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

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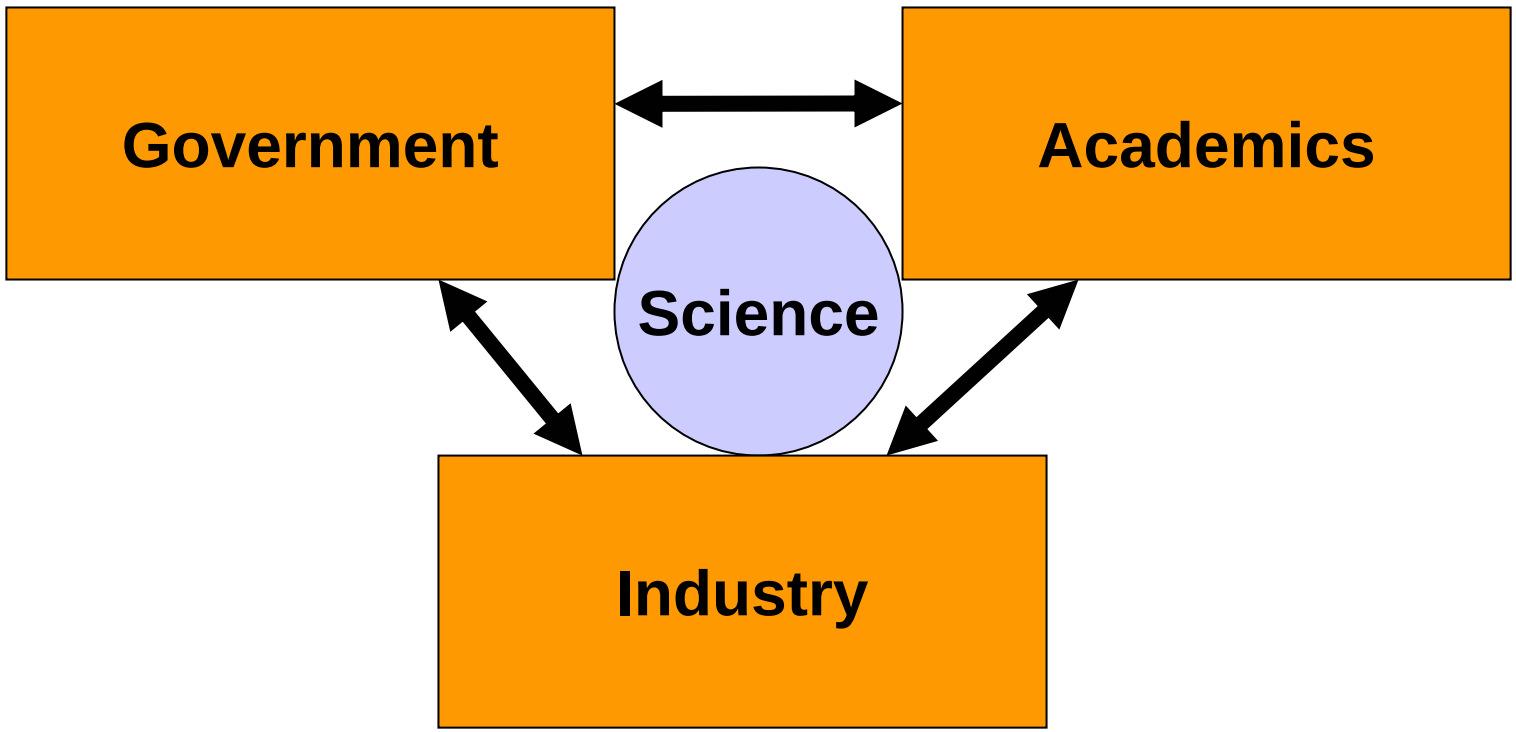
ILSI HESI Mission:
Issue Identification and Resolution

Tripartite Approach



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

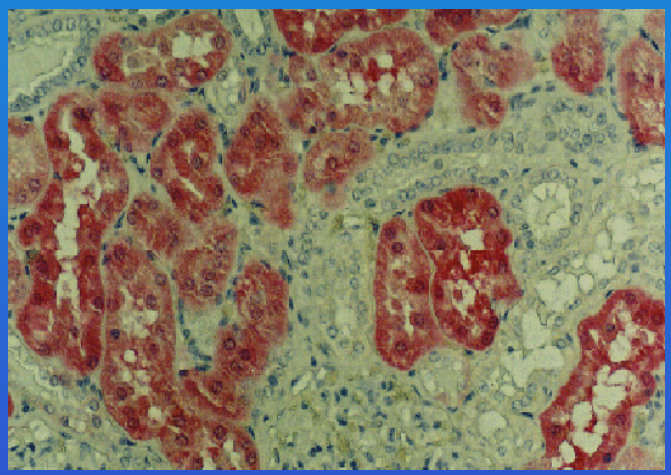
- ICCVAM Liaisons to subcommittee identified
  - Dr. Karen Hamernik (US EPA Office of Pesticide Programs)
  - Dr. Leonard Schechtman (ICCVAM Chair; US FDA, NCTR)
- Second Biomarkers Subcommittee meeting – July 21-22, 2003. Three proposals selected for evaluation:
  - Biomarkers of Nephrotoxicity
  -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

Biomarkers of Nephrotoxicity Working Group

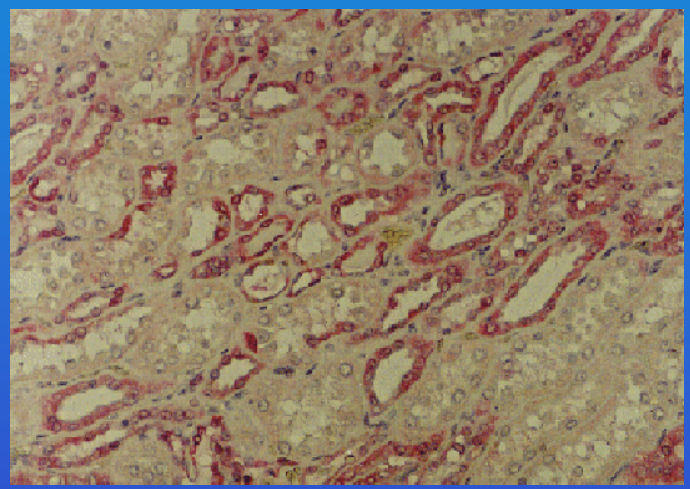
Biomarkers of Nephrotoxicity

Biomarker	Localization	AB availability	Source
αGST	Proximal Tub	Rat, Hu	Biotrin
Kim1	Proximal Tub	Hu Moab, rat, dog	TBD (Bonventre)
GST π	Distal Tub	Rat, Hu	Biotrin
PAP1	Papilla	Moab	Biotrin
Clusterin	General	Commercial	TBD

Immunohistochemical Localisation of GST Isoforms In The Human Kidney



αGST in Proximal Tubules



πGST 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.

Criteria for Model Toxicant Selection

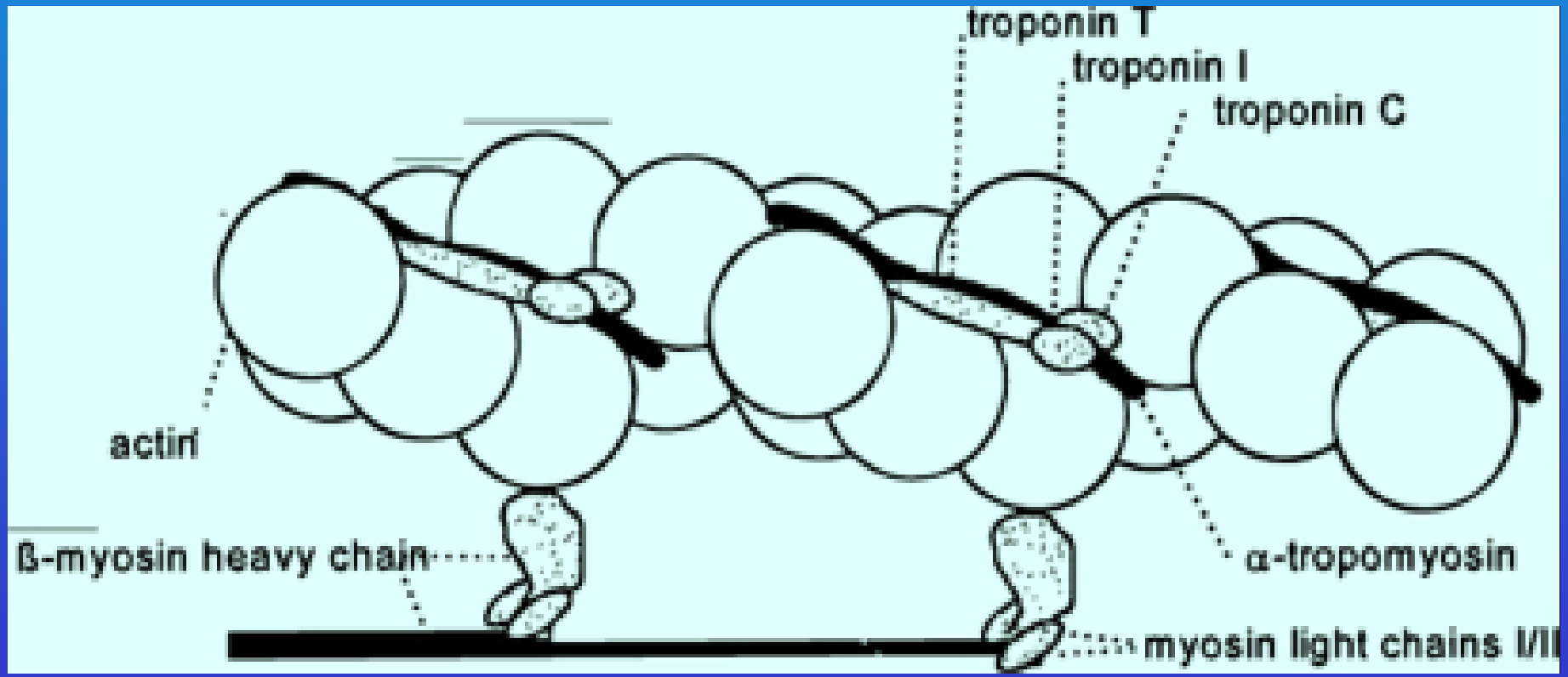
- Specificity: Ideal toxicant should target selected regions of the kidney.
  - Proximal tubule damage - gentamicin, cisplatin or p-aminophenol
  - Distal tubule damage - amphotericin B
  - Papillary damage - indomethacin, BEA
- Formulation: Ideally toxicants should be prepared in identical vehicle.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

Serum Cardiac Troponins Working Group

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

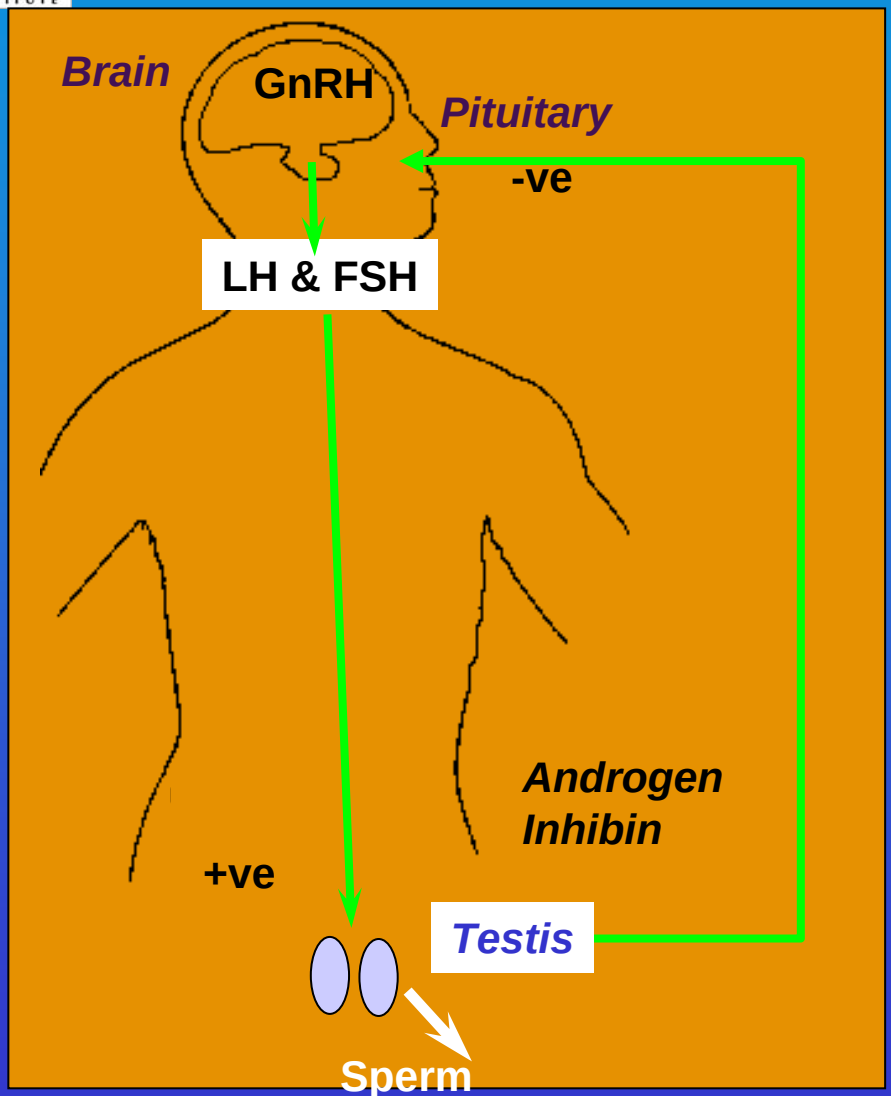
1. Evaluate kinetics of release and return to baseline (diagnostic window) of serum cardiac troponins and correlate with histopathology for compounds that cause cardiac injury.
2. Determine if there is a threshold for the increase in serum troponins below which there is no substantial or sustained cardiac 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 Working Group

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.

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

For More Information on ILSI HESI Biomarkers Subcommittee

Visit http://hesi.ilsi.org/

or

Email: hesi@ilsi.org