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

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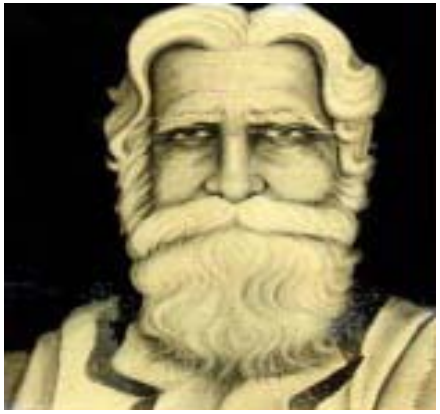
First diagnostic biomarker?



Sushruta (clinician in India, 600 B.C.)
Recorded that urine of diabetic patients attracted ants

= Diagnostic biomarker for diabetes

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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

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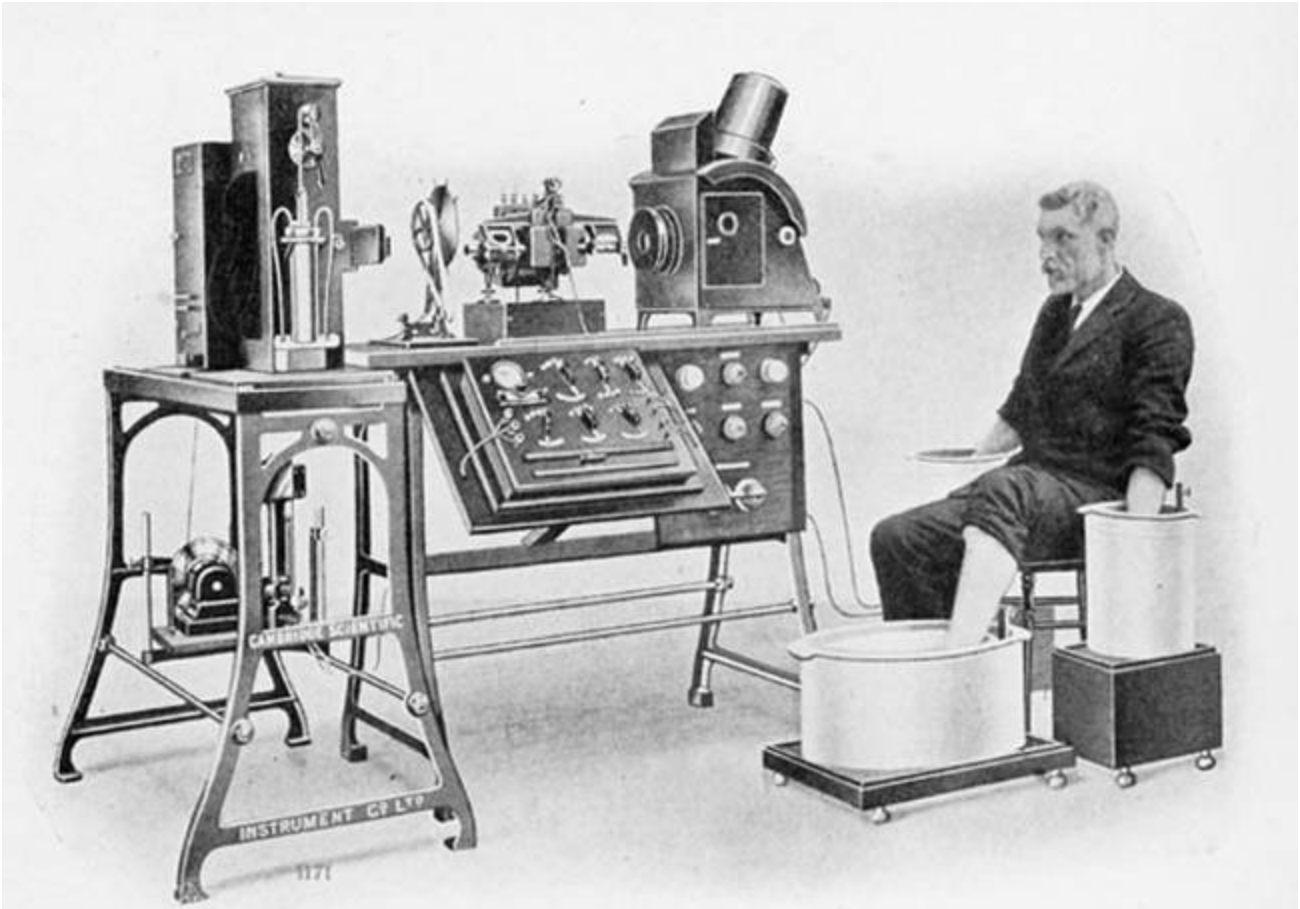
An early ECG device



PHOTOGRAPH OF A COMPLETE ELECTROCARDIOGRAPH, SHOWING THE MANNER IN WHICH THE ELECTRODES ARE ATTACHED TO THE PATIENT, IN THIS CASE THE HANDS AND ONE FOOT BEING IMMERSED IN JARS OF SALT SOLUTION

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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 invention"

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

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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

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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.

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"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

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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

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Cardiovascular Injury Biomarkers

QT Safety Biomarkers

Nonclinical risk assessment:

- In vitro I_{Kr}
- In vivo QT

Ref: ICH S7B

Clinical risk assessment:

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

Ref: ICH E14

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QT Safety

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

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QT Safety Challenges Remaining

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

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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.

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

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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.

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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.

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Feb 2012 – FDA decision

Not enough data and inconsistent results in non-human primates.

FDA encourages voluntary collection of cTn data especially for NHP.

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- 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?

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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

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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.

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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

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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, β2-Microglobulin, Cystatin C, Clusterin, Trefoil Factor 3

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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, β2-Microglobulin, Cystatin C as biomarkers of acute drug-induced glomerular alterations/damage and/or impairment of kidney tubular reabsorption

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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

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

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- 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 reversibilty after exposure to known nephrotoxicants such as aminoglycosides
- Sufficiently validated assays need to be available.

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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)

lamividine
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.

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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.

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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.

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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

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Novel Biomarkers of Drug-Induced Liver Injury

- Liver-enriched microRNAs were shown to be promising serum biomarkers of acetaminophen-induced acute liver injury in mice[1]
- miR-122 and miR-192 were detected earlier than ALT and at lower doses.
- miR-122 had improved liver tissue specificity vs ALT.

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

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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 controls[2]

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

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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

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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.

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Male Reproductive Safety – Preclinical Evaluation

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

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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

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Testicular Safety Assessment during Clinical Drug Development

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

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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

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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.

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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

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Testicular Safety Monitoring during Clinical Studies

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

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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; histopath changes included degeneration of spermatocytes and dilatation of seminiferous tubules
- FDA placed a Partial Clinical Hold on development

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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.

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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. $\geq$ 90 days.

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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

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Design of Clinical Study

- Phase I, randomized, double-blind, placebo-controlled, non-inferiority, multiple oral dose study in healthy male volunteers.
- Number of subjects: 110
- 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.

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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.

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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.

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