

Report from the ILSI/HESI  
Subcommittee on the  
Development and Application of  
Biomarkers of Toxicity

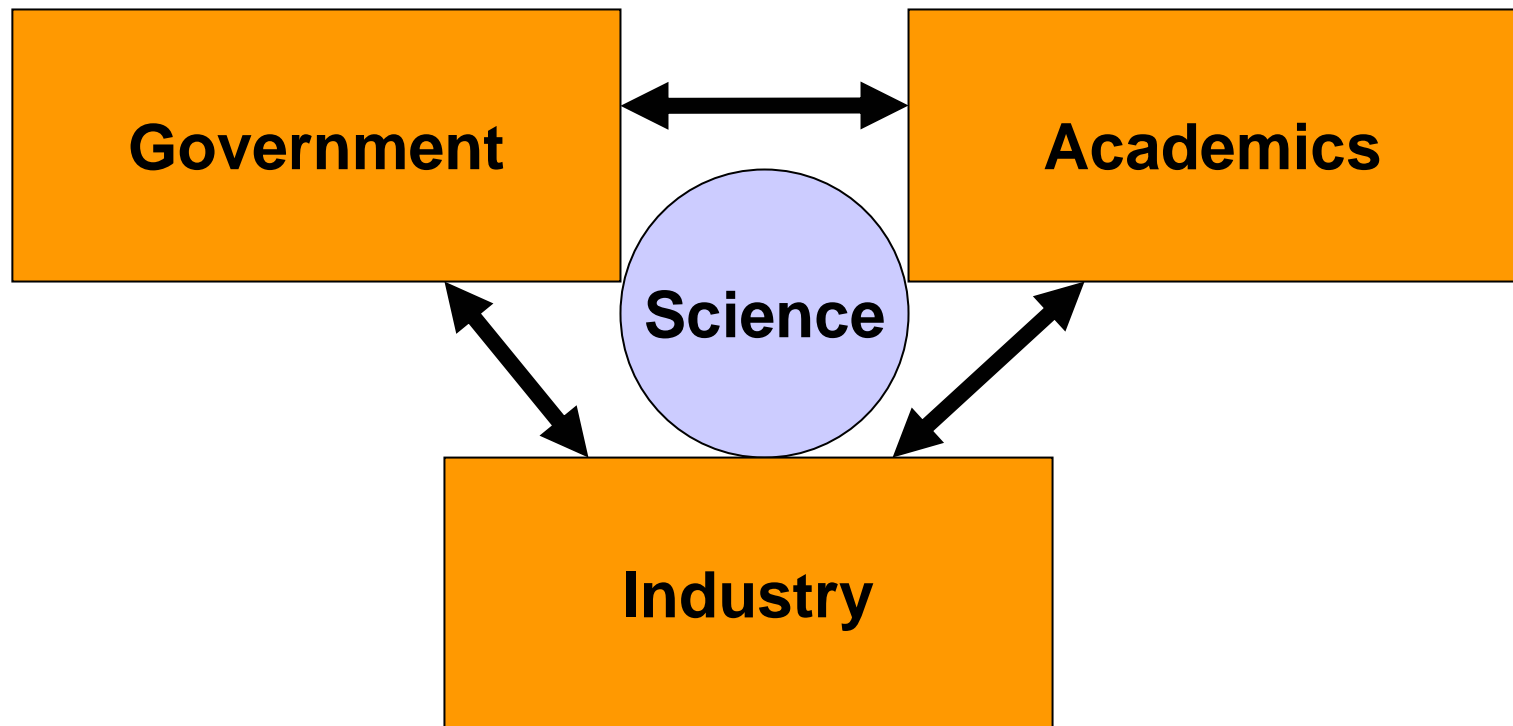
Jack H. Dean, Ph.D, D.A.B.T., Fellow A.T.S.  
Sanofi-Synthelabo Research  
Malvern, PA 19355

# ILSI/HESI Strategic Objectives

- Play a lead role in identifying and addressing key scientific issues.
- Ensure impact of ILSI HESI's scientific contributions through partnerships and communication.
- Increase recognition of ILSI HESI as a global scientific organization.
- Ensure transparency of scientific procedures and communications as each priority is addressed.

ILSI HESI Mission:  
Issue Identification and Resolution

**Tripartite Approach**



# ILSI HESI's Current Membership

- Pharmaceuticals
- Agricultural Chemicals
- Consumer Products
- Biotechnology Products (Pharma and Ag)
- Chemical Industry

46 corporate members in 2003,  
representing 10 countries on 3 continents

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Participation from academic advisors and major scientific government agencies in U.S., Europe, Canada, and Japan (e.g., NIH, FDA, EPA, DOD, Japan NIHS, EMEA, RIVM, PMRA, etc)

# ILSI HESI Biomarkers Subcommittee Mission

To advance the scientific basis for the **development and application of biomarkers** of target organ toxicity; to develop a **systematic approach** based on newly available technologies for the **identification of biomarkers** that **bridge** from the pre-clinical to clinical stages of drug development; and to provide a **scientific forum** for building **consensus** regarding how to apply biomarkers of toxicity in risk assessment.

# OBJECTIVES

- Engage in a broad-based, multinational collaborative research program to **advance** the development and **application of biomarkers** of target organ toxicity.
- Identify **accessible** biomarkers with the **potential to bridge** from the preclinical to the clinical stages of drug development.
- Evaluate the potential **application of proteomic technologies** for the identification and development of markers of tissue injury.
- Build **consensus** regarding how to apply **new biomarkers** of toxicity in risk assessment.
- Provide an ongoing **scientific forum** to facilitate discussion of the evolving state of the science related to biomarkers and proteomic technologies.

## Evolution of Biomarkers Subcommittee

- Topic selected as a top emerging issue by the ILSI HESI membership (Jan 2002) - subcommittee formed.
- First exploratory meeting of the Biomarkers Subcommittee – November 4-5, 2002.
- Project proposal was developed soliciting biomarker candidates for evaluation (April/June 2003) – seven proposals received.

# Biomarkers Project Proposal

- **Biomarkers Selection:** List of biomarkers chosen through a nominating process (*one quarter*).
- **Assay Development:** Analytical methods developed for biomarkers evaluation protocols (*up to one year*).
- **Interim Evaluation:** Review of Assay(s); examine in-life studies feasibility and projected cost (*one day meeting*).



# Biomarkers Project Proposal (cont.)

- **Biomarkers Evaluation:** Protocols will include in-life studies for testing in multiple laboratories of members (*up to one year*).
  - Sensitivity
  - Specificity
  - Reproducibility
  - Predictive Value
- **Analysis and Publication:** Results of evaluations to be published in peer-reviewed literature, and as appropriate, submitted to ICCVAM for review (*three quarters*).

## Evolution of Biomarkers Subcommittee

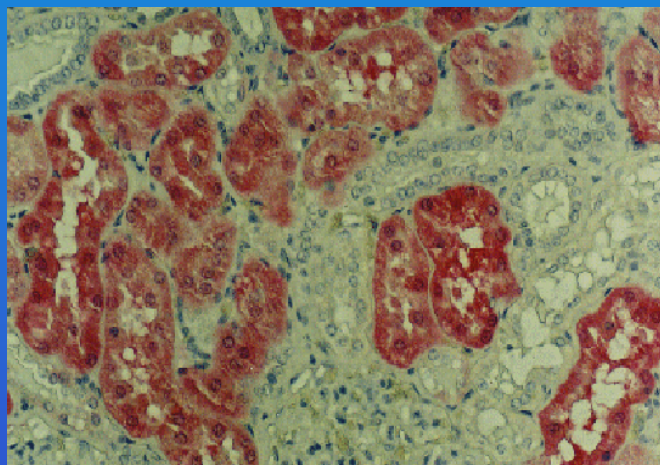
- **ICCVAM Liaisons to subcommittee identified**
  - Dr. Karen Hamernik (US EPA Office of Pesticide Programs)
  - Dr. Leonard Schechtman (ICCVAM Chair; US FDA, NCTR)
- **Second Biomarkers Subcommittee meeting** – July 21-22, 2003. Three proposals selected for evaluation:
  - Biomarkers of Nephrotoxicity
  - Serum Cardiac Troponins
  - Inhibin B for testicular toxicity
- **Future Steps:** Expert Working Groups to be formed around selected proposals.

# Biomarkers of Nephrotoxicity

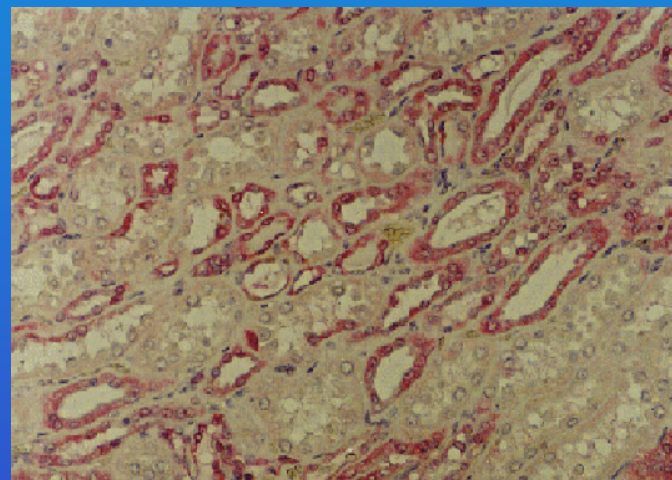
# Biomarkers of Nephrotoxicity

<b>Biomarker</b>	<b>Localization</b>	<b>AB availability</b>	<b>Source</b>
<b><math>\alpha</math>GST</b>	<b>Proximal Tub</b>	<b>Rat, Hu</b>	<b>Biotrin</b>
<b>Kim1</b>	<b>Proximal Tub</b>	<b>Hu Moab, rat, dog</b>	<b>TBD (Bonventre)</b>
<b><math>\mu</math>GST</b>	<b>Distal Tub</b>	<b>Rat, Hu</b>	<b>Biotrin</b>
<b>PAP1</b>	<b>Papilla</b>	<b>Moab</b>	<b>Biotrin</b>
<b>Clusterin</b>	<b>General</b>	<b>Commercial</b>	<b>TBD</b>

# Immunohistochemical Localisation of GST Isoforms In The Human Kidney



$\alpha$ GST in Proximal Tubules



$\pi$ GST in Distal Tubules

# Experimental Design

- Assays needing development and validation (Kim1 and clusterin). Lead time 6-9 months.
- Pilot studies to establish stability of biomarker and limits of detection.
- GLP in-life studies. At least 3 sites to assess site variability (ICCVAM).
- Assay analysis. At three sites to assess operator variability (ICCVAM).
- Histopathology with peer review.

# Biomarkers of Nephrotoxicity

## Comparative Assays:

- NAG
- GGT
- Total Protein
- Creatine
- BUN
- Routine Urinalysis
- Microscopics

## Criteria for Model Toxicant Selection

- **Specificity:** Ideal toxicant should target selected regions of the kidney.
  - **Proximal tubule damage** - gentamicin, cisplatin or p-aminophenol
  - **Distal tubule damage** - amphotericin B
  - **Papillary damage** - indomethacin, BEA
- **Formulation:** Ideally toxicants should be prepared in identical vehicle.
- **Route of Administration:** Ideally, toxicants should be delivered orally, IV or SQ.

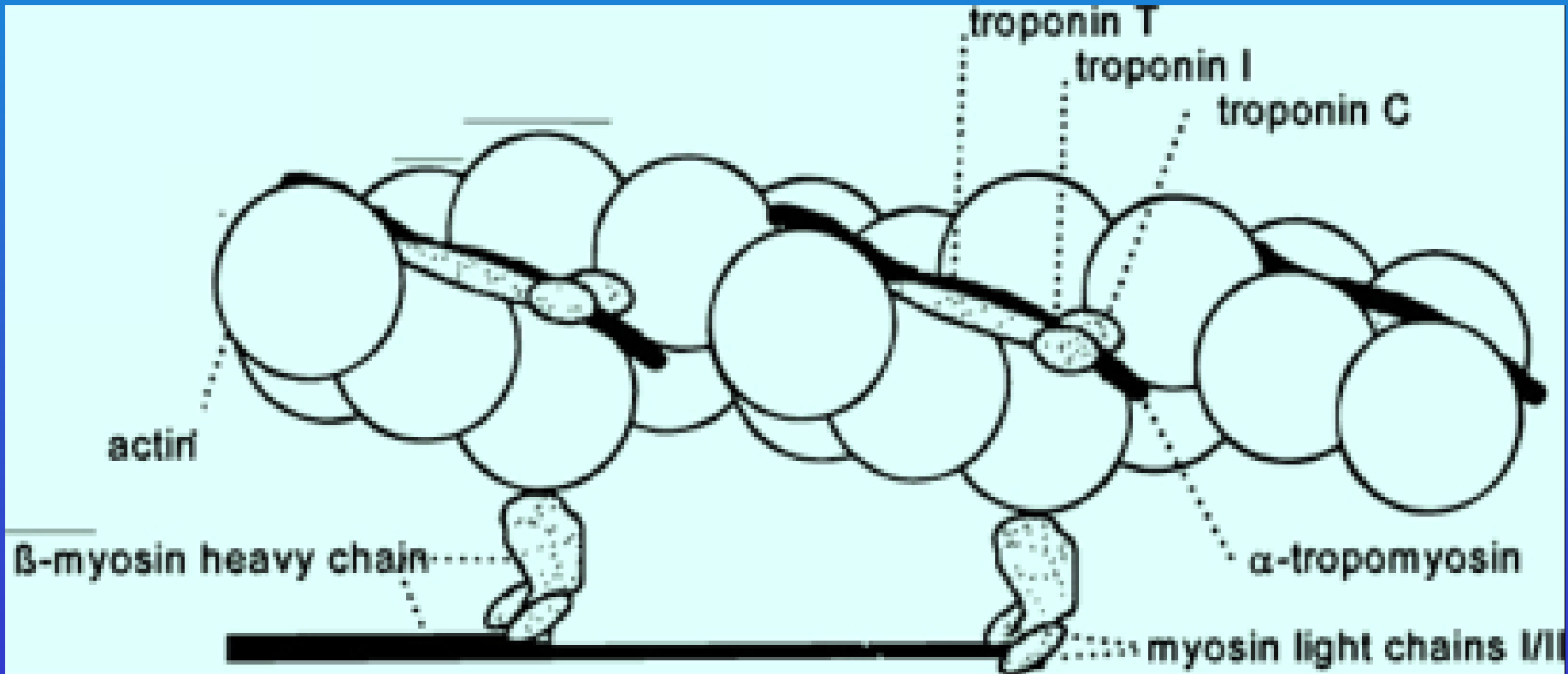


# Serum Cardiac Troponins

# Serum Cardiac Troponins

**Hypothesis:** Troponins serve as an early indicator of myocyte injury in man and can be expanded to include other species. Changes in troponin levels correlate with the development of drug-related cardiotoxicity.

# The Troponin Complex located on the thin filament of striated muscle



# Potential Goals of Research

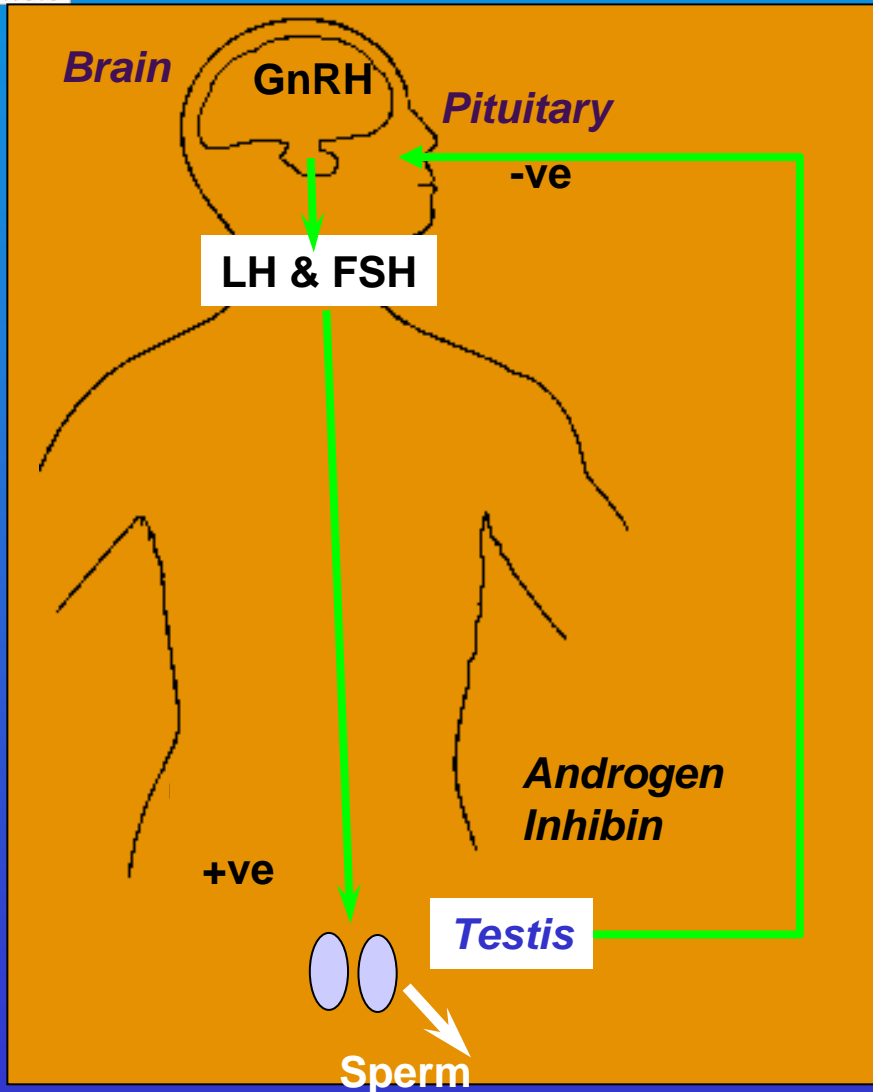
1. Evaluate kinetics of release and return to baseline (diagnostic window) of serum cardiac troponins and **correlate with histopathology** for compounds that cause cardiac injury.
2. **Determine if there is a threshold** for the increase in serum troponins below which there is no substantial or sustained cardiac injury.
3. Determine if there is a **diagnostic advantage** to measuring serum cTnI, cTnT, or both.
4. Establish whether variation in the cTnI assay platforms will influence **diagnostic sensitivity** within and across laboratories and animal species.

# Testing Paradigm

- **Species** – Rat to begin
  - Determine baseline levels representative of inter-individual variation
- **Preliminary issues** – Ability to detect rat troponins using commercial kits, dose-response, limits of detection, correlation of results with pathology, baseline levels, troponin stability, effects of sample collection and processing.
- **Reference standard** – recombinant protein is available for rat.
- **Assay results to be benchmarked with established clinical chemistry parameters.**

# Inhibin B as a Biomarker of Testicular Toxicity

## Endocrine role of Inhibin B in males



- **Inhibin B** is a Sertoli cell product
- Sertoli cells are critical cells in spermatogenesis which cannot divide post puberty
- Inhibin B production stimulated by LH and FSH.
- Inhibin has a preferential control of FSH secretion with elevated Inhibin B reducing plasma FSH in adults
- Likely to have developmental role pre-pubertally with very high levels in infant males.
- **In man, inhibin B already used to monitor reproductive function**

# Inhibin B – Phase I Studies

- Establish that commercial kits cross-react specifically with rat inhibin B protein.
- Examine analytical validation for both commercially available assays (OBI, DSL).
- Characterize normal reference ranges for SD and Wistar rats
- Select test compounds.
- Design in-life testing protocols.



# Reference Male Repro-toxicants

- Ethane dimethane sulphonate –Leydig cells
- Phthalate esters, 2,5 hexane dione - Sertoli cells
- Glycol ether - Spermatocytes
- Cadmium chloride - Causes vascular hypoxia
- Bicalutamide – Induces androgen deficiency
- Goserelin - LH-RH agonist

# Inhibin B – Phase II Studies

## Standardized Protocol:

- Aim to cover full dose-response (low, mid, and high doses, plus control).
- Necropsies at early, peak and recovery phases, plus intermediate bleeds
  - Histopathology, endocrinology (FSH, LH, T, DHT), sperm counts/functional evaluation, organ weights.

## Output of ILSI/HESI Biomarkers Project

- Provide framework for collaboration (industry, government, academia).
- Evaluate sensitivity and specificity of biomarker(s).
- Establish correlations between biomarkers and changes in histomorphology.
- Establish bridge between non-clinical and clinical data.
- Use “omics” approach to identify newer markers or clusters.
- Assist with regulatory acceptance of proven biomarkers.

# Anticipated Impact

- Identification and acceptance of new biomarkers of toxicity
  - Reduced time and cost for drug discovery/development
  - Improved post-marketing surveillance
  - Improved surveillance of populations suspect of environmental exposure to toxicant

# Companies Interested in Biomarkers Subcommittee Project

- Abbott Laboratories
- Amgen, Inc.
- AstraZeneca Pharmaceuticals
- Aventis Pharmaceuticals
- Biogen, Inc.
- Bristol-Myers Squibb Co.
- CuraGen Corporation
- Dow AgroSciences
- Dow Chemical Company
- Eisai Co., Ltd.
- Eli Lilly and Company
- Georgia-Pacific Corporation
- GlaxoSmithKline
- Hoffmann-La Roche, Inc.
- Johnson & Johnson Pharm.
- Merck and Co., Inc.
- Novartis Pharmaceuticals Corp.
- N.V. Organon
- Pfizer Global Res. & Devel.
- Pharmacia Corporation
- Purdue Pharma, L.P.
- Rohm and Haas Company
- Sanofi-Synthelabo, Inc.
- Schering AG
- Schering-Plough Res. Institute
- Syngenta
- Tanabe Seiyaku Co., Ltd.
- Wyeth Research

# Public Sector Parties Interested in Biomarkers Subcommittee Project

- Johns Hopkins University
- Medical College of Wisconsin
- National Institute of Health Sciences (Japan)
- NIEHS
- RIVM
- University of Minnesota
- U.S Army Center for Environmental Health Research
- U.S. EPA
- U.S. FDA, NCTR
- Wayne State University



*ILSI Health and Environmental Sciences Institute*

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# For More Information on ILSI HESI Biomarkers Subcommittee

Visit <http://hesi.ilsi.org/>

or

Email: [hesi@ilsi.org](mailto:hesi@ilsi.org)