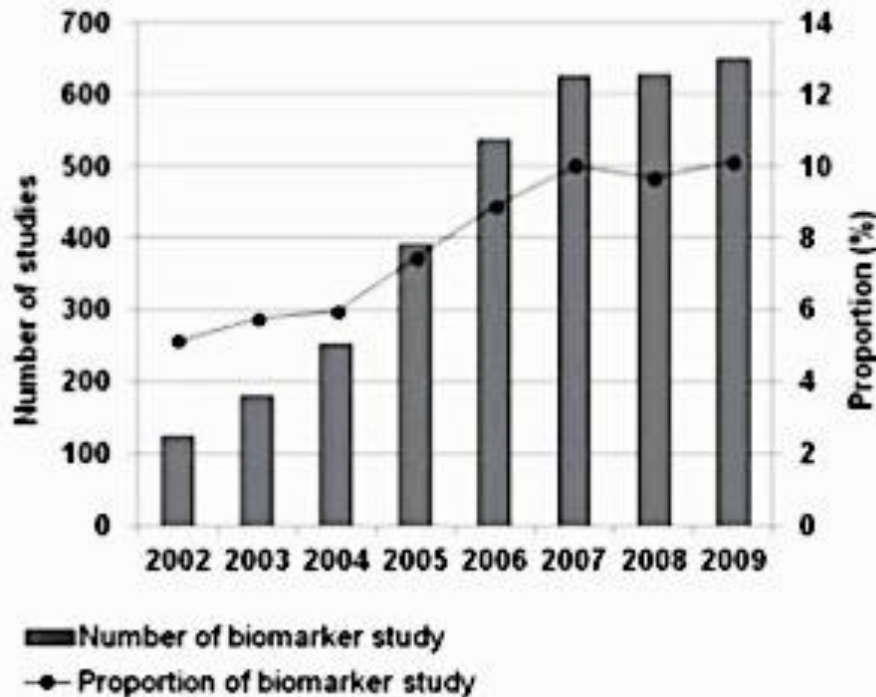
# Clinical Use of Biomarkers

Utility of Biomarkers



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

# Use of Biomarkers in Clinical Trials



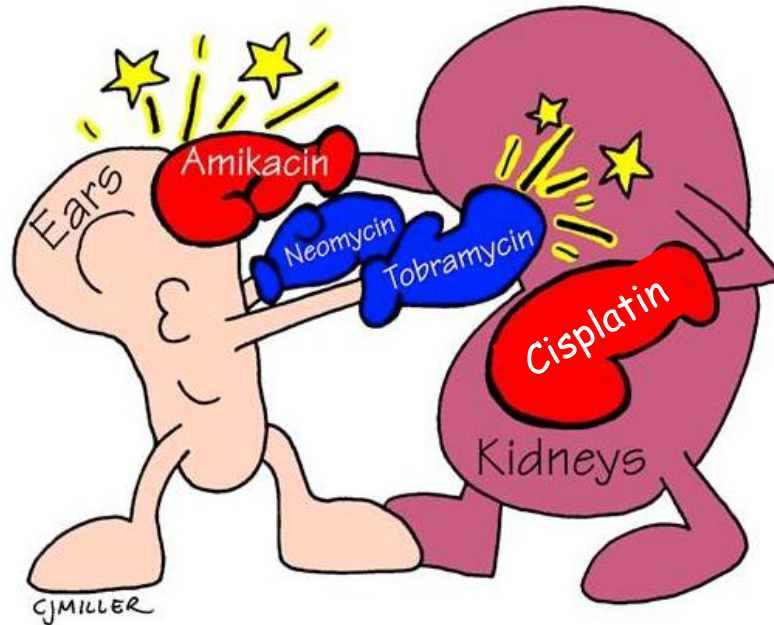
- Over the past 10 years the number of studies using biomarkers increased **6-fold**
- Biomarker studies are relatively **small** (< 20%)
- 70% of biomarker studies were in phase 1-2
- Biomarkers used in 37% of **oncology clinical studies**
- Most common use was for **patient selection**

# Newer Urinary Biomarkers of Acute Renal Injury: Site and Mechanism

BIOMARKER	TYPE OF INJURY AND LOCATON
Cystatin C	Functional marker (glomerular filtration)
Albumin	Functional marker (glomerular filtration)
Total protein	Up-regulated proteins (glomerular filtration)
Kidney injury molecule (KIM-1)	Up-regulated protein (proximal tubule)
Beta-2-microglobulin	Functional marker (proximal tubule reabsorption)
Clusterin	Functional marker (distal tubule epithelium)
Trefoil factor-3	Functional marker (tubular reabsorption)

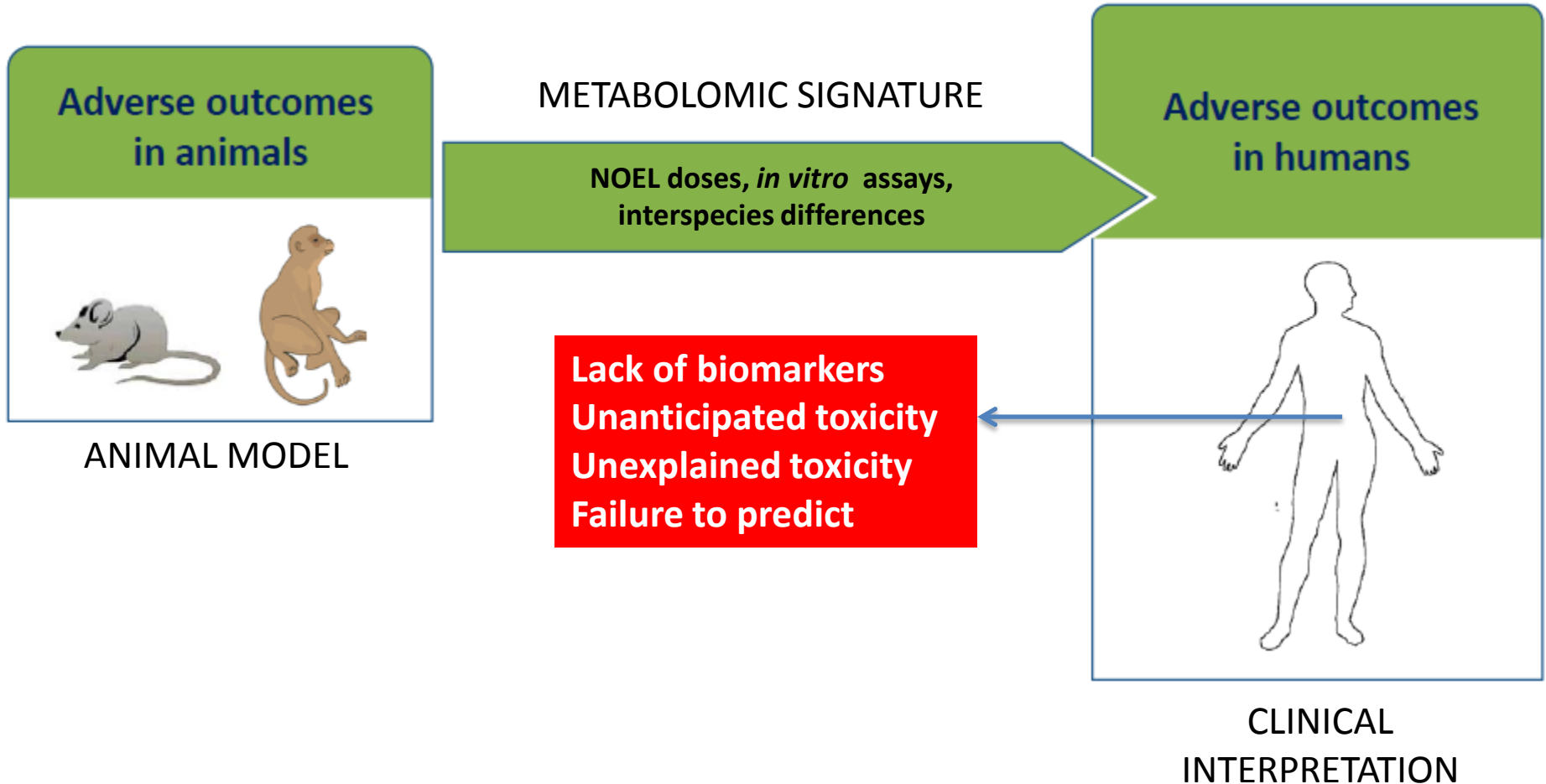
“The more novel the biomarker, the more difficult validation (linking biomarker change to histological change) can become”

## OUR CISPLATIN TOXICITY STUDY



Major toxic effects of cisplatin are nephrotoxicity and ototoxicity

# Metabolomics: Alternative Approach to Renal Drug Safety Biomarkers



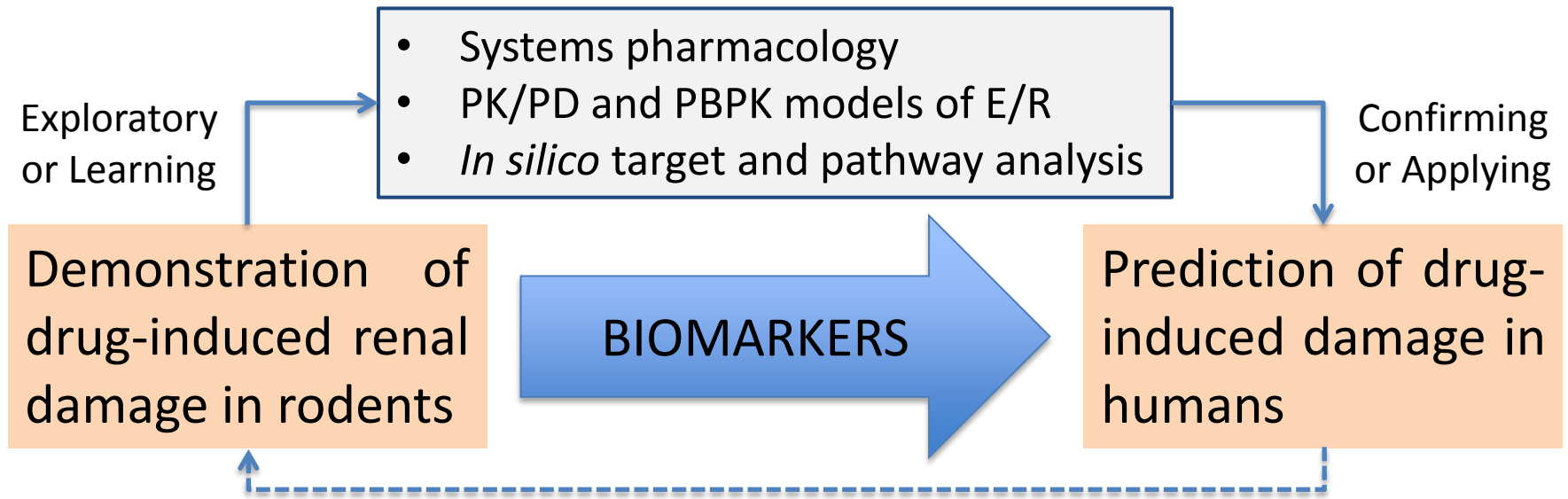
# Why Metabolomics?

- 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

# 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 *di-peptides*; 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

Today ▼	N=4-6	N=3-5	N=2-4	N=1-3
Baseline	Day 1	Day 7	Day 14	Day 28

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 for 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

**Feature Finding Quantitate**

**MassHunter Qual**  
*AMDIS or Find by chromatographic deconvolution*

**MassHunter Qual**  
*MFE, Find by Formula, Find by Ion*

# Metabolite Identification Challenge

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

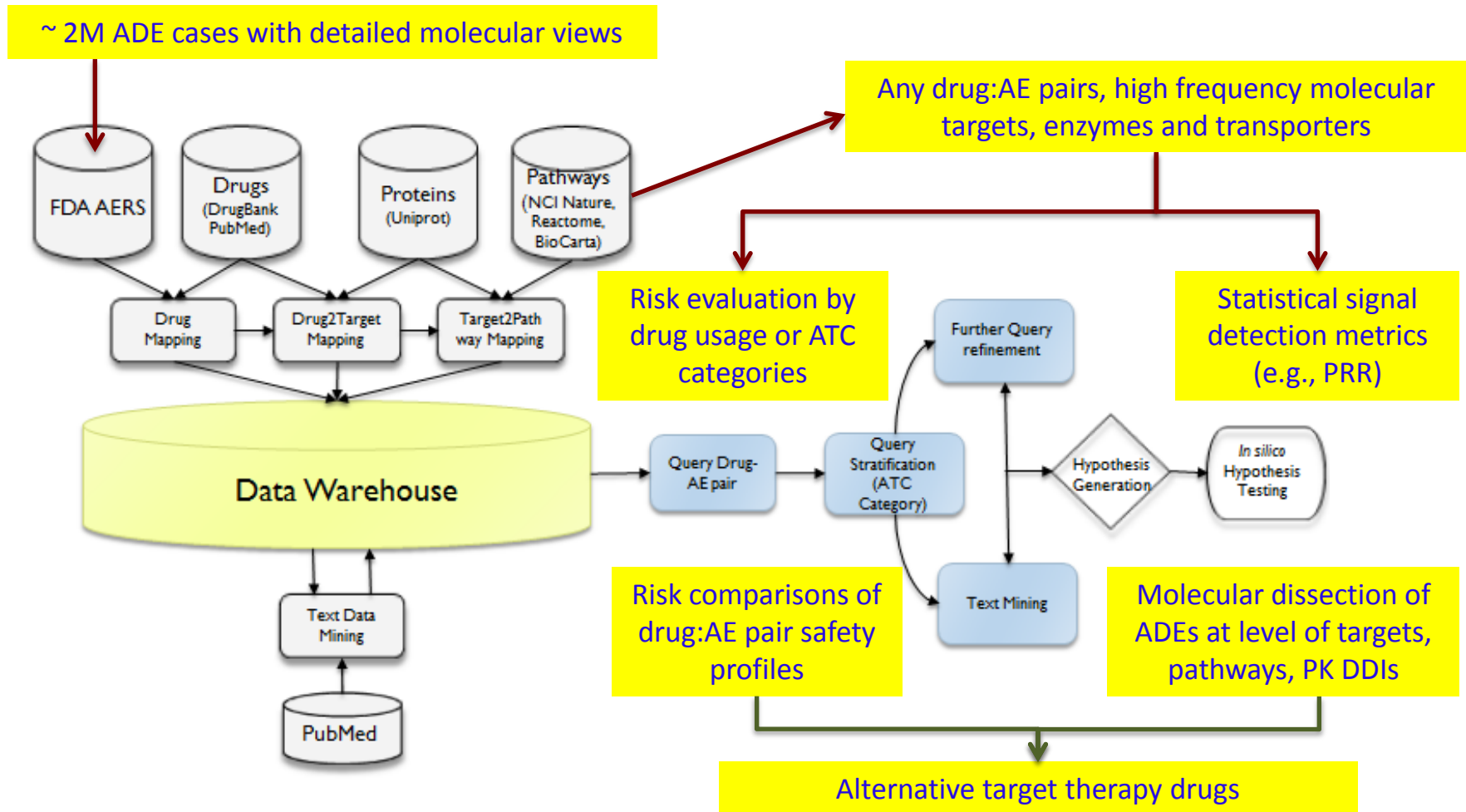
2. Published studies of cisplatin rendered as many as 232 metabolites in kidney tissue and 192 in urine

Automated comparison  
to reference library using  
Agilent proprietary  
software

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

# Target Identification and Pathway Analysis: MASE<sup>R</sup>



# 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

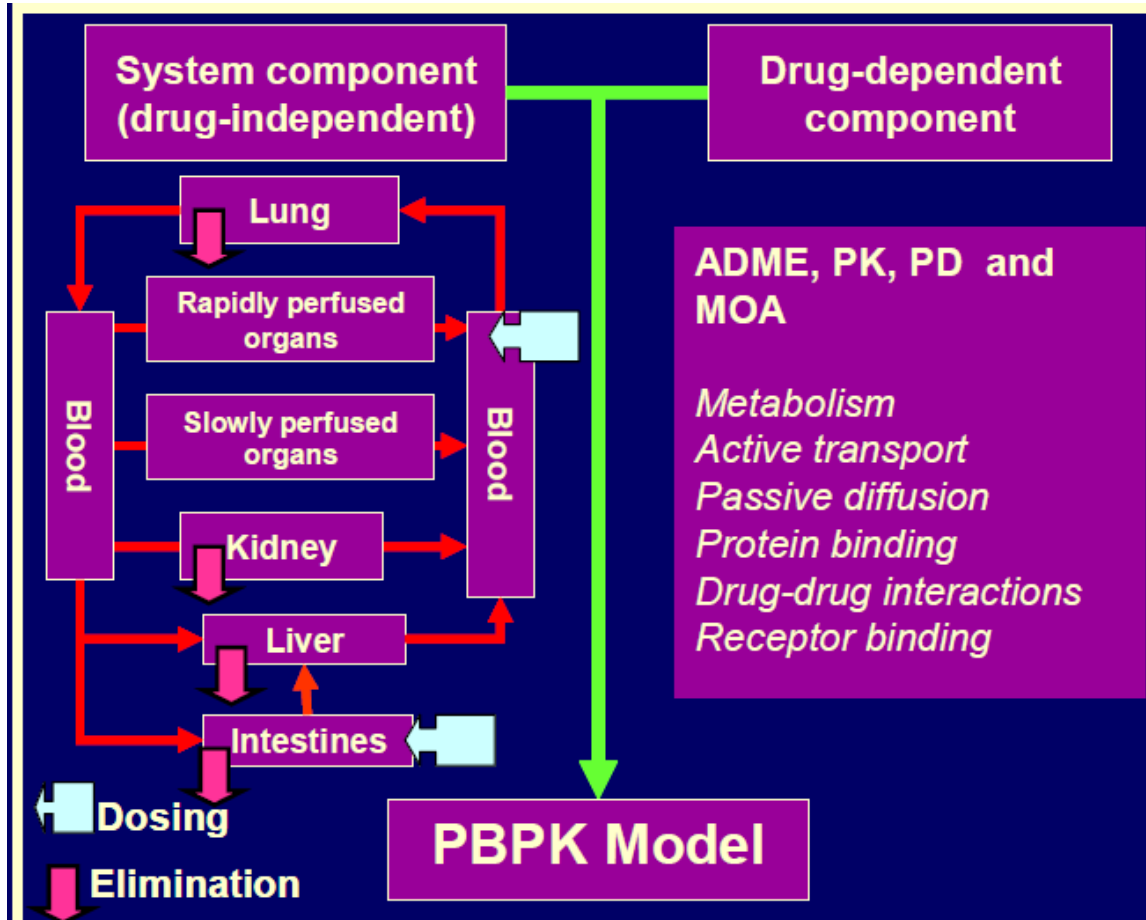
# Model Building: Built “Fit for Purpose”



Stan Gudder  
Professor of Mathematics  
University of Denver

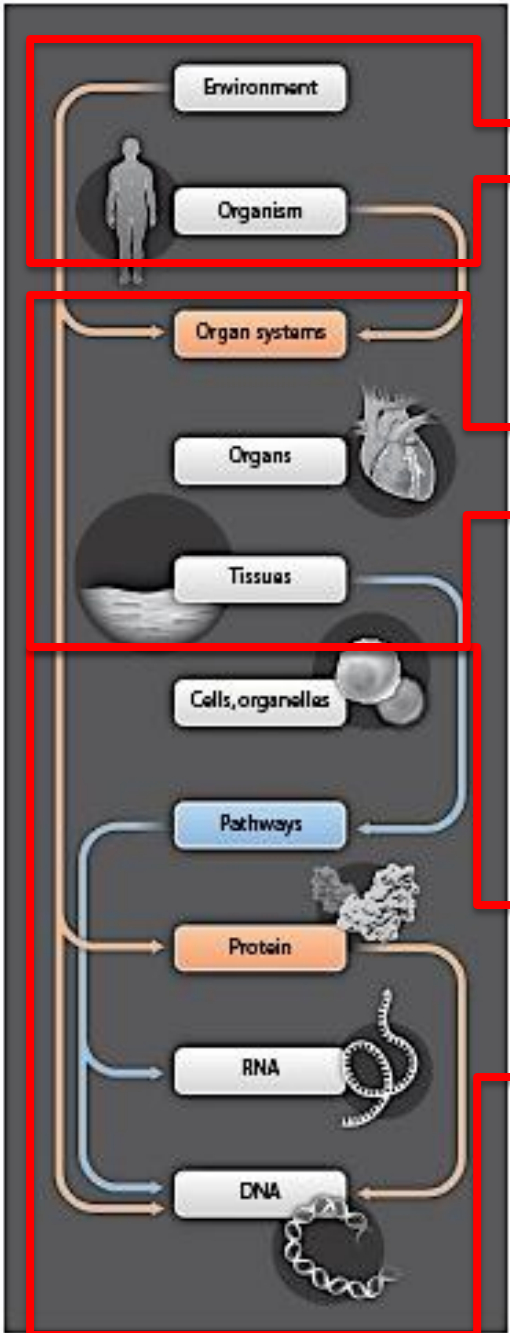
“The essence of mathematics is not to make simple things complicated, but to make complicated things simple”

# Mathematical Physiologically-Based PK Model



Generate PK, urine and kidney tissue concentration-time profiles to provide mechanistic insights for untested scenarios

Multiple Omics -- Multiple Models



## PK/PD

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

## PBPK

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

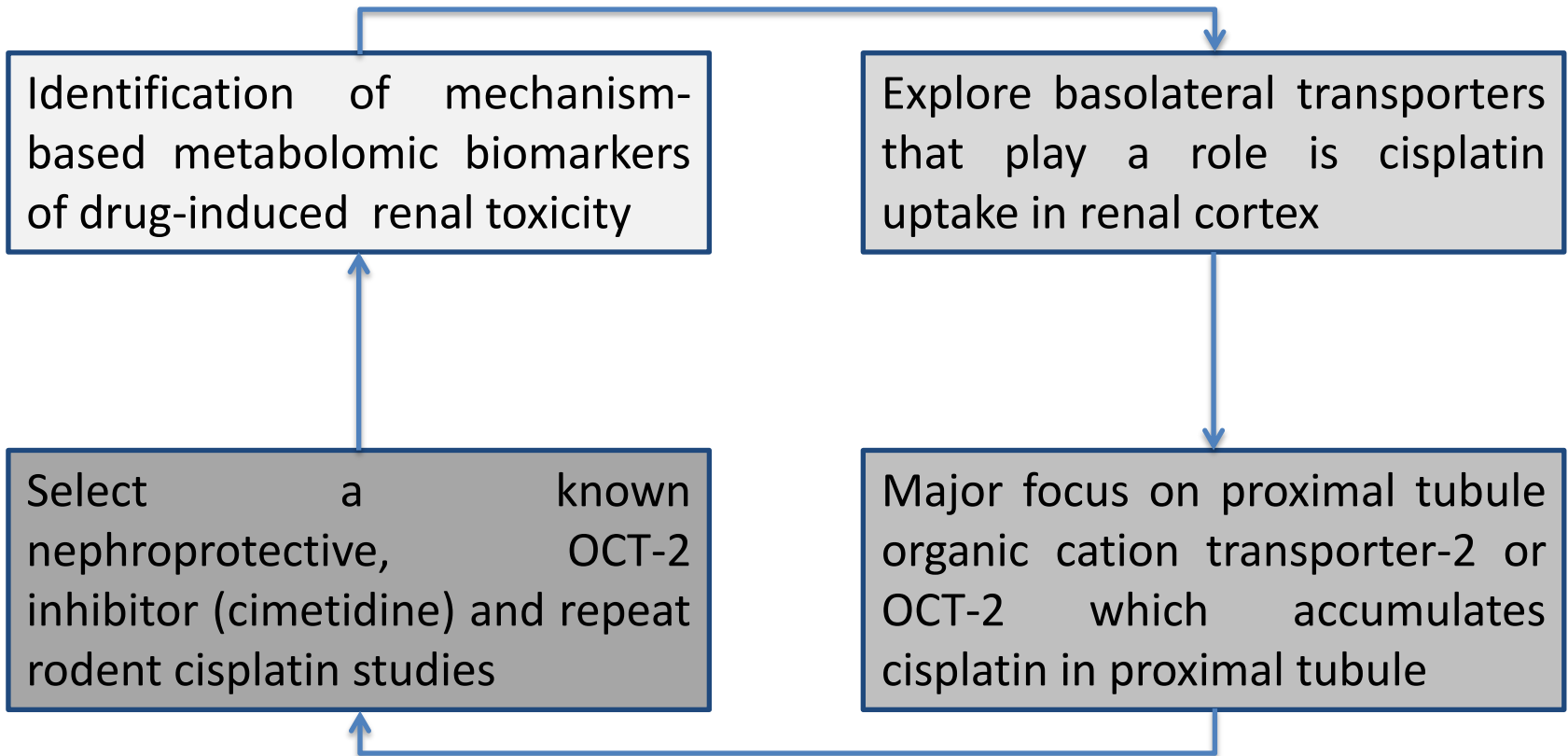
## Systems Pharmacology

*Mechanistic -- Mechanism of off-target drug effects, metabolomics and integrated "omics"*

## 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



# 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

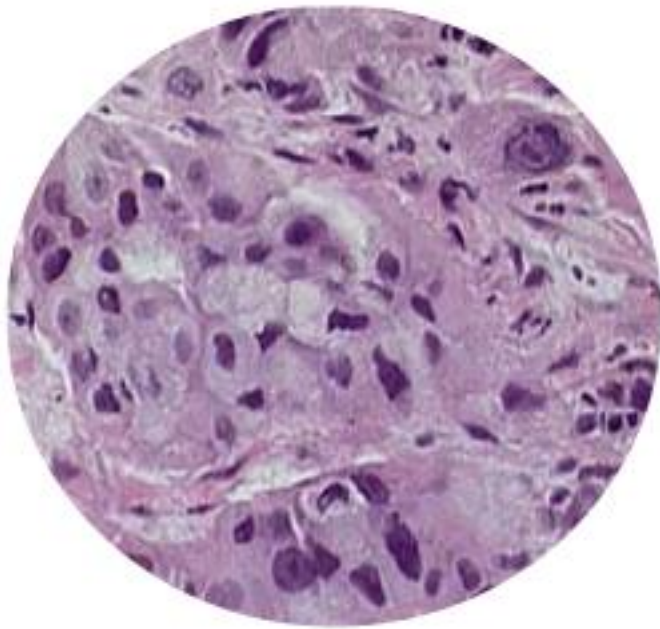
**How can we identify the 1 in 7 cancer patients that get renal toxicity?**

## **FEMALES**

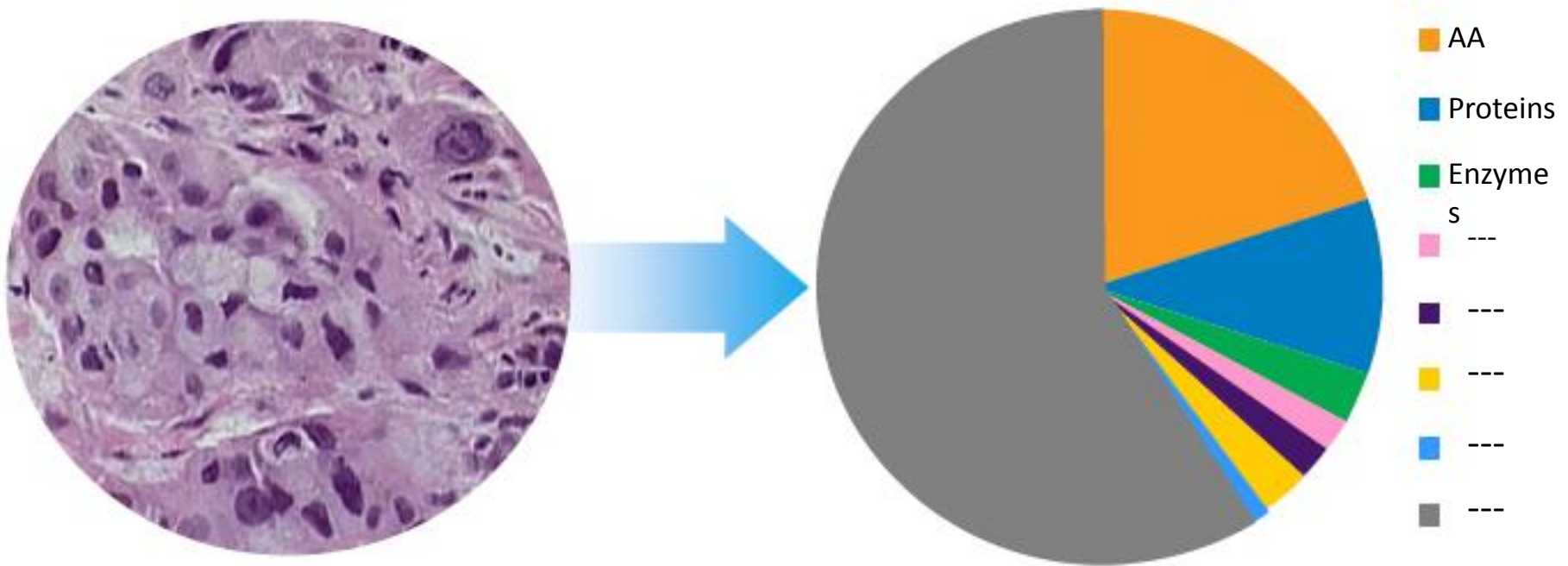
Breast, uterine, colon, melanoma, thyroid and non-Hodgkin lymphoma account for 75% of cancers

# Metabolomics Can Redefine Our Future Thinking About **Diseases and Treatments**

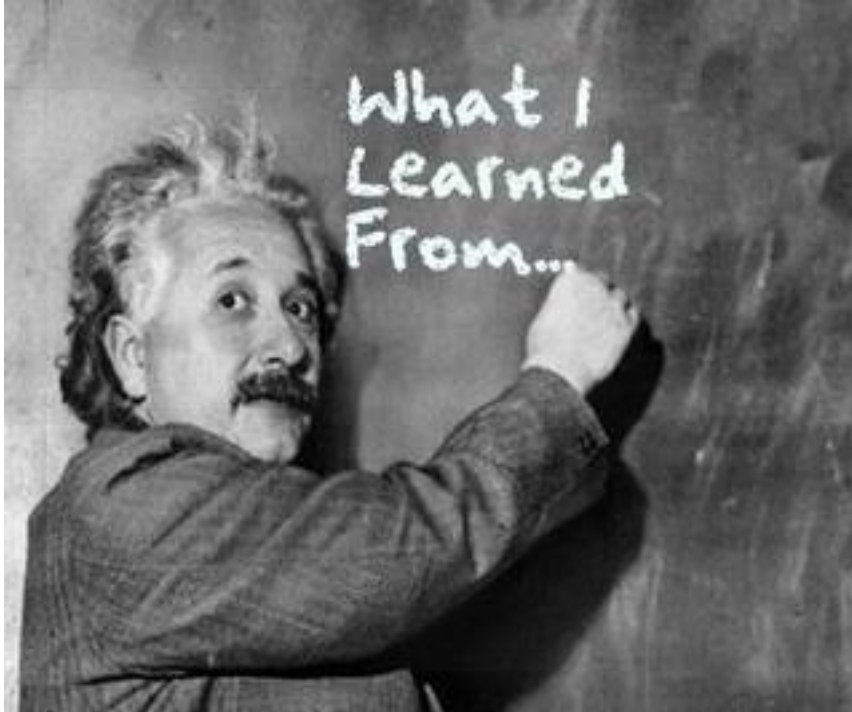
Adenocarcinoma (1999): classified by anatomic and histologic criteria



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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407-313-7008

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Questions?

