
Collaborative Approaches to Genomic Biomarker Evaluation



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ILSI Health and
Environmental Sciences
Institute

APPLICATION OF GENOMICS TO MECHANISM- BASED RISK ASSESSMENT 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.



Committee Participation Affiliations

- Abbott Laboratories
- Actelion Pharmaceuticals Ltd.
- Allergan Inc.
- Amgen Inc.
- Astellas Pharma Inc.
- AstraZeneca Pharmaceuticals
- Bayer HealthCare Pharmaceuticals
- Boehringer Ingelheim GmbH
- Bundesinstitut fuer Arzneimittel und Medizinprodukte
- Daiichi Sankyo Co. Ltd.
- Eli Lilly and Company
- European Medicines Agency
- Exiqon
- Georgetown University
- Health Canada
- Institute de Recherches Internationales SERVIER
- Johnson & Johnson Pharmaceuticals
- Maastricht University
- Merck & Co. Inc.
- Michigan State University
- Novartis Pharmaceuticals
- Pfizer Inc.
- sanofi-aventis
- Sumitomo Chemical Co. Ltd.
- Syngenta Ltd.
- Takeda Pharmaceutical Company Limited
- University of Minnesota
- US Army
- US Department of Agriculture
- US Environmental Protection Agency
- US Food and Drug Administration
- US National Institute of Environmental Health Sciences



HESI Genomics Committee Activities Addressing Biomarkers

2 experimental programs:

- **Qualification of a genomic biomarker approach to provide context to positive findings in *in vitro* chromosome damage assays**

- **Use of microRNAs for toxicological applications**



Genomic Biomarkers

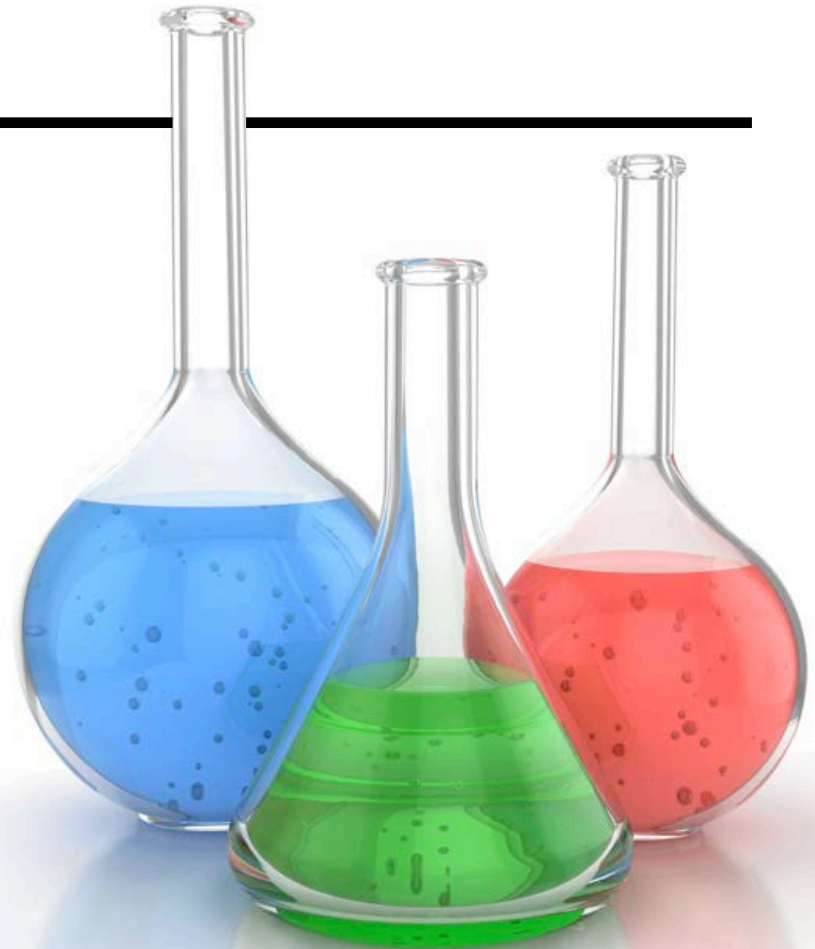
- The 2007 EMA guidance on definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories defines a genomic biomarkers as:
 - **A measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other interventions**

2 Genomics Committee projects → mRNA & microRNA expression



I. Genotoxicity Working Group:

Qualification of a Genomic Biomarker Approach to Provide Mechanistic Context to Positive Findings in In Vitro Chromosome Damage Assays



II.

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Genotoxicity Testing



Standard Genotoxicity Test Battery includes:

- a) Assessment of mutagenicity in a bacterial reverse mutation test.
- b) Genotoxicity evaluation in mammalian cells *in vitro* and/or *in vivo*.
 - Includes the *in vitro* chromosome damage assay

Highly sensitive
Low specificity
Limited mechanistic information



Issues to Address

- Assessment of risk and relevance of positive findings in *in vitro* chromosome aberration assays presents a challenge, and requires additional mechanistic studies *in vitro* and *in vivo*
- Keep high sensitivity with the current testing battery
- Address low specificity via toxicogenomic analysis of the genotoxic stress response (genomic biomarker approach)
 - Gaining insights into mechanisms (pathways)
 - Differentiate thresholded vs. non-thresholded dose response (DNA reactive vs. non-reactive)



Approach

- Combined QRT-PCR and microarray-based approach
- Genotoxic stress genes can help to facilitate differentiation of direct and indirect acting genotoxins in *in vitro* assays



3 Phases

- 1 – Initial comparability assessment (against previously obtained data {VXDS})
- 2 – Main genomics qualification study (assessment of approx. 50 compounds across mechanistic classes)
- 3 – Metabolic activation scoping study (testing of additional compounds requiring S9 metabolism)

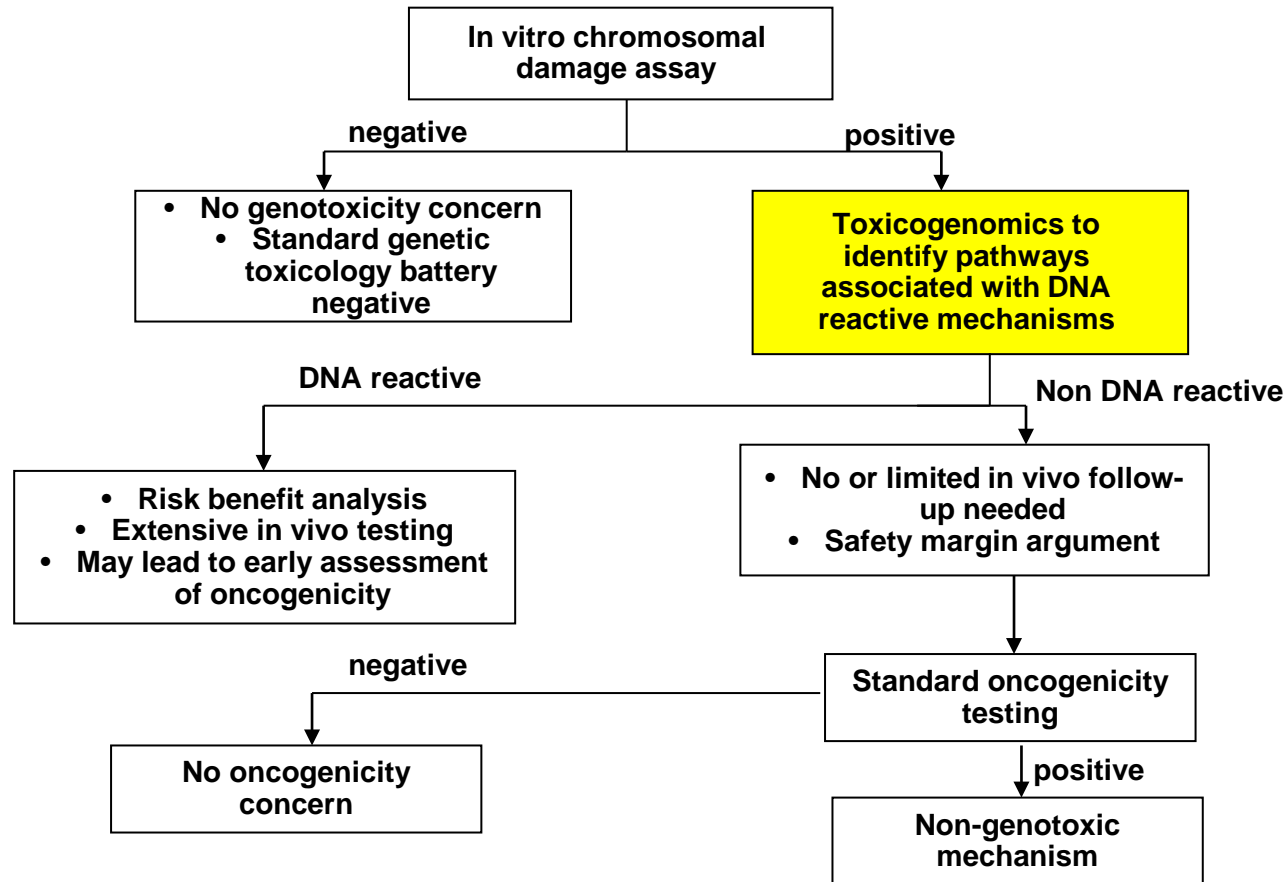


Proposed context of use

- Genomic biomarker approach capable of differentiating DNA reactive vs. DNA non-reactive mechanisms in TK6 cells.
- Application of the genomic biomarker approach for providing a mechanistic context to positive findings in the chromosome damage assays in mammalian cells *in vitro*.



Proposed Application of Toxicogenomic analysis for Risk Assessment of Genetox *in vitro* Positive Findings*



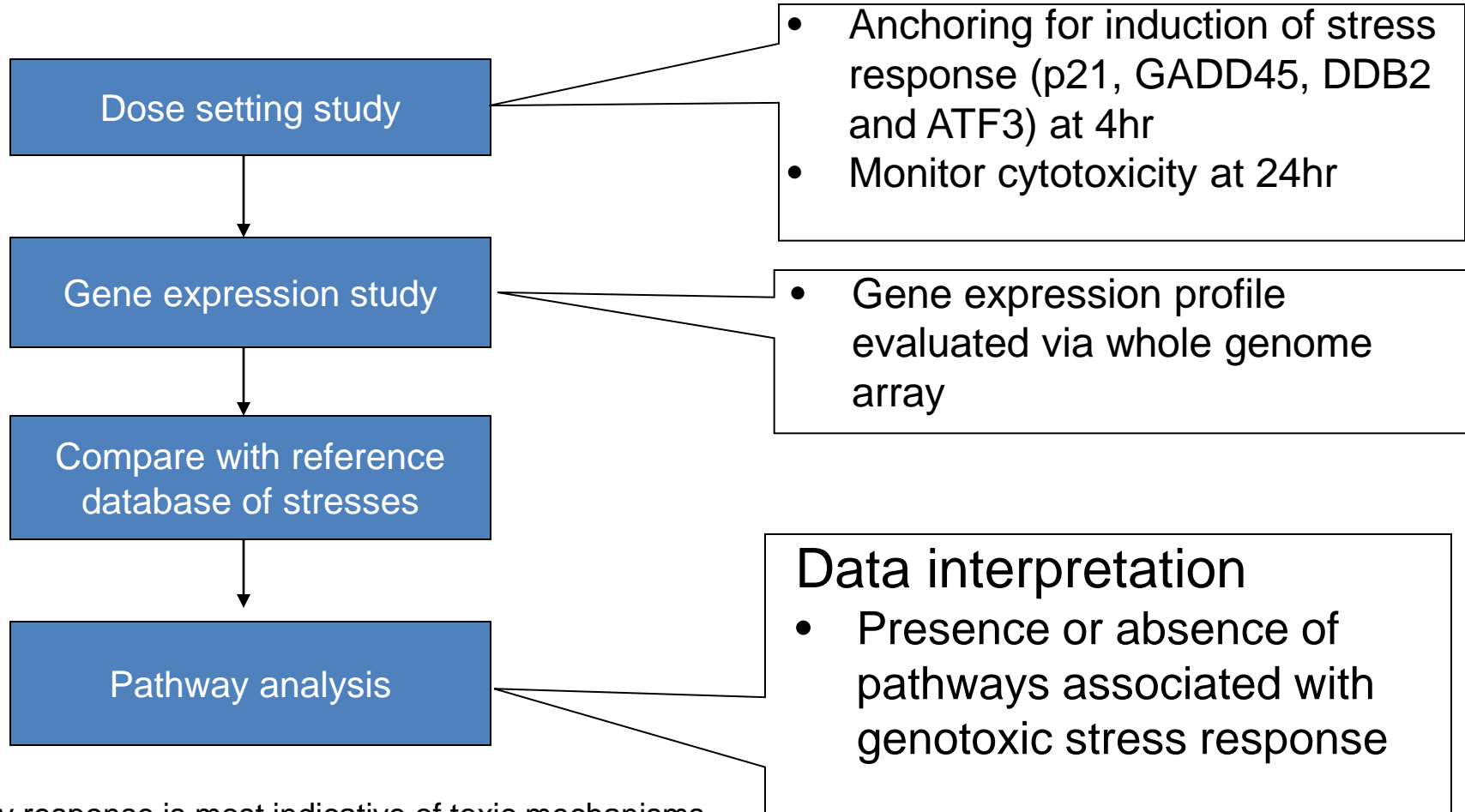
- Genomic biomarker approach capable of differentiating DNA reactive vs. DNA non-reactive mechanisms in TK6 cells.
- Application of the genomic biomarker approach for providing a mechanistic context to positive findings in the chromosome damage assays in mammalian cells *in vitro*.

VXDS (Voluntary Exploratory Data Submission)- Case Study

- A prior VXDS (J. Aubrecht and colleagues) on caffeine (positive in in vitro chrom damage assay) included approx. 25 compounds
- Demonstrated caffeine does not activate genotoxic stress response (by expression profiling vs. known DNA damaging agents)
 - The positive chromosome aberration findings of the test compound are of a non-DNA reactive mechanism.
- Building on the VXDS...
- A study involving approximately 50 additional compounds was designed by the Genomics Committee
- Intended for submission via the FDA Biomarker Qualification process



Toxicogenomic Study Design



- Early response is most indicative of toxic mechanisms
- Since gene tox profile of compounds is known thus no additional IVMN assay needed
- Focus on compounds that do not require metabolic activation, if successful S9 assay bridging studies needed

Genomic Biomarker Qualification Proposal: Compound Selection

Compounds in 6 mechanistic classes
will be evaluated for biomarker qualification

1 - Genotoxins that interact directly with DNA

2 – Genotoxins that interact indirectly with DNA
(topoisomerase inhibitors, DNA intercalators,
nucleoside analogues)

3 – Genotoxins that interact indirectly with DNA
(effect on cell cycle and mitotic apparatus)

4 – Non-DNA reactive chemicals (in vitro negative)

5- Irrelevant positives

6 – Pathway compounds

- Direct = DNA reactive = agents directly interacting with DNA (e.g. adduct formation, alkylation)
 - Linear dose response assumed
- Indirect = DNA non-reactive = agents causing genotoxicity via generation of radicals, inhibition of DNA synthesis, interaction with enzymes such as topoisomerases, etc. including cytotoxicity or cellular stress
 - Thresholded dose response

Current Status

Phase 1

➤ *Objective:*

To confirm the technical robustness and reproducibility of cell culture exposure conditions, microarray data and overall comparability with previously obtained data

➤ *Overall findings:*

- Good reproducibility with high correlation coefficient
- Four test treatments clustered with expected category by 2D clustering on genotoxic classifier

Phase 2

In progress. 20 compound data set anticipated by April 2012.



Anticipated Impact

- A toxicogenomics approach able to differentiate subsets of DNA non-reactive mechanisms of genotoxicity
- Reduction of “irrelevant positives”
- A qualified assay for follow up to positive findings in chromosome damage assays



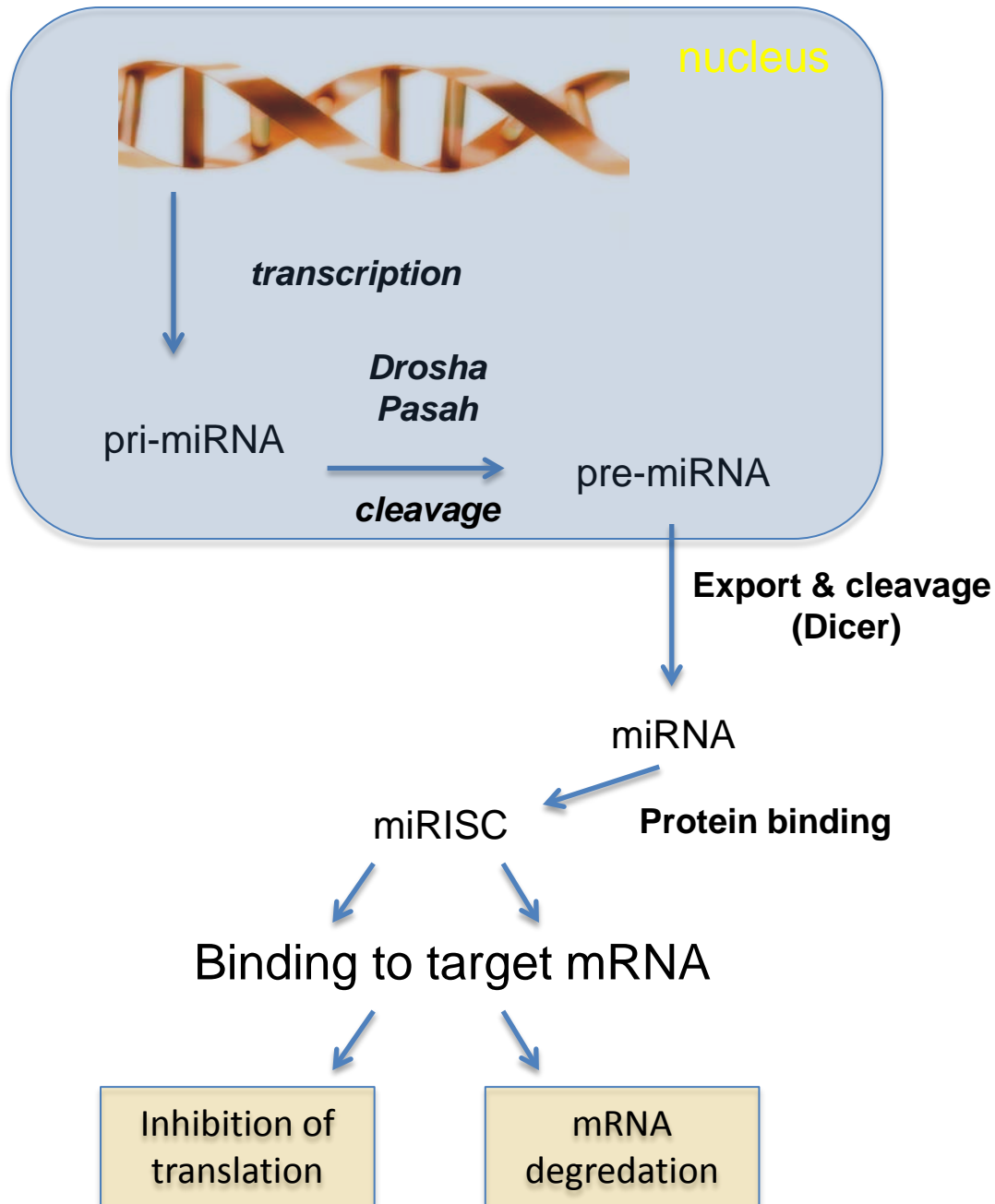
II. Use of MicroRNAs in Toxicological Applications



microRNAs

- **Short, non-coding RNAs**
- **Control gene expression on a post-transcriptional level**
- **Intracellular**
- **Also detected in biofluids**
- **May be useful as biomarkers (injury, disease)**





Potential for Application of MicroRNAs as Injury Biomarkers

- Small total number of microRNAs compared to mRNAs
- A few microRNAs exhibit highly specific tissue expression
- Tissue-selective microRNAs may be useful circulating biomarkers of tissue injury at specific sites
- Release of microRNAs from tissues into circulation (may be correlated with injury)
- Conservation across species



Questions

- Can microRNAs be applied as site-specific markers of injury/effect?
- Is expression of specific microRNAs associated with tissue pathologies?
- Will microRNAs be an effective tool for earlier and more accurate detection of risk to human health?



October 2010: Use of MicroRNAs in Toxicological Applications

Goal: To assess the state-of-the-science on use of microRNAs in toxicological applications

Program:

I. Progress on the use of microRNAs as biomarkers of injury

- Evaluation of techniques for genome-wide miRNA measurements
- Issues associated with microRNA measurements
- MicroRNAs as injury markers in urine
- MicroRNAs as injury markers in tissue.

II. Design of studies to assess microRNAs as injury markers

- Biomarkers of cardiotoxicity
- Biomarkers of nephrotoxicity
- miR-122 as a hepatotoxicity biomarker
- Biomarkers of testicular toxicity

Workshop Outcomes

- Challenges were identified at the workshop that informed design of a collaborative study, including:
 - Heterogeneity in microRNA length and sequence; Hybridization specificity due to short sequence length
 - Normal levels of injury-induced microRNAs in biofluids may be too low to quantitate
 - MicroRNAs in blood cells can contaminate biofluid samples
 - Preamplification PCR steps required to measure in biofluids – potential introduction of bias?
 - No consensus on endogenous microRNA controls in biofluids
 - Non-homogenous physical state of microRNAs in circulation - associated with protein and/or lipid vesicles
 - Reports of quantitative or qualitative difference in microRNAs recovered from serum and plasma



Collaborative Study on the Use of MicroRNAs in Toxicological Applications

- The committee has initiated conducting an inter-laboratory study to:
 - i) identify variables in quantitation of injury-related microRNAs in serum, plasma, and urine in toxicological studies, and
 - ii) to inform best approaches/methods for pre-clinical toxicology studies

Project involves:

- A drug induced (isoproterenol) model of cardiac injury in rats
- Generation of samples via an in-life study run at a central site
- Distribution of samples to multiple laboratories for analysis
- Comparison of results across sites



Study Stages

- Phase 1: Dose selection study
- Phase 2: Serum vs Plasma comparison in an injury model
 - Multi-site analysis of several cardiac microRNAs and controls
- Phase 3: Urine vs. plasma/serum sample in injury model
 - Multi-site analysis of several cardiac microRNAs and controls



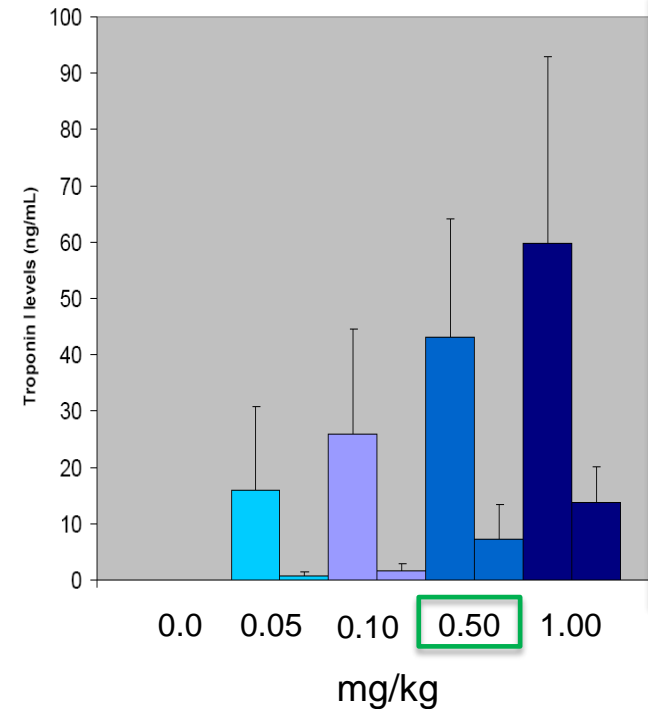
Phase 1

Study Design

- Administer a single sc dose of 0, 0.05, 0.1, 0.5, or 1 mg/kg isoproterenol to male Hanover Wistar rats (n=5/group)
- Plasma cardiac troponin I assessment at T+4 hr and T+24
- Heart histopathology at T+48 hr

Preliminary Results

- Mortality at 0.1 and 0.5 mg/kg, clinical signs in all isoproterenol-treated groups
- Increases in plasma cTnI at T+4 and T+24 hrs
- Myocardial necrosis/fibrosis at T+48 hr with dose-related severity



Phase 2

Study Design

- Administration of isoproterenol at 0 and 0.5 mg/kg (n=12/dose)
- At T+4 hr:
 - Plasma for cTnl evaluation
 - Plasma for miRNA assessment
 - Serum for miRNA assessment
- Plasma and serum sent to participating labs for processing and analysis
 - 12 participating organizations (North America, Europe, Asia)
 - All labs conduct standard protocol; also optional pre-defined protocol variations

- Standard Protocol

- (1) microRNA isolation
- (2) Multiplexed Reverse Transcription
- (3) Multiplexed Preamplification
- (4) Real Time PCR Amplification

Phase 2

- Several miRNAs selected for evaluation based on evidence in prior studies as circulating markers of acute drug-induced cardiac injury

miR-208 (Cardiac muscle specific)

miR-499 (Cardiac muscle enriched)

miR-1 (Enriched in muscle - not selective for cardiac)

(and an internal reference)



Analysis of Results from Phase 2

Analysis of Phase 2 results in progress across the contributing labs

Comparisons include:

- Standard protocol vs variations
- Site to site (12 sites)
- Reproducibility between technical replicates
- Serum vs plasma
- Treatment effect - saline vs isoproterenol
- microRNA targets (for cardiac injury)
- Normalization methods
- Protein-associated vs lipid vesicle-associated targets (optional targets)



Study Phase 3: Urine vs Blood sample in injury model

- Can injury-associated microRNAs detected in plasma also be detected in urine?
- What is the optimal protocol for detection of injury-associated microRNAs in urine?
- Plasma selected for phase 3 with urine arm of study
- Phase 3 anticipated to initiate in 1Q 2012



Anticipated Results

- Better understanding of preanalytical steps affecting microRNA detection and quantification in biofluids in drug-induced injury models
- Establish reference data set for comparing the sensitivity of methods for measuring injury-associated microRNAs in blood
- Important first steps in the exploration of the utility of circulating microRNAs as biomarkers of drug-induced injury beyond “proof of concept” studies



CONCLUSIONS

- Genomics holds promise for new approaches to biomarker development and optimization for toxicology studies
- Gene expression profiling approaches have potential for differentiating DNA reactive vs. DNA non-reactive genotoxic mechanisms
 - Program to qualify a genomic biomarker approach
- Circulating microRNAs may serve as markers of injury in toxicological studies
 - Work toward assay optimization/standardization



Acknowledgements

HESI Genomics Committee

- Leadership: Drs. Jiri Aubrecht and Richard Paules

microRNA Work Group

- Leadership: Drs. Philippe Couttet and Karol Thompson

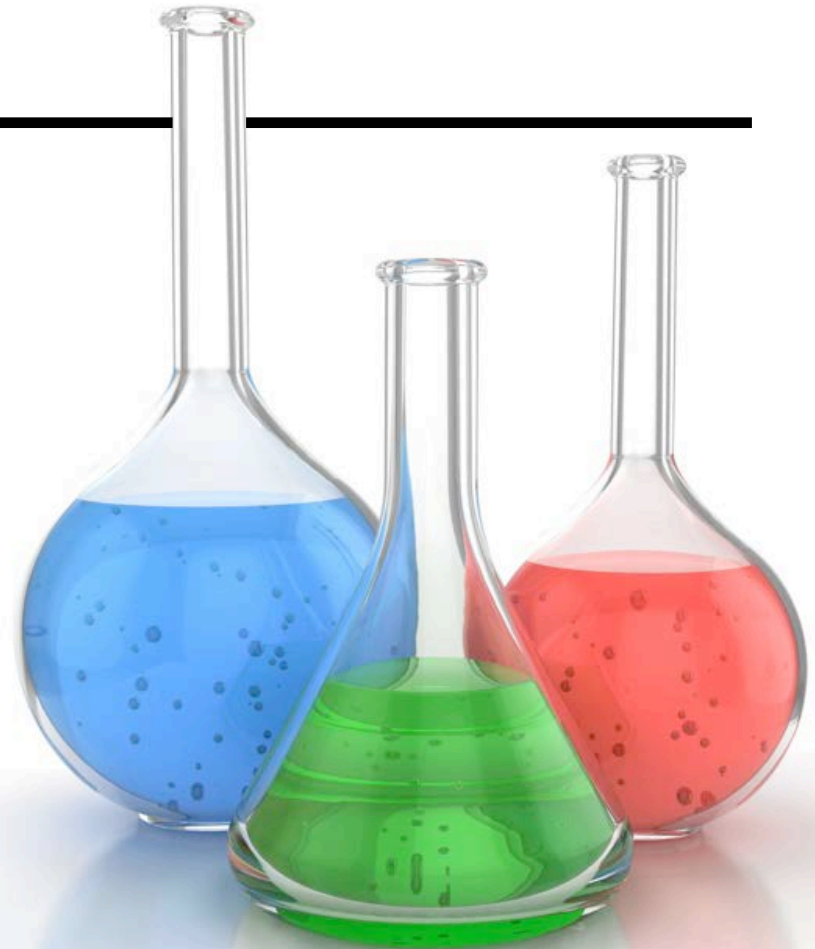
Genotoxicity Work Group

- Leadership: Drs. Jiri Aubrecht, Eric Boitier, Heidrun Ellinger, Roland Froetschl, Warren Ku, Carole Yauk



THANK YOU!

Questions?



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