New Strategies for the Use of Genetic Toxicology Data in Human Risk Assessment

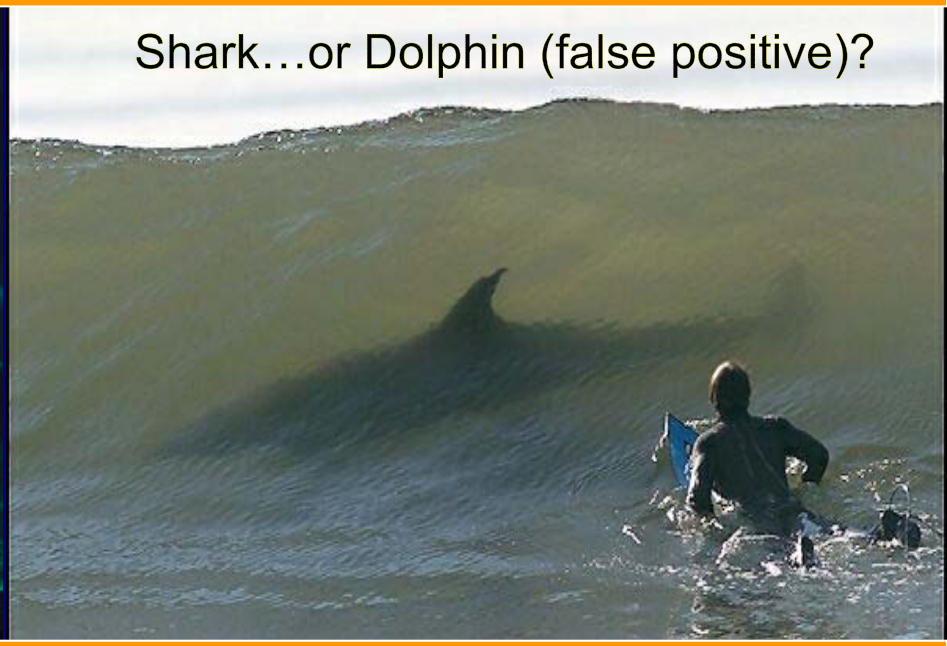
Organized by the HESI Project Committee
Relevance and Follow-Up of Positive Results in
In Vitro Genetic Toxicity Testing (IVGT)

HESI Annual Meeting
May 12, 2010
Reston, Virginia

James Kim, PhD, DABT

Risk Assessment

HAZARD VERSUS RISK



Hazard Identification → Hazard Characterisation → Dose Response Analysis → Risk Evaluation → Risk Communication

Mission of this Committee

- Improve the scientific basis of the interpretation of results from *in vitro* genetic toxicology tests for purposes of more accurate human risk assessment

- Develop follow-up strategies for determining the relevance of *in vitro* test results to human health

- Provide a framework for integration of *in vitro* testing results into a risk-based assessment of the effects of chemical exposures on human health

Membership

Industry Participation

AstraZeneca
Bayer Healthcare Pharma
Bristol-Myers Squibb
Coca-Cola
Covance
Dow Chemical
GlaxoSmithKline
Johnson & Johnson
L'Oreal
Mitsubishi Tanabe Pharma
Novartis
Pfizer Inc.
Procter & Gamble
sanofi-aventis
Schering-Plough
SERVIER
Takeda

Total = 17

Government / Research Institution Participation

Federal Institute for Drugs and Medical Devices (BfArM, Germany)
Health Canada
National Center for Toxicological Research, U.S. FDA
National Institute for Public Health and the Environment (RIVM, NL)
National Institute of Environmental Health Sciences (USA)
National Institute of Health Sciences (Japan)
U.S. Department of Agriculture
U.S. Environmental Protection Agency
U.S. Food and Drug Administration

Academic Participation

New York Medical College
Swansea University
University of Ottawa
University of Surrey
St. George's University of London

Consultant Participation

Errol Zeiger Consulting
David Kirkland Genetox Consulting
Marilyn Aardema Consulting
Jim MacGregor Toxicology Consulting Service

IVGT Project Committee Organization

IVGT Project Committee Members
Industry, Academia, Government
Leadership team
Chair: Veronique Thybaud
Vice Chair: Bhaskar Gollapudi
HESI manager: Jim Kim

IVGT Steering Committee in March 2010
Anthony Lynch (since 2010)
Bhaskar Gollapudi
David Jacobson-Kram
David Kirkland (since 2010)
Elisabeth Lorge (since 2010)
George Douglas
Jim MacGregor
Jennifer Sasaki
Kerry Dearfield
Marilyn Aardema
Masamitsu Honma
Peter Kasper
Veronique Thybaud

Review WG (2008-2010)
Co-leader: Veronique Thybaud
Co-leader: Kerry Dearfield
Sunset upon manuscript publication in 2010

New/Emerge Tech WG (2008-2010)
Co-leader: David Jacobson-Kram
Co-leader: Jennifer Sasaki
Sunset upon manuscript publication in 2010

Pig-a Assay Validation WG
Leader: Jennifer Sasaki
Advisor: Steve Dertinger

Bhaskar Gollapudi
Bob Heflich
David Lovell – stats lead
George Johnson
Jan van Benthem
Jim MacGregor
Vasily Dobrovolsky
Veronique Thybaud

Others
BioReliance
BMS
Covance
GSK
Pfizer
Teijin
U.S. FDA / NCTR

Pig-a Stats WG
Leader: D.Lovell
Advisor/Co-leader: TBD
Participants: BMS, Covance, FDA, GSK, Pfizer

Quantitative WG (2008-2012)
Co-leader: Bhaskar Gollapudi
Co-leader: Veronique Thybaud (2010)
Advisor: Jim MacGregor

Database
Jim MacGregor
Bhaskar Gollapudi
Veronique Thybaud
Beth Julien
QA/QC Reviewers

Data Collection
Leaders:
Beth Julien
Andreas Czich

Participants: TBD

Data Analysis
Leaders:
Lynn Pottenger
Paul White

Participants: TBD

Health Canada Project
Co-leaders:
Paul White
George Douglas

Project Steering Committee:
Beth Julien
Bhaskar Gollapudi
Jim MacGregor
Peter Kasper
Veronique Thybaud

Co-investigators
Andrew Williams
Bette Meek
David Phillips

New Approaches WG (2010-2012)
Co-Leader: Anthony Lynch
Co-Leader: Masa Honma
Workshop in 4Q11- 1Q12?

Improving Existing Assays WG (2010-2012)
Co-Leader: David Kirkland
Co-Leader: Elisabeth Lorge
Experimental work in 2010-11?

Genetox of Nanoparticles (Not confirmed as IVGT WG)
Workshop at EMS in October 2010
Follow-up HESI actions: TBD

Quantitative Working Group

Bhaskar Gollapudi (Dow Chemical Co.), Co-leader

Veronique Thybaud (sanofi-aventis), Co-leader (since 2010)

Jim MacGregor (Toxicology Consulting Services), Advisor

Quantitative Working Group

Database

- Need to collect data to be used for quantitative analysis
- Need for financial support to develop the database
- Application to 3 yr Health Canada Grant “to develop improved understanding of the relationship between in vitro and in vivo genetic toxicology assay”
- Collaboration with HESI to:
  - Develop the database and conduct analyses
  - Support additional laboratory work (mainly in Health Canada)
- Grant was approved and funded in August 2008: $130,000 CAD

Project Team (Health Canada, HESI, Inst. Cancer Res., UK)

Paul White, George Douglas (Co-Principal Investigators)

Beth Julien (Database manager)

B. Meek, A. Williams, J. Kim, M. Holsapple, D. Phillips

Steering Committee

B. Gollapudi, P. Kasper, D. J.-Kram, J.MacGregor, V.Thybaud

Quantitative Working Group

Database: Current status

- Access Database has been developed
- "G4" chemicals
  - ethyl methane sulfonate (EMS) and methyl methane sulfonate (MMS)
  - ethyl nitroso urea (ENU) and methyl nitroso urea (MNU)
- List of assays
  - *In vitro* cytogenetics (CA and MN)
  - *In vitro* gene mutation (MLA and HGPRT)
  - *In vivo* cytogenetics (CA and MN)
  - Transgenic rodent mutation models
  - Rodent cancer bioassay results
  - DNA adducts and other exposure metrics (*in vitro* and *in vivo*)
  - *Ames, Pig-A and Comet assays are subsequently added*
- Literature data on G4 chemicals have been collected and are being reviewed by experts (data collection subgroup)
- Unpublished data (e.g., pig-a) are being collected

Quantitative Working Group

Database: Next steps

- G4 dose-response data will be analyzed with various approaches
- Potential data gaps will be identified
- Experimental work might be needed to complete the analysis and finalize the proof of concept
- Additional chemicals with different modes of action will be selected up to G8-12 to evaluate and validate the concept (e.g. aflatoxins, polycyclic hydrocarbons, “non-DNA reactive” chemicals)
- Data will be collected and analyzed

Improving Existing Assays Working Group

David Kirkland (Consultant), Co-leader

Elisabeth Lorge (Servier), Co-leader

Improving Existing Assays Workgroup

Objectives

- Continue discussions on issues identified at the IWGT* 2009 workshop
  - Cell-type comparisons
  - Issues of genomic stability of currently used cell models in genetox
  - Top concentration for *in vitro* assays
  - Metabolism
  - Cytotoxicity
  - Possible new cell types for genetox
- Identify avenues for IVGT to contribute to resolution of these issues
  - HESI is uniquely qualified because of its diverse membership, the sweat equity of its members, funding mechanisms, and ability to perform/fund laboratory research to fill data gaps

***IWGT: International Workshop on Genotoxicity Testing, one meeting every four years.**

Improving Existing Assays Workgroup

Cell type comparisons

Question: Is p53 gene status or human/rodent nature of cells the decisive factor for relevant results?

- Experimental work needed:
  - TK6 (human/p53+) vs WIL2-NS (human/p53-) vs L5178Y (rodent/p53-)
  - Using same endpoint/protocol like COLIPA project (i.e., 6/7 ‘false positive’ compounds in *in vitro* micronucleus assay)
  - Possible second stage: testing of real positives on WIL2-NS and L5178Y
- Possible laboratories:
  - Covance, Bioreliance or G. Johnson (University). RFP process needed
- Timelines:
  - 9 months total (3 months to RFP decision, 6 months for laboratory work).
  - Results expected by the end 2010/beginning 2011.

Improving Existing Assays Workgroup

Good practices for cell culture

Question: Are specific recommendations, and good quality repository needed for cells used in genotoxicity tests?

- Review already available guidelines and define if specific aspects are missing
- Prepare a publication:
  - to describe which factors related to culture conditions and cell line stability are important and how can they be controlled
  - to provide guidelines how to establish cell line for genetox (karyotype, cell cycle time, cloning efficiency, background range, diagnostic chemicals?)
- Identify good quality repository interested by the project
  - find original cell isolates of the currently used cell lines within IVGT Committee or well-known laboratories
  - identify appropriate repository (commercial supplier? Check with ECVAM, Harlan, Covance UK, Bioreliance, others)
  - provide good quality cells through repository

Timing, cost and feasibility: to be evaluated and drafted by end 2010

Improving Existing Assays Workgroup

Top concentration for *in vitro* assays

Question: Can the top concentration be lowered (e.g., 1 mM vs 10 mM) without impacting hazard identification and risk assessment?

- On hold until further information are available and distributed to the group (ECVAM review data, similar exercise on *in vivo* genotoxins, review of NTP data, etc.)
- Determine if experimental work is needed when data available.

Metabolism

Question: Would human S9 be more appropriate? Should phase II and detoxification be evaluated?

- Literature review needed on the use of human S9 and Phase II detoxification pathways
- Determine if experimental work is needed after literature review.

Improving Existing Assays Workgroup

Cytotoxicity

Question: Could cytotoxicity parameters recently defined for *in vitro* micronucleus assay be used for chromosome aberration assay?

- The group agreed. This statement will be added into IWGT publication.
- No experimental work needed.

Possible new cell types

Question: What is the status of new cell types under evaluation in genetic toxicology?

- Their status should be clarified before being used by IVGT Committee for any experimental work[e.g., AHH1 (unstable), MCL5 (unstable, lose C-DNA), HepaRG (patent issue and stability unclear to be checked), FE1 (p53 status unknown, work ongoing in Health Canada)].

New Approaches Working Group

Anthony Lynch (GSK), Co-leader

Masa Honma (NIHS, Japan), Co-leader

New Approaches Working Group

Objectives (Kick-off meeting in March 2010)

o Horizon scan outside genetic toxicology – How genetic toxicology could benefit from new advances in biology and technology - where should genetox testing be in the 21st Century?

- New biology – potential impacts on genetox testing
  - Stem cells, organ culture
- New technology – potential impacts on genetox testing
  - Whole genome sequencing, imaging technologies, infrared microscopy, germ cell mutagenesis assays, transcriptomics, high throughput assays, biomarkers of genetic damage, DNA adductome
- New strategies for risk assessment
  - Translational methods
  - Epigenetics

o Two-day Workshop in 4Q2011-1Q2012: Open to a large audience (possible CTT workshop)

HESI is uniquely qualified because of its diverse membership to reach out into other non-genetox disciplines, resources to organize a workshop.

Genotoxicity of Nanomaterials

(Not part of IVGT activities)

Stefan Pfuhler (Procter & Gamble), Co-Leader

Marilyn Aardema (Consultant), Co-Leader

Genotoxicity of Nanomaterials
(Not part of IVGT activities)

Objectives (First Planning meeting in March 2010)

- o Take advantage of the strong interest among IVGT members especially cosmetic and chemical industries, as well as regulatory scientists
  - Not considered a direct IVGT activity (currently)
- o Workshop being organized for the EMS 2010 meeting
  - Overview of the many ongoing efforts worldwide
    - Define HESI (IVGT) potential role in these efforts
  - Issues:
    - is the current genetox battery appropriate?
    - need for particle characterization
    - need for exposure characterization

HESI is uniquely qualified because of its ability to leverage sweat equity and funding from diverse sources (e.g., Health Canada) for this workshop.

IVGT Project Committee Outreach and Perspectives for 2010-2012 Period

Future workshops / symposia:

- [Workshop on Nanoparticles (2010)]
- Workshop on New Approaches (2011 or 2012)
- Japan SOT and other (Genetic) Toxicology Society meetings

Future publications:

- Proceedings of workshop on New Approaches
- Data and recommendations from Improving Existing Assays WG
- Data and recommendations from Quantitative WG

Other activities requiring partnership:

- G8/12 database
- Cell repository

Petition for Technical Committee Status at end of 2012?

Objectives of this Symposium

- Provide an overview of the IVGT's projects
- Provide an opportunity for Committee leaders to present their own perspectives
- Stimulate discussion on issues in genetic toxicology
  - Systematically examine the state of the science in genotoxicity assessment
  - Assess the utility of new and emerging genetic toxicology tools
  - Address a shift away from qualitative genetox assessment to a quantitative approach – from hazard identification to more accurate human risk assessment

Agenda for this Symposium

- Current Strategies in Assessing Genotoxic Risk - Kerry Dearfield, PhD (USDA)
- Need for a New Approach to Genetic Toxicity Assessment : Lessons Learned and New Opportunities – Jim MacGregor, PhD (Toxicology Consulting Services)
- Approaches to Follow-Up on Positive Results in Genetic Toxicology Tests in the Context of Human Risk Assessment – Veronique Thybaud, PhD (sanofi-aventis)
- New Technologies to Predict Genotoxic Risk in Humans – David Jacobson-Kram, PhD (U.S. FDA)
- Beyond Positive or Negative: A Quantitative Approach for Interpreting Genotoxicity Data – Bhaskar Gollapudi, PhD (Dow Chemical Co.)
- Optimal Design for in Vivo Mutation Studies to Inform Cancer Mode-of-Action Assessment – Martha Moore, PhD (U.S. FDA / NCTR)