



Nonclinical Biomarkers and their Translation to the Clinic

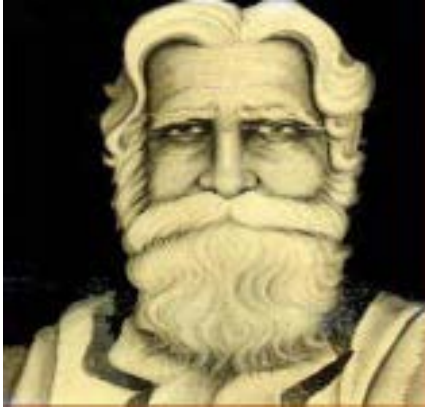
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Overview of Presentation

- Biomarkers – some background
- Why we need better safety biomarkers
- Cardiovascular biomarkers
- Kidney injury biomarkers
- Liver injury biomarkers
- Case study: testicular biomarkers
- Q&A

First diagnostic biomarker?



Sushruta (clinician in India, 600 B.C.)

Recorded that urine of diabetic patients attracted ants

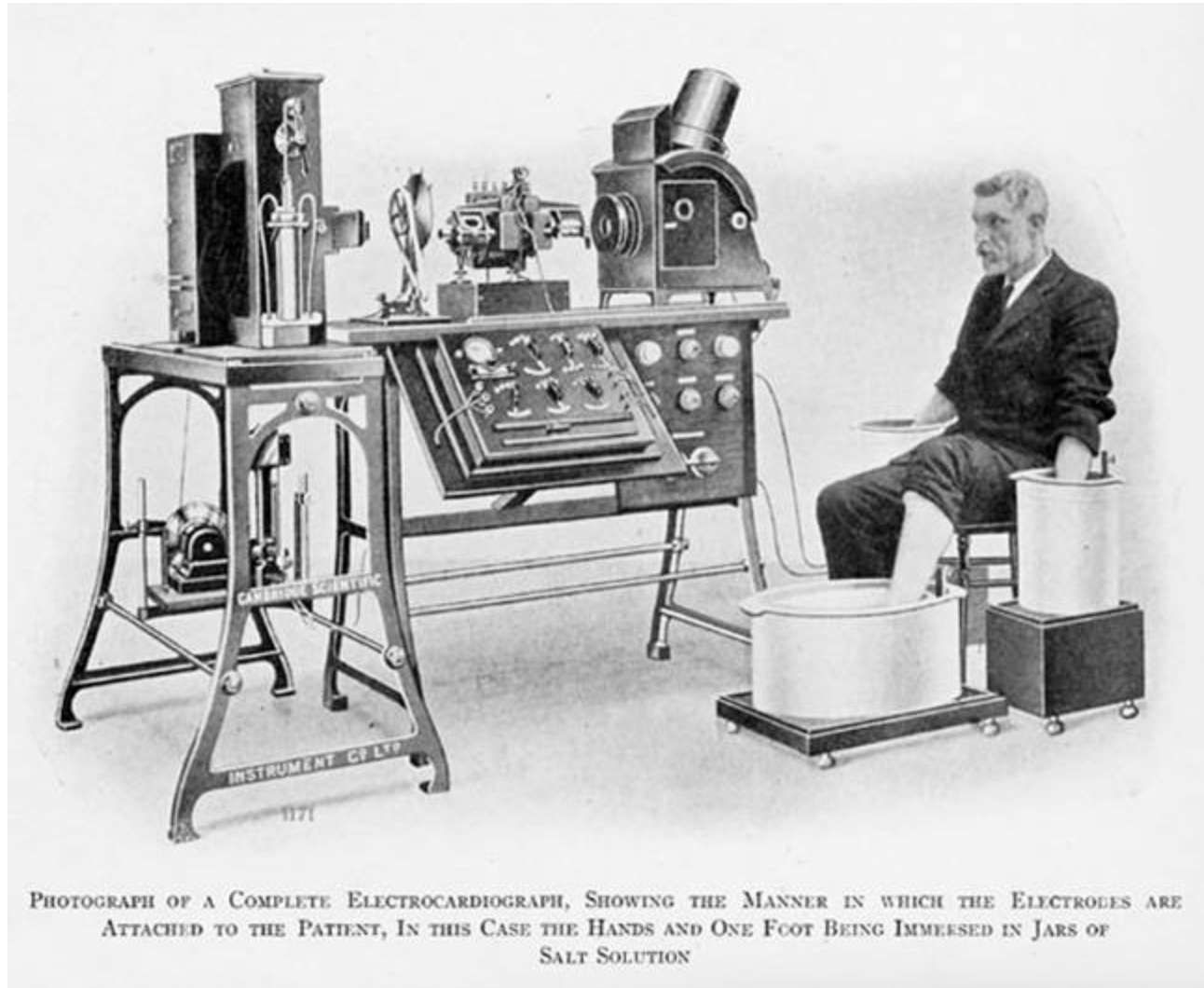


= Diagnostic biomarker for diabetes

Other early milestones in biomarker development

- **1555** Józef Struś first measured blood pressure
(by placing increasing weights on the skin over an artery until the pulse no longer lifted the weight)
- **1895** Wilhelm Röntgen discovered x-rays → imaging biomarkers
- **1896** Henri Becquerel discovered radioactivity → radiodiagnostics
- **1901** Willem Einthoven invented the first ECG apparatus

An early ECG device



Definition of a Biomarker

- “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention”

Atkinson AJ et al. (2001) Clin Pharmacol Ther 69: 89-95.

Why do we need better Safety Biomarkers in Drug Development?

- Minimize risk to subjects/patients
- Avoid costly late stage failures
- Avoid market withdrawals
- Better understanding of mechanisms of toxicity

Why do we need better Safety Biomarkers in Drug Development?

- Press release August 24, 2012:
 - “Heart, Kidney Failure Mark End of the Road for BMS HCV Drug”
- Clinical testing of BMS-986094, a nucleotide polymerase inhibitor, was suspended after one trial participant died of heart failure and 8 others were hospitalized due to heart and kidney toxicity.

- “The safety issue in question was seen in some preclinical animal studies at higher exposures than those being studied in humans. **In addition, biomarker indicators seen in preclinical studies were not seen in the clinical case in question.** The relationship of the safety issue in question to our preclinical data is unclear.”

BMS spokesperson

What are the Features of an Ideal Biomarker?

- Specific
- Sensitive
- Known and stable baseline values
- Relationship to injury established
- Translatable between species
- Non- or minimally invasive
- **Easily and reliably measureable (assay)**
- Inexpensive

Cardiovascular Injury Biomarkers



QT Safety Biomarkers

Nonclinical risk assessment:

- In vitro IKr
- In vivo QT

Ref: ICH S7B

Clinical risk assessment:

- ECGs in Phase I - II
- Thorough QT/QTc study

Ref: ICH E14

QT Safety

- **During Discovery** - Purpose is hazard identification and elimination
- **Before FIH** – Risk assessment
- **Clinical Development and Life Cycle Management** – Risk management and mitigation

Atkinson AJ et al. (2001) Clin Pharmacol Ther 69: 89-95.

QT Safety Challenges Remaining

- Discrepancies between non-clinical assays and clinical outcome
- Non-hERG mediated QT prolongation
- QT interval shortening

Myocardial Injury

- Traditional nonclinical cardiac biomarkers, LDH and CK, lack sensitivity and specificity.
- In humans, cardiac troponin (cTn) has replaced CK-MB isoenzyme analysis.
- In 2000, the American College of Cardiology and the European Society of Cardiology declared cTn as the biomarker of choice for acute MI.

- Cardiac Troponin is an example of **reverse translation** from human into animal use as a safety biomarker.

Development of Troponin as a Non-Clinical Biomarker

- HESI Cardiac Troponins Biomarker Working Group evaluated different automated instrument assays developed for use in humans to determine which were best for cTn analysis in rats, dogs and monkeys.
- 2008 – Submission of a Request for Qualification by FDA of Cardiac Troponin as a Blood Biomarker for Non-clinical Toxicology Studies.

Feb 2012 – FDA decision

Serum cTnT and cTnI are qualified biomarkers in rats and dogs:

1. When there is previous indication of cardiac structural damage in preclinical studies, cTn can be used to help choose safe doses for human clinical studies.
2. When there is a known drug class effect and histopathology does not indicate structural damage, cTn may be used to support or refute cardiotoxic potential.
3. When unexpected cardiac structural toxicity is found in a nonclinical study, retroactive cTn analysis can be used to help determine a NOAEL or LOAEL.

Feb 2012 – FDA decision

- Not enough data and inconsistent results in non-human primates.
- FDA encourages voluntary collection of cTn data especially for NHP.

- HESI Cardiac Troponins Biomarker Working Group is currently evaluating new high-sensitivity assays capable of detecting cTn in the low pg/mL range (vs ng/mL)
- Biomarkers of very early signs of potentially reversible cardiac injury?

Kidney Injury Biomarkers



Biomarkers of Drug-Induced Kidney Injury

- Classic biomarkers include blood urea nitrogen (BUN) and serum creatinine
 - Increased levels are indicative of impedance in the glomerular filtration rate (GFR)
 - Simple tests to perform but...
 - Lack sensitivity and specificity

- Two consortia, led by C-Path and ILSI-HESI, and aligned with academia, industry and FDA experts, set up an evaluation of newly identified biomarkers of nephrotoxicity for use in preclinical safety studies.

Criteria for Evaluation and Development of New Kidney Injury Biomarkers

- Preference for non-invasive samples
- Translatable from preclinical use to the clinic
- Assays should be robust and kits readily available
- Assays should be multiplexed to minimize cost and expedite sample analysis
- Biomarkers should predict or report out site-specific injury
- Biomarkers must be more sensitive and specific of kidney injury than existing standards
- Biomarkers should be predictive of kidney injury in the absence of histopathology

New Kidney Injury Biomarkers

- In 2007, C-Path Predictive Safety Testing Consortium (PSTC) submitted data to the FDA and EMA to support the use of 7 novel urinary nephrotoxicity biomarkers in preclinical GLP rat studies:
- KIM-1, Albumin, Total Protein, β 2-Microglobulin, Cystatin C, Clusterin, Trefoil Factor 3

- April 2008 – FDA and EMA qualify novel urinary biomarkers of drug-induced nephrotoxicity in rats:
 - KIM-1, Albumin, Clusterin, Tff-3 as biomarkers of drug-induced acute kidney tubular alterations
 - Total Protein, β 2-Microglobulin, Cystatin C as biomarkers of acute drug-induced glomerular alterations/damage and/or impairment of kidney tubular reabsorption

- Sept 2010 – FDA qualifies additional novel urinary biomarker of drug-induced nephrotoxicity in rats: Renal Papillary Antigen (RPA-1)
 - For detecting acute drug-induced renal tubule alterations, particularly in the collecting duct, in male rats

- α -GST was not qualified due to differing behaviour depending on location of renal injury:
- \uparrow α -GST detected proximal tubule injury
- \downarrow α -GST detected collecting duct injury

- None of these urinary biomarkers are currently qualified for routine monitoring of drug-induced kidney injury in the clinic.
- Human qualification studies are needed.
 - e.g. evaluation of pattern of elevation, timeframe, and reversibility after exposure to known nephrotoxicants such as aminoglycosides
- Sufficiently validated assays need to be available.

Drug-Induced Liver Injury Biomarkers



Regulatory Action on Marketed Drugs due to DILI (1995-2010)**

**Partial List

Withdrawals (US &/or other countries+)*

troglitazone*
bromfenac*
trovofloxacin*
tilbroquinol+
pemoline+
tetrabamate+
ebrotidine+
nefazodone+
tolrestat+
droxicam+
niperotidine+
chlormezanone+
ximelegatran+
lumaricoxib+
gemtuzumab+

Restricted (US)

trovofloxacin
felbamate
pemoline

Boxed Warnings (US)

lamivudine
leflunomide
propylthiouracil
lapatanib
pazopanib
sunitimib
tenofovir
tipranavir
tolcapone
bosentan
deferasirox
ambrisentan
acitretin
cytarabine
maraviroc
eltrombopa
acetaminophen (Rx)

Drug-Induced Liver Injury Biomarkers

- Classic serum chemistry tests include total bilirubin (TBL) and enzyme activities of ALP, AST and ALT.
- These biomarkers have been “validated” by more than 60 years of clinical use.
- Serum ALT has become leading biomarker for acute liver injury or disease.

Drug-Induced Liver Injury Biomarkers

- However, ALT (as well as AST and ALP) are not tests of liver function.
- Serum bilirubin and prothrombin time do measure functions of the liver.
- Combined biomarkers of ALT and TBL is the current standard for biomarkers of liver injury.
- ALT has high sensitivity; TBL has high specificity.

Limitations of Current Drug-Induced Liver Injury Biomarkers

- Do not discriminate between drug & non-drug etiologies.
- Are not early predictors of DILI outcomes (resolution vs acceleration of injury).
- Enzymes sensitive but not specific for serious DILI
- Definition of “normal” not agreed upon, i.e. different “normal” ranges.

Drug-Induced Liver Injury Biomarkers

- There is a need for DILI biomarkers to predict
 - Clinical resolution vs progression at an early stage of mild liver injury
 - which drugs can cause idiosyncratic DILI
 - which patients are susceptible to develop DILI

*From Mark Avigan, CDER, FDA, presentation at FDA/C-Path/PhRMA
HepTox Steering Committee Meeting, 15 March 2012*

Novel Biomarkers of Drug-Induced Liver Injury

- Liver-enriched microRNAs were shown to be promising serum biomarkers of acetaminophen-induced acute liver injury in mice¹
- miR-122 and miR-192 were detected earlier than ALT and at lower doses.
- miR-122 had improved liver tissue specificity vs ALT.

¹Wang K et al. Proc Natl Acad Sci USA 106: 4402-4407 (2009)

Translation to the Clinic?

- There is a high degree of cross-species conservation of microRNA sequences.
- Serum miR-122 was significantly higher in patients with acetaminophen acute liver injury and in patients with different liver injury etiologies compared to healthy controls²

²Starkey Lewis PJ et al. Hepatology 54: 1767-1776 (2011)

Other Exploratory Biomarkers of DILI Currently under Investigation

- Albumin mRNA
- α -glutathione S-transferase
- High-mobility group box 1
- Cytokeratin 18
- Glutamate dehydrogenase
- Sorbitol dehydrogenase
- F-protein

Testicular Toxicity Biomarkers



Male Reproductive Safety – Preclinical Evaluation

- Preclinical general toxicology studies are the most frequent source of concern regarding the potential effect of a drug on the testis.
- Concern is less likely to arise from the animal fertility study (usually carried out later in development).
- In rodents, histopath is more sensitive biomarker than effects on fertility.

Male Reproductive Safety – Preclinical Evaluation

- Preclinical toxicology studies may demonstrate testicular histopathological abnormalities in one species or in multiple species.
- There is no single species that is best for prediction of human risk.
- Abnormalities in any species may be a cause for concern.

Testis is a Dual Organ in Function and Structure

Interstitial Compartment

- Endocrine Function
- Leydig cell
- Low metabolic rate
- Fibroblast stem cells
- Resistant to toxicity

Seminiferous Compartment

- Exocrine Function
- Sertoli and germ cells
- Active turnover rate
- Spermatogonial stem cells
- Sensitive to toxicity

Testicular Safety Assessment during Clinical Drug Development

- What biomarkers are available to monitor testicular safety during a clinical study?

Evaluation of Spermatogenesis during Drug Development

- Hormonal Evaluation
 - LH
 - Testosterone
 - FSH
 - Inhibin B
- Advantages
 - Easily incorporated into clinical studies
 - Acceptable to subjects, no recruitment issues

But...

- **LH** and **testosterone** mainly reflect Leydig cell function and are poor biomarkers for spermatogenesis.
- **FSH** stimulates spermatogenesis and is generally elevated when spermatogenesis has been impaired, however, it is variable and lacks sensitivity.

Inhibin B is a potential biomarker for evaluating drug-induced injury to spermatogenesis

- **Inhibin B** is produced exclusively by the testis (Sertoli cells); serum inhibin B levels are strongly positively correlated with testicular volume and sperm counts in humans.
- Further validation is needed to qualify inhibin B as a clinical biomarker of drug-induced testicular toxicity.
- HESI is evaluating the use of inhibin B as a potential biomarker for testicular toxicity in rats.
 - Human inhibin B ELISA kits can be used to detect rat inhibin B

Testicular Safety Monitoring during Clinical Studies

- Semen analysis is the best measure of spermatogenesis available, despite its shortcomings.

Case Study: Clinical Biomarkers of Testicular Safety Risk

- Novel anti-viral drug
- 1 month oral toxicity studies in rat and dog revealed histopathological changes in the testes.
- Reproductive toxicity studies in rat demonstrated ↓fertility, ↓ spermatogonia, ↑morphologically abnormal sperm
- Subsequent testicular toxicity studies were conducted in mice, rats, rabbits and monkeys
- Rat was the most sensitive species
- FDA placed a Partial Clinical Hold on development

How to design a clinical study to evaluate testicular safety?

- First, sponsor conducted a Phase I multiple dose safety – PK study in a healthy volunteer population of vasectomized males and postmenopausal women .
- Data used to select dose for testicular safety study, ensuring adequate safety margins.

Design of Clinical Study

- Sperm concentration (biomarker) is the most commonly used endpoint.
- Define “responder” as an individual with a 50% reduction in sperm conc.
- Non-inferiority analysis used.
- Study duration must include at least one spermatogenic cycle, i.e. ≥ 90 days.

Design of Clinical Study

- Goal is to reduce “noise” while having sufficient numbers to obtain a statistically valid result.
- Control factors that contribute to variation
 - Limit # sites to control geographical variation
 - Limit # laboratories to control inter-laboratory variation
 - Multiple samples per time point
 - Control period of abstinence

Design of Clinical Study

- Phase I, randomized, double-blind, placebo-controlled, non-inferiority, multiple oral dose study in healthy male volunteers.
- Primary endpoint: sperm conc. at Day 95
- Semen analysis conducted at baseline, Day 65, Day 95 and Day 125.
- 3 samples per time point 48-hour abstinence between samples; clinic confinement during sample collection periods.
- 2 clinical sites; single laboratory conducted semen analysis.
- DSMB reviewed semen and laboratory safety data at Day 65, 95 and 125.

Clinical Study Results

- Responder rate following drug treatment was not inferior to placebo re: semen biomarkers (sperm conc., motility and morphology).
- Drug and placebo were comparable re: endocrine biomarkers (LH, testosterone, FSH and inhibin B).
- FDA removed PCH.
- Sponsor could proceed to Phase II studies in the US.

Summary



Summary

- There is a need for better safety biomarkers.
- Novel urinary biomarkers of drug-induced nephrotoxicity in rats have been qualified.
- Cardiac troponin is an example of reverse translation of a biomarker from clinic to non-clinical use.
- Biomarker consortia are identifying and evaluating promising new biomarkers for drug-induced tissue-specific injuries.