



***TECHNICAL COMMITTEE ON H E S I
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



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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



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- Actelion
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- 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
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- Syngenta
- Takeda

Genomics Technical Committee:

Public Participation



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- 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



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- *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



H E S I

- 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...



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- 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



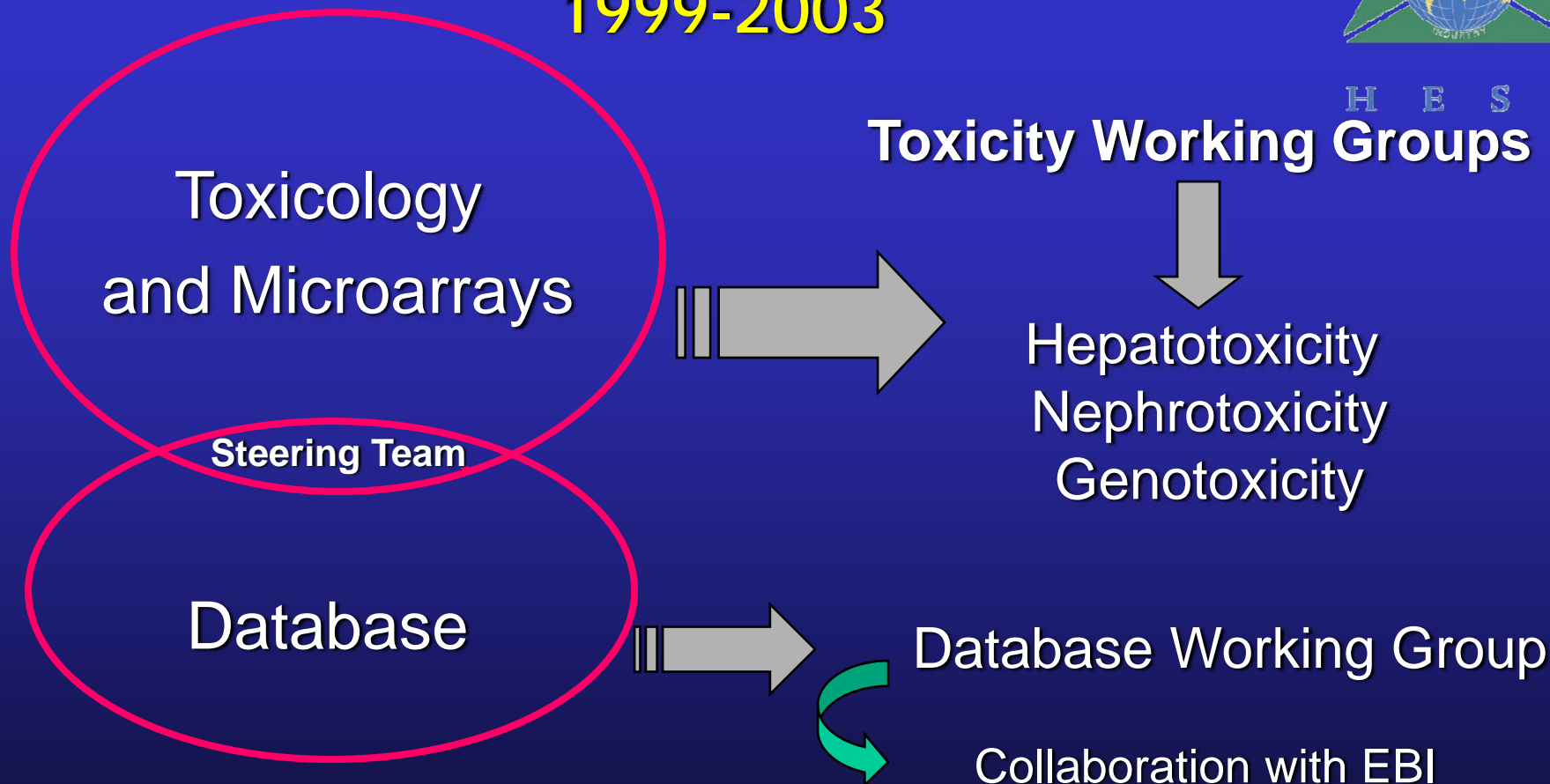
HESI COMMITTEE ON GENOMICS

Initial Program Activities

1999-2003



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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



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- ❖ 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

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Baseline Animal Database Program

Karol Thompson, Chairperson
CDER, US FDA

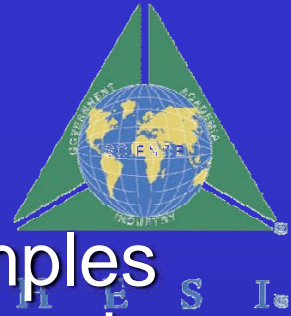
Key Objectives



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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/>



BID (Biomedical Investigation Database)

BID (Biomedical Investigation Database)

Home > Search

Home

Search >>

My Genes List

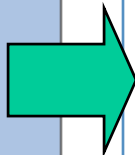
Logout

All Data

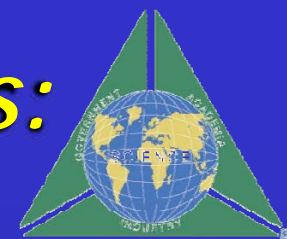
Hide: Investigation Study Groups

Investigation Study Groups Subjects

| | | | | |
|--------------------------|-------------------------|----------------------------|----------------------|--|
| <input type="checkbox"/> | Details | Conclusion | Data | ICI-60 |
| <input type="checkbox"/> | Details | Conclusion | Data | Mouse_Recombinant_Strains |
| <input type="checkbox"/> | Details | Conclusion | Data | Hyperoxia_Mouse_2004 |
| <input type="checkbox"/> | Details | Conclusion | Data | Pfizer-hepatocarcinogen panel |
| <input type="checkbox"/> | Details | Conclusion | Data | J&J Hepatotoxicant Library |
| <input type="checkbox"/> | Details | Conclusion | Data | HESI BaseLine |
| <input type="checkbox"/> | Details | Conclusion | Data | HESI Hepatotoxicity |
| <input type="checkbox"/> | Details | Conclusion | Data | HESI Nephrotoxicity |
| <input type="checkbox"/> | Details | Conclusion | Data | Iconix_Hepatotoxicants_Acute_SD |
| <input type="checkbox"/> | Details | Conclusion | Data | GeneAtlas |
| <input type="checkbox"/> | Details | Conclusion | Data | Sankyo Phenobarbital 2004 |



And Mirrored at Additional Sites:



Chemical Effects on Biological Systems:

<http://cebs.niehs.nih.gov/>

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EBI ArrayExpress:

<http://www.ebi.ac.uk/microarray-as/ae>

Experiment E-TOXM-39

You are logged in as *guest* [Login](#) » ArrayExpress [Help](#)

User *guest*, your query for Experiments
with accession = E-TOXM-39 produced
1
match

| | | | |
|-------|------------------------|---------------------|---|
| 1 / 1 | Experiment : E-TOXM-39 | Submitter(s) : HESI | Lab : Health and Environmental Sciences Institute |
|-------|------------------------|---------------------|---|

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 RAE230A [RAE230A], Affymetrix GeneChip® Rat Genome U34A [RG_U34A]], producing 517 raw data files and 3 transformed and/or normalized data files.

Conclusions



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- 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

Study Completed and Published



Publication Success - Highly Accessed!

“Sources of variation in baseline gene expression...”
BMC Genomics, 9:285, 2008.

BioMed Central | Full text | Sources of variation in baseline gene expression levels from toxic - Microsoft Internet Explorer

Address: <http://www.biomedcentral.com/1471-2164/9/285>

4th EMBO Conference
From Functional Genomics to Systems Biology
EMBL Heidelberg, Germany, 15 - 18 November 2008

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Research article

Sources of variation in baseline gene expression levels from toxicogenomics study control animals across multiple laboratories

Highly accessed Open Access

Michael J Boedigheimer¹, Russell D Wolfinger², Michael B Bass¹, Pierre R Bushel³, Jeff W Chou³, Matthew Cooper⁴, J Christopher Corton⁵, Jennifer Fostel³, Susan Hester⁵, Janice S Lee⁵, Fenglong Liu⁶, Jie Liu⁷, Hui-Rong Qian⁸, John Quackenbush^{6,9}, Syril Pettit¹⁰ and Karol L Thompson¹¹

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8 Eli Lilly and Co., Indianapolis, IN 46285, USA
9 Harvard School of Public Health, Boston, MA 02115, USA
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BMC Genomics 2008, **9**:285 doi:10.1186/1471-2164-9-285

The electronic version of this article is the complete one and can be found online at: <http://www.biomedcentral.com/1471-2164/9/285>

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Abstract

Background

The use of gene expression profiling in both clinical and laboratory settings would be enhanced by better characterization of variance due

Viewing options:

- Abstract
- Full text
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Associated material:

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Post to:

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- Connotea
- Del.icio.us
- Digg
- Facebook

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Genotoxicity Working Group

Dr. Jiri Aubrecht, Chairperson
Pfizer

Key Objectives



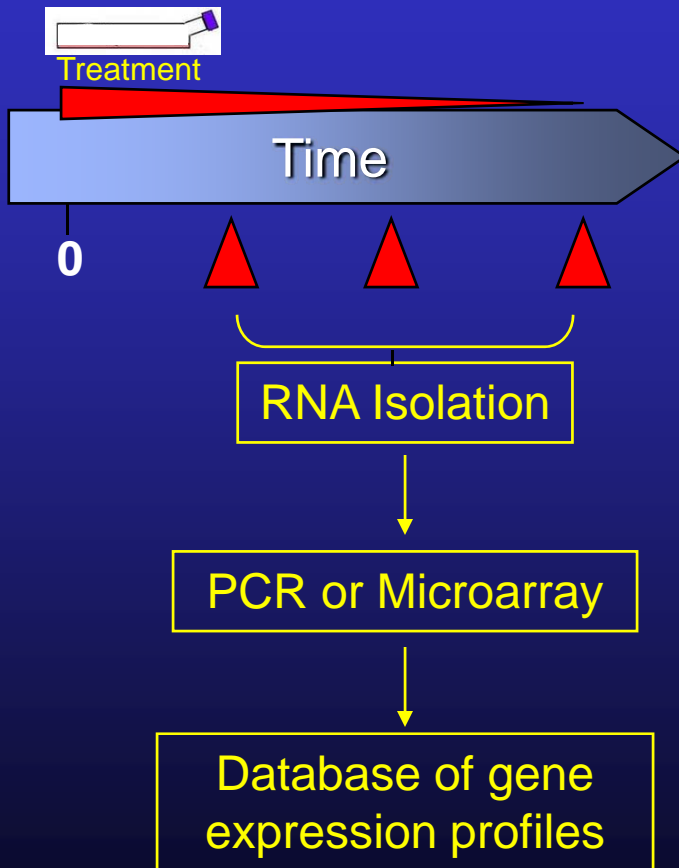
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1. Determine whether PCR- and microarray-based approaches can facilitate differentiation of direct and indirect acting genotoxins in *in vitro* assays
 - *Participating labs ran studies in-house using custom PCR assays and/or Affymetrix or Agilent 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. Microarrays**
 - QRT-PCR and Array data are comparable
 - Subset of genes measured with PCR is feasible but microarrays provide better mechanistic insight

Project Completion



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- 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.

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State of Science Survey Working Group

Alison Vickers, Chairperson
Allergan

Goals of Toxicogenomics Survey



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- ❖ 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



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- ❖ Manuscript undergoing final revisions.
- ❖ Anticipated to be submitted to *Environmental Health Perspectives* in early 2009.

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**Mechanism-Based Markers of Toxicity
Working Group**

Co-chairs: Dr Hisham Hamadeh (Amgen Inc.)
Dr Jon Lyon (GlaxoSmithKline)

Mechanism-based Markers of Toxicity Working Group Aims



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- 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...?



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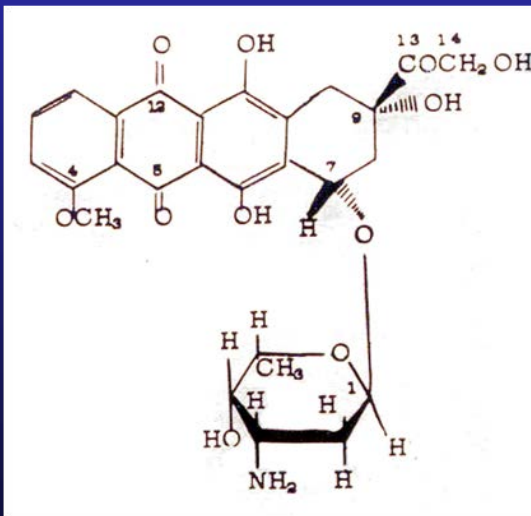
- Economy of scale
 - nature of study outside usual scope of individual organizations
- Open discussion between/within industry and regulators - identify optimum features
- Unique breadth of expertise to aid in design, execution and interpretation of the study

Doxorubicin (Adriamycin)



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- Treatment of solid and hematologic neoplasms but efficacy limited by delayed cardiotoxicity (significant clinical issue)
- Cardiomyopathy directly related to total cumulative dose



Doxorubicin

- 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



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

Protective intervention
*[Doxorubicin vs
Doxorubicin+Dexrazoxane]*

Tissue Specificity
*[Cardiac vs
Gastrocnemus]*

**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



- Set doses & dosing methods
- Check pharmacology & tox markers
 - Check TK
 - Samples preserved



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

Many Data Were Collected

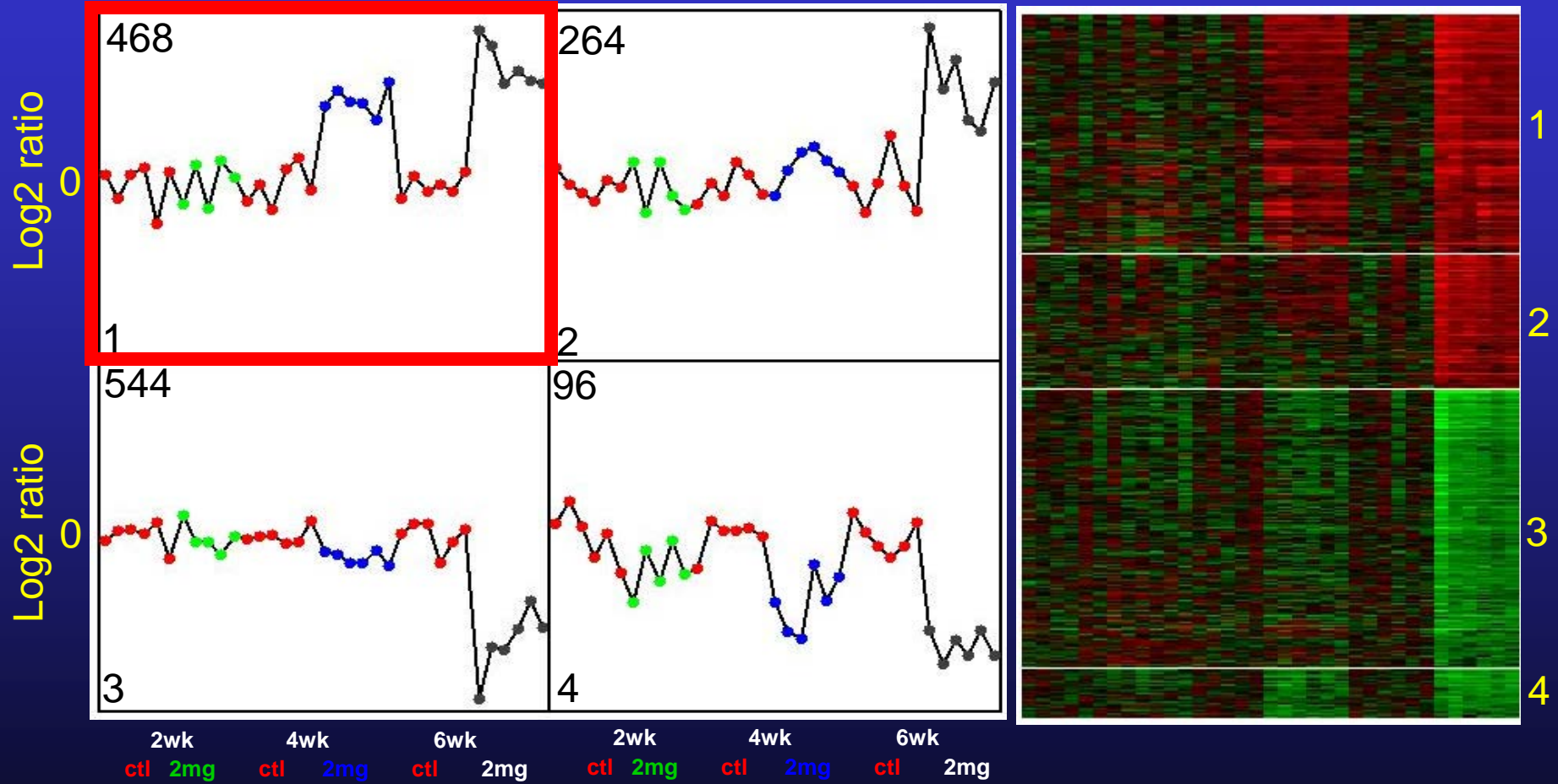


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- **Toxicokinetics**
- **Micronucleus**
- **Clinical pathology**
- **Histopathology**
 - **Full Heart Pathology**
 - **Gastrocnemus Path**
 - **Diaphragm Path**
- **Troponin I & T**
- **Gene expression**
 - **Agilent on heart and gastrocnemus (288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

Time-Dependent Gene Expression Increases at 2 mg/kg with Doxorubicin

Pattern number



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.

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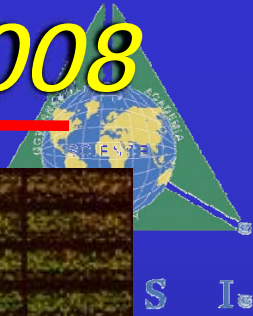


H E S I

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.

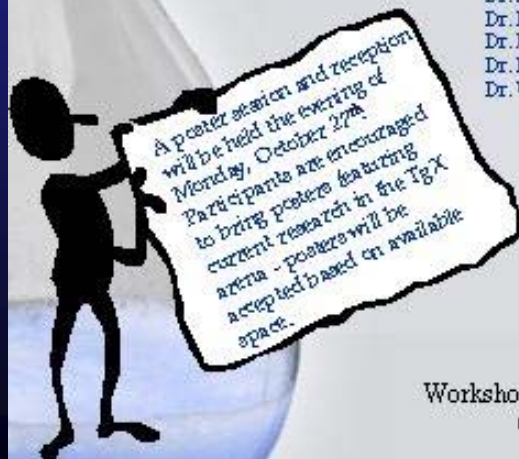
Workshop presenters include:

| | |
|---|---|
| Dr. Cynthia Afshari, Amgen Inc. | Dr. Donald Robertson, Bristol-Myers Squibb |
| Dr. Jiri Aubrecht, Pfizer | Dr. Allen Roses, Duke University |
| Dr. William Benson, U.S. Food & Drug Administration | Dr. Frank Sistare, Merck |
| Dr. Bruce Car, Bristol-Myers Squibb | Dr. Craig Thomas, Lilly Research Lab |
| Dr. Frederica Goodsaid, U.S. Food & Drug Administration | Dr. Russell Thomas, The Hammer Institutes for Health Sciences |
| Dr. Lois Lékman-McKeeman, Bristol-Myers Squibb | Dr. Jonathan Tugwood, AstraZeneca Pharmaceuticals |
| Dr. Ruth Lightfoot-Duran, Amgen Inc. | Dr. Paul Watkins, University of North Carolina |
| Dr. James MacDonald, Schering-Plough | Dr. Patrick Wier, GlaxoSmithKline |
| Dr. Rick Paules, National Institutes of Health | Dr. Douglas Wolf, U.S. Environmental Protection Agency |
| Dr. William Penne, Pfizer | |

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



Successful Workshop Held in Oct. 2008



- 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.

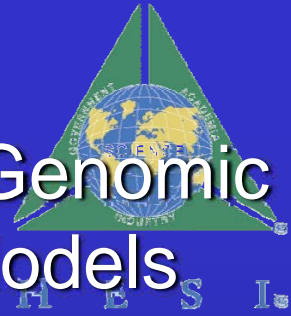
Manuscript Being Drafted And To Be Submitted To
Peer-Reviewed Journal In Early To Mid 2009

Programs in Development



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.

Programs in Development



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



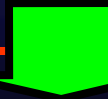
***TECHNICAL COMMITTEE ON H E S I
APPLICATION OF GENOMICS TO
MECHANISM-BASED RISK ASSESSMENT***

***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



HESI COMMITTEE ON GENOMICS

Key team members



H E S I

- 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
- Cyril Pettit, HESI



2008 Publications Overview



Sources of variation in baseline gene expression...

❖ Status: Published, *BMC Genomics*, 9:285, 2008.

❖ 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