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Workshop: "Genetic Toxicology: Opportunities to Integrate New Approaches"

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Lincoln Ballroom
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Sponsored by:
ILSI Health and Environmental Sciences Institute
Project Committee on Relevance and Follow-up of Positive Results in In Vitro Genotoxicity Assays (IVGT)

Speakers and Abstracts

Organizing Committee

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Masa Honma	National Institute of Health Sciences (Japan)
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Manju Manjanatha	U.S. FDA / NCTR (USA)
Stefan Pfuhler	Procter & Gamble (USA)
Leon Stankowski	BioReliance (USA)
Véronique Thybaud	Sanofi (France)
Jan van Benthem	RIVM (Netherlands)
Paul White	Health Canada (Canada)
Kristine Witt	NTP / NIEHS (USA)
Errol Zeiger	Consultant (USA)

IVGT Session Leaders

- **James H. Kim**

Jim graduated from the Johns Hopkins Bloomberg School of Public Health with a Ph.D. in Toxicology in 2001. For almost 7 years, Jim worked as a toxicology consultant and project director at Tetra Tech Sciences. Since coming to the ILSI Health and Environmental Sciences Institute in 2007, he has managed the Developmental and Reproductive Toxicology (DART) Technical Committee, the Project Committee on the Relevance and Follow-up of Positive Results from In Vitro Genetic Toxicity Testing (IVGT), the Biological Significance of DNA Adducts Project Committee, the Nonclinical/Clinical Safety Correlations Technical Committee, and will soon be taking on the Imaging Project Committee. He is an active member of the Society of Toxicology, the Teratology Society and the Environmental Mutagen Society. Jim became a Diplomate of the American Board of Toxicology in 2007.

- **Véronique Thybaud**

Véronique Thybaud, head of genetic toxicology at sanofi aventis's Paris Research Center for many years, is currently preclinical scientific advisor in Drug Disposition, Preclinical Safety and Animal Research, in sanofi aventis. She gained her PhD in fundamental and applied toxicology from the University of Paris, Villejuif Institute of Cancer Research. Following postdoctoral fellowships at the University of Leuven (Belgium) and at the Institut Curie in Orsay (France) she joined Rhône-Poulenc in 1986 where she became Head of Genetic Toxicology in 1989. In her current role, she is closely involved in the development and validation of new technologies such as transgenic animal for the detection of gene mutations, the comet assay, the in vitro micronucleus and toxicogenomics as well as discussions on the new guidelines, strategy and risk assessment. For example, she is an OECD expert for genotoxicity, a European industry representative in the revision of ICH S2 (International Conference on Harmonization for pharmaceuticals). She was also several times involved as chair person in the International Workshop Genotoxicity Testing meetings. She has published over 45 scientific papers, gives regular lectures for five different universities and supervises students. She is Past President of the French Genetic Toxicology Society (SFTG), and served as council member of the Environmental Mutagen Society, and European Environmental Mutagen Society. She is the chairperson of the HESI-ILSI Project Committee on the Relevance and Follow-up of Positive Results in In Vitro Genetic Toxicity (IVGT) Testing.

- **Marilyn Aardema**

Dr. Marilyn Aardema is the Chief Scientific Officer for Toxicology at the BioReliance Corporation. Marilyn is responsible for providing scientific leadership within BioReliance, for setting business strategy, and providing regulatory and scientific guidance to colleagues and clients. Marilyn was

previously at The Procter & Gamble Company in the Central Product Safety organization where she managed overall genetic toxicology battery design and risk assessment/external defense of key ingredients for diverse products worldwide. Marilyn has been involved in various research projects including studying mechanisms and thresholds for genotoxicity, aneuploidy, and development of improved genotoxicity testing approaches including most recently the development of a novel micronucleus assay in 3D human skin models

Marilyn received her Ph.D. in Genetics from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences. She is active in a number of organizations including the Environmental Mutagen Society, the Society of Toxicology, the Genetic Toxicology Association, ILSI-HESI, The American Society for Cellular and Computational Toxicology.

- **Bhaskar Gollapudi**

B. Bhaskar Gollapudi, Ph.D. is the Scientific Director of Mammalian Toxicology at The Dow Chemical Company's global Toxicology & Environmental Research laboratory located in Midland, MI. He is currently serving as an Associate Editor of the journal Toxicological Sciences, Editorial Board member of Journal of Toxicology, and a member of Faculty 1000 Biology. He is as an adjunct associate professor of toxicology in the Department of Public Health at the University of Michigan, Ann Arbor, MI. He has been an active participant in the International Sciences Institute's Health and Environmental Sciences Institute, currently serving on its Board of Trustees and the Co-Chair of the project committee on the Relevance and Follow-up of Positive Results in *In Vitro* Genotoxicity Testing. He is also a member of the Steering Committee of the International Workshops on Genotoxicity Testing. He has mentored a number of undergraduate, graduate, and post-doctoral students in his laboratory. His current research activities are in the areas of mode of action and dose-response in chemical mutagenesis and carcinogenesis. He has authored/co-authored more than 90 scientific publications in peer reviewed journals. He received his B. Sc (Chemistry) and M.Sc. (Genetics) degrees from Osmania University, Hyderabad, India, and a Ph.D. in Biology from Dalhousie University, Halifax, NS, Canada.

- **Kristine Witt**

Kristine Witt is Group Leader of Genetic Toxicology in the Biomolecular Screening Branch of the Division of the National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIH/NIEHS). She manages the NTP's genetic toxicology testing contract, provides genetic toxicology data evaluation for NTP Technical Reports and study designs, and manages NIEHS research studies on cytogenetic endpoints in populations exposed to agents with known or suspected genetic toxicity. She also is involved in the NTP's High-Throughput Screening initiative where she serves as co-chair of the Assays and Pathways Working Group for the Tox21 collaboration and chairs the NTP's High-Throughput Screening Faculty.

Witt received a B.S. in zoology and an M.S. in genetics from The Ohio State University, and completed additional graduate study in Human Genetics at the University of Chicago. She serves on the Editorial Board of Mutation Research — Genetic Toxicology and Environmental Mutagenesis, and is a Councilor and active committee member of the Environmental Mutagen Society. She has authored or co-authored 55 peer-reviewed papers and book chapters on topics related to genetic toxicology and high throughput screening.

- **Manju Manjanatha**

Dr. Mugimane (Manju) G. Manjanatha received his Ph.D. in Microbiology and Genetics from Iowa State University in 1990. After training as an Oak Ridge Institute of Science and Engineering postdoctoral fellow at the National Center for Toxicological Research (NCTR), Jefferson, AR, Manju joined the FDA staff at NCTR in 1994. Manju is a recognized leader in the field of genetic toxicology especially in the use of transgenic animals for hazard identification, characterization, and risk assessment. His research interests also extend to modification of Comet assay for assessment of genotoxic and epigenetic mode of action of FDA regulated agents, and gene expression profiling to determine mode of action as a tool for human risk assessment. Most recently, he is involved in the development of a transgenic albino and hairless mouse model for Phototoxicology studies. Manju serves on many FDA, lLSI, and EMS committees that address the application of genetox model systems in hazard characterization and mode of action of FDA relevant agents. Manju has been an active member of the EMS since the mid 90s and served on the Alexander Hollaender, the Publication and Policy, and the EMS Program Committees for 2011 and 2012. Since 2008, he has been serving as the Chair of the EMS Transgenic and In vivo Mutagenesis Special Interest Group and as the Chair of the EMS Public Relations and Communication Committee. Manju also served in the working groups of genotoxicity of nanoparticles and in vitro and in vivo genetox at ILSI/HESI.

Manju has more than 55 publications in peer-reviewed toxicology journals, book chapters and mini reviews. H serves in the editorial boards of Molecular Biology Journal of International Scholarly Research and Toxicology Journal of the Scientific World. He also reviews articles for many toxicology journals including *Environmental and Molecular Mutagenesis, Mutation Research, Carcinogenesis,* and *Toxicological Sciences*, etc. He has received several major awards and recently the FDA Commendable Service Award.

Speakers and Abstracts

❖ Stefan Pfuhler

Dr. Stefan Pfuhler received his Ph.D. in Biology from the department of Pharmacology and Toxicology of The University of Ulm in 1997. After heading the genotoxicity lab of BSL Bioservice, a CRO in Munich (Germany) for 2 years he joined his current employer, The Procter and Gamble Company were he now serves as the global company expert for genetic toxicity. In the recent years Dr. Pfuhler's research has been focusing on the genotoxic properties of aromatic amines, genotoxicity testing strategies, and on alternatives to animal testing. He is chairing The European Cosmetics Industries' (COLIPA) Task Force Genotoxicity since 2004 and has been chairing the GUM Working Group Testing Strategies until 2009, which he still serves as a member. Dr Pfuhler is a member of the Environmental Mutagen Society (EMS), the European EMS, serves on ECVAM's Genotoxicity and Carcinogenicity Expert Team and is actively involved in the In Vitro Genotoxicity Testing (IVGT) project committee of ILSI-HESI and the ongoing OECD genetox guideline revision efforts.

Overview of the use of 3-dimensional tissue constructs for genotoxicity testing

Alternatives to animal testing are increasingly being utilized by the scientific community for a variety of reasons which include the versatility of *in vitro* methods, considerations of cost and time, ethical concerns, as well as regulatory restriction of animal use. The EU Cosmetics Directive, e.g., prohibits the use of in vivo genotoxicity tests in safety assessments for cosmetic ingredients. *In vitro* genotoxicity test have a long history of use, however, the predictive capacity of a battery of such assays seems to be sub-optimal. This triggers the need for follow-up studies which usually would be done in animals. Taking the mentioned limitation of animal studies into account, three dimensional tissue constructs come to mind as logical follow-up tools for genotoxicity testing as they resemble *in vivo*-like conditions. 3D skin models have been successfully established for testing for genotoxic properties of dermally applied compounds and the status of the ongoing validation exercise will be briefly presented. Data from a variety of additional tissue constructs will be shown that demonstrate the applicability of the same endpoints (micronucleus and comet assays) to tissues like lung and oral mucosa. Reconstructed lung models which provide improved metabolic competency are now commercially available and data will be shown that demonstrate the utility of such models for genotoxity testing.

❖ Seiichi Ishida

Dr. Seiichi Ishida earned his Ph.D. in Pharmaceutical Science from The University of Tokyo in 1993. Dr. Ishida completed a post-doctoral fellowship at The Cancer Institute in Tokyo, working on transcript targets of c-MYC protein. This was followed by a research fellowship in the laboratory of Dr. J.R. Nevins, Howard Hughes Medical Institute, Duke University Medical Center, Durham, where he worked on identification of transcription targets of E2F during the cell cycle progression and development of bioinformatic analysis methods for DNA microarray data. Dr. Ishida is currently a Section Chief, Division of Pharmacology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan, where his research interests are reconstruction of liver/hepatocyte function, evaluation of synthetic retinoids by global gene expression analyses, and development of bioinformatic analysis methods for DNA microarray data.

Development of in vitro toxicity tests using hepatocyte differentiated from human stem cells

The evaluation of hepatotoxicity of drug candidates is important because liver plays major roles in the process of their absorption, distribution, metabolism, and excretion. Hepatotoxicity tests depend on animal model in many aspects. However, species differences often limit the extrapolation of results obtained in animal tests to human. Thus, the establishment of the hepatotoxicity tests using human tissues has been desired. Human primary hepatocytes are widely used for this purpose these days, although they still have problems such as inter-individual differences or stable supply. Thus, many studies have been done so far to get hepatocytes by differentiating human stem cells. After the establishment of human iPS cells (Yamanaka *et al.* 2008), the speed of such study accelerated.

We have promoted studies to develop *in vitro* toxicity test using the cells differentiated from human iPS cells. iPS cells are the promising source of an alternative to primary human cells. However, several difficulties exist, *e.g.* it is difficult to go through the complicated differentiation process with reproducibility; it is still insufficient to get the matured cell phenotype, *etc*.
I will introduce recent progress of studies in this field and would like to discuss what is required to establish iPS derived hepatocyte suitable for hepatotoxicity tests.

❖ **Darrell Boverhof**

Dr. Boverhof is a Senior Toxicology Specialist with The Dow Chemical Company's Toxicology & Environmental Research and Consulting (TERC) group in Midland, MI. He earned a B.Sc. in Biomedical Toxicology from the University of Guelph, Canada, and a Ph.D. in Biochemistry/Toxicology from Michigan State University. Prior to his graduate work, he was employed as a Toxicologist with Health Canada where he was involved the categorization and evaluation of chemicals on the Canadian Domestic Substances List. Dr. Boverhof joined Dow in 2006 and is responsible for testing, research, and consulting in the areas of immunotoxicology, respiratory allergy and sensitization, nanotechnology and toxicogenomics. Dr. Boverhof also serves as a representative on the ILSI-HESI Immunotoxicology Technical Committee, the American Chemistry Council Nanotechnology Panel and is currently serving as chair of the ILSI NanoRelease project. He also serves on the Medical Committee for the Elsa U. Pardee Foundation and on the Editorial Board for *Toxicological Sciences* and has previously served as a Councilor for the Michigan Society of Toxicology. He has authored/coauthored over 25 peer reviewed research publications, review articles, and book chapters in the area of toxicology and toxicogenomics in addition to recently co-editing a book on the application of toxicogenomics to risk assessment.

Humanized models in toxicology and their applications to hazard characterization and risk assessment

The toxicity testing paradigm is at a turning point. Many are calling for the implementation of new approaches, models, and technologies in order to enhance and refine the hazard and risk assessment process. Important components to the emerging views are implementation of mode/mechanism-of-action data, a greater focus on human models/relevance, and a reduction in the use of animals. One important emerging tool that has the potential to advance the field is the use of humanized animal models. The use of humanized models and their resultant data has the potential to further enhance mechanism- based assessment approaches while inherently providing data relevant to human risk. Furthermore, if implemented strategically into the hazard and risk assessment paradigms, such models could obviate the need for large-scale animal testing thereby reducing animal use. However, the application of these data to human risk assessment requires appropriate and consistent interpretation of the data and acceptance of a given mode-of-action and its relevance to humans. Such acceptance requires discussion and consensus among all relevant stakeholders. This talk will provide an overview of the key areas where humanized models are being applied in toxicology and risk assessment and the associated challenges and opportunities.

❖ Peter Dedon

Peter Dedon is the Underwood-Prescott Professor of Biological Engineering and Toxicology in the Department of Biological Engineering at MIT, Singapore Research Professor of Infectious Disease, and the Deputy Director of the MIT Center for Environmental Health Science. Following graduation with a B.A. in Chemistry from St. Olaf College in 1979, he obtained an M.D. and a Ph.D. in Pharmacology from the University of Rochester in 1987, and joined the Toxicology faculty at MIT in 1991. The Dedon research group seeks to understand the chemical etiology of human disease, with the long-term goals of developing diagnostic tools, biomarkers and therapies. Using both data- and hypothesis-driven approaches, the research team develops ultra-sensitive bioanalytical tools to characterize and quantify normal and damaged biomolecules in cells and tissues and to interrogate biochemical networks and systems. The current research program has a broad theme of nucleic acid chemistry and biology with a focus on microbial pathogens, inflammation and cancer. As part of the Singapore-MIT Alliance for Research and Technology, his research group is studying the role of translational control mechanisms in the pathophysiology of microbial pathogens and cellular stress responses.

A new paradigm for epigenetic control of cell phenotype: Dynamic reprogramming of tRNA modifications and ribosomes controls selective translation of stress response proteins

Cells respond to environmental changes by altering gene expression at several levels, with translational control mechanisms being poorly understood. Emerging evidence points to complex interactions between tRNA, mRNA and ribosomes that control the rate and fidelity of translation. Contributing to this complexity are ~200 genes encoding tRNAs in humans, dozens of genes encoding ribosomal RNA and proteins, and >100 different ribonucleoside modifications in tRNA and rRNA across all organisms in addition to the canonical A, C, G and U. While RNA modifications are emerging as critical players in tRNA stability, stress response and cell growth, information about the higher-level biological function of ribonucleoside modifications is lacking.

To explore the biological function of RNA modifications, we recently developed a platform for purification of individual RNA species and quantification of the full set of RNA modifications in an organism by liquid chromatography-coupled mass spectrometry. This platform revealed that the dozens of modified ribonucleosides in tRNA behave as a system, with reprogramming of the modifications in response to different types of cell stress. This behavior was observed across the spectrum of living organisms, including bacteria, yeast and mammalian cells. When cells were exposed to a diverse set of toxicants, such as hydrogen peroxide, peroxynitrite, hypochlorous acid, ionizing radiation and a series of alkylating agents, multivariate statistical analysis revealed dynamic shifts in the population of RNA modifications as part of the response to damage, with signature changes for each agent and for different doses of each agent. Further, cells lacking the enzymes involved in synthesizing the tRNA modifications that changed significantly following toxicant exposure proved to be hypersensitive to cytotoxicity caused by the toxicant. Analysis of

tRNA modifications affected by hydrogen peroxide exposure revealed a codon-specific translational bias favoring synthesis of specific ribosomal proteins and other stress response proteins. These results suggest a step-wise mechanism of cell response involving reprogramming of tRNA modifications that leads to reprogramming of ribosome structure in the translational control of cellular stress responses.

❖ **Jennifer Marlowe**

Jenny is Head of the Molecular Toxicology group in Cambridge (Discovery & Investigative Safety, Preclinical Safety). Jenny began her career at Novartis in Investigative Toxicology in Basel, where she headed a laboratory focused on molecular mechanisms of carcinogenesis as well as epigenetic mechanisms of adverse drug effects. She transitioned in 2009 to Cambridge in order to initiate and head a new Molecular Toxicology group focused on molecular on- and off-target mechanisms of toxicity as well as understanding mechanisms of adverse effects of systemically delivered siRNA therapeutics. She also represents Preclinical Safety for early oncology and metabolism programs, and serves as the Science and Portfolio Manager for cardiovascular and metabolism projects. Prior to joining Novartis, Jenny earned a PhD in Molecular Toxicology, with an emphasis in cellular and molecular mechanisms of carcinogenesis, from the Department of Environmental Health Sciences at the University of Cincinnati. She also holds a Bachelor of Science degree in Zoology (Miami University, Oxford, Ohio).

Epigenomics and Impact for Drug Safety Sciences

It is becoming increasingly clear that some of the earliest events preceding the development of overt pathologies, including those arising from the exposure to environmental and pharmaceutical agents, involve perturbations of the epigenome. Recent advances in technologies for mapping and characterizing the mammalian epigenome are generating new opportunities for exploring the relationship between epigenetic modifications, human disease and the therapeutic potential of pharmaceutical drugs. While the best examples for xenobiotic-induced epigenetic perturbations come from the field of non-genotoxic carcinogenesis, there is growing evidence for the relevance of epigenetic mechanisms associated with a wide range of disease areas, targets, and drug exposures. The application of epigenomic profiling technologies to drug safety sciences has great potential for providing novel insights into the molecular basis of long-lasting cellular perturbations, including increased susceptibility to disease and/or toxicity, memory of prior immune stimulation and/or drug exposure, and transgenerational effects.

❖ Igor Pogribny

Igor Pogribny is a Senior Research Investigator in the Division of Biochemical Toxicology at the FDA-National Center for Toxicological Research (Jefferson, AR). Dr. Pogribny received his M.D. (1982) and Ph.D. (1986) in Biochemistry and Oncology in Ukraine. His research focuses on biochemical and molecular aspects of carcinogenesis, particularly on identifying early biomarkers of tumorigenesis and developing strategies from the early detection of carcinogenic potential of various compounds, including components of the diet. Dr. Pogribny has authored more than 130 peer-reviewed articles.

Epigenetic traits as biomarkers of carcinogenesis

Environmental exposure to the natural and man-made chemical and physical agents is one of the major causes of human cancer. Therefore, the need for the rapid identification and appropriate regulation of human carcinogens before their dissemination into society is of prime importance for the primary prevention of neoplasia in humans. Recent advances in the field of cancer research have established that all major human cancers, in addition to having a large number of genetic alterations, exhibit prominent epigenetic abnormalities. This presentation highlights evidence indicating that epigenetic alterations are the early events in carcinogenesis that may be used as indicators of malignant transformation for both genotoxic and non-genotoxic carcinogens and as biomarkers in the evaluation of the carcinogenic potential of various chemical and physical agents. Additionally, the cellular epigenomic status of target tissue may be one of the key factors determining individual susceptibility to carcinogenic process.

❖ **Andrea Kasinski**

Dr. Kasinski earned a Ph.D. from Emory University in 2009. She is currently a post-doctoral associate in the Department of Molecular, Cellular and Developmental Biology at Yale University in the lab of Frank Slack. She is currently funded by the American Cancer Society.

MIR-34 prevents in vivo lung tumor initiation and progression in the therapeutically resistant KRAS;TRP53 mouse model

Lung cancer represents the leading cause of cancers deaths in men and women worldwide, and current therapies fail to treat this disease in the overwhelming majority of cases. The RAS and p53 pathways are two of the most frequently genetically modified pathways in lung cancers. Alterations in both result in loss of responsiveness to current therapies leading to decreased overall patient survival. Because the microRNA, *mir-34* is a downstream transcriptional target of p53, which is reduced in its expression in p53 null tumors, we hypothesized that targeting *Kras;p53* tumors with miR-34 would represent a novel yet powerful therapeutic to suppress lung tumorigenesis. To this end we have made use of the therapeutically resistant $Kras^{LSL\text{-}G12D/+};p53^{LSL\text{-}R172H/+}$ mouse model of lung cancer. In this work we characterized the tumor progression in these mice following transgene activation specifically in the lung through intratracheal administration of adenoviral particles expressing cre recombinase. We note that adenocarcinomas are evident as early as 10 weeks following cre-recombination with severe lung inflammation presenting at 22 weeks. Epithelial cells from these tumors were harvested and cultured *in vitro* where they remain propagating, currently at passage 45. All three lines generated support growth in soft agar assays and are invasive based on transwell migration assays. Because of the pro-apoptotic effects of miR-34, we transduced the cells with miR-34-expressing lentivirus. Transduced cells responded with reduced proliferation and decreased migration. Based on these promising results we began two series of *in vivo* experiments. We first assessed the contribution of miR-34 to <u>prevent</u> tumor formation in $Kras^{LSL\text{-}G12D/+};p53^{LSL\text{-}R172H/+}$ mice. Animals that were treated with miR-34 at the same time as cre-induced recombination of transgenes show little to no evidence of tumorigenesis at 19 weeks, while control animals had multiple nodules that represented ~8% of the total lung area. The second series of *in vivo* experiments evaluated the ability of miR-34 to act as a <u>treatment</u> in pre-formed tumors. While miR-34 was unable to reduce the preformed tumors in these animals it was able to prevent further tumor growth. These data support the use of miR-34 as a tumor preventive mechanism and suggest that miR-34 may be useful in sensitizing tumors to other conventional therapeutics.

❖ **Bill Slikker**

Dr. William Slikker, Jr. is the Director of the FDA's National Center for Toxicological Research (NCTR). Bill received his Ph.D. in Pharmacology and Toxicology from the University of California at Davis. Dr. Slikker holds Adjunct Professorships in the Departments of Pediatrics, and Pharmacology and Toxicology at the University of Arkansas for Medical Sciences. He is currently Associate Editor for NeuroToxicology and Toxicological Sciences and Vice President of the Society of Toxicology. Dr. Slikker has authored or co-authored over 300 publications in the areas of transplancental pharmacokinetics, developmental neurotoxicology, neuroprotection, systems biology, and risk assessment.

Imaging as an approach to safety assessment

Although the use of imaging in the clinical setting is well established, the use of imaging for pre-clinical assessments is infrequent. Even though the advantages such as using the animal as its own control, longitudinal study design and minimal invasiveness are now well recognized, standardization of approach, resolution and quantization have, until recently, slowed acceptance of imaging as a routine assessment tool. MicroPET now offers functional resolution of 1.5 mm and application of imaging to assessment of any target organ can deliver quantitative information in a minimally invasive manner and in parallel with other endpoint requirements. We conducted microPET imaging to evaluate the effects of general anesthetics, such as ketamine, isoflurane (ISO) and nitrous oxide (N_2O) on developing brain. On postnatal day (PND) 7, rat pups were exposed to either six injections of ketamine (20 mg/kg at 2-h intervals) or saline. On PND 35, 37 MBq (1 mCi) of ^{18}F -Annexin V was injected into the tail vein of these rats, and static microPET images were obtained over 2 h following the injection. The uptake of ^{18}F -Annexin V was significantly increased in the ROI of ketamine-treated rats.

The effect of ISO/N_2O on the uptake and retention of [^{18}F]-DFNSH in the brains of different aged rats and the potential protective effect of acetyl-L-carnitine (ALC) on anesthetic-induced neuronal cell death were investigated using microPET imaging. On PND 7, rat pups were exposed to either a mixture of 70% N_2O/30% oxygen plus 1% ISO for 8 hours with or without ALC, or to room air only (control). On PNDs 14, 21 and 28, [^{18}F]-DFNSH (18.5 MBq) was injected i.p. and 30 minutes later microPET images were obtained over 90 minutes. In PND 14 rats the uptake of [^{18}F]-DFNSH was significantly increased and the duration of tracer wash-out was prolonged in ISO/N_2O-treated rats. No significant difference was found in radiotracer uptake in the frontal cortex of the brains of PND 21 and 28 rats compared with same aged controls.

In addition, our animal experiments showed that ketamine caused substantial neuronal cell death in developing monkeys. We also observed ketamine significantly increased neuronal cell death in frontal cortex of PND 7 rats by EM and cell death detection ELISA. The cell death in rats seemed to be apoptotic in nature. *In situ* hybridization demonstrated that NMDA receptor NR1 subunit

expression was dramatically increased in the frontal cortex of ketamine-treated rats. Microarray analysis revealed altered expression of apoptotic-relevant genes and increased NMDA receptor gene expression in brains of ketamine-treated animals. Quantitative RT-PCR confirmed the microarray results.

We determined the dose response to ketamine using newborn rat forebrain cultures and tested if co-administration of L-carnitine and NR1 antisense, could protect or reverse ketamine-induced cell death. Neuronal cells collected from the rat forebrain were incubated for 24 h with 0.1, 1, 10 or 20 uM ketamine or 10 uM ketamine plus L-carnitine or NR1 antisense. 10 uM ketamine caused an increase in DNA fragmentation and immunoreactivity to nitrotyrosine. Ketamine-induced neurotoxic effects were effectively attenuated by L-carnitine and NR1 antisense. The data of calcium imaging supports the hypothesis that continuously blocking the NMDA receptors by ketamine can cause compensatory up-regulation of NMDA receptors, which allows the accumulation of toxic levels of intracellular Ca^{2+} after ketamine withdrawal, subsequently may trigger apoptosis in neurons.

❖ **Ray Tice**

Dr. Tice received his Ph.D. in Biology in 1976 from Johns Hopkins University (Baltimore, MD). He was employed by the Medical Department at Brookhaven National Laboratory (Upton, NY) from 1976-1988, and by Integrated Laboratory Sciences, Inc. (Durham, NC) from 1988 to 2005, where his last position was Senior Vice-President for Research and Development. He joined the National Institute of Environmental Health Sciences (NIEHS) in 2005 as the Deputy Director of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and in 2009 became the Chief of the Biomolecular Screening Branch within the Division of the NTP. This Branch is responsible for coordinating NTP's activities in the U.S. Tox21 consortium, the purpose of which is to prioritize chemicals for more extensive toxicological evaluation; identify mechanisms of chemically-induced biological activity in order to characterize toxicity pathways, facilitate cross-species extrapolation, and provide input to models for low-dose extrapolation; and develop predictive models for biological response in humans.

Dr. Tice has served as President of the Environmental Mutagen Society (EMS) and as Vice-President of the International Association of Environmental Mutagen Societies. He is the recipient of NIH Director's Group Awards for activities associated with the NIH Molecular Libraries Initiative and with the development of the ICCVAM Five-Year Plan (2008-2012). In 2008, he (along with Dr. Christopher Austin of the NIH Chemical Genomics Center and Dr. Robert Kavlock of EPA's National Center for Computational Toxicology) received the North American Alternative Award from the Humane Society of the United States and Proctor & Gamble for "outstanding scientific contributions to the advancement of viable alternatives to animal testing". In 2009, Dr. Tice received the EMS Alexander Hollaender Award in recognition of outstanding contributions in the application of the principles and techniques of environmental mutagenesis to the protection of human health. During his career, he has served on over 50 international expert panels and committees related primarily to genetic toxicology and more recently to the validation of alternative test methods. He has published 140 scientific papers and book chapters, contributed to 23 electronic review publications in support of the NTP chemical nomination process and to 35 NICEATM-ICCVAM publications, and has edited 4 symposia proceedings. Dr. Tice has been a member of the editorial boards of Mutation Research and Environmental and Molecular Mutagenesis.

The Tox21 strategy for detecting genotoxicants

In 2008, the National Institute of Environmental Health Sciences/National Toxicology Program (NTP) entered into a Memorandum of Understanding with the NIH Chemical Genomics Center and the Environmental Protection Agency's (EPA) National Center for Computational Toxicology on the research, development, validation, and translation of new and innovative *in vitro* and lower organism test methods that characterize key steps in toxicity pathways. This collaborative effort, known informally as Tox21, was expanded in 2010 with the addition of the U.S. Food and

Drug Administration. During the Tox21 pilot phase (2005-2011), ~1400 (NTP) or ~2800 (NTP/EPA) compounds were screened against ~100 quantitative high throughput screens (qHTS), predominantly reporter gene assays, at 14 concentrations (0.5 nM to ~92 μM) in 1536-well format on an automated platform. The qHTS assays included several that related to the detection of genotoxic compounds (ATAD5, p53, DNA repair-deficient chicken DT40 cell lines). In late 2011, we began screening a 10K compound library against a set of nuclear receptor and stress response pathway assays, including some of those used in Phase I to detect genotoxic compounds. Follow-up studies on identified subsets of compounds with defined activity in the genetox assays will include micronucleus and H2AX assays employing image analysis technologies. The lack of xenobiotic metabolism is the single, major limitation of the qHTS assays. Data being generated by these assays are being compared against existing in vitro genotoxicity data as a first step in determining the relevance of the qHTS data to the detection of genotoxic compounds.

❖ **Scott Auerbach**

Dr. Auerbach received a dual BS from The Pennsylvania State University in Physiology and Biochemistry/Molecular Biology in 1998. He then went on to receive his Ph.D. in Pharmacology from the University of Washington in 2004. From 2005 to 2007 Dr. Auerbach was a postdoctoral fellow at Duke University and then NIEHS under the direction Dr. David Schwartz where he undertook genetic studies of human pulmonary fibrosis. Subsequently he went on to a fellowship at the National Toxicology Program. Dr Auerbach became a staff scientist at the National Toxicology Program in 2009 and became a Diplomat of the American Board of Toxicology the same year. Also in 2009, Dr. Auerbach's SOT abstract that described an approach to application of genomics to hazard identification was awarded the Perry J. Gehring Best Postdoctoral Fellow Abstract Award. Dr. Auerbach has been a longstanding member of the Society of Toxicology. His research interests include the application of genomic tools and in vitro screening to predictive toxicology and genetic susceptibility to toxic challenge.

The behavior of genomic signatures of genotoxicity: Effect of dose level and exposure duration

The discipline of toxicogenomics for most of its existence has been focused on interpreting the qualitative nature of the molecular biology that underlies single, high-dose level toxicity. In addition, most of the studies published are also short in duration ranging from one day to five days. While this approach has been helpful for mode-of-action toxicological assessments it not helpful in identifying no effect levels that can be used for risk assessment. A critical question that needs to be asked in order to start implementing quantitative genomic metrics in risk assessment is how these metrics scale against traditional measures of toxicity. To evaluate the behavior of genomic signatures over time and dose we first developed multiple, mechanism focused models of genotoxic and non-genotoxic liver carcinogenesis using transcriptomic data from the rat toxicogenomics database, Drugmatrix. Using the most informative features of these models in combination with transcriptomic data from the Japanese Toxicogenomics Database we evaluate the impact that dose level and study duration have on transcript-based benchmark dose (BMD) estimates. Further we assess the relationship between the transcript BMDs and BMDs that were derived from cancer bioassay, rat liver tumor incidence data. The results from this analysis suggest that direct-acting genotoxins produce a cumulative effect over time which cause transcript BMDs to become progressively lower as the duration of study increases. Furthermore, in the case of direct acting genotoxins, transcript BMDs from short-term studies underestimate rat liver tumor BMDs. Taking into account the dose-response behavior of genomic signatures over time will be essential in effectively applying such metrics to set exposure limits that protect public health.