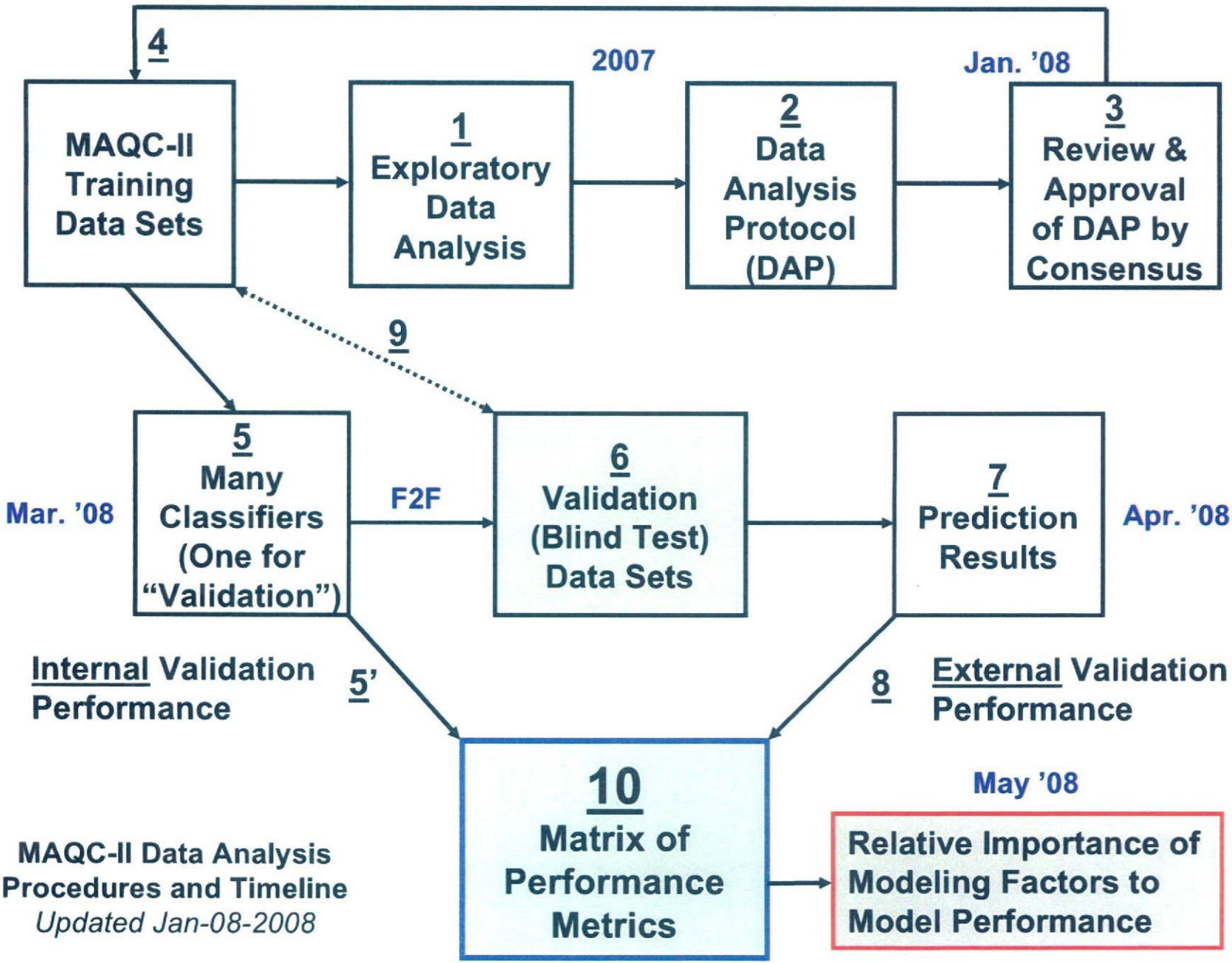
Summary of MAQC-II Data Sets

Leming.Shi@fda.hhs.gov Tel: +1-870-543-7387 Updated: January-17-2007

Table 1 A: MAQC-II Data Analysis Teams Are Required to Predict the 13 Endpoints from Six Data Sets

No.	Date Set Code	Endpoint Code	Endpoint Description	Excel Column Header*	Excel Column*	Number of Samples	Positives	Negatives	P/N Ratio
1	Hamner	A	Lung Tumor	Class_LT_NLT	C	70	26	44	0.59
2	Iconix	B	Liver Carcinogen	Class	B	216	73	143	0.51
3	NIEHS	C	Overall Necrosis Score	Class	C	214	79	135	0.58
4	BR	D	Treatment Response	pCR	O	130	33	97	0.34
5		E	Estrogen Receptor Status	erpos	H	130	80	50	1.6
6	MM	F	Overall Survival Milestone Outcome	OS_MO	AB	340	51	289	0.18
7		G	Event-free Survival Milestone Outcome	EFS_MO	AA	340	84	256	0.33
8		H	Clinical Parameter S1	CPS1	S	340	194	146	1.33
9		I	Clinical Parameter R1	CPR1	T	340	200	140	1.43
10	NB	J	Overall Survival Milestone Outcome	OS_MO	AM	238	22	216	0.10
11		K	Event-free Survival Milestone Outcome	EFS_MO	AL	239	49	190	0.26
12		L	Newly Established Parameter S	NEP_S	AN	246	145	101	1.44
13		M	Newly Established Parameter R	NEP_R	AO	246	145	101	1.44

*See Excel files listed in Table 1B. Endpoints (OS and EFS related) described in red have been updated based on the outcome of a "milestone" survey time; note that these endpoints are unbalanced.


4
2007
Jan. '08
MAQC-II Training Data Sets
1 Exploratory Data Analysis
2 Data Analysis Protocol (DAP)
3 Review & Approval of DAP by Consensus
9
5 Many Classifiers (One for "Validation")
Mar. '08
F2F
6 Validation (Blind Test) Data Sets
7 Prediction Results
Apr. '08
Internal Validation Performance
5'
8
External Validation Performance
10 Matrix of Performance Metrics
May '08
Relative Importance of Modeling Factors to Model Performance
MAQC-II Data Analysis Procedures and Timeline
Updated Jan-08-2008

ILSI HEALTH & ENVIRONMENTAL SCIENCES INSTITUTE (HESI)

PLENARY MEETING

OF THE

HESI TECHNICAL COMMITTEE ON GENOMICS IN MECHANISM-BASED RISK ASSESSMENT

NOVEMBER 7-8, 2007

WESTIN WASHINGTON DC CITY CENTER HOTEL
CONFERENCE ROOMS: NATIONAL B AND C
1400 M STREET, N.W.
WASHINGTON, DC

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The ILSI Health and Environmental Sciences Institute (HESI): The Global Branch of ILSI

Overview

HESI's programs bring together scientists from around the world from academia, industry, and regulatory agencies and other governmental institutions, to address and reach consensus on scientific questions that have the potential to be resolved through creative application of intellectual and financial resources. This "tripartite" approach forms the core of every HESI scientific endeavor. As a non-profit organization, HESI provides a unique, objective forum for initiating dialogue among scientists with different perspectives and expertise. Industry members provide primary financial support for HESI programs, but HESI also receives financial and in-kind support from a variety of U.S. and international government agencies.

HESI was established in 1989 as a global branch of the International Life Sciences Institute (ILSI) to provide an international forum to advance the understanding of scientific issues related to human health, toxicology, risk assessment, and the environment. In 2002, HESI was recognized by the United States government as a publicly supported, tax-exempt organization, independently chartered from ILSI. HESI draws its membership from the chemical, agrochemical, petrochemical, pharmaceutical, biotechnology and consumer products industries, with member companies based in the United States, Europe, and Japan. Additional information on HESI can be found on the website at www.hesiglobal.org.

HESI's Mission

The mission of the ILSI Health & Environmental Sciences Institute (HESI) is to stimulate and support scientific research and educational programs that contribute to the identification and resolution of health and environmental issues of concern to the public, scientific community, government agencies, and industry.

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HESI Technical Committee on Genomics in Mechanism-Based Risk Assessment
Plenary Meeting
November 7-8, 2007

Agenda

November 7

8:30 a.m.	Continental Breakfast
9:15 a.m.	Welcome and Introduction to Session– C. Afshari, Amgen, Genomics Committee Chair

Presentation of Results from HESI Genomics Research Programs 2004-2007

9:30 a.m.	Baseline Animal Database Program – K. Thompson, FDA; J. Chou, NIEHS
11:00 a.m.	State-of-the-Science Survey Results – A. Vickers, Allergan
12:00 p.m.	Lunch
1:00 p.m.	Results of the *In Vitro* Genotoxicity Program – J. Aubrecht, Pfizer
2:30 p.m.	Break
2:45 p.m.	A 6-Week Study of Rodent Cardiotoxicity: Exploring Mechanisms of Toxicity via Traditional Toxicity Endpoints, Cardiac Troponin, and Microarray – H. Hamadeh, Amgen; J. Lyon, GlaxoSmithKline
4:00 p.m.	Wrap-Up Discussion and Adjourn Day 1

Agenda (Cont'd)

November 8, 2007

7:45 a.m.	Continental breakfast
8:00 a.m.	Welcome and Review of Day One
8:15 a.m.	Presentation of New Program Proposals for HESI Genomics Committee 2008: Description of Process and Timelines
8:45 a.m.	Proposal 1: Moving from Rodent to Non-rodent Expression Profiling in Preclinical Safety Assessment. Presenter: Dr. Jim Stevens, Lilly
9:15 a.m.	Proposal 2: Practical Experiences in Applying Toxicogenomics to Risk Assessment: A Workshop/Case-Study Approach. Presenter: Dr. Cynthia Afshari, Amgen
9:45 a.m.	Proposal 3: Predictive Cardiovascular Risk Assessment by Genomic Methods. Presenter: Dr. Brian Berridge, GlaxoSmithKline
10:15 a.m.	BREAK
10:30 a.m.	Introduction to Carcinogenicity-Related Proposals – C. Afshari, Amgen
10:45 a.m.	Proposal 4: Embryonic Model Systems as a Surrogate Assay for Proliferative Potential of Test Compounds. Presenter: Dr. Kim Brannen, Bristol-Meyers Squibb
11:15 a.m.	Proposal 5: Validation of a New *In Vitro* Testing Paradigm for Detecting Chemical Carcinogenicity and Development of Biomarkers for Chemical Carcinogenesis Applicable to Risk Assessment. Presenter: Dr. Jiri Aubrecht, Pfizer
11:45 a.m.	Proposal 6: Genomic Analysis of Cancer Signaling (Hedgehog, Notch, Wnt) Important in Stem Cell Maintenance and Renewal as a Predictor of Cancer Risk Following *In Vivo* and *In Vitro* Exposure to Environmental Carcinogens. Presenter: Dr. Don Delker, U.S. Environmental Protection Agency
12:15 p.m.	Discussion of Proposals Presented and Additional Input

Agenda (Cont'd)

12:45 p.m. LUNCH
(Steering Committee to meet in Closed Session)

1:45 p.m. Other Ongoing Activities in the Field of Toxicogenomics

a. FDA Research and Regulatory Priorities – Dr. Felix Freuh, FDA, CDER (20 min. update)

b. EPA Research and Regulatory Priorities – Dr. Norman Birchfield, EPA's Office of the Science Advisor (20 min. update)

c. EMEA Research and Regulatory Priorities – Dr. Jean-Marc Vidal, EMEA (20 min. update)

d. Japan NIH Research and Regulatory Priorities – Dr. Jun Kanno (30 minutes)

e. C-Path Institute Activities – Dr. William Mattes (C-Path Institute) – (15 min)

f. European Inno-med Initiative Update – Dr. Heidrun Ellinger, Bayer (15 min)

3:45 p.m. Perspectives on the Application of Genomics to Risk Assessment: *This session will feature panelists discussing their current experience with TGx and Risk Assessment in a moderated panel discussion: (Chair- Dr. Jiri Aubrecht, Pfizer)*

a. What are current regulatory requirements/experience around the incorporation of these data?

b. Where does the technology show the greatest value?

c. Where are you seeing genomic data applied?

d. Where are there needs in the field?

e. Are TGx improving the ability to do risk assessment?

4:45 p.m. Adjourn

5:00 p.m. Opportunity for HESI Genomics Committee working groups to convene at HESI offices for discussion if requested.

5:30 p.m. Networking Dinner (location to be announced)

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Establishing a Public Baseline Gene Expression Database

Baseline Animal Microarray Database Group of the HESI Genomics Committee

ABSTRACT

A new project area was initiated within the Health and Environmental Sciences Institute (HESI) Genomics Committee to establish a publicly accessible dataset of control animal microarray data. It was thought that meta-analysis of microarray data from untreated or vehicle-treated animals within the control arm of toxicogenomic studies could provide useful information to the toxicogenomics community on baseline fluctuations in gene expression, although this type of data had not been collected on a scale and in a form best served for data-mining. The objective of this working group was to develop a public resource that would allow for assessments of variability in baseline gene expression and of the primary biological or technical factors that contribute to variation.

Voluntary contributions of control animal microarray data were requested from member companies in the Health and Environmental Sciences Institute (HESI) Genomics Committee. Solicitations were limited to rat liver and kidney samples run on Affymetrix GeneChips to increase the probability of acquiring a dataset large enough to assess the feasibility of comparing signal data across multiple studies and sources. The data was requested in the CEL file format to allow for uniform data processing. Sample annotation was requested for animal biometric data (e.g. age, weight, gender, source), as well as specific parameters relating to housing and feeding, dosing, tissue collection, and sample processing conditions, and collected in a controlled vocabulary context.

Data from over 500 Affymetrix RGU-34A or RAE230 series microarrays were collected from 16 different institutions for control rat liver and kidney. Thirty-five biological and technical factors were obtained for each animal, describing a wide range of study characteristics. This dataset has been anonymized and deposited in the Chemical Effects in Biological Systems Biomedical Investigation Database (CEBS-BID) and the EBI ArrayExpress public database.

A subset of study factors were evaluated in detail for their contribution to total variability using multivariate statistical and graphical techniques. Certain factors that emerged as key sources of variability include gender, organ section, strain, and fasting state. Genes that are the most and least variable, gender-selective, or altered by fasting were also identified and categorized by functional annotation.

Another outcome from this project was the identification of key descriptors that should be included in the minimal information about a toxicogenomic study needed for interpretation of

results by an independent source. Among the key descriptors are those that were identified as prominent sources of variability in baseline gene expression. Better characterization of gene expression variability in control animals should also aid in the design of toxicogenomic studies and in the interpretation of their results.

The Committee submitted a manuscript to a peer-reviewed journal in Q3 2007 that describes the findings from analysis of the baseline expression dataset. The dataset will be released to the public upon acceptance of the manuscript.

State-of-the-Science Working Group

ABSTRACT

An on-line survey to probe the current status and future challenges facing the field of toxicogenomics for drug and chemical evaluation was taken by scientists and scientific decision/policy makers actively engaged in the field of toxicogenomics within industrial, academic, and regulatory sectors of the U.S., Europe, and Japan. The goal of this working group is to facilitate the public understanding of and to facilitate the discussion around the current and future practical uses of toxicogenomic data for decision making within organizations and by policy makers. For the purpose of this survey toxicogenomics relates specifically to the use of gene expression responses (transcriptomics) to evaluate xenobiotic exposure in experimental and pre-clinical models. The survey covers the following general areas: a) technical /utilization strategies for toxicogenomics, b) organizational capacity and resource allocation, c) toxicogenomics for preclinical assessment, and d) data storage and exchange. The survey identifies key areas in which toxicogenomics will have an impact in the next 2-5 years, as well as the key hurdles in applying toxicogenomic data to address issues. The outcomes of this survey will be presented and compiled in a white paper/peer-reviewed publication.

Genotoxicity Working Group of the HESI Genomics Committee

ABSTRACT

The risk assessment of genotoxic agents is typically based on linear extrapolation methods. However, there is substantial evidence that some chemicals may exhibit a clear thresholded dose-response. It is generally accepted that characterizing clastogenic mechanisms, is essential for proper risk assessment of compounds with unexpected findings in the in vitro chromosome damage assays. The availability of discriminatory assays is limited and currently available methods are not effective at distinguishing chromosome damage arising secondarily from cytotoxicity and that arising from direct DNA reactivity or from interruptions of normal DNA metabolic processes. This results in laborious and time consuming follow-up strategies causing delays in the introduction of vital therapeutics to patients. Therefore, the development of alternative experimental approaches capable of evaluating a whole range of clastogenic mechanisms is extremely important.

Genotoxic stress triggers a variety of biological responses including the transcriptional activation of genes regulating DNA repair, cell survival and cell death. The genotoxic stress-associated gene expression profile analysis has been evaluated as a tool for investigating genotoxic mechanisms. Although the published data are promising, systematic studies are necessary to fully evaluate this approach, develop appropriate experimental protocols and achieve consensus in the scientific community on interpretation of the data in light of risk assessment.

The primary objective of the HESI genotoxicity working group is to develop a gene expression profile-based approach that is capable of differentiating cytotoxic, DNA reactive and DNA non-reactive genotoxic mechanisms for compounds with positive findings in the *in vitro* chromosome damage assays. Since achieving this goal requires significant research investments, the proposed collaborative project is focused and staged. The objective of the project is to establish experimental conditions, develop suitable protocols and evaluate inter-laboratory variability of the data.

In this study we conducted fundamental studies characterizing time course and dose response in two cell lines (TK6, mouse lymphoma) using a limited number of well-studied compounds (Cisplatin, Taxol, NaCl, Etoposide). The working group selected a set of 47 genes that might provide discriminatory information for use in RT-PCR based on the literature. The studies incorporated assessments of dose response and time course effects and considered cytotoxicity at these points as well. Studies were conducted in nine laboratories in the U.S., Europe, and Japan. Two of the participating laboratories used microarray technology to evaluate the gene expression patterns.

The study demonstrates that microarray data provide insights into genotoxic mechanisms (relevant biological pathways) and that trends in gene expression signatures can be observed at the individual gene level and over dose/time across laboratories. There was generally good reproducibility in the study across laboratories, even without highly controlled conditions from lab to lab. The study supports the hypothesis that a limited set of genes measured via RT-PCR can provide biologically relevant data and does have predictive value. Data analysis continues and manuscript development is currently in progress.

Mechanism-Based Markers of Toxicity Working Group

ABSTRACT

The goal of this working group is to highlight the use of genomics to enhance the mechanistic understanding of chemical/compound toxicities and ultimately, their risk assessment. The group has undertaken a purpose designed toxicogenomic study of "significant depth" to act as a case study for discussion regarding technical and interpretive practices. The study, of doxorubicin cardiotoxicity in rats, includes multiple timepoints, doses, controls/comparators and "recovery" groups. The aim is to generate new molecular information about this clinically important toxicity and in light of the comprehensive study design, to provide insight into how time and dose alter gene expression and the relationship to the onset of toxicity (molecular threshold). The study includes full toxicological context, including histopathology, clinical pathology and TK, in addition to cardiac troponins (T and I) and reticulocyte micronuclei as markers of cardiac injury and compound pharmacology respectively. Gene expression data is being generated using both the Affymetrix and Agilent array platforms and plasma samples have also been collected for future metabonomic profiling by NMR. Etoposide has been included as a control compound that also targets topoisomerase pharmacologically but does not cause cardiotoxicity. This, along with analysis of non-target tissues (e.g. diaphragm muscle) and also the effect of an adjunctive treatment (dexrazoxane) which reduces the oxidative stress element of doxorubicin toxicity, will allow further dissection of the gene effects specific to doxorubicin toxicity in the heart. The inclusion of "recovery" groups will allow exploration of changes that become fixed at an early stage of doxorubicin treatment and may contribute to the cumulative nature of the cardiomyopathy observed. Overall, the committee approach to this project offered an economy of scale and a unique breadth of expertise to aid in study design, execution and interpretation of the data. It is hoped that the opportunity for all sectors (industrial, regulatory and academia) to collectively discuss the study design and data will allow the optimum features from each perspective to be identified.

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PROJECT PROPOSAL

Topic: Moving from Rodent to Non-rodent Expression Profiling in Preclinical Safety Assessment

Submitted by: James L. Stevens, Ph.D., Lilly Research Laboratories, Greenfield, IN

Application of toxicogenomics to preclinical safety assessment is largely focused on the rodent. Despite the fact that non-rodent animal models play a prominent role in preclinical safety assessment, non-rodent toxicogenomic applications have received much less attention. The dog is the most common non-rodent species used in preclinical drug development and has been reported to be more predictive than rodent for adverse drug reactions in human (Olsen et al. 2000). The publication of a high-quality draft (7.5X) of the dog genome (Ostrander and Wayne, 2005) and the availability of commercial canine microarrays provide new tools that could accelerate the application of transcript profiling and genomic analysis to preclinical development. Nonetheless, this approach is underutilized in part due to technical issues, including limited functional annotation of dog genome, the higher proportion of poorly annotated EST's on commercial arrays, and a lack of bioinformatics tools that allow for facile comparative genomics applications hamper progress. In addition, non-technical issues, such as a lack of focus on canine genomics research in the scientific community as well as cost and ethnical issues related to running non-rodent studies (particularly outside the US), have slowed progress.

The ILSI-HESI Toxicogenomics Committee could bring much needed focus to these issues. If toxicogenomic approaches are to be deployed in ways that support preclinical safety assessment and mechanism-based risk assessment for human, then they must be applied to the appropriate species that drive these decisions. A three-tiered approach could help focus attention on the application of toxicogenomics to the dog:

Step 1: Assemble a small working group of interested members to identify key stakeholders and define the opportunities. At some point in this process adding an academic advisor(s) would be helpful in this evaluation phase.

Step 2: Establish a workshop to bring together key stakeholders (e.g. NIH, pharma, FDA) to determine the major needs in non-rodent genomics applications. Technology companies could and should be involved at various steps in the process.

Step 3: Present a focused project proposal for endorsement for ILSI/HESI funding.

Maintaining focus will be important, therefore, the output from each step will determine next step. The longer term deliverables from the project would include:

1. More accurate EST annotation and gene ontology for canine microarrays.

2. Improved approaches to comparative genomics and transcript profiling, e.g. consistent target and pathway content across species.
3. Increase utilization of non-rodent transcript profiling in non-clinical safety assessment.

References:

1. Ostrander, E.A. and Wayne, R.K. (2005) The canine genome. *Genome Research.* 15:1706-1716.
2. Olsen et al. (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology and Pharmacology.* 32:56-67.

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PROJECT PROPOSAL

Topic: Use of Toxicogenomics: Case Study Workshop and Publication

Submitted by: Cynthia Afshari, Ph.D., Amgen, Inc., Thousand Oaks, CA

Application of microarray technology has increased within many companies and organizations for both investigative and predictive studies. While the toxicology community has had opportunities to share technical experience with the use of microarrays, there have been fewer opportunities to obtain broad feedback and discussion on the biological application and interpretation of these tools. One of the primary tools for sharing research-based discovery and experience is publication. However, the role for publication in this field has been somewhat limited because a segment of the current activity in toxicogenomics is occurring in sectors (e.g., industry) that typically publish infrequently due to the low priority assigned for this kind of activity. There is an opportunity to increase the impact of this technology by sharing experiences, positive and negative, among scientists. In order to facilitate this activity, we are proposing to hold a workshop where industrial and other scientists will present case examples that illustrate how microarray analyses are being applied in a predictive mode, or how they were used to increase understanding for issues or risks. In addition to hosting a workshop to exchange experiences and ideas, this proposal also suggests the development of a publication from the workshop material that captures the case examples and could be disseminated to promote the 'lessons learned' at the workshop.

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PROJECT PROPOSAL

Topic: Predictive Cardiovascular Risk Assessment by Genomic Methods

Submitted by: Brian R. Berridge, Ph.D. and J. Greg Falls, Ph.D., GlaxoSmithKline Safety Assessment, Research Triangle Park, NC

The drug development community has recently been plagued by a number of high-profile, non-QT cardiovascular safety issues in late-stage or marketed compounds. Though our preclinical safety assessment paradigms provide a fair opportunity to identify acute cardiovascular effects of novel therapeutic compounds, they are not as useful for identifying long-term risk associated with insidiously progressive alterations in cardiac physiology. Nor are they crafted to understand the effects of xenobiotics in the context of pre-existing cardiovascular disease. Paradoxically, this limitation is in the context of target patient populations with a significant background of cardiovascular disease. Predictive cardiac genomics could provide an opportunity to identify risky drug-related changes in cardiac physiology (particularly energy metabolism and myocardial remodeling) long before these changes result in a clinically detectable endpoint.

This effort would fall squarely within the stated mission of the HESI Genomics Committee to "advance the scientific basis for the development and application of genomic methodologies to mechanism-based risk assessment". It would also build on current efforts of this group evaluating a doxorubicin model of cardiac injury in rodents. Additionally, this work could link efforts in and outside HESI focused on various aspects of cardiovascular safety and biomarker development (i.e., ILSI-HESI Cardiac Troponin Working Group, Critical Path Institute's Cardiac Safety Research Consortium). Lastly, this effort could bring together experts in the fields of experimental, clinical, and preclinical cardiac disease to progress the understandings of cardiac physiology, pathophysiology, toxicity, and morpho-functional-genomic correlates.

This effort might begin with a workshop to convene relevant experts in the fields of preclinical and clinical cardiac disease/toxicity to understand the background of cardiovascular disease in target patient populations as well as commonalities in the adverse cardiovascular events that have complicated development and application of these efficacious molecules. A consequent expert working group would convene to identify relevant animal models and test compounds. Transcriptional data would be gathered in these models in the context of more routine endpoints like morphology and cardiac function to provide the appropriate biological context. Those data sets could be interrogated in a discovery paradigm to uncover previously undescribed relationships but could also be evaluated in a supervised way to identify well-recognized pathologic pathways (e.g., perturbations in energetics, myocardial remodeling, etc.).

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PROJECT PROPOSAL

Topic: Embryonic Model Systems as a Surrogate Assay for Proliferative Potential of Test Compounds

Submitted by: Kim Brannen, Ph.D., Bristol-Myers Squibb, Drug Safety Evaluation

The ILSI-HESI Toxicogenomics Committee (technical committee for the "application of genomics to mechanistic based risk assessment") has focused its previous efforts largely on in vivo expression profiling associated with target organ toxicity in rat liver, kidney and heart. These efforts have provided relevant fundamental data that has contributed to the growing use of transcriptional profiling data in hazard identification. In many drug safety evaluation groups, transcriptomic profiling in short term repeat-dose toxicology studies has become a routine component of non-clinical safety assessment, and when compared to in-life, hematology, clinical chemistry, and histopathology results, transcriptional profiles show some utility for predicting target organ toxicities. Moreover, transcriptional profiles can also be utilized in short term studies to make decisions on back-up compounds without completion of full toxicologic assessments.

While transcriptional profiling has shown utility in assessing target organ toxicities, there has been less effort directed toward evaluating transcriptomic profiles that may predict chronic toxicity (including carcinogenicity) or efforts to utilize in vitro models to evaluate or predict both acute and chronic toxicity. To that end, there is an opportunity to expand the application of transcriptional profiling data to safety evaluation and risk assessment by determining whether transcriptomic profiles from shorter term repeat dose studies can be correlated with more chronic toxicities. Proactive identification of these liabilities would enable strategic decisions on the timing and design of non-clinical studies to address such safety concerns.

The proposed work will focus on hyperplasia or cell proliferation as a major endpoint, with the overarching objective being to determine whether hyperplastic potential can be predicted by development of in vitro model systems capable of confirming cellular proliferative responses in vivo. Embryonic cell model systems are potentially robust in vitro models for such purposes, given the broad pluripotency of cell populations, their high proliferative capacity, and the expression of pathways associated with the balance between proliferation and differentiation (e.g., protooncogenes, growth factors, tyrosine kinases, wnt pathway, Homeoboxes,)[1-8].

Research efforts would be initiated with exploratory work to determine whether in vitro developmental models are suitable for verifying proliferative potential of compounds and to determine whether this liability can be identified from transcriptomic profiles. The test systems

would include rat whole embryo culture and/or mouse embryonic stem cells, and the study will include cell cycle and proliferation measurements (using multiple methods and markers) following treatment with reference compounds known to produce positive or negative proliferative responses. Once methods have been optimized and proof-of-concept achieved, additional studies will be conducted with a small set of tool compounds that have been profiled by transcriptomics and have produced hyperplastic responses in representative target organs including liver or mammary gland (and potentially other tissues) in repeat-dose toxicology studies.

The proposed work is exploratory in nature, but if successful, would add to our scientific understanding of hyperplasia, a common event seen in many toxicology studies. Furthermore, a major benefit derived from the focus on in vitro models is the possible application of these tools for hazard identification and risk assessment relative to current regulatory restrictions on the use of animals in toxicology testing (i.e. REACH legislation in the EU). Finally, although the collaborative work is consistent with the ILSI-HESI efforts in toxicogenomics, it is also applicable to and consistent with efforts to predict long-term outcomes from shorter-term studies, a collaborative effort presently ongoing within the ILSI-HESI Cancer Hazard Identification Committee. As such, a focused, collaborative effort on the application of embryonic model systems to transcriptional profiling and hazard identification relative to hyperplasia and carcinogenic outcome is consistent with broad initiatives within ILSI. A collaborative research effort to assess the feasibility and predictivity of this experimental approach would greatly facilitate the generation of data, and the body of work in its totality would provide important new information on the application of in vitro test systems for transcriptional profiling evaluation along with the prediction of chronic toxicities from short-term and/or in vitro studies.

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PROJECT PROPOSAL

Topic: Validation of a New *In Vitro* Testing Paradigm for Detecting Chemical Carcinogenicity and Development of Biomarkers for Chemical Carcinogenesis Applicable to Risk Assessment

Submitted by: Jiri Aubrecht, Pharm.D., Ph.D., Pfizer, Groton CT; Albert Fornace Jr., MD, Georgetown University, Washington, DC; Robert H. Schiestl, Ph.D., UCLA, Los Angeles, CA

The assessment of cancer risk associated with exposure to chemicals relies on the genotoxicity testing battery followed by the 2-year rodent carcinogenicity bioassay. In this paradigm, the genotoxicity testing battery enables relatively simple, rapid and inexpensive hazard identification and the 2-year rodent carcinogenicity bioassay provides mainly an assessment of a cancer risk in vivo. Because of the long term nature of the carcinogenicity testing and significant cost limitations, the genotoxicity testing battery is relied on as a surrogate marker of carcinogenicity in early drug development and evaluation of chemicals. Although the link between genotoxicity and carcinogenicity is well documented, this relationship is complicated due to the impact of non-genotoxic mechanisms of carcinogenesis and by nature of the *in vitro* genotoxicity assays and endpoints. Therefore, the predictivity of the current in vitro testing paradigm for carcinogenicity is challenging. Thus several positions and guidance have been published [1-3] resulting in the development of a mode-of-action framework [4]. Improving the predictivity of the genetox battery is also a subject of current discussions on the ICH testing guidelines and the crucial need for development of appropriate scientific approaches is fully recognized by the EU REACH initiative [5].

Here we propose to evaluate a new in vitro testing paradigm published by Ku et al 2007[6] consisting of a single test that addresses the relevant principal genetic lesions covered in the current test battery (DEL recombination assays, [7]) followed by toxicogenomic analysis to differentiate genotoxic and carcinogenic mechanisms [8, 9]. The advantage of the DEL assay resides in its ability to detect cancer-relevant changes in a single test system amenable to higher throughput and automation. In fact, recently the EPA in their ToxCast testing scheme as well as the NTP proposed incorporating the automated version of the DEL assay into their HTS screening. Currently available data indicate that the DEL assay has accuracy for predicting carcinogenicity of 92% compared to 62% with the Salmonella assay. Furthermore, the shape of the dose response for DEL recombination induction in relation to cytotoxicity could be used as a first criterion to differentiate direct (DNA reactive) from indirect genotoxic mechanisms, and thereby serve to prioritize the need for further toxicogenomic evaluation. The subsequent toxicogenomic analysis is directed to investigate the nature and biological relevance of DEL