# *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

Collaboration with EBI

**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*

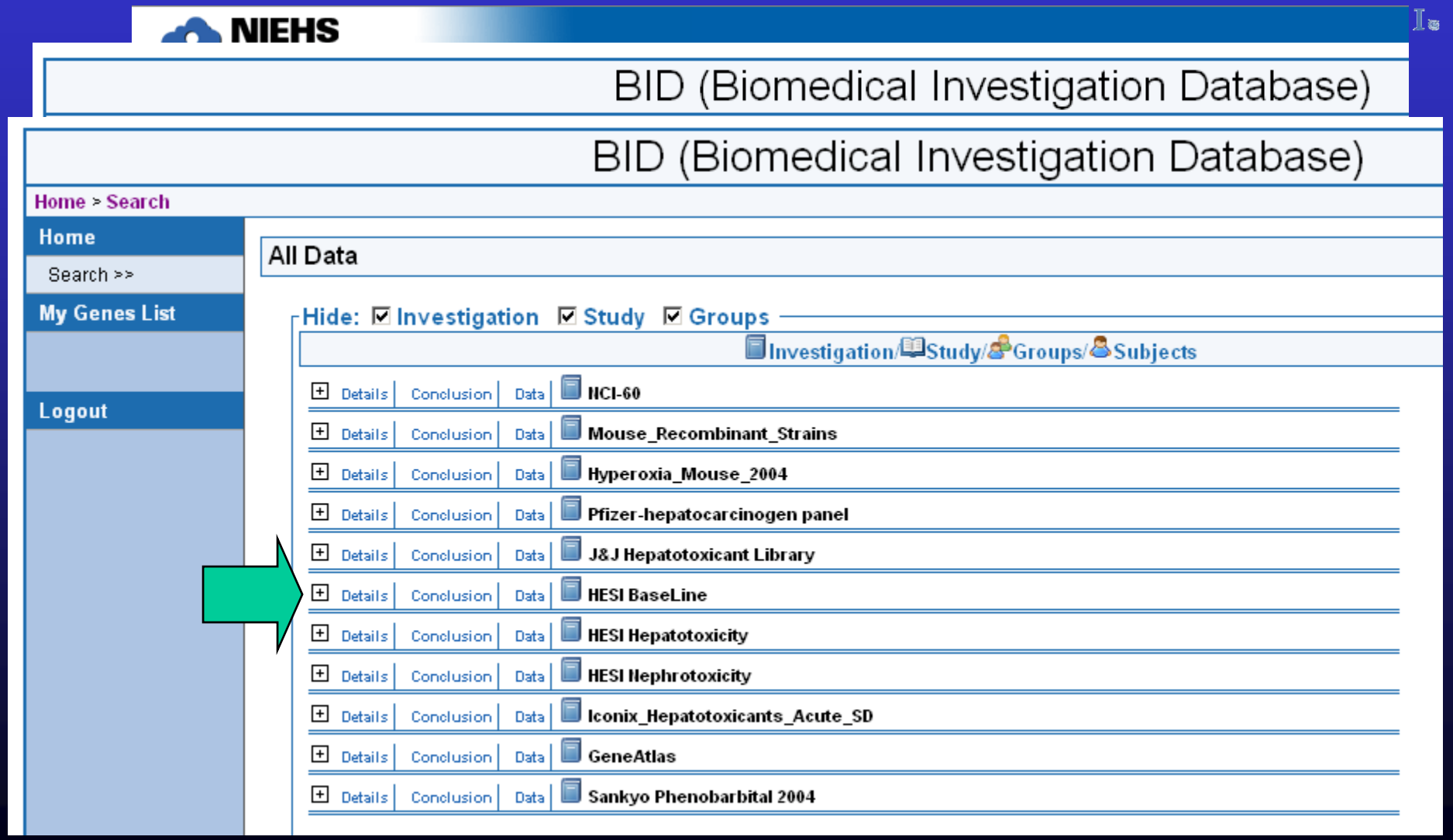
# Data Collection

- 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

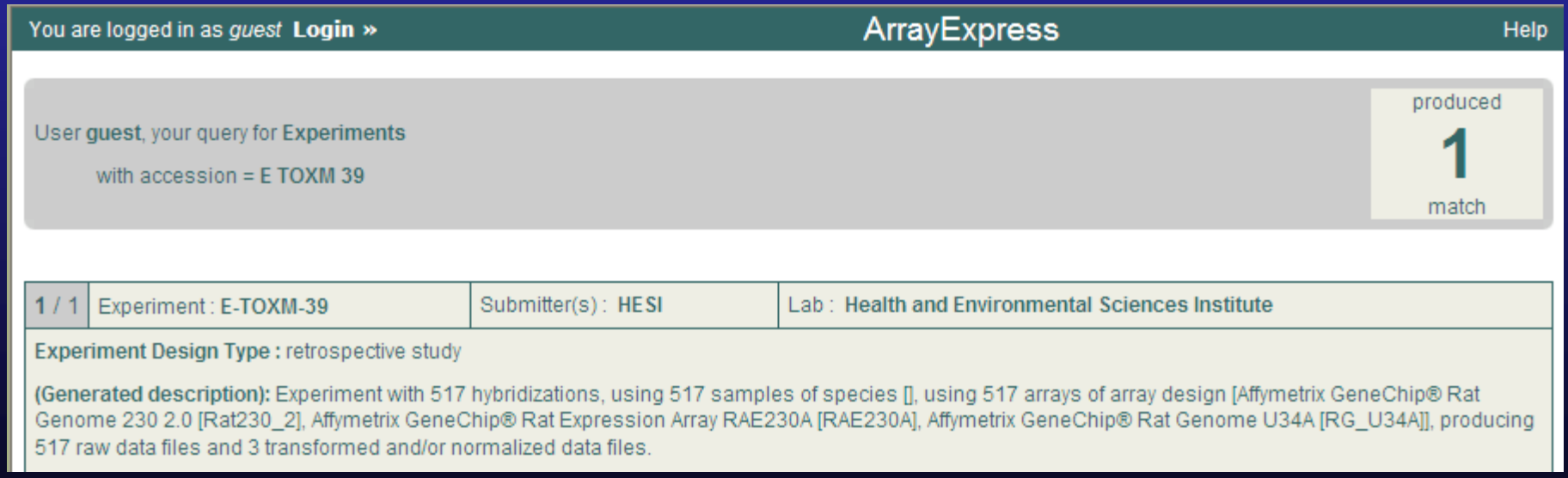
https://dir-apps.niehs.nih.gov/arc/



# *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



# Conclusions

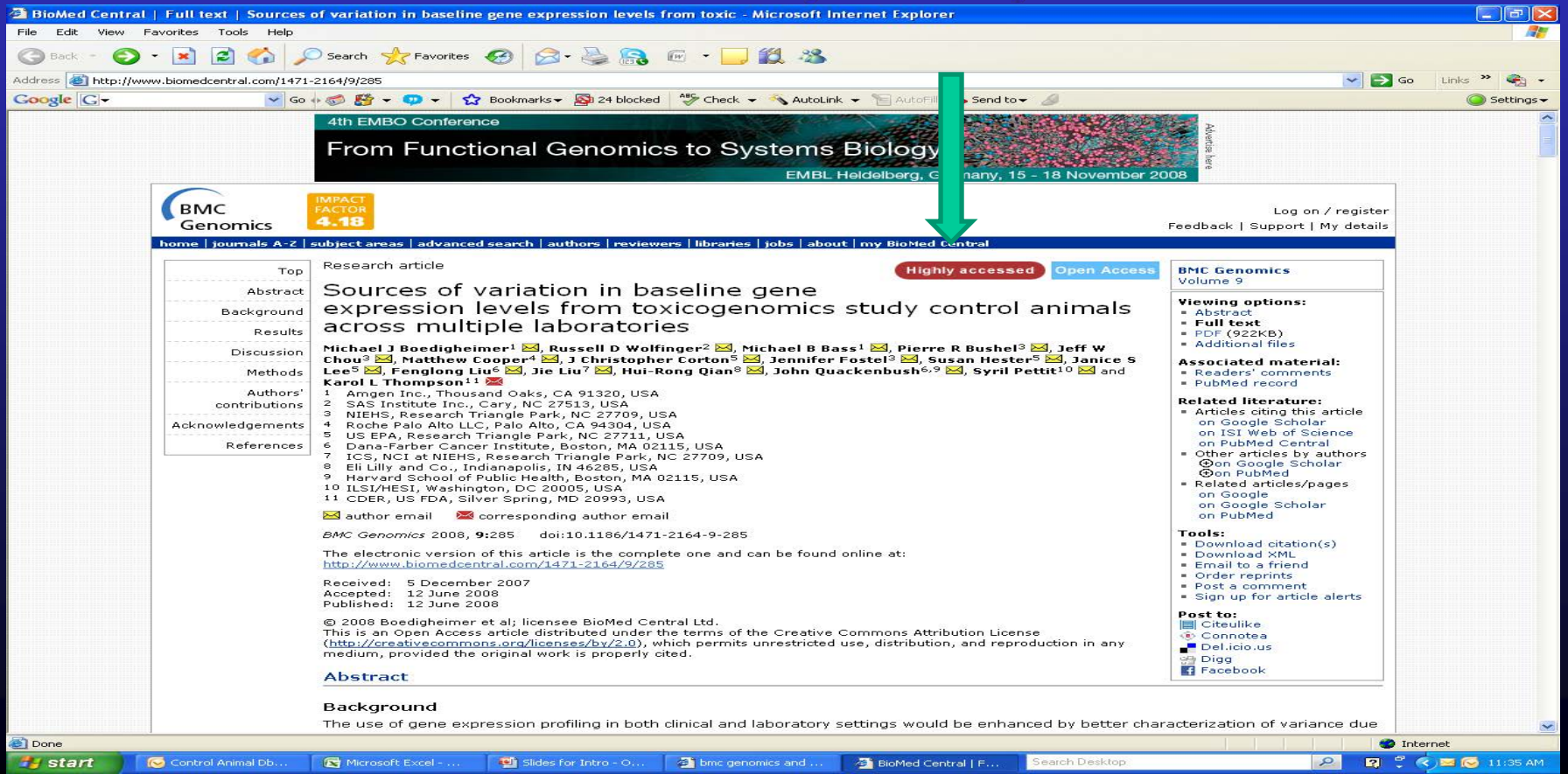
- A large dataset of control rat data from multiple sites that is linked to study parameter annotations can be used to:
  - Examine associations between study factors and gene expression variability
  - Identify genes with high and low variance in baseline expression
  - Identify pathways that contain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

# Study Completed and Published

## Publication Success - *Highly Accessed!*

"Sources of variation in baseline gene expression…"

*BMC Genomics, 9:285, 2008.*



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# Genotoxicity Working Group

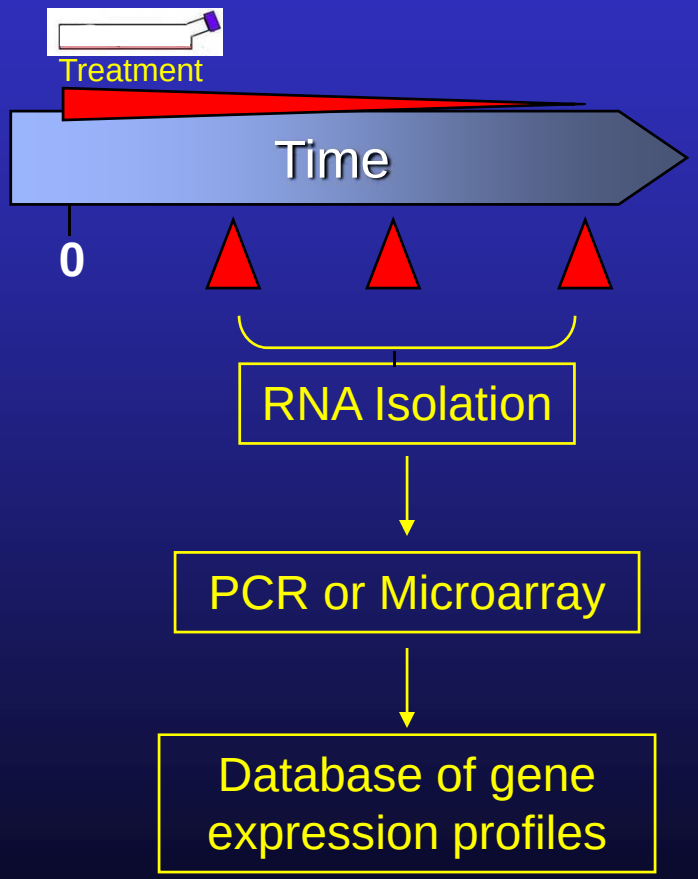
Dr. Jiri Aubrecht, Chairperson
Pfizer

# 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

# *Project Completion*

- 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*

- 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.

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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*

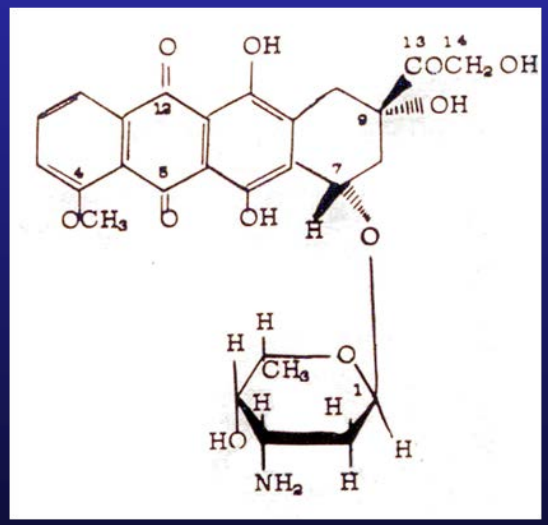
- Generate new molecular data on a toxicity of importance
  - move the science forward
  - potential to identify novel markers of toxicity
- Investigate wider toxicological concept - molecular threshold
  - study design to provide insight into relationship between time/dose, gene expression and onset 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 <u>but</u> efficacy limited by delayed cardiotoxicty (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


**Protective intervention**
***[Doxorubicin vs Doxorubicin+Dexrazoxane]***

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

**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

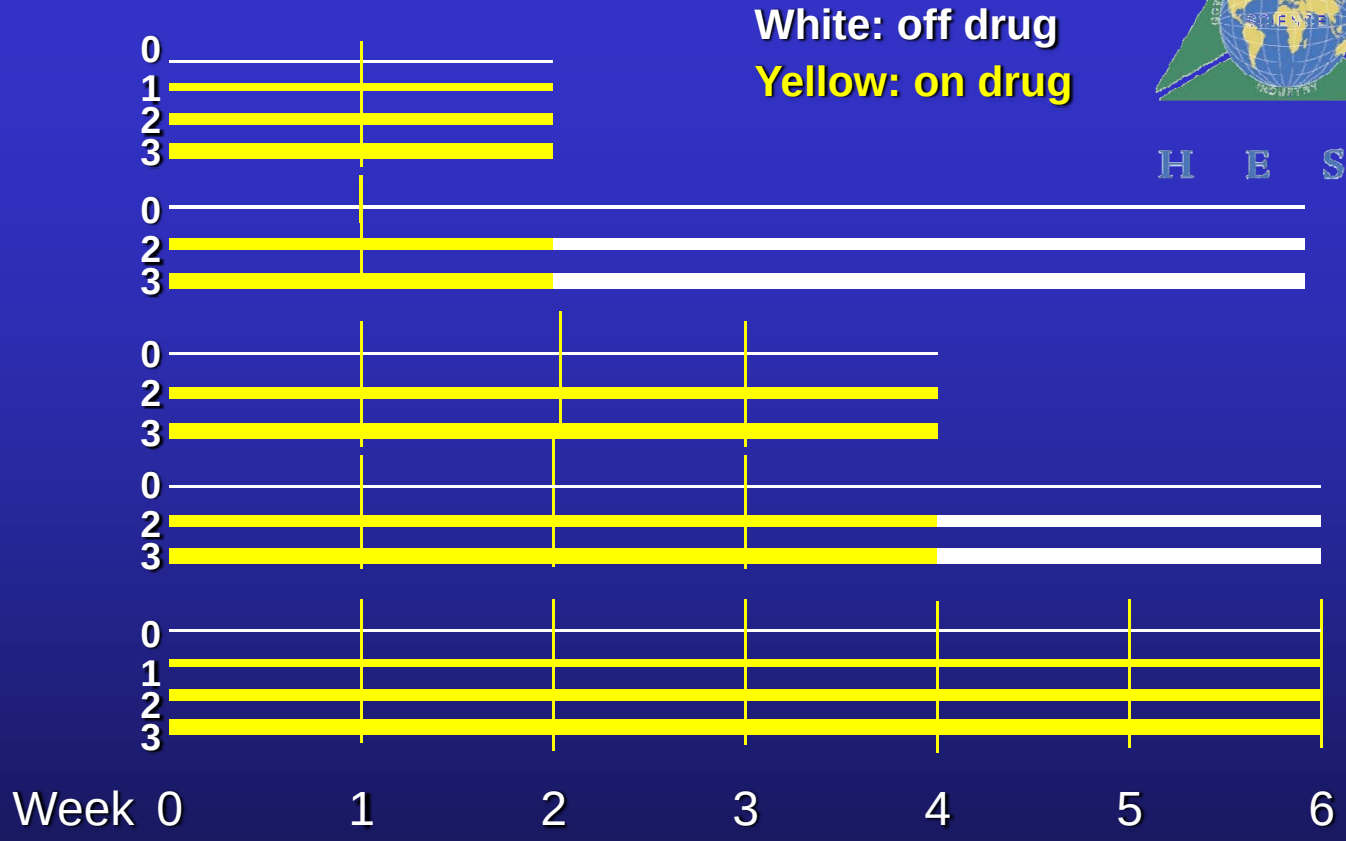
- 6 weekly doses, sacrifice 1 week after last dose
- Doxorubicin **0.5, 1, 2, 3** mg/kg/week
- Etoposide **0.5, 1, 3** mg/kg/week
- Dexrazoxane **50** mg/kg/week
- Doxo **2, 3** + Dexra **50** mg/kg/day

- 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

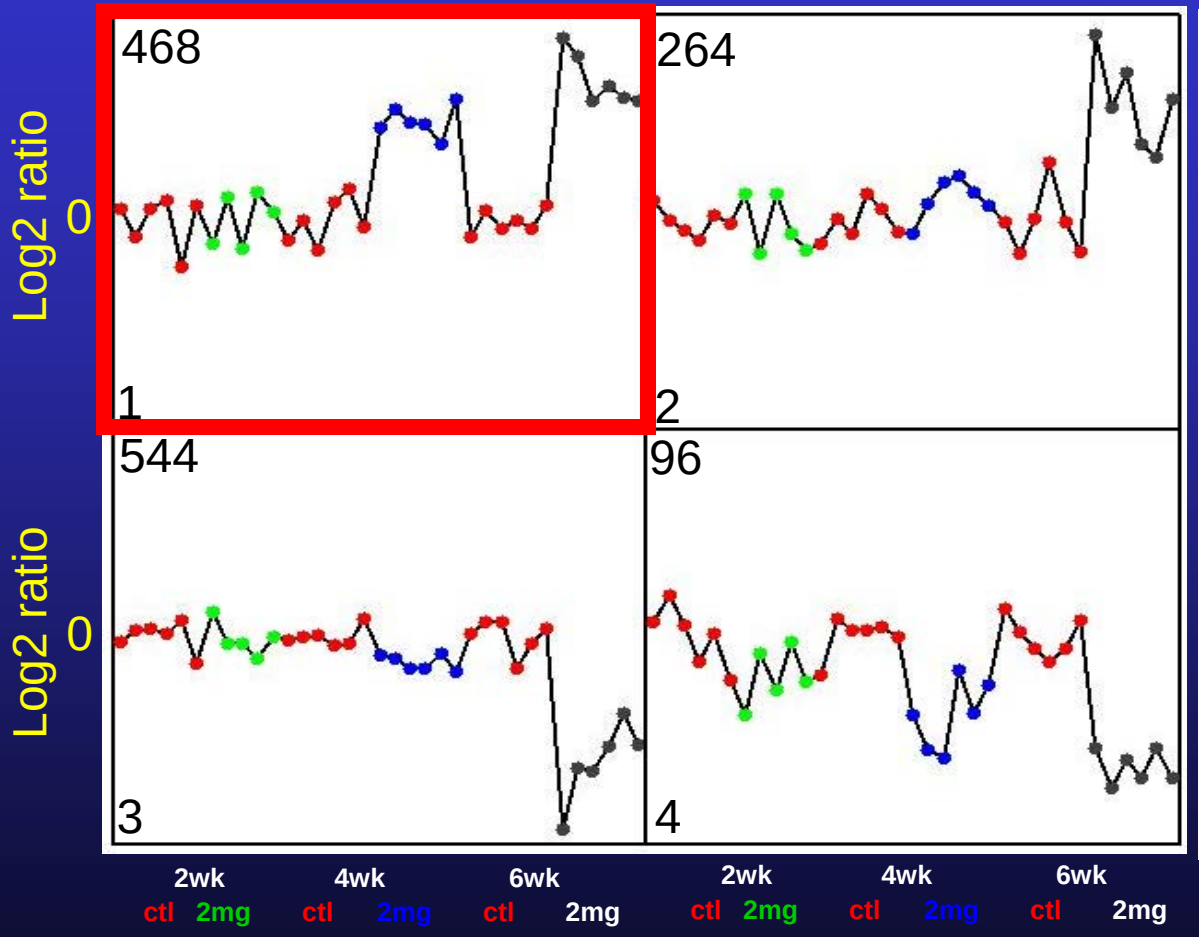
# Main Study Design

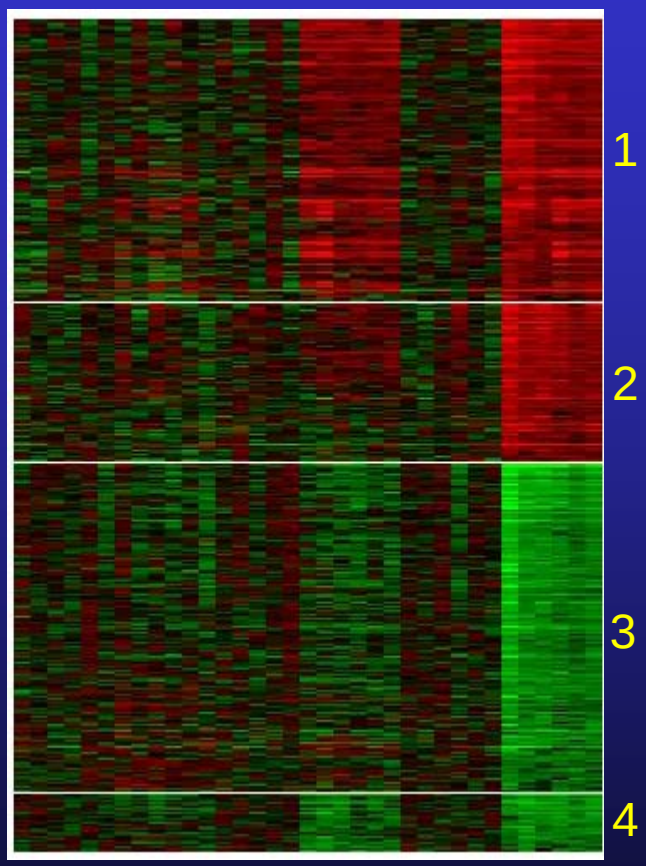


# *Many Data Were Collected*

- 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

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# *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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# Genomic Applications in Safety Studies - A Case Study Workshop

Dr. Cindy Afshari, Organizer
Amgen

# Successful Workshop Held in 2008

# *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 APPLICATION OF GENOMICS TO MECHANISM-BASED RISK ASSESSMENT

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***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

- 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…

- ❖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