TECHNICAL COMMITTEE ON APPLICATION OF GENOMICS TO MECHANISM-BASED RISK ASSESSMENT

Presented by:
Richard S. Paules, Ph.D.
(Vice-Chair, Genomics Technical Committee)

January 20, 2009
Genomics Technical Committee:

Leadership

Current:

Dr. Jiri Aubrecht, Chair
(Pfizer)

Dr. Richard Paules, Vice-Chair
(National Institute of Environmental Health Sciences, NIH)

Past:

Dr. Cindy Afshari, Chair
(Amgen)

Dr. George Orphanides, Vice-Chair
(AstraZeneca)

HESI Program Manager:  Raegan O’Lone (Syril Pettit)
Genomics Technical Committee:
2008 Industry Members

- Actelion
- Allergan
- Amgen
- Astellas Pharma
- AstraZeneca
- Bayer HealthCare
- Biogen Idec
- Boehringer Ingelheim
- Bristol-Myers Squibb
- Daiichi Sankyo
- Dow Chemical
- Eli Lilly
- GlaxoSmithKline
- Hoffman-La Roche
- Institute de Recherches Internationales SERVIER
- Johnson & Johnson
- Meiji Seika Kashi
- Novartis
- Pfizer
- sanofi-aventis
- Schering Plough
- Sumitomo
- Syngenta
- Takeda
**Genomics Technical Committee: Public Participation**

- Georgetown University
- Harvard University
- Michigan State University
- University of Minnesota
- University of Surrey (United Kingdom)
- European Medicines Agency
- National Institute of Health Sciences (Japan)
- National Institute for Public Health and the Environment (RIVM, the Netherlands)
- US Department of Agriculture
- US Environmental Protection Agency
- US Food and Drug Administration
- US National Cancer Institute
- US National Center for Toxicological Research
- US National Institute for Environmental Health Sciences
Genomics Technical Committee:

Mission

• To advance the scientific basis for the development and application of genomic methodologies, and

• To facilitate public discussion and information dissemination on the use of genomics as a tool to characterize mechanism of action and facilitate safety assessment of drugs and chemicals.
About The Genomics Committee

• One of HESI’s longest standing and largest projects, ongoing since 1999

• Large, international group of participants allows for broad potential impact

• Emphasis on technology evaluation, original data generation, and application of data and experience to the practice of risk and safety assessment.
Barriers to Toxicogenomics in Risk Assessment

In 1999 - we saw...

- Lack of publicly available databases
- Lack of validation of available technologies
- Lack of comparable tools, methods, study designs
- Lack of robust tools for data analysis
- Lack of knowledge - how transcriptional changes relate to toxicity
- Uncertain regulatory applications

HESI COMMITTEE ON GENOMICS
Initial Program Activities
1999-2003

Toxicity Working Groups
- Hepatotoxicity
- Nephrotoxicity
- Genotoxicity

Database Working Group

Proof of Concept Projects to Focus on:
- inter and intra-lab variability and reproducibility, and
- development of public database (create & populate)
Committee Consensus:
Technical / Biological Interpretation
2004 Study Conclusions

- Gene expression analysis using microarrays is a valuable tool for identifying alterations in biological pathways of interest.

- Pathway-level analysis is consistent across laboratories and platforms; gene-by-gene comparisons are challenging.

- Genomic data is not a ‘stand-alone’ - Critical to place data in context of other biological findings (e.g., exposure, clinical chemistry, histopathology, protein expression, etc.) for interpretation.

- Changes in gene expression as measured on a microarray platform do not in themselves equate to single biological endpoints (adverse or adaptive).
Research from 2000 - 2004

- Publication of 12 technical and overview articles in mini-monograph of EHP Toxicogenomics (March ’04) + 3 articles in May ’04 Issue of Mutation Research

- > 1000 hybridizations and related tox data entered into publicly accessible ArrayExpress dbase via European Bioinformatics Institute
5 Programs in 2005 → 2008

- Baseline Animal Database Working Group
- State of Genomics Survey Working Group
- Genotoxicity Working Group
- Mechanism-Based Markers of Toxicity Working Group / Doxorubicin Study
- Case Study Workshop
ILSI Health and Environmental Sciences Institute (HESI)

Baseline Animal Database Program

Karol Thompson, Chairperson
CDER, US FDA
Key Objectives

1. Establish a public dataset of microarray data on baseline expression levels in the rat
   • Voluntary contributions of genomic data from control animals in toxicogenomic studies of liver and kidney

2. Demonstrate utility of control dataset for evaluating sources of variance
   • Focus on impact on study design and data interpretation
Data Collection

• Affymetrix array data for liver or kidney samples from rats in the control groups of toxicogenomics studies

  – Results: >500 CEL files from 16 institutions (US & EU)

  • Amgen
  • Astra Zeneca
  • Bayer Healthcare
  • Biogen Idec
  • Boehringer Ingelheim GmbH
  • EPA
  • FDA
  • GlaxoSmithKline
  • Johnson & Johnson
  • Lilly
  • NIEHS
  • Novartis
  • Pfizer
  • Sankyo
  • Sanofi-Aventis
  • Schering-Plough
## Data Available in BID

[https://dir-apps.niehs.nih.gov/arc/](https://dir-apps.niehs.nih.gov/arc/)

### BID (Biomedical Investigation Database)

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<thead>
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<td>Sankyo Phenobarbital 2004</td>
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**And Mirrored at Additional Sites:**

**Chemical Effects on Biological Systems:**
http://cebs.niehs.nih.gov/

**EBI ArrayExpress:**
http://www.ebi.ac.uk/microarray-as/ae

Experiment E-TOXM-39

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<tr>
<th>1 / 1</th>
<th>Experiment : E-TOXM-39</th>
<th>Submitter(s) : HESI</th>
<th>Lab : Health and Environmental Sciences Institute</th>
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**Experiment Design Type:** retrospective study

*(Generated description):* Experiment with 517 hybridizations, using 517 samples of species [], using 517 arrays of array design [Affymetrix GeneChip® Rat Genome 230 2.0 [Rat230_2], Affymetrix GeneChip® Rat Expression Array RA230A [RAE230A], Affymetrix GeneChip® Rat Genome U34A [RG_U34A]], producing 517 raw data files and 3 transformed and/or normalized data files.
Conclusions

• A large dataset of control rat data from multiple sites that is linked to study parameter annotations can be used to:
  – Examine associations between study factors and gene expression variability
  – Identify genes with high and low variance in baseline expression
  – Identify pathways that contain genes with high intrinsic variability among control animals
  – Identify robust changes in expression associated with certain study factors
Sources of variation in baseline gene expression...”

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Genotoxicity Working Group

Dr. Jiri Aubrecht, Chairperson
Pfizer
Key Objectives

1. Determine whether PCR- and microarray-based approaches can facilitate differentiation of direct and indirect acting genotoxins in *in vitro* assays
   - *Participating labs ran studies in-house using custom PCR assays and/or Affymetrix or Agilent arrays*

2. Build on outcomes of 2000-2004 genotox project
   - *Focus on mechanistic information derived from selected gene sets and pathways from arrays*
### Basic Experimental Approach

#### Experimental Design

- **TK-6** and **L5178Y**
  - (p53-proficient and p53-deficient)

#### Model compounds
- Direct genotoxins: Cisplatin, Etoposide
- Indirect genotoxin: Taxol
- Cytotoxic clastogen: NaCl

Dose response and time course performed
Dose selection based on cytotoxicity

Gene set of 47 genes selected based on literature and previous data

Gene expression changes detected by QRT-PCR TaqMan® Assays-on-Demand™ or Microarrays: Agilent and Affymetrix

Data collected in CEBS
Results

• Trends between labs are similar
  – Early profiles at 4hr when cytotoxicity has not yet developed, appear to differentiate among direct and indirect genotoxins
  – Direct DNA damaging Compounds →
    • Early upregulation of p53 target genes
    • Early downregulation of anti-apoptosis and cell cycle progression genes
  – Indirect Genotoxin → Later upregulation of p53 target genes
  – Cytotoxic clastogen → Deregulation inconsistent or only at high cytotox
  – General stress response genes deregulated early and late +/- by all compounds

• QRT-PCR vs. Micoarrays
  – QRT-PCR and Array data are comparable
  – Subset of genes measured with PCR is feasible but microarrays provide better mechanistic insight
Manuscript entitled, “Characterization and interlaboratory comparison of a gene expression signature for differentiating genotoxic mechanisms” complete and in HESI peer review process currently.

Manuscript to be submitted to Tox. Sci. in January 2009.
ILSI Health and Environmental Sciences Institute (HESI)

State of Science Survey
Working Group

Alison Vickers, Chairperson
Allergan
Goals of Toxicogenomics Survey

- Probe current and future uses of Toxicogenomics for drug and chemical evaluation
  - Identify hurdles & key enablers for moving field forward
- Multi-sector survey of scientists and decision/policy makers active in Toxicogenomics
  - Public resource to facilitate discussion amongst academia, industry, regulatory sectors

- For informational purposes only and not attributed to a company or agency
Study Completion

- Manuscript undergoing final revisions.

- Anticipated to be submitted to *Environmental Health Perspectives* in early 2009.
Mechanism-Based Markers of Toxicity

Working Group

Co-chairs: Dr Hisham Hamadeh (Amgen Inc.)
Dr Jon Lyon (GlaxoSmithKline)
Generate new molecular data on a toxicity of importance
- move the science forward
- potential to identify novel markers of toxicity

Investigate wider toxicological concept - molecular threshold
- study design to provide insight into relationship between time/dose, gene expression and onset of toxicity
Why use a committee approach...?

- Economy of scale
  - nature of study outside usual scope of individual organizations

- Open discussion between/within industry and regulators - identify optimum features

- Unique breadth of expertise to aid in design, execution and interpretation of the study
Doxorubicin (Adriamycin)

- Treatment of solid and hematologic neoplasms **but** efficacy limited by delayed cardiotoxicity (significant clinical issue)

- Cardiomyopathy directly related to total cumulative dose

- Many mechanisms of toxicity and protective strategies have been proposed

- Pharmacological action – DNA intercalation and inhibition of topoisomerase II complex
Questions and Aims

• Generate hypotheses of mechanism(s) of progressive damage associated with Doxorubicin
  • Doxorubicin-associated changes not observed with Etoposide
  • Heart-specific changes vs changes in negative tissue (skeletal muscle, gastrocnemus)
• Investigate cumulative effect and reversibility
  • Is there evidence of cumulative effect of doxorubicin at gene level (e.g. 1 mg/kg for 6 weeks vs. 3 mg/kg for 2 weeks)
  • Are there genes changes which become fixed or progress during drug-free phase
• Identification of early and persistent changes in cardiac tissue
  • Comparison of genomic endpoints with other measurable effects (e.g. Histopath and troponins) on individual animal basis
• Understand what Dexrazoxane reverses/prevents and what it does not
  • Clinical implications for Doxorubicin co-treatment
**Overall Strategy and Goals**

- **Cardio toxic vs non-cardio toxic comparison** 
  *Doxorubicin vs Etoposide*

- **Protective intervention** 
  *Doxorubicin vs Doxorubicin+Dexrazoxane*

- **Tissue Specificity** 
  *Cardiac vs Gastrocnemius*

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**Doxorubicin Cardiotoxicity Mechanisms & Thresholds**

- **Persistent vs Transient**
- **Time Course and Dose Response**
In-Life Study Design/Execution

Two in vivo studies in male SD rats (Covance, USA)

Dose Range Finder
- 6 weekly doses, sacrifice 1 week after last dose
- Doxorubicin 0.5, 1, 2, 3 mg/kg/week
  - Etoposide 0.5, 1, 3 mg/kg/week
  - Dexrazoxane 50 mg/kg/week
- Doxo 2, 3 + Dexra 50 mg/kg/day

Main Study
- Doxorubicin 1, 2, 3 mg/kg/week
- Etoposide 1, 3 mg/kg/week
- Dexrazoxane 50 mg/kg/week
- Doxo 2 + Dexra 50 mg/kg/day

- Set doses & dosing methods
- Check pharmacology & tox markers
  - Check TK
- Samples preserved
**Main Study Design**

- **Doxorubicin** (mg/kg/week):
  - Week 0: 0
  - Week 1: 1
  - Week 2: 2
  - Week 3: 3
  - Week 4: 0
  - Week 5: 2
  - Week 6: 3

- **Etoposide** (mg/kg/week):
  - Week 0: 0
  - Week 1: 1
  - Week 2: 3
  - Week 3: 1
  - Week 4: 3
  - Week 5: 1
  - Week 6: 3

- **Dexrazoxane** (mg/kg/week):
  - Week 0: 0
  - Week 1: 2
  - Week 2: 50
  - Week 3: 50
  - Week 4: 2 + 50
  - Week 5: 50
  - Week 6: 2 + 50

- **Dox + Dex** (mg/kg/week):
  - Week 0: 0
  - Week 1: 0
  - Week 2: 2
  - Week 3: 0
  - Week 4: 2
  - Week 5: 0
  - Week 6: 2

Legend:
- White: off drug
- Yellow: on drug
Many Data Were Collected

- Toxicokinetics
- Micronucleus
- Clinical pathology
- Histopathology
  - Full Heart Pathology
  - Gastrocnemus Path
  - Diaphragm Path
- Troponin I & T
- Gene expression
  - Agilent on heart and gastrocnemus (288 samples)
  - Affymetrix on heart and gastrocnemus (288 samples)
**Time-Dependent Gene Expression**

*Increases at 2 mg/kg with Doxorubicin*
Preliminary Conclusions on Mechanism(s) of Doxorubicin from Genomic Analysis

- Disruption of calcium homeostasis
- Generation of ROS
- Release of vasoactive amines
- Impairment of mitochondrial activity
- Inhibition of nucleic acid and protein synthesis
- Induction of NOS
- Apoptosis
- Altered immune functions
Study Status

- Troponin and histopath analyses done
- Microarray analysis on both cardiac and gastrocnemus tissue complete
- 2 day data review meeting held in Oct 08.
- Analysis to Date Indicates High Quality Dataset….lots of opportunities for insights both technical and biological
- Final analyses and publication outlines in progress.
- 2-4 manuscripts expected by Year End 2009.
Genomic Applications in Safety Studies - A Case Study Workshop

Dr. Cindy Afshari, Organizer
Amgen
Successful Workshop Held in 2008

Save the Date!

Genomics Applications in Safety Studies – Case Study Workshop

This international workshop will feature leading scientists from industry, regulatory agencies, and government presenting specific examples of the successes and challenges associated with use of toxicogenomics for safety and risk evaluation. The workshop program will cover both chemical and pharmaceutical applications and offer ample opportunity for discussion and interaction.

Workshop presenters include:

Dr. Cynthia Ashburn, Amgen Inc.
Dr. Jiri Nekvindova, Pfizer
Dr. William Benson, U.S. Food & Drug Administration
Dr. Bruce Car, Bristol-Myers Squibb
Dr. Frederica Godbold, U.S. Food & Drug Administration
Dr. Lois Lehman-McKeeman, Bristol-Myers Squibb
Dr. Ruth Lightfoot-Dress, Amgen Inc.
Dr. James MacDonald, Schering-Plough
Dr. Rick Pantaleo, National Institutes of Health
Dr. William Pennie, Pfizer
Dr. Donald Robertson, Bristol-Myers Squibb
Dr. Allen Ross, Duke University
Dr. Frank Sitarz, Merck
Dr. Craig Thomas, Lilly Research Lab
Dr. Russell Thomas, The Hammersmith for Health Sciences
Dr. Jonathan Tugwood, AstraZeneca Pharmaceuticals
Dr. Paul Wehrli, University of North Carolina
Dr. Patrick Wier, GlaxoSmithKline
Dr. Douglas Wolf, U.S. Environmental Protection Agency

For more information or to register, please visit the HESI website at: www.hesiglobal.org and click on the “Events” tab.

October 27-28, 2008
Arlington, Virginia

Workshop organized by the ILSI Health and Environmental Sciences Institute Committee on Application of Genomics in Risk Assessment
Almost 100 participants from industry, academia and government

Many senior regulatory and industry scientists in attendance (including Dr. Janet Woodcock, Director of CDER)

‘Real-world’ unpublished case studies presented – stimulated challenging discussions on exploratory, mechanistic, screening uses, etc.

Manuscript Being Drafted And To Be Submitted To Peer-Reviewed Journal In Early To Mid 2009
1. Proposal: “Qualification of genomic biomarker for providing mechanistic context to positive findings in *in vitro* chromosome damage assays”

- Proposal drafted by Jiri Aubrecht, Pfizer, and David Jacobsen-Kram, FDA.
- Proposal vetted on multiple conference calls and at Oct. 08 Technical Committee meeting.
- Strong support across many organizations, including FDA.
- Further planning in Dec. 08 and early 2009 to define and refine protocols.
2. Proposed Workshop: ‘Implementation of Genomic Approaches with both In Vitro and In Vivo Models for Safety Assessment’
   • Co-organized with EU Carcinogenomics Committee and ECVAM
   • Satellite to 10th ICEM Meeting
   • Planned for August 27-28, 2009, Italy

3. Additional follow-up Workshops, Research, or Discussion Forums Related to Oct. 2008 Case Study Workshop
   - Workshop generated enthusiastic feedback
   - Committee Steering Team evaluating options
TECHNICAL COMMITTEE ON APPLICATION OF GENOMICS TO MECHANISM-BASED RISK ASSESSMENT

Thank You for Your Time and Interest
We still face many of same challenges

- Lack of **(well-populated)** publicly available databases
- Lack of validation of available technologies
- Lack of comparable tools, methods, study designs - *do we agree on need for this?*
- Lack of robust tools for data analysis - *tools are out there - but which ones to use? what assumptions? Are there enough people and storage resources to house data and process?*
- Lack of knowledge - how transcriptional changes relate to toxicity
- Uncertain regulatory applications
Key team members

- Kazuyuki Hiratsuka, Meiji
- Aruga Chinami, Tanabe
- Gotaro Tanaka, Taiho
- Ron Snyder, Schering-Plough
- Eric Boitier, sanofi-aventis
- Jean-Christophe Hoflack, Roche
- Heidrun Ellinger-Ziegelbauer, Bayer Health Care
- Catherine Spire, Servier
- Jiri Aubrecht, Pfizer
- Jennifer Fostel, NIEHS
- Daniel Bauer, Novartis
- Syril Pettit, HESI
2008 Publications Overview

Sources of variation in baseline gene expression...


- Design and interlaboratory comparison of a gene expression signature for differentiating genotoxic mechanisms.
  - Status: *In HESI Peer Review*

- Results Summary of a Survey from the HESI Genomics State of Science Subcommittee.
  - Status: *To HESI Peer review by year end.*

- Summary and Recommendations from a Workshop on Case Studies for Toxicogenomics in Safety Assessment
  - Status: Initial draft done, to HESI peer review in early 2009.

- Multiple publications from doxorubicin study anticipated in 2009