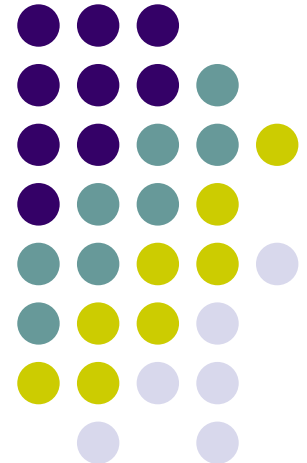


Experience With a Biomarker Consortium

William B. Mattes, PhD, DABT
Critical Path Institute
Rockville, MD / Tucson, AZ



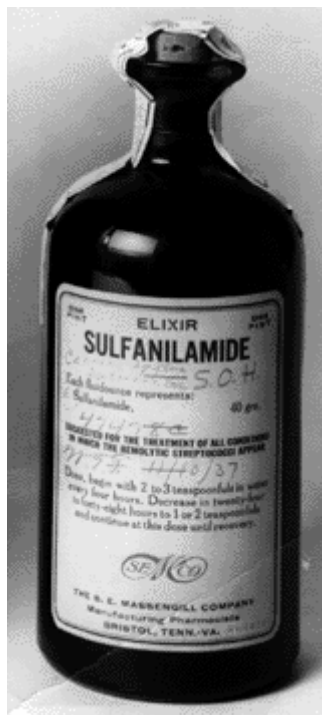
Outline

- Background: Preclinical Safety & Biomarkers
- Consortia and All That
- The Predictive Safety Testing Consortium
- PSTC Progress
- Translational Strategies and Considerations

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Pre-clinical Safety Testing?



1937 Massengill distributed **Elixir Sulfanilamide** without testing for safety (which was not required by law). Because it contained diethylene glycol as a vehicle, 107 people died, many of whom were children.

1938 Federal Food, Drugs and Cosmetic Act

New drugs are required to be safe before marketing.

“The Problem”

Results of Current
Pre-clinical Safety Testing

**Clear Signals of
Toxicity
(Rat Poisons)**

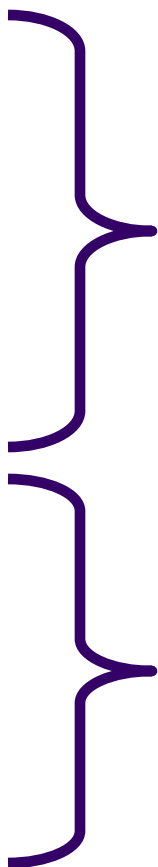
**Ambiguous Signals
of Toxicity**

**Ambiguous Signals
of Toxicity**

**No Apparent
Signals of Toxicity**

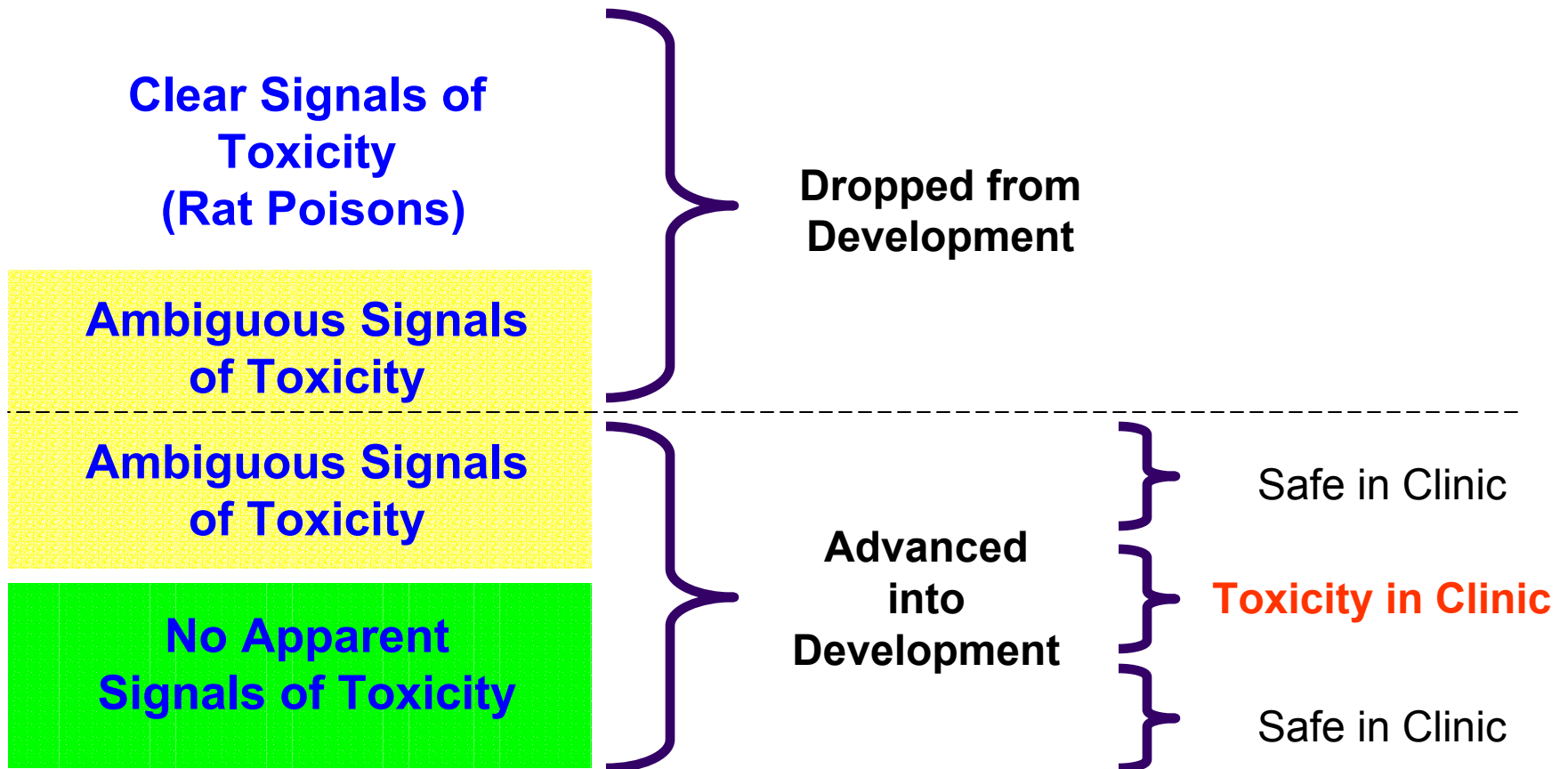
**Dropped from
Development**

**Advanced
into
Development**



“The Problem”

Results of Current
Pre-clinical Safety Testing



“The Problem”

Results of Current
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**Clear Signals of
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**Ambiguous Signals
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**Ambiguous Signals
of Toxicity**

**No Apparent
Signals of Toxicity**

**Dropped from
Development**

**Advanced
into
Development**

How many of
these were
actually safe?

Safe in Clinic

Toxicity in Clinic

Safe in Clinic

“The Problem”

Results of Current
Pre-clinical Safety Testing

**Clear Signals of
Toxicity
(Rat Poisons)**

**Ambiguous Signals
of Toxicity**

**Ambiguous Signals
of Toxicity**

**No Apparent
Signals of Toxicity**

**Dropped from
Development**

**Advanced
into
Development**

How could
these have
been clearly
detected
earlier?

Safe in Clinic

Toxicity in Clinic

Safe in Clinic

“Current” Biomarkers of Safety

- Urine creatinine
 - introduced 1904
- Blood creatinine, urea, glucose, cholesterol
 - Introduced ~1914
- Alanine Transaminase (ALT)
 - Described in 1954

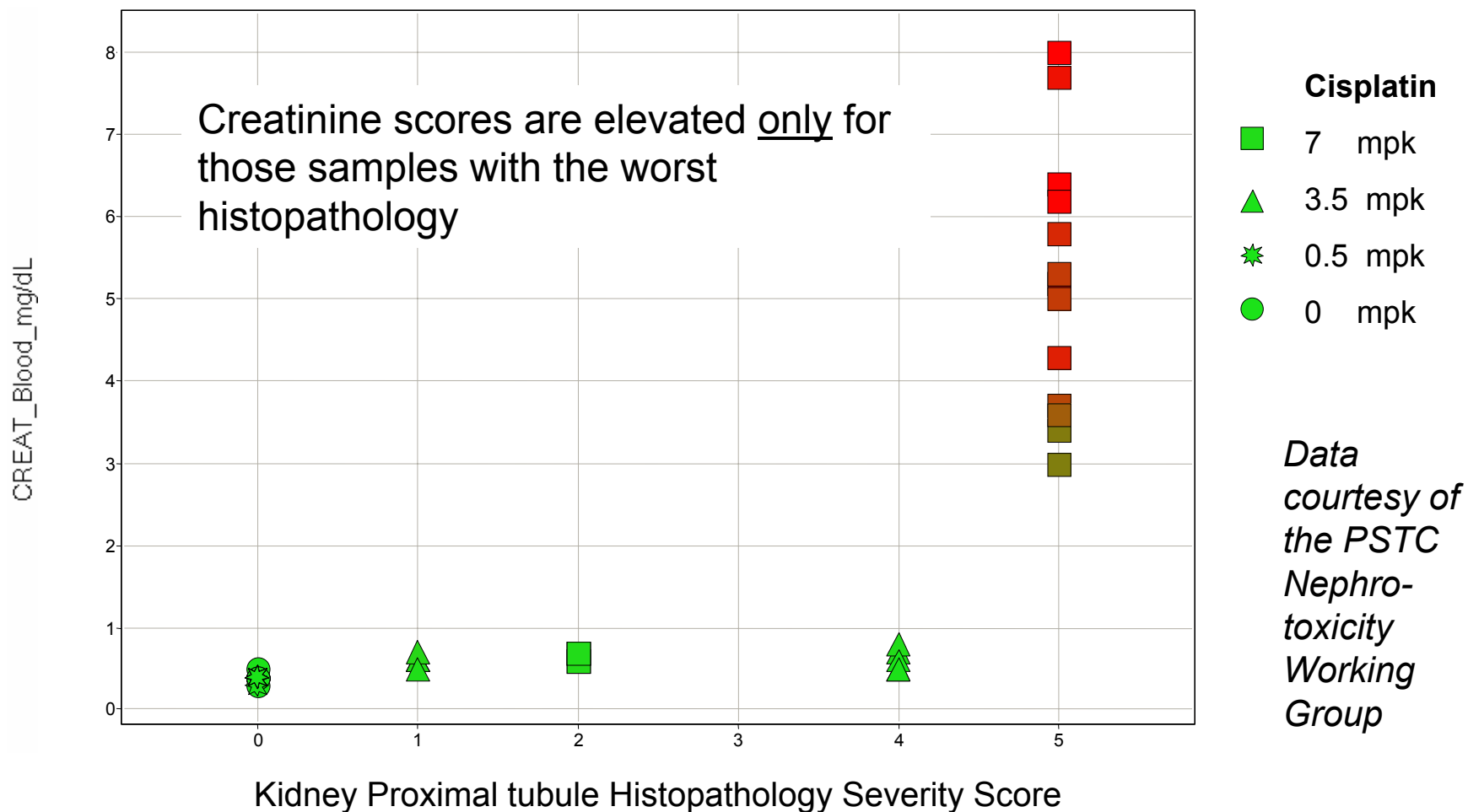
Why New Safety Biomarkers?

- Nephrotoxicity

- Correct assessment of kidney function is important both for dosage adjustment of renally excreted drugs and for early detection of drug nephrotoxicity, that mostly is reversible if the offending agent is discontinued. ...**Serum creatinine is a late marker of nephrotoxicity** that does not reflect rapid changes in renal function.

- M. Schetz, J. Dasta, S. Goldstein, T. Golper, *Curr Opin Crit Care* **11**, 555-65 (Dec, 2005).

Creatinine vs Histopathology



Why New Safety Biomarkers?

● Vascular Damage

Table 1
Approved or approvable drugs that cause arterial toxicity in animals

Drug	Mechanism	Preclinical cardiovascular effects
Milrinone	PDE 3 inhibitor	Decreased MABP/reflex tachycardia
Fenoldopam	DA1 agonist	Decreased MABP
Theophylline	PDE 3 inhibitor/adenosine antagonist	Decreased MABP/reflex tachycardia
Minoxidil	Potassium channel opener	Decreased MABP/reflex tachycardia
Adenosine	A1 agonist	Vasodilator
Hydralazine	?	Decreased MABP/reflex tachycardia
Bosentan	Endothelin receptor antagonist	No significant change in MABP or HR
Cilomilast	PDE 4 inhibitor	No significant change in MABP or HR
Nicorandil	Potassium channel opener/nitrate	Decreased MABP/reflex tachycardia

Yet NONE of these have demonstrated clear vascular damage in human experience

From W. Kerns *et al.*, *Toxicol Appl Pharmacol* **203**, 62-87 (Feb 15, 2005).

Why NOT New Safety Biomarkers?



The screenshot shows the PubMed website interface. At the top, the PubMed logo is displayed alongside the text "A service of the National Library of Medicine and the National Institutes of Health" and the URL "www.pubmed.gov". Navigation tabs for "Nucleotide", "Protein", "Genome", "Structure", "OMIM", "PMC", and "Journals" are visible. A search bar contains the query "biomarkers of toxicity" with "Go", "Clear", and "Save" buttons. Below the search bar, there are buttons for "Limits", "Preview/Index", "History", "Clipboard", and "Details". The "Display" dropdown is set to "Summary", "Show" is set to "20", and "Sort by" is set to "Relevance". A summary bar indicates "All: 9418" and "Review: 1058". The results list shows "Items 1 - 20 of 9418" with "Page 1 of 471 Next". The first result is a paper by Gluckmann M, Fella K, Waidelich D, Merkel D, Kruft V, Kramer PJ, Walter Y, Hellmann J, Karas M, Kroger M, titled "Prevalidation of potential protein biomarkers in toxicology using iTRAQtrade mark reagent technology." with a PMID of 17443845.

You would think we would have lots of new safety / toxicity biomarkers!

Issues with New Biomarkers

- **Compound Diversity**
 - Most studies investigate new biomarkers with only a few compounds as positive and negative examples
- **Study Diversity**
 - Rarely are more than a few studies conducted
- **Species Diversity**
 - Most of the studies reported in the literature look at rats

Biomarker Bottom-Line

- **For a biomarker to be considered useful it takes a LOT of resources to develop a data set**
 - Convincing to the larger scientific community
 - Convincing to the regulatory community
 - Convincing even for internal decision making

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Collaboration



Barn-Raising

Also evokes the Finnish concept of **Talkoot**:

a group of people gathering to work together, to build or repair something. by definition voluntary, and the work is unpaid.

One's honor and reputation may be severely damaged if one doesn't show up — or proves to be a poor worker.

- From Wikipedia

Safety Biomarker Consortia

- Acute Kidney Injury Network (AKIN)
- Drug-Induced Liver Injury Network (DILIN)
- Serious Adverse Events (SAE) Consortium
- InnoMed PredTox
- ILSI HESI Biomarkers Technical Committee
- Predictive Safety Testing Consortium (PSTC)

Acute Kidney Injury Network

- Clinical focus
- Interdisciplinary group of adult and pediatric nephrologists and critical care physicians
- Clinical studies examining several aspects of kidney injury, not just biomarkers

Drug-Induced Liver Injury Network

- Clinical focus
- Created by the National Institute of Diabetes and Digestive and Kidney Diseases
- Currently five clinical sites
- Research goals include:
 - Create a registry of carefully documented DILI cases and corresponding controls
 - Identify clinical, immunological, and environmental risk factors for drug-mediated hepatotoxicity
 - Create a bank of biological specimens consisting of DNA, plasma, and immortalized lymphocytes to facilitate detailed genetic analyses

SAE Consortium

- Clinical focus
- 501(c)3 organization; CEO: Arthur L. Holden
- Identifying and validating DNA-variants useful in predicting the risk of drug-related serious adverse events
- Seven pharmaceutical company participants
- Focused on identifying the genetic markers associated with rare drug-related liver toxicity and Stevens-Johnson Syndrome (SJS)

InnoMed PredTox

- Preclinical focus
- Joint Industry and European Commission collaboration
- 14 pharmaceutical companies, three academic institutions and two technology providers
- Use of multiple ‘omic technologies
- Focus on a comparably restricted number of different compounds.
- Questions addressed include:
“What combination of methods / technologies delivers the best predictive results for hepatotoxicity and / or nephrotoxicity?”

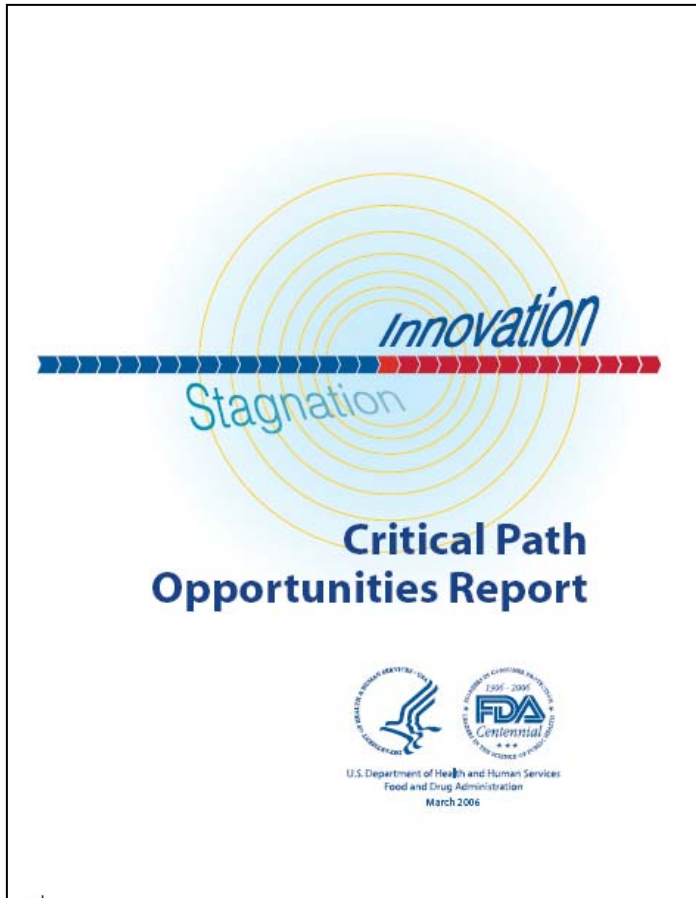
HESI Biomarkers Committee

- Preclinical focus
- 17 pharmaceutical companies, 5 public organizations
- Goals include:
 - Identify accessible biomarkers with the potential to bridge from the pre-clinical to the clinical stages of drug development;
 - Evaluate the potential utility of newly identified markers of tissue injury for preclinical safety studies;
 - Build consensus regarding how to apply newly identified biomarkers of toxicity in risk assessment
- Working Group Accomplishments:
 - Nine troponin assays evaluated for assessing cardiotoxicity
 - Several region-specific assays (clusterin, GST, and RPA) for nephrotoxicity evaluated and scheduled for FDA submission

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Critical Path Opportunities



March 2006

This FDA report Critical Path Report identifies six priority public health challenges. An accompanying **Critical Path Opportunities List** identifies 76 specific opportunities that can address those challenges.

CPI Opportunity # 46

- 46. Identification and Qualification of Safety Biomarkers.
 - “Collaborative efforts to pool and mine existing safety and toxicology data would create new sources for identification and qualification of safety biomarkers.”

Predictive Safety Testing Consortium (PSTC)



- Announced by HHS/FDA on March 16, 2006 as part of Critical Opportunities List
- The Critical Path Institute (C-Path) is a third party, partner, and project catalyst.
 - A non-profit, publicly-funded Institute that serves as a “neutral ground”
 - Founding partners include: FDA, SRI International, and The University of Arizona
 - Executed Memorandum of Understanding with the Food and Drug Administration Dec 16, 2005
 - FDA and EMEA scientists serve as advisors

PSTC Goals

- To cross-qualify pre-clinical animal model biomarkers aimed at reducing the cost and time of pre-clinical safety studies
- To use the **combined resources, sample sets, novel compounds, and expertise** to generate a biomarker data package convincing enough for FDA/EMEA qualification as an approved biomarker
- To provide potential early indicators of clinical safety in drug development and post-marketing surveillance.
 - Note the goal of translational biomarkers
- To develop new tools for FDA and EMEA to assist in regulatory decision making.

Consortium Members

- ✧ Abbott
- ✧ Amgen, Inc
- ✧ Astra Zeneca
- ✧ Boehringer Ingelheim
- ✧ Bristol-Myers Squibb
- ✧ GlaxoSmithKline
- ✧ Iconix Pharmaceuticals
- ✧ Johnson & Johnson
Pharmaceutical R&D
- ✧ Eli Lilly, Inc
- ✧ Merck & Co., Inc.
- ✧ Novartis
- ✧ Pfizer, Inc.
- ✧ Roche
- ✧ Sanofi-aventis U.S. Inc
- ✧ Schering Plough
Research Institute
- ✧ Wyeth

An International Endeavor



PSTC Organizational Aspects

- Member companies are bound by a Consortium Agreement that covers, among other things:
 - Intellectual Property
 - Confidentiality
 - Information Sharing
 - Anti-Trust
- Projects are formally described and legally detailed with Project Agreements
 - Cover IP created in the course of the project
 - Detail specific responsibilities of project members
 - Describe the roles of non-member collaborators

Role of FDA and EMEA

- Regulatory body participation is key to interactively develop a cross-validation process leading to regulatory qualification
- FDA is *not* a member, but acts as an advisor and has advisors on all the working groups except the Membership Subcommittee
- The FDA may be consulted for input on decisions of the Advisory Committee
- When appropriate agreements with the FDA (e.g. CRADA) may be developed for specific projects
- Representatives from the EMEA are also participating as advisors

Working Groups and Teams

- Five Injury Area Working Groups
 - Nephrotoxicity
 - Hepatotoxicity
 - Vascular Injury
 - Carcinogenicity
 - Focus on signatures of non-genotoxic carcinogenicity
 - Myopathy
- Data Management Team
- Translational Team

Types of Analytes Considered

- Enzymatic assays
 - E.g. GLDH
- ELISA assays
 - Preference for multiplex assays
- Small molecules
 - E.g. F(2)-isoprostanes
- Genomic Signatures
 - Useful for assessing non-genotoxic carcinogenicity
 - Genomics useful for identifying other candidate biomarkers

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Carcinogenicity WG Status

- Two mRNA signatures of non-genotoxic hepatocarcinogenicity selected for initial evaluation
 - Uses mRNA from a short term (<14 day) rat study to predict induction of liver tumors in the 2-year rat bioassay
- Evaluation of these signatures by member companies using internal data bases is complete
- Conversion to qRT-PCR assay is complete, and reevaluation is in progress

Vascular Injury WG Progress

- Numerous candidate biomarkers considered; final selection nearly complete
- Samples have been exchanged for qualification of two biomarkers, Caveolin-1 (Cav-1) and von Willebrand Factor pro-peptide (vWFpp)
- Late-stage discussions with Vasculitis Clinical Research Consortium (VCRC) for access to samples for clinical translation

Candidate Biomarkers of Drug-Induced Vascular Injury

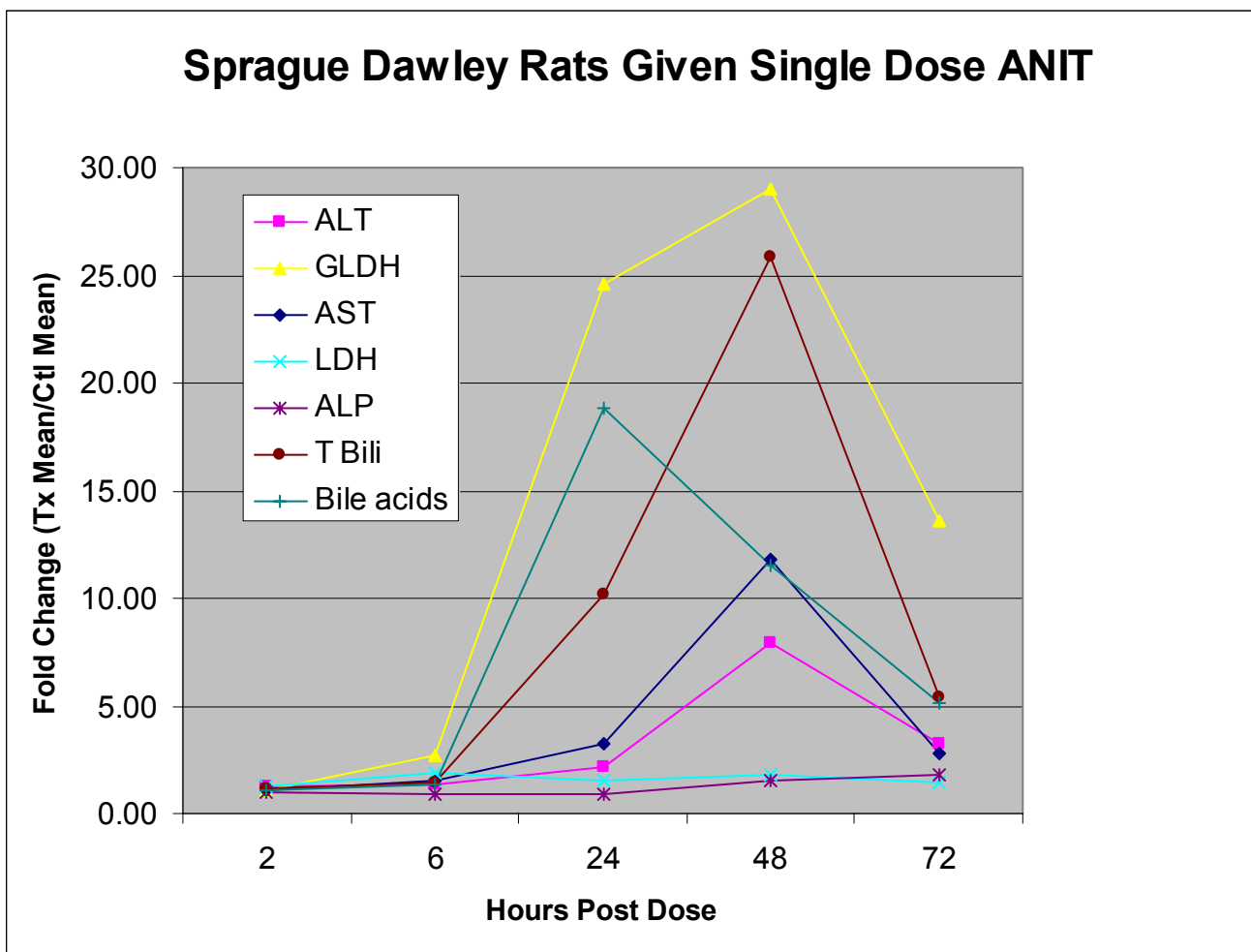


- Acute Phase Response genes
- Von Willebrand's Factor pro-peptide
- Serum nitrite as measure of iNOS
- Endothelial cell gene markers: iNOS, Pla2g2a, S100A8/9, properdin, PTN
- Selectins, ICAMs
- Mast cell activation markers
- Caveolin-1
- VEGF

Hepatotoxicity WG Progress

- Four candidate biomarker assays identified for initial cross-qualification
 - PON-1, MDH, PNP, and GLDH
 - Standard auto-analyzer enzymatic assays
 - Significant preliminary internal data
- Three member laboratories identified for assay-cross-qualification
- Cross-qualification samples inventoried
- Further discussions on potential candidate biomarkers ongoing

GLDH vs ALT



Data Sharing from 23 Proposed Exploratory Biomarkers of Kidney Toxicity – 9 Overlapped

- Albumin
- B2-microglobulin
- Calbindin d28
- Clusterin
- Cystatin C
- EGF
- GSTa
- GSTmu
- Kim-1
- Lipocalin2 (NGAL)
- NAG
- Osteoactivin
- Osteopontin
- Podocin
- RPA1
- TFF3
- Timp1
- Total Urinary Protein
- Uromodulin (Tamm-Horsfall)
- VEGF
- Macrophage Migration Inhibitory Factor
- Monokine Induced by Interferon Gamma
- Interferon Gamma Induced 10Kda Protein

Data Sharing from 23 Proposed Exploratory Biomarkers of Kidney Toxicity – 7 Prioritized

- ➔ ● Albumin
- ➔ ● B2-microglobulin
- Calbindin d28
- ➔ ● Clusterin
- ➔ ● Cystatin C
- EGF
- GSTa
- GSTmu
- ➔ ● Kim-1
- Lipocalin2 (NGAL)
- NAG
- Osteoactivin
- Osteopontin
- Podocin
- RPA1
- ➔ ● TFF3
- Timp1
- ➔ ● Total Urinary Protein
- Uromodulin (Tamm-Horsfall)
- VEGF
- Macrophage Migration Inhibitory Factor
- Monokine Induced by Interferon Gamma
- Interferon Gamma Induced 10Kda Protein

Biomarker Qualification Submission



- Data from 23 compounds for 7 biomarkers
- First submission of its kind under the FDA Voluntary Data Submission (VXDS) process
- Joint submission on June 15, 2007 to both FDA and EMEA
- Face to face meeting on July 12, 2007
- Final face to face meeting on October 9, 2007
- All outstanding issues addressed
- Decision on submission claims expected by end of year

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Translational Considerations

- The first focus is on pre-clinical biomarkers
 - This allows a robust correlation between biomarker performance and histopathology
- If a biomarker is to be used to support safety in Phase-1 clinical trials:
 - It's performance in pre-clinical studies must be understood
 - *It's performance in clinical settings must also be understood*

Translational Strategies

- Key to developing strategies is a Translational Team that eliminates silos
 - Clinical scientists from member organizations
 - Preclinical scientists
 - Regulatory scientists
 - Outside advisors as determined



Ending Thoughts

- New safety biomarkers require an intentional qualification process to assure both scientific and regulatory acceptance
- C-Path's Predictive Safety Testing Consortium is a forum for industry members to share efforts toward biomarker qualification
- Involvement of regulatory scientists in Consortium discussions is critical for final regulatory acceptance of biomarkers
- A translational strategy that integrates pre-clinical and clinical scientists is essential for supporting biomarkers as robust drug development tools

I'm Done

Thank You.
Questions?

