Genomics as a Tool in Toxicology – Present and Future

WB Mattes, PhD, DABT

Senior Scientific Director, Toxicogenomics

Gene Logic, Inc

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

immunotoxicogenomics

Resources

- National Center for Biotechnology Information
    - *Education:*
      http://www.ncbi.nlm.nih.gov/Education/index.html
    - *Mini-Courses:*
      http://www.ncbi.nlm.nih.gov/Class/minicourses/=
    - *Science Primer:*
      http://www.ncbi.nlm.nih.gov/About/primer/index.html

- Other
    - *Genes in Action (Special Science Issue:*
      http://www.sciencemag.org/sciext/genome2004/
    - *Learning About Genomics:*
      http://www.123genomics.com/files/learning.html

- Genetics:
  - *The study of inheritance patterns of specific traits.*
    - (Human Genome Project – Genome Glossary)
      - http://www.ornl.gov/sci/techresources/Human_Genome/glossary/

- # Genomics

  - *The study of genes and their function.*
    - (Human Genome Project – Genome Glossary)

  - *The study of genomes, which includes genome mapping, gene sequencing and gene function.*
    - (Biotech Life Science Dictionary)
      - http://biotech.icmb.utexas.edu/search/dict-search.html

  - *Genomics is trying to look at all the genes as a dynamic system, over time, to determine how they interact and influence biological pathways, networks and physiology, in a much more global sense.*
    - (CHI Genomic Glossary)
      - http://www.genomicglossaries.com/content/Basic_Genetic_Gloss aries.asp

  - *"Transcript Profiling": studies determining the levels of tens to thousands of mRNAs (transcripts) under experimental conditions*
    - (Mattes)

- Bioinformatics
  - *Roughly, bioinformatics describes any use of computers to handle biological information. In practice the definition used by most people is narrower; bioinformatics to them is a synonym for "computational molecular biology"- the use of computers to characterize the molecular components of living things.*
    - (Bioinformatics Web)
      - http://www.geocities.com/bioinformaticsweb/definition.html
  - *"The mathematical, statistical and computing methods that aim to solve biological problems using DNA and amino acid sequences and related information.*
    - (Fredj Tekaia at the Institut Pasteur)
      - http://www.geocities.com/bioinformaticsweb/definition.html

- Microarray

  - *A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide containing samples of many genes arranged in a regular pattern.*
    - (NCBI – Microarrays Factsheet)
      - http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html

  - *A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated.*
    - (NCBI – Microarrays Factsheet)

References

- Mechanistic Toxicology: The Molecular Basis of How Chemicals Disrupt Biological Targets by Urs A. Boelsterli. CRC Press; 2002, 312 pages ISBN: 0415284597

- Toxicogenomics: Principles and Applications Hisham K. Hamadeh (Editor), Cynthia A. Afshari (Editor) ISBN: 0-471-43417-

- Toxicogenomics and Proteomics Volume 356 NATO Science Series: Life and Behavioural Sciences Edited by: J.J. Valdes 2004, 216 pp., Hardcover ISBN: 1 58603 402 2

- An Introduction to Toxicogenomics by Michael E. Burczynski: 348 pages ; CRC Press; (March 1, 2003) ISBN: 084931334

- Comprehensive Toxicology, Volume 14: Cellular and Molecular Toxicology, Edited by J.P. Vanden Heuvel, W.F. Greenlee, G.H. Perdew, and W.B. Mattes Elsevier, 2002 648 pages, ISBN: 0-444-50868-6

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Nuts and Bolts – Recipe for a Microarray Experiment

- Get genes (and sequences)

- Prepare a cDNA or oligonucleotide microarray

- Run your experiment

- Hybridize RNA samples to your microarray

- Finally analyze your results

Recipe: Get genes (and sequences)

1. Grind up a biological sample in something like phenol and isolate RNA

2. Make a pot of complementary DNA: Use Oligo-dT and Reverse Transcriptase to make a DNA copy of each and every mRNA

3. Ligate each individual cDNA molecule into a plasmid vector, so as to clone it into E. coli (the plasmid carries an antibiotic resistance gene as well)

4. Introduce this pot of cDNA-containing plasmids to receptive E. coli, such that no more than one molecule gets into a single bacterium

5. Plate the bacteria onto antibiotic-containing medium – only those bacteria with a plasmid grow

6. Next morning you hopefully have thousands of colonies – each with a different cDNA in it. Transfer each colony to a tube, grow enough to isolate plasmid DNA.

7. Subject each separate plasmid DNA to automated DNA sequencing

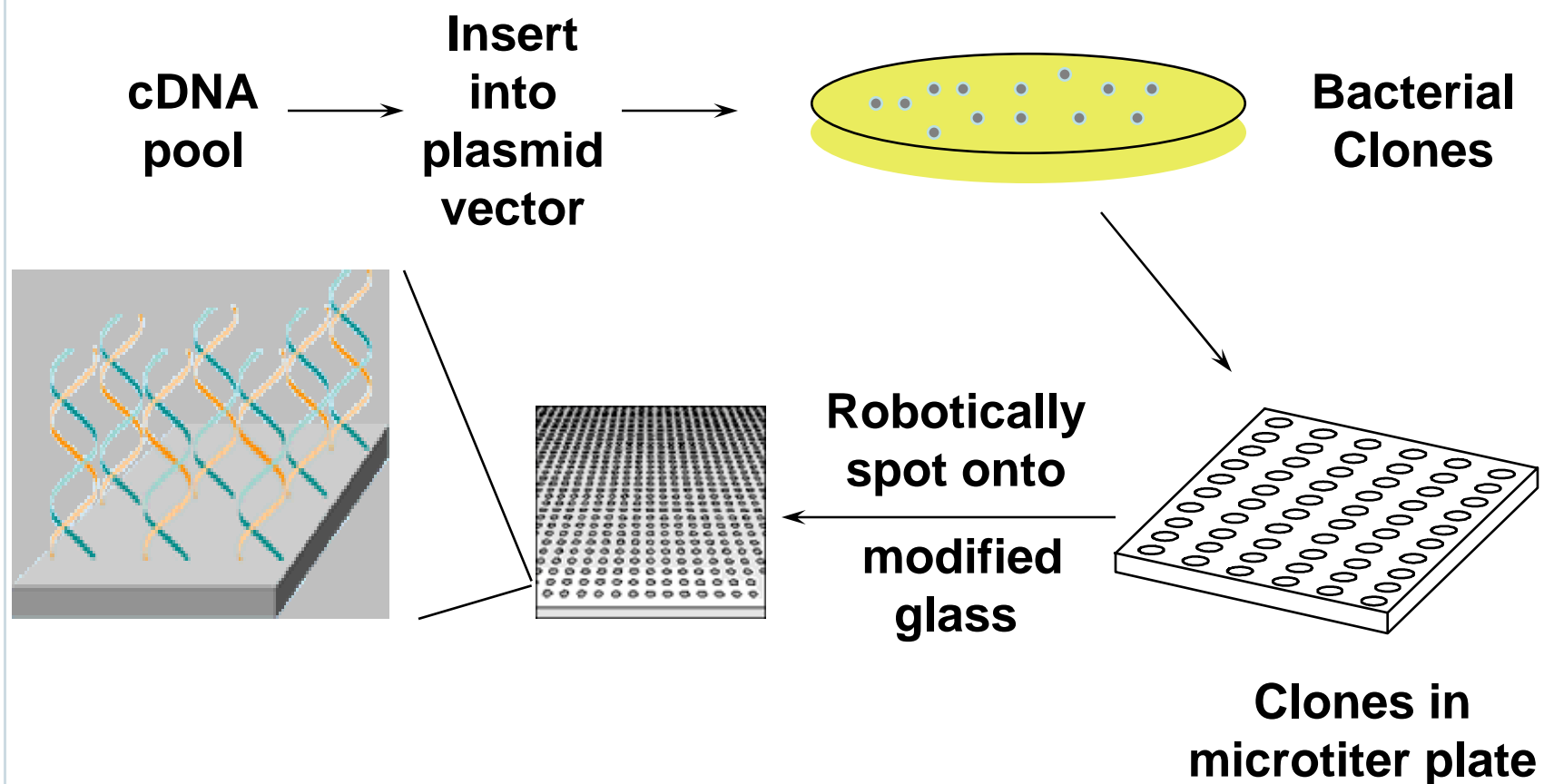8. Result: thousands of plasmid-cDNA samples of known sequence

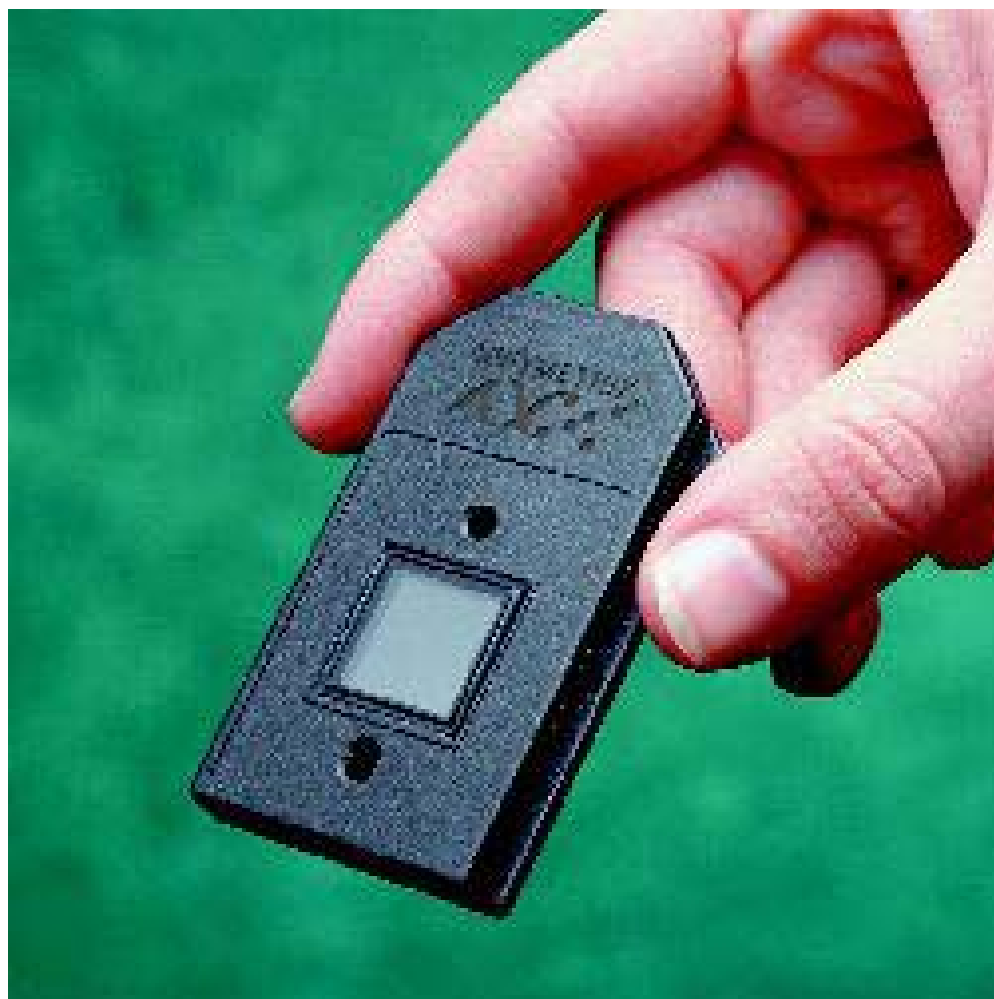# Recipe: Prepare a cDNA or oligonucleotide microarray

1. **cDNA Microarray**: start with the cDNA samples

2. Use a robotic "arrayer" that places nL aliquots of each cDNA sample onto specially-treated glass slides

3. **Oligonucleotide microarray**: start with the sequences of the cDNA samples

4. Oligonucleotides corresponding to 25-60 bases of the cDNA sequence are actually synthesized on a glass slide or silicon chip (Affymetrix)

5. **Either way** you end up with a glass substrate with thousands of smalls pieces of DNA, each of precisely known sequence, and located at a precisely known position (spot) on the array

cDNA Libraries & Arrays

cDNA pool → Insert into plasmid vector → Bacterial Clones

Robotically spot onto modified glass ← Clones in microtiter plate

Affymetrix GeneChip®

innunotoxicogenomics

Recipe: Now run your experiment

1. Run control and experimental samples, <u>with replicates</u>

2. Isolate RNA from each sample

3. QC step: Make sure the RNA is of good quality – not degraded!

4. Using a protocol not detailed here, convert your RNA to cDNA and then tag it with a fluorescent tag
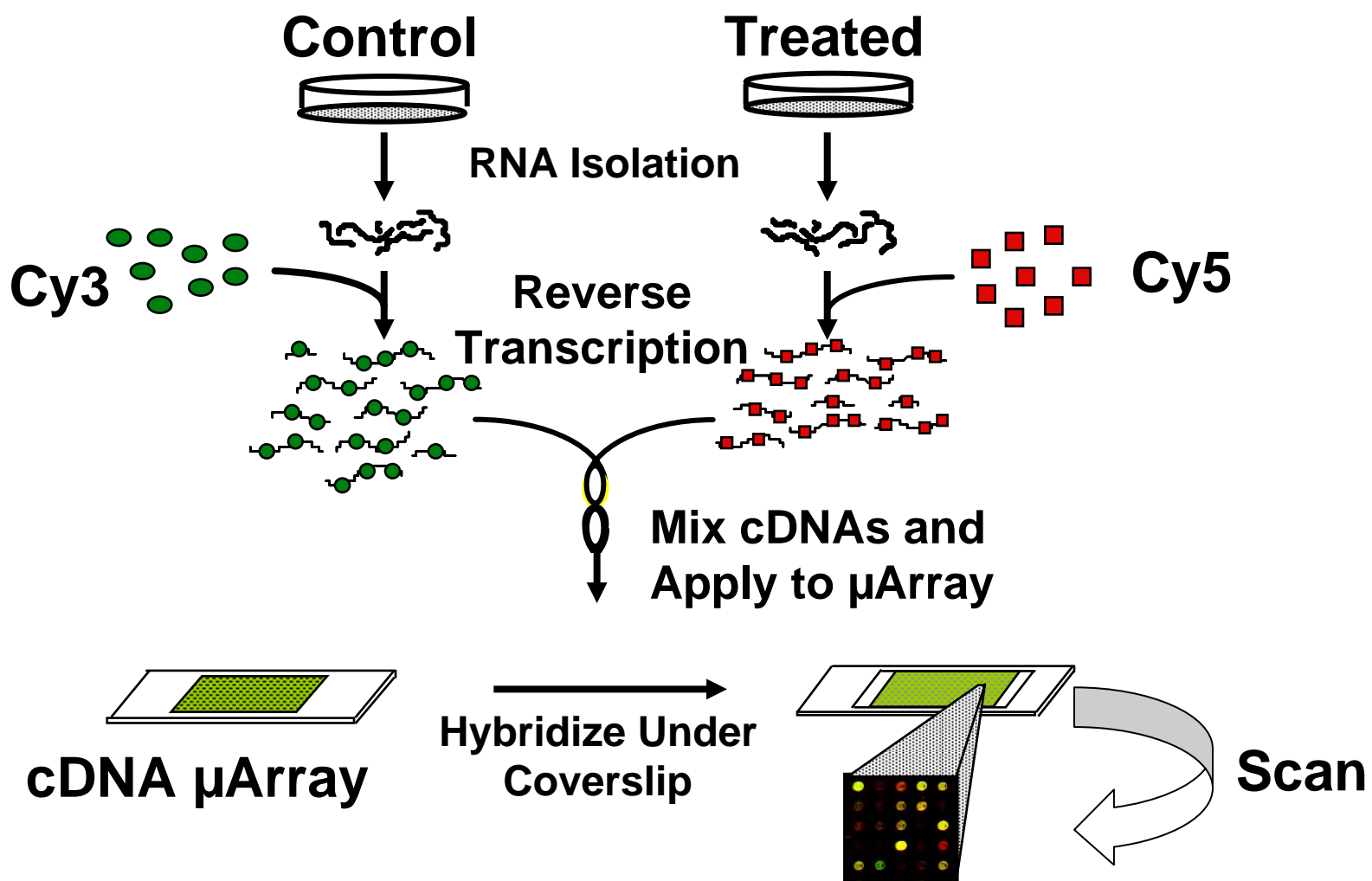
1. **cDNA Microarray**: the control sample is labeled with a green dye, the experimental sample with a red dye

2. The two samples are mixed, applied to the microarray slide, and competitively hybridized to the DNA bound to the slide

3. The ratio of red to green at each spot indicates the ratio of the levels of RNA in the experimental sample to that in the control sample

4. **Oligonucleotide Microarray**: each sample is labeled with the same dye

5. The two samples are applied to two different slides (chips) and hybridized to the oligonucleotides bound to the slide

6. The fluorescent intensity at each spot indicates the level of mRNA in the sample being measured

7. **Both Arrays**: The fluorescence at each spot is determined with a confocal laser scanner. The output is analyzed by a computer and sophisticated software is required to convert the confocal laser image to numbers for individual spots

Gene Expression Analysis Using cDNA Microarrays

Control

Treated

RNA Isolation

Cy3

Cy5

Reverse Transcription

Mix cDNAs and Apply to µArray

cDNA µArray

Hybridize Under Coverslip

Scan

immunotoxicogenomics

Recipe: Analyze your results

1. The data from one slide / chip must be normalized to data from other chips (and sometimes to other experiments)

2. Quality steps include checking for bad chips / bad samples by high level statistical approaches, such as Hierarchical Cluster Analysis (HCA) or Principal Component Analysis (PCA). Both these approaches take into account ALL the data at once and look for "similarities" and differences.

3. Approaches to analyses vary widely, and remain a wonderful source of study and employment for statisticians. The basic problem is that if you are measuring the changes in mRNA levels for e.g. 8800 genes, by chance, the levels for several hundred will appear changed!

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Risk Assessment

- Hazard Identification
  - SAR
  - In vitro tests
  - Animal bioassays
  - Epidemiology

- Risk Characterization
  - Dose-response assessment
  - Exposure assessment
  - Cross-species comparisons (XSC)
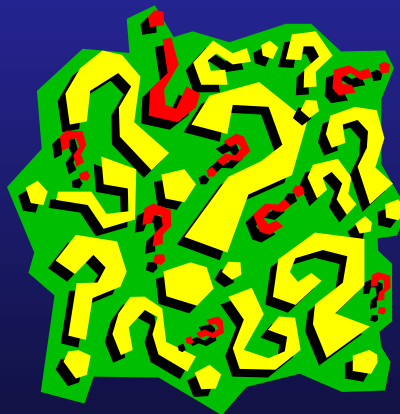 - Susceptibility in limited populations

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

How does HESI Work?

- **International, non-profit scientific organization**

- **A global branch of the ILSI organization**

- **Multi-sector committees address leading scientific issues.**

- **Coordinates production of high quality scientific papers, meetings, and research. HESI itself is not a laboratory.**

- **Transparent - All work is published and publicly available.**

- **Diverse - Academic advisors and government scientists are critical participants in projects and have significant input.**

24

HESI Genomics Committee is…

- **FIRST** large-scale collaborative experimental program on toxicogenomics
- Large number of participants (~30 organizations) from regulated industry, academic, and gov't labs
- Offers practical insights into data exchange issues
- Cross platform comparison
- Cross laboratory comparisons
- Database/Data Exchange Experience
- Multi-Sector Discussion Forum

26

Committee Partic...
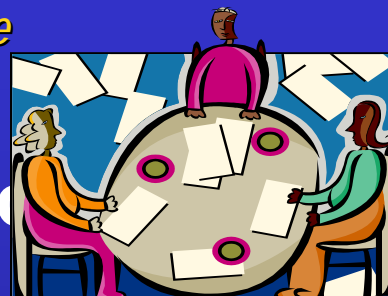
- **Private Sector:** 21 Pharmaceutical, 4 Chemical/Ag Chemical, 1 Consumer Product

- **Academia:** TIGR, Michigan State, U. Surrey, U. Wisconsin

- **Government:** EPA, FDA, NIH, NIEHS, Air Force, EMEA, Japan NIHS, RIVM

In 1999, the HESI Genomics Committee formed to address leading issues in the emerging field of toxicogenomics…

Many similar issues to those facing new field of immunomics now….
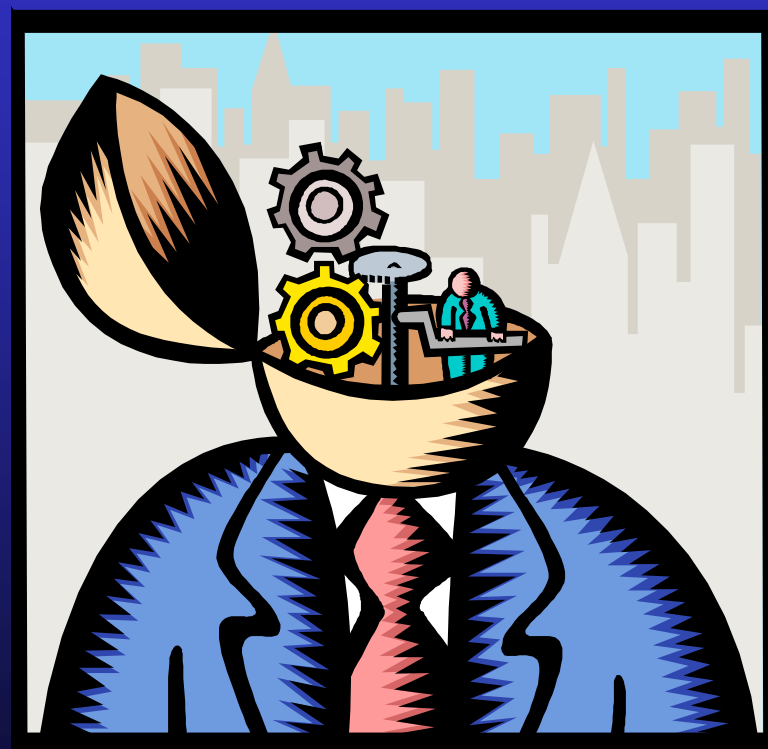
Barriers to Toxicogenomics in Risk Assessment

1999

- 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 transcription products relate to toxicity
- Uncertain regulatory applications

HESI COMMITTEE ON GENOMICS

29

How did phase one of the HESI program operate (1999-2003)?
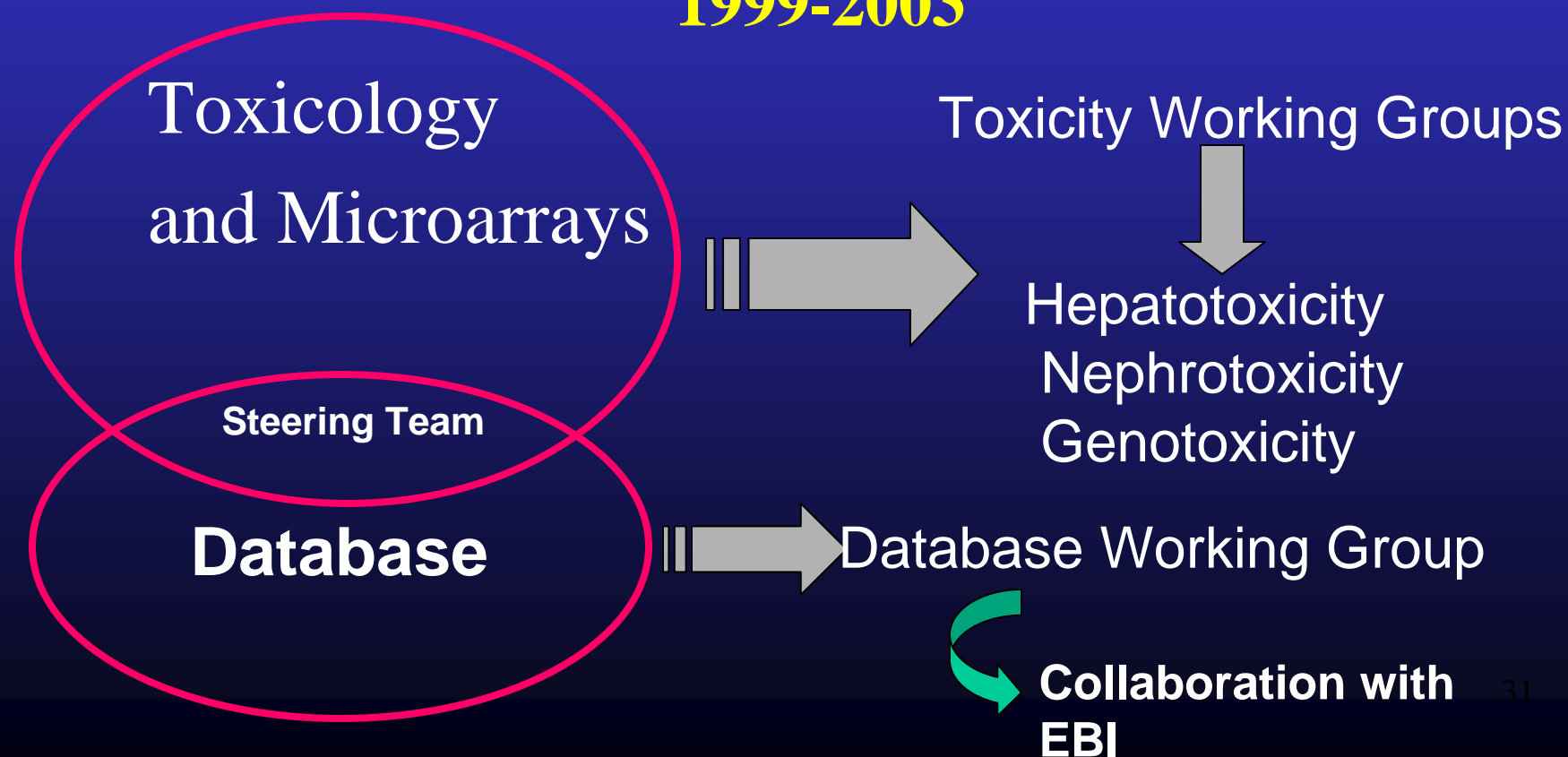
Experimental Component:
Common Design Features

- Well-studied compounds - known toxicity profiles/biological parameters

- Investigate temporal relationships

- Low vs. high dose response relationships – multiple dose levels

- Variability – how many replicates needed?

- Compare SOPs from lab to lab

- Compare different types of platforms

Committee Consensus: Technical and Biological Interpretation -1

- **Pathway level analysis was consistent across laboratories and platforms; gene by gene comparisons challenging**

- **Gene expression analysis a valuable tool for identifying biological pathways of interest**

- **Genomic data is not a 'stand-alone' – follow-up required.**

33

Committee Consensus: Technical and Biological Interpretation -2

- 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 only do not in themselves equate to meaningful biology (either adverse or adaptive)

The Collaborative Approach…

Benefits

- Opportunity to build scientific consensus & ID disagreements
- Many replicates and pooled resources
- Parallels 'real world' variation in lab conditions, experimental approaches, and analytical techniques
- Diverse expertise and perspectives shared
- Offers insights into challenge of multi-site info exchange

Drawbacks

- More coordination required
- Can be a lengthy process

35

For More Information….

➤ Publication of <u>12 technical and overview</u> 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 ArrayExpress dbase – public release pending

36

New Programs Underway in

2005

– **Survey on State of Application of Genomics**

- Activity will undertake a broad multi-sector survey to identify perceptions and experience in the application of genomics to safety evaluation. Results published, public discussion.

– **Genomics for Identifying Markers of Toxicity**

- A novel experimental program that will focus on a genomics study of cardiac toxicity in rodents with sufficient depth to explore gene expression and other measurable changes (pathology, toxicokinetics, troponin levels, etc.) at the 'point of departure'.

37

New Programs for 2004-2005, cont'd

– **Database of Baseline Genomic Animal Data**

- Project will compile and analyze baseline (e.g., untreated or control) microarray data from rats to characterize 'normal' patterns of gene expression.

– **Genomics for Elucidating Genotoxic Mechanisms**

- Experimental program will use RT-PCR and microarray to facilitate characterization of genotoxic and non-genotoxic mechanisms of toxicity following positive response in *in vitro* chrom ab assay;

38

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Cost

- Toxicogenomics experiments are <u>very</u> expensive:
  - A <u>single</u> microarray (chip) alone costs ~$1000
  - Such costs do not include:
    - Reagents
    - Equipment (scanners, hybridization ovens)
    - **Analysis software (~$10,000 or more)**
    - **Analysis time**

- **No Gut$, No Glory**
  - Standard study: **>$18,000 in chips alone!**
    - Control group, two treatment groups (two doses)
    - Two time points (sacrifice times)
    - Replicates of three

- Replicates <u>are required</u>, as in any good study!

Study Design

- **Toxicogenomics experiments require good study design!**
  - Biological replicates ($\geq$3)
  - **Time <u>and</u> dose ranging**

  - Certain gene expression responses may be transitory
    - Early gene expression may be part of an "initiating" event
  - Certain doses may not elicit a gene expression response
    - Gene expression <u>does</u> have a NOEL

    Parfett et al 1998 In Vitro Toxicology 1996;9:403-417.
    Tygstrup etl al Biochem Biophys Res Commun. 2002 Jan 11;290(1):518-25.
    Hamadeh etl al Toxicol Sci. 2002 Jun;67(2):219-31.
    Boverhof et al Toxicol Sci. 2005 Jun;85(2):1048-63.

Sample Collection

- Considerations with "heterogeneous" tissues
  - Different cell populations may have different gene expression profiles

    Forsberg etl al PLoS Genet. 2005 Sep 2;1(3):e28

  - Global tissue changes in gene expression may reflect changes in cell populations
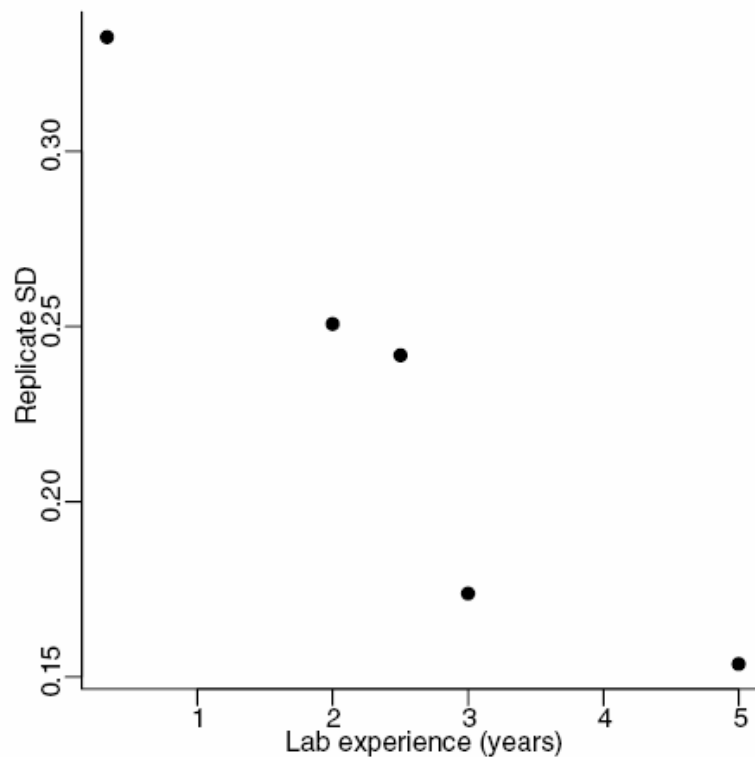
    Hamadeh et al 2002 (BioTechniques 32:322-329 (February 2002)

    Mallakin et al Am J Respir Cell Mol Biol. 2005 Sep 15

- Necropsy procedures need to accommodate rapid tissue freezing and preservation
  - Good RNA <u>can</u> be obtained even from pancreas!

- One simple theme is important:

Experience Matters

See Irizarry et al
Nat Methods. 2005
May;2(5):345-50.

- "It's the informatics, period!"

INFORMATICS → Biological Explanation

Experiment ? ⇢
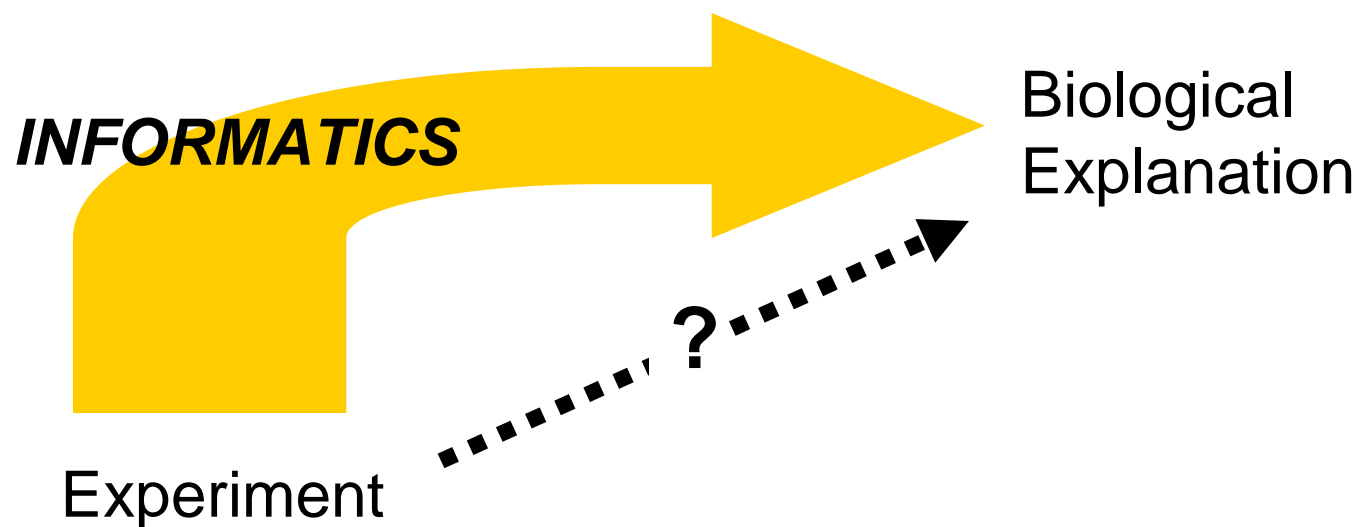
- And it's awfully tempting to take shortcuts!

Informatics – Not Just One Area

- Statistical analysis and filtering
  - What signals are "real"

- Sequence QC and annotation
  - What do these signals mean

- Data storage / database
  - How can I handle all this data

Statistical Analyses

- Many good articles on analytical approaches are available

    Imbeaud and Auffray Drug Discov Today. 2005 Sept 1;10(17):1175-1182.

    Butte Nat Rev Drug Discov. 2002 Dec;1(12):951-60.

- Bad analyses can lead to bad conclusions, i.e. microarrays are unreliable!

    Shi et al BMC Bioinformatics. 2005 Jul 15;6 Suppl 2:S12.

Statistical Analyses (cont)

- Good analyses show microarray experiments to be comparable
  - Shi et al BMC Bioinformatics. 2005 Jul 15;6 Suppl 2:S12.
  - Irizarry Nat Methods. 2005 May;2(5):345-50. Epub 2005 Apr 21.
  - Larkin et al Nat Methods. 2005 May;2(5):337-44. Epub 2005 Apr 21.
  - Bammler et al Nat Methods. 2005 May;2(5):351-6. Epub 2005 Apr 21.

- A defined data set suggests that <u>both</u> a statistical filter <u>and</u> a fold-change cut-off is important for assuring "real" results
  - Choe et al Genome Biol. 2005;6(2):R16. Epub 2005 Jan 28.

- At Gene Logic we employ <u>both</u> a statistical filter <u>and</u> a fold-change cut-off for mechanistic analysis

Array Annotation

- Example: Probe-set rc_AA800054_at

  - Affymetrix annotation:
    EST189551 Rattus norvegicus cDNA, 3 end

  - Updated annotation:
    Rpl19, ribosomal protein L19

- *Accurate annotation avoids misleading interpretations of data*

Array Quality Check

- Example: Affymetrix probe set 99172_at
  - Annotated as "Transcription factor A, mitochondrial (TFAm)"
  - Compare to Reference Sequence NM_009360 (TFAm)

```
                    460         470         480         490
    NM_009360   AGCTAACTCCAAGTCAGCTGATGGG-TATGGAGAAGGAGGCC
                :::: : :::: : ::::: : ::  :::: :: :: : :::
    99172_at    AGCTTA-TCCATGACAGCTAAAGGCCTATGCAGGAGAAAGCC
                 30          40          50          60
```

  - Clearly signals from this probe set are NOT TFAm

Essential Informatics Steps

1. Have replicate samples !!
   - No guts ($$), no glory !
2. Compare array output for good behavior
   - Inspect image
   - Check overall signal intensity
   - Scatterplot matrix
   - Correlation coefficient
   - Principle Component Analysis
3. Normalize array signals

iMMUNOTOXiCOGENOMiCS

Essential Informatics Steps *(cont)*

4.   Examine arrays for patterns of variability
    –   Principle component analysis
    –   Global clustering (hierarchical, k-means)
5.   Use distinction calculation to identify transcriptional events unique to a given group
6.   Use profile searches to identify transcriptional events with a desired pattern
    –   Dose response
    –   Temporal response
    –   Tissue response

immunotoxicogenomics

Essential Informatics Steps *(cont)*

8. Confirm and update annotation for probes / probe-sets of interest
9. Confirm sequence accuracy for for probes / probe-sets of interest
10. THEN …infer biology.

Challenges of Toxicogenomics

- Dose makes the poison: $\Rightarrow$ ALL 'drugs' are toxic!

- Toxicogenomic experiments include biologically active (ie toxic) doses

- Challenge can be correlating transcription changes with biological events related to toxicity
  - TxP responses participating in cellular toxicity
  - TxP responses secondary to cellular toxicity
 - TxP responses *unrelated* to cellular toxicity

Non-Specific Responses – Example

- Wanted to determine mechanism of toxicity for a compound class with a defined target organ

- Toxicity may be modeled in an *in vitro* cell system with 24 hour treatment

- Cells were treated with a model compound or *it's toxicologically inactive enantiomer*

- Samples were collected at several times for toxicogenomic analysis with Affymetrix microarrays
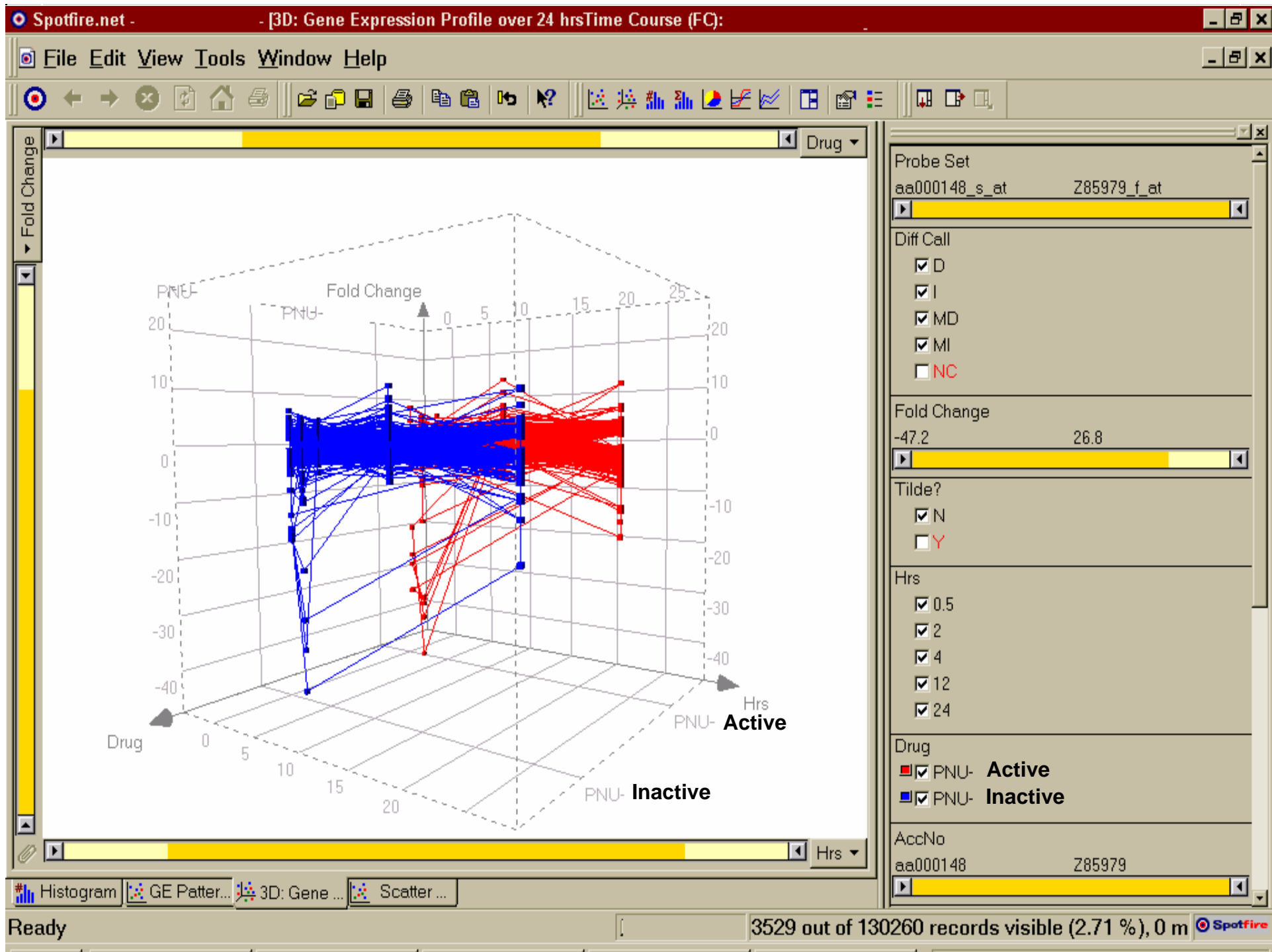
Results of Custom Analysis
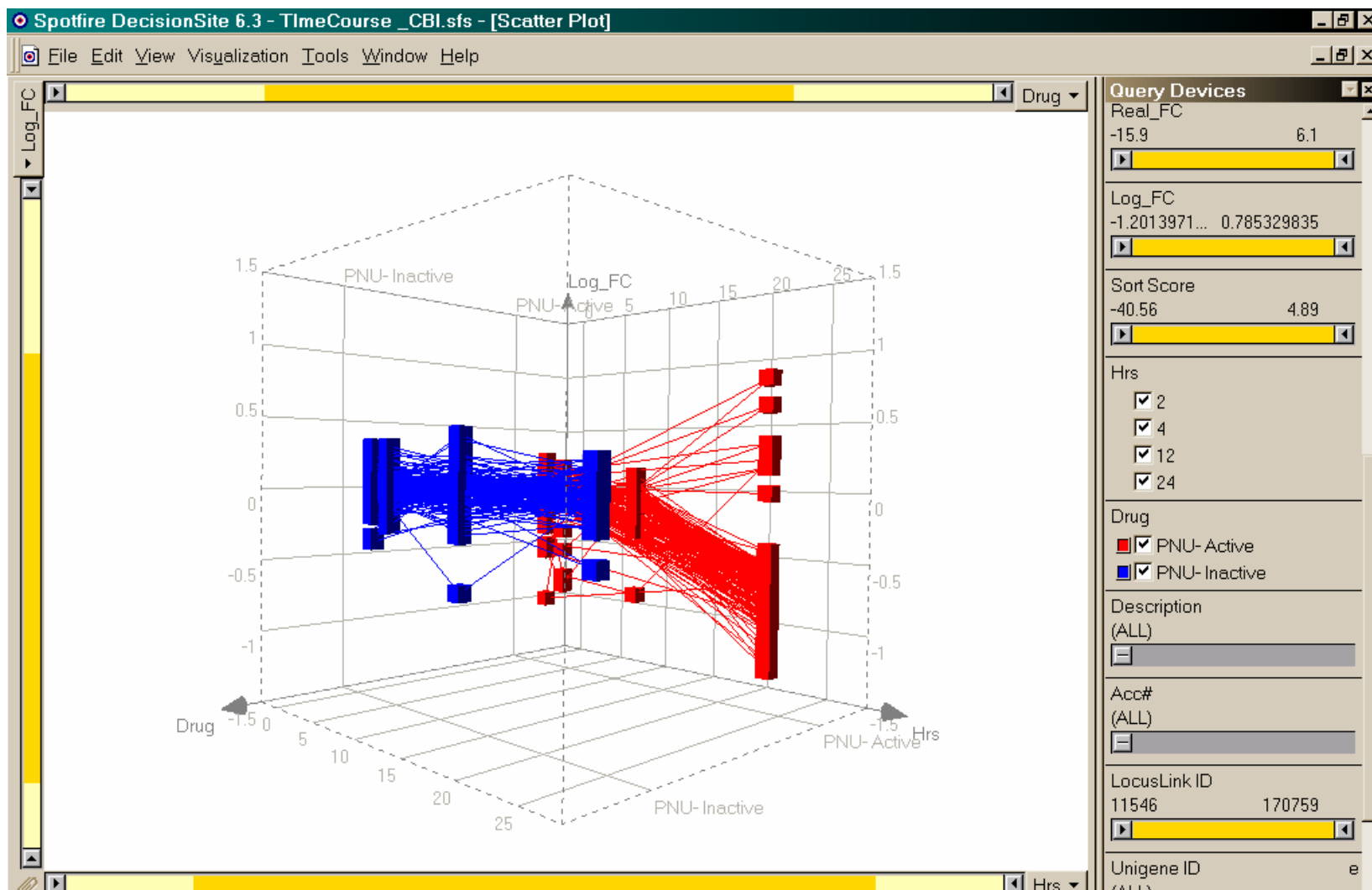
Non-Specific Responses– Example *(cont)*

- Conclusions:
  - Transcript changes can be induced by chemical treatment irregardless of biological activity
  - Control or reference compounds are required to sort out non-specific effects
  - Such toxicogenomics experiments may often require custom analytical approaches and /or a reference database

Confirmation of Microarray Results

- Several papers note the correlation between microarray results and quantitative RT-PCR results _if appropriate analyses are followed_.

- Protein levels and transcript (mRNA) levels for a particular gene <u>will probably be temporally discordant</u>
  - mRNA may be increased transiently followed by an increase in protein

- More important may be an independent (biochemical?) confirmation of the _biology_ inferred by the microarray results

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Activities Toward Risk Assessment

- Prediction of Toxicity

- Mechanism of Toxicity

- Biomarker Discovery

- Cross-Species Analysis

References for Predictive Toxicogenomics

- *In vitro*
  - Burczynski, M.E., et al., Toxicogenomics-based discrimination of toxic mechanism in HepG2 human hepatoma cells. *Toxicological Sciences*, 2000. 58(2): p. 399-415.
  - Mendrick, D.L. et al., Using gene markers identified from a large databaswe built with primary rat tepatocytes fro prediction of human hepatotoxicity. *Toxicologist* 2003 72:244
  - Boess F, et al, Gene Expression in Two Hepatic Cell Lines, Cultured Primary Hepatocytes and Liver Slices Compared to the in Vivo Liver Gene Expression in Rats: Possible Implications for Toxicogenomics Use of in Vitro Systems. *Toxicological Sciences*, 2003

- Short-term *in vivo*
  - Waring, J.F., et al., Clustering of hepatotoxins based on mechanism of toxicity using gene expression profiles. *Toxicology and Applied Pharmacology*, 2001. 175(1): p. 28-42.
  - Thomas, R.S., et al., Identification of toxicologically predictive gene sets using cDNA microarrays. *Mol Pharmacol*, 2001. 60(6): p. 1189-94.
  - Hamadeh, H.K., et al., Prediction of compound signature using high density gene expression profiling. *Toxicol Sci*, 2002. 67(2): p. 232-40.
  - Porter, M.W., et al., Predictive Toxicogenomics, in An Introduction to Toxicogenomics, M.E. Burczymski, Editor. 2003, CRC Press: Boca Raton. p. 225-259.

What Might We Accomplish?

- Prediction of Toxicity
- Mechanism of Toxicity
- Biomarker Discovery
- Cross-Species Analysis

Mechanism of Ranitidine / LPS Synergy

- Animal model of idiosyncratic toxicity
  - Developed by Robert Roth (MSU)
    - Buchweitz, JP et al, Underlying Endotoxemia Augments Toxic Responses to Chlorpromazine: Is There a Relationship to Drug Idiosyncrasy? JPET 2002 300:460–467
  - Involves treatment with a non-toxic doses of LPS combined with an otherwise non-hepatotoxic toxic drug

Mechanism of Ranitidine / LPS Synergy
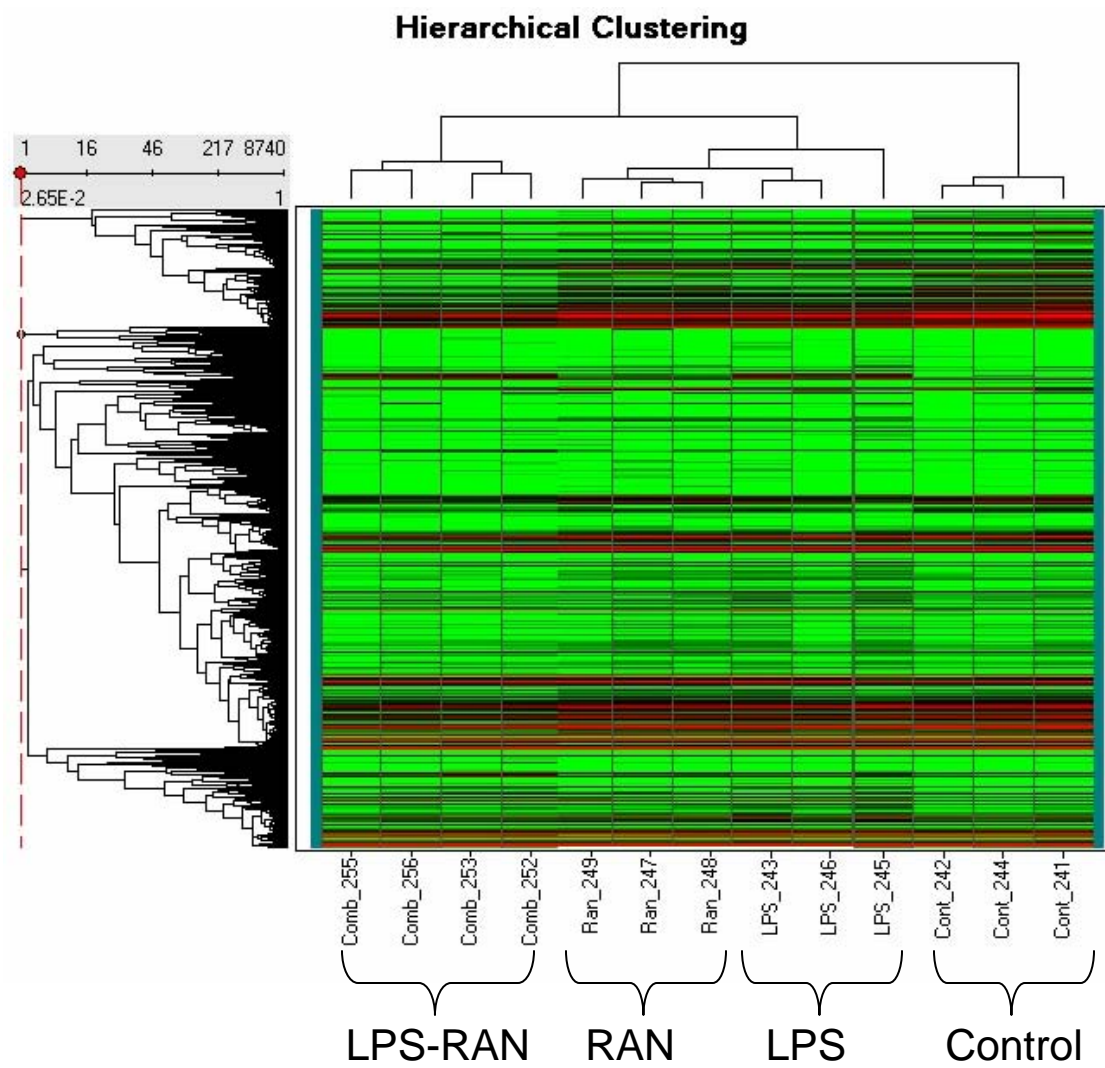
Clustering by Expression of Thousands of Genes Identifies Treatment Groups

Selected Genes Dysregulated in Treatments

Average Signals (MAS 5.0)

Cont	LPS	Ran	LPS-Ran	Locus	Description
15	139	94	276	Copeb	core promoter element binding protein
9	238	38	775	Pai1	serine (or cysteine) proteinase inhibitor, member 1
77	774	680	3795	Igfbp1	insulin-like growth factor binding protein 1
134	668	256	1624	Btg2	B-cell translocation gene 2
16	353	108	683	Cxcl10	small inducible cytokine B subfamily (Cys-X-Cys), member 10
32	43	126	319	Egr1	Early growth response 1

Selected Genes Dysregulated in Treatments

Average Signals (MAS 5.0)

Cont	LPS	Ran	LPS-Ran	Locus	Description
15	139	94	276	Copeb	core promoter element binding protein
9	238	38	775	Pai1	serine (or cysteine) proteinase inhibitor, member 1
77	774	680	3795	Igfbp1	insulin-like growth factor binding protein 1
134	668	256	1624	Btg2	B-cell translocation gene 2
16	353	108	683	Cxcl10	small inducible cytokine B subfamily (Cys-X-Cys), member 10
32	43	126	319	Egr1	Early growth response 1

Genes Induced in Hypoxia

Mechanism of Ranitidine / LPS Synergy

- "Consistent with the antifibrinolytic activity of PAI-1, significant fibrin deposition occurred only in livers of LPS/RAN-treated rats."

- "The results suggest the possibility that expression of PAI-1 promotes fibrin deposition in liver sinusoids of LPS/RAN-treated rats and are consistent with the development of local ischemia and consequent tissue hypoxia."
  - Gene Expression Analysis Points to Hemostasis in Livers of Rats Cotreated with Lipopolysaccharide and Ranitidine; Luyendyk, JP et al (2004) *Tox. Sciences* 80, 203–213

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Toxicogenomics So Far

- Toxicological principles are essential in study design and analysis
  - Dose, time, and exposure
  - Replicates !

- Rigorous informatics and careful analysis are essential for meaningful results

Toxicogenomics So Far – *(cont)*

- Transcript changes may be due to non-specific responses
  - Requires care in study design
  - May be de-convoluted with the use of non-active compounds

Toxicogenomics So Far – *(cont)*

- Transcript changes can be associated with pathological changes
    - Can give insight into mechanism of toxicity
    - May be due to altered regulation
    - *May be due to altered cell population*

- Transcript changes may precede biochemical / pathological changes
    - Can provide opportunities for predictive models or early screening
    - Can provide opportunities for biomarker discovery

Outline of Talk

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

Questions For the Future

- What are the relationships between:
  - high dose/ short term gene expression changes
  - Low dose / long term gene expression changes
  - Low dose / long term *biological* changes

- How might gene expression changes be extrapolated between species?

- How might we better describe gene expression changes in terms of interacting pathways?

- How will address the inherent unknowns in "patterns" (signatures or fingerprints) that contain un-annotated sequences?

Where We've Been

- Background and Definitions
- Nuts and Bolts – A "Quick" Toxicogenomics Recipe
- What do we want to accomplish?
- What did the ILSI effort cover?
- What are the issues to address?
- Examples along the way.
- What have we learned so far?
- Questions for the future.

End of Slides

immunotoxicogenomics