NorCal SOT Fall Symposium 2017

The 3R's

September 28th, 2017
South San Francisco Conference Center, 255 South Airport Boulevard,
South San Francisco, CA 94080

Morning Session	
7:30 am – 8:30 am	**Registration & Breakfast**
8:30 am – 8:45 am	**Opening message from The NorCal SOT President** *Doris Tham Zane, PhD, DABT, Sr. Director of Preclinical Development, Intarcia Therapeutics, Inc.*
8:45 am – 9:30 am	**Integrated toxicology (3Rs in action) and how *in vitro* toxicology is critical for success for a dermal IND** *Clive Roper, PhD, Head, In Vitro Sciences, Charles River*
9:30 am – 10:15 am	**Adopting a Culture of Care by Implementing the 3Rs in Toxicology Studies** *Lisa Wong, Non-clinical Study Monitor, Genentech, Inc.*
10:15 am – 10:30 am	**Coffee Break**
10:30 am – 11:15 am	**Modernizing the "six-pack" testing strategy: influx of modern *in vitro* techniques** *Gertrude-Emilia Costin, PhD, MBA, Study Director, Institute for In Vitro Sciences, Inc.*
11:15 am – 12:00 am	**To Each Their Own: Molecular Mechanisms of Inter-Individual Variability in Toxic Exposure Effects** *Shaun D. McCullough, PhD, Principal Investigator, US Environmental Protection Agency*
12:00 pm – 1:15 pm	**Lunch Break: Lunch with Experts**
Afternoon Session	
1:15 pm – 1:30 pm	**Chapter announcements and acknowledgements**
1:30 pm – 2:15 pm	***In Silico* Toxicology Protocols** *Glenn J. Myatt, Chief Scientific Officer, Leadscope Inc.*
2:15 pm – 2:30 pm	**Coffee Break**
2:30 pm – 3:15 pm	**Microphysiological Organ Systems (Organs on Chips) for Drug Discovery, Disease Modeling, and Precision Medicine** *Kevin E. Healy, PhD, Jan Fandrianto and Selfia Halim Distinguished Professorship in Engineering, Professor of Bioengineering, Professor of Materials Science and Engineering, University of California at Berkeley*
3:15 pm – 3:20 pm	**Closing Remarks**
3:20 pm – 5:00 pm	**Reception & Networking**

DIRECTIONS TO SSF CONFERENCE CENTER

Abstracts

Integrated toxicology (3Rs in action) and how *in vitro* toxicology is critical for success for a dermal IND

Clive S Roper, PhD, Head, In Vitro Sciences, Charles River, Edinburgh, UK

In vitro testing has been mainstream in toxicology since the 1970's. The hERG channel test is well integrated into the safety pharmacology testing paradigm. A genetic toxicology programme utilises screening in vitro assays then progresses to in silico, GLP in vitro bacterial and mammalian cellular and finally rodent in vivo. In discovery, on and off target efficacy screens are performed in cellular, and increasingly, computational (in silico) models.

In vitro skin penetration/ distribution studies using human skin are used to screen in new actives at early discovery and then in formulation development and selection at lead optimisation. Increasingly, full GLP mass balance studies are utilised to provide justification for clinical trials or even to replace them (Mitra et al, 2016). These tests may be used in support for choosing if the drug should be for topical, dermal or transdermal use and to repurpose drug candidates (e.g. that have been deselected due to, for example, poor oral bioavailability). Increasingly, there is focus on translation from toxicology species in vivo to human in vitro to human in vivo. Skin from toxicology species are tested alongside human skin to derive estimates or to confirm translational safety and efficacy.

Local effect risks must be quantified or, ideally, ruled out. In silico QSAR models (e.g. Derek Nexus) are predicting these, and other, toxic outcomes. The in vitro human tissue tests are increasingly replacing the in vivo animal tests. Examples include; ocular irritation and severe damage (BCOP and EpiOcular versus ocular Draize), skin irritation and corrosion (EpiSkin versus dermal Draize), and skin sensitization (DPRA, KeratinoSens, U Sens/ hCLAT versus LLNA or Buehler). Where a regulator requires the animal test, these tests will screen out the positives so ensuring only negatives are tested in vivo resulting in improvements in animal welfare. This is already an accepted reality in phototoxicology (3T3-NRU before in vivo).

A typical example for a dermal drug (i.e. intended to be active in the skin) could be as follows. Genetox: in vitro screen (BlueScreen, Ames MPF), in silico Leadscope/ Derek), in vitro (Ames, MLA/ CA/ in vitro MNT), in vivo (rodent MNT with or without Comet) and pigA could be added onto a chronic toxicology test. Safety pharmacology: in vitro (screening ion channels, hERG), in vivo (rodent respiratory function, Irwin test, non rodent cardiovascular telemetry). DMPK: in vitro (hepatic metabolism species selection, induction/ inhibition, PPB, met profiling, toxic metabolites), in vivo (screening PK, PK). Formulation selection: in vitro (skin penetration/ distribution). Translation: in vitro (human versus rat/ pig). General toxicology: local toxicology (as above), rodent IV/SC DRF, rodent IV/SC 28 day toxicity, non rodent dermal MTD, non-rodent 28 day dermal toxicity. These could also be with TK. A testing programme should be planned on a case-by-case basis and, if possible, reviewed by a regulator.

In conclusion, a well planned and executed integrated toxicology testing programme will apply many aspects of the 3Rs whilst delivering reduced costs, improved regulatory compliance and he best chance of success for efficacy and safety. This is already a reality in modern toxicology.

Mitra A, Kim N, Spark D, Toner F, Craig S, Roper C and Meyer T (2016). Use of an in vitro human skin permeation assay to assess bioequivalence of two topical cream formulations containing butenafine hydrochloride (1%, w/w). Regulatory Toxicology and Pharmacology 82; 14-19.

Adopting a Culture of Care by Implementing the 3Rs in Toxicology Studies

Lisa Wong, Non-clinical Study Monitor, Genentech, Inc.

The 3Rs vision at Genentech/Roche is to continue to increase awareness of the 3Rs principles, drive innovation, and influence best practices to reduce animal use and distress in nonclinical studies. This presentation will focus on the advancement of methods and approaches implemented at Genentech/Roche to REDUCE, REPLACE, and REFINE the use of animals in research. Case studies will be provided to illustrate the refinements in housing and enrichment, study designs, and in vitro and in silico models that we have implemented.

Modernizing the "six-pack" testing strategy: influx of modern *in vitro* techniques

Gertrude-Emilia Costin, PhD, MBA, Study Director, Institute for In Vitro Sciences, Inc.

In the current global regulatory climate favorable for further advancement of in vitro testing methods, joint efforts by industry, regulatory agencies and animal welfare organizations are the key to successful legislative changes for the safety assessment and labeling of chemicals. In the United States, the latest endeavor of the Environmental Protection Agency (US EPA) targets the modernization of the battery of toxicity tests classically known as the "six-pack" (acute oral, dermal and inhalation toxicity; primary ocular and dermal irritation; and dermal sensitization).

To help with the reinvention of the six-pack known as an "all in vivo" testing strategy for products registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), several validated in vitro test methods (many of which are adopted OECD Test Guidelines) have been considered for evaluation of their capacity to address US EPA hazard categories. The modernization of the six-pack has started with the use of non-animal test methods for classification and labeling for ocular irritation. The policy is the outcome of a multi-year project between the US EPA, Institute for In Vitro Sciences, Inc. (IIVS), industry stakeholders, and coordinated by The Accord Group, to utilize non-animal (in vitro/ex vivo) test methods in place of the rabbit test to determine the eye irritation potential of commonly used household cleaning products with anti-microbial claims. In 2015, the US EPA updated this policy for the use of an alternate testing framework for classification of eye irritation potential of more conventional EPA pesticides products. A similar program focused on the use of in vitro test methods for classification and labeling for dermal irritation is currently ongoing. Other efforts within industry with support from

US EPA are utilizing complementary in vitro test methods in concert with in silico prediction tools in a weight of evidence approach to address the rest of the endpoints included in the six-pack.

This presentation will provide the current status of the six-pack modernization from the perspective of the testing laboratory specialized in non-animal testing, outreach and education activities promoting in vitro testing strategies for global regulatory acceptance.

To Each Their Own: Molecular Mechanisms of Inter-Individual Variability in Toxic Exposure Effects

Shaun D. McCullough, PhD, Principal Investigator, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency

Traditional approaches to identifying susceptible populations have relied on factors such as age, genotype, and disease status to explain variability in exposure outcomes; however, these are neither sufficient to faithfully identify differentially responsive individuals nor are they modifiable factors that can be leveraged to mitigate the effects of toxic exposures. Unlike these factors, the epigenome is dynamic and shaped by an individual's environment. We characterized the relationship between the baseline abundance of six epigenetic markers with established roles as key regulators of gene expression – trimethyl histone H3 lysine 4 (H3K4me3), acetyl H3K27 (H3K27ac), pan-acetyl H4 (H4ac), di/trimethyl histone H3K27 (H3K27me2/3), unmodified H3, and 5-hydroxymethylcytosine (5-hmC) - and the variability in the O3-induced expression of pro-inflammatory and oxidative stress genes in an air-liquid interface model using primary human bronchial epithelial cells from a panel of donors. The relationships that we observed led to our proposal of the "Epigenetic Seed and Soil" model in which the baseline abundance of particular chromatin modifications within the regulatory regions of specific toxicant-responsive genes correlates with the magnitude of their exposure-mediated induction. While proposed with data collected from airway epithelial cells, the model is also applicable to the use of baseline epigenetic data to predict exposure responses in cell and tissue types throughout the body. Identifying the role of the epigenome in toxicant responsiveness will provide an additional dimension to our understanding of the mechanisms underlying inter-individual variability in exposure effects and provide new insight into identifying populations.

In Silico Toxicology Protocols

Glenn J. Myatt, Chief Scientific Officer, Leadscope Inc.

In silico toxicology is an important alternative approach to *in vivo* testing that provides a fast and inexpensive prediction of toxicity. Although running the models is fast, the whole process of making predictions, including selecting and acquiring the models, interpreting the results, performing an expert review, and documenting the results, can be time-consuming and difficult to repeat. It is also challenging to defend the results, primarily due to a lack of published procedures for performing an *in silico* assessment. To support the development of such protocols, a 52-member consortium has been

assembled and includes representatives from international regulatory agencies and government research laboratories in the United States, Canada, Japan and Europe, as well as companies from major industrial sectors (e.g., pharmaceuticals, cosmetics, food), academic groups and other stakeholders. The protocols will ensure any *in silico* assessments are performed in a consistent, repeatable, and well-documented manner to support wider uptake and acceptance of the approaches.

Microphysiological Organ Systems (Organs on Chips) for Drug Discovery, Disease Modeling, and Precision Medicine

Kevin E. Healy, PhD, Jan Fandrianto Professor, Bioengineering and Materials Science & Engineering, University of California at Berkeley

Drug discovery and development are hampered by high failure rates attributed to the reliance on non-human animal models employed during safety and efficacy testing that poorly reflect human disease states. With the discovery of human induced pluripotent stem cells (hiPSCs), bioengineers can now develop in vitro disease specific tissue models to be used for high content drug screening and precision medicine. Combining the genetic background of human cells with appropriate biophysical tissue architecture and “tissue-like” drug gradients can recapitulate a minimal human organoid sufficiently to allow accurate prediction of the toxicity of drugs. This presentation will discuss our progress in developing integrated in vitro models of human cardiac and liver tissue based on populations of normal and patient specific hiPSCs differentiated into cardiomyocytes, hepatocytes, or supporting cells. The benefits of our approach include: 1) robust microengineering platforms that control microtissue organization and function; 2) precise delivery of molecules (e.g., drugs) in a computationally predictable manner; 3) ability to model human disease; 4) cost efficient and high content characterization of an integrated multi-organ drug response; and, 5) reduction in use and refinement of animal experiments.

Biographies

Clive Roper, BSc (Hons), PhD, CBiol, CSci, MRSB, CRL

Bio for Clive S Roper BSc (Hons) PhD CBiol CSci MRSB

Dr Roper graduated with a degree (1990) and PhD (1994) from Newcastle University, followed by a postdoctoral position at Newcastle University. His Unilever funded PhD assessed and developed in vitro methods to determine absorption and metabolism of ethoxylates in skin as a replacement for in vivo testing. His papers are cited in OECD Guidance Document No. 28. In 1996, Dr Roper joined Inveresk Research (later to become Charles River Laboratories) as a Research Scientist, Study Director and Scientific Manager. Within these roles, he set up and grew the in vitro skin penetration service, created an in vitro nail absorption model and was a driver for the introduction and development of the in vitro toxicology service. In 2010, Dr Roper was promoted to Department Head, In Vitro Sciences where his sphere of influence widened to include in vitro DMPK, in vitro safety pharmacology, genetic toxicology and investigational and mechanistic toxicology. Dr Roper is an active member of Charles River's In Vitro Toxicology, 3Rs, Safety Pharmacology and Laboratory Sciences Leaders Working Groups and advises corporate Charles River on in vitro and integrated toxicology strategy. Dr Roper has been an advisor for biocides, cosmetic and chemical industry bodies as well as an individual companies. He was a committee member for Skin Forum (2008-2015) and served on the scientific organising committee for many meetings since 1995, most notably Skin Forum, Skin Metabolism and WC9. Dr Roper is also a member of the NIH/PETA Inhalation Working Group. Dr Roper is one of the founder members of the North American 3Rs Collaborative (www.na3rsc.org) which was launched at WC10 in Seattle in August 2017.

Lisa Wong, Non-clinical Study Monitor, Genentech

Lisa Wong is a Senior Nonclinical Study Manager at Genentech with over 20 years' experience in diverse roles supporting nonclinical development of biopharmaceuticals. She co-leads the 3Rs Expert Working Group at Genentech and is responsible for operationalizing Genentech's toxicology strategy and policy on animal husbandry, such as space allocation, social housing needs, and environmental enrichment. Lisa holds a Bachelor of Science degree in Biochemistry from the University of California at Riverside.

Gertrude-Emilia Costin, PhD, Institute for *In Vitro* Sciences, Inc.

Dr. Gertrude-Emilia Costin received her Ph.D. (Cum laude) in 2001 from the Institute of Biochemistry of the Romanian Academy. She continued her work on intracellular trafficking and maturation of melanosomal proteins during postdoctoral training at the National Cancer Institute, National Institutes of Health (NIH). After completing her postdoctoral fellowship at NIH, Dr. Costin worked as Senior Research Scientist for Avon Products, Inc. – Global R&D. She joined The Institute for In Vitro Sciences, Inc. (IIVS) in 2007 and currently works as Manager of Scientific Services and Study Director. She is in charge of a wide range of safety and efficacy commercial studies and research projects using in vitro

testing strategies. Her main area of expertise is in the use of in vitro test methods for the dermal safety assessment of ingredients and final formulations manufactured by the personal care or pharmaceutical industry as well as products to be registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which is regulated by the U.S. Environmental Protection Agency (EPA). Furthermore, Dr. Costin specializes in the use of non-animal methods to address the safety assessment of feminine care products or medical devices registered under the Food and Drug Administration (FDA). As part of IIVS' mission in education, Dr. Costin is involved in educational workshops focused on non-animal research and testing using diverse in vitro assay systems to assist the needs of the pharmaceutical and personal care industry as well as chemical manufacturers. In the last eight years, Dr. Costin has been the coordinator of the review process for the Alternatives Research Grants awarded annually by the Alternative Research and Development Fund (ARDF). Dr. Costin is currently serving as Board member and Newsletter Editor for the National Capital Area Chapter of Society of Toxicology (NCAC-SOT) and the PanAmerican Society for Pigment Cell Research (PASPCR), and is a Board Council member of the American Society for Cellular and Computational Toxicology (ASCCT).

Shaun D. McCullough, PhD, US EPA

Dr. Shaun D. McCullough is a Principal Investigator in the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency where he leads a research laboratory that focuses on exploring the molecular and epigenetic mechanisms of inter-individual variability in adverse health effects of inhaled toxicants. After earning his Ph.D. in Biochemistry and Molecular Genetics from the University of Virginia School of Medicine, Dr. McCullough completed his postdoctoral training in the EPA's Clinical Research Branch where he identified novel molecular pathways of single and multi-toxicant mediated stress and specific epigenetic markers that can be used to predict the cellular response to toxicant exposure. As a result of his postdoctoral work, he received the EPA Superior Accomplishment Award, the Gabriel L. Plaa Education Award and the Molecular and Systems Biology Specialty Section postdoctoral fellow award, and was featured in an article on postdoctoral fellows in the journal Science. Dr. McCullough is a regular peer-reviewer for a wide range of journals, serves on the editorial boards of Environmental Epigenetics and the Journal of Toxicology and Environmental Health, and serves as a Subject Matter Expert in epigenetics and in vitro models for the EPA review of guidance and policy documents. An active leader in the toxicology community, Dr. McCullough is Chairman of the EPA's National Health and Environmental Effects Research Laboratory Epigenetics Workgroup, a member of the Board of Directors for the American Society for Cellular and Computational Toxicology, a founding member of the University of North Carolina at Chapel Hill's Program in Chromatin and Epigenetics, and the Task Leader for EPA initiatives to develop concordance between in vitro and human in vivo toxicity testing. Dr. McCullough is also involved in the Society of Toxicology (SOT) as the Vice President of the Molecular and Systems Biology Specialty Section, Co-Chair of SOT's Career Resources and Development Committee, and he has chaired three scientific sessions at the SOT Annual Meeting in the last three years as well as a recent Contemporary Concepts in Toxicology meeting on toxicoepigenetics. Additionally, Dr. McCullough plays an active role in science education a

mentor to trainees ranging from high school students to postdoctoral fellows, including a recipient of the 2017 SOT Pfizer Undergraduate Award and a 2017 Carl C. Smith Award winner. Dr. McCullough also engages his local community in toxicology as the Director and Co-Founder of Tarheel Tox Talks, a public outreach partnership between the UNC Curriculum in Toxicology and the North Carolina regional chapter of SOT.

Glenn J. Myatt, Leadscope Inc.

Dr. Myatt is one of the founders and is currently the Chief Scientific Officer of Leadscope, Inc. He has over 25 years of experience researching and developing *in silico* solutions. He is currently the principal investigator on a US National Institutes of Health research grant and has co-authored 21 publications (including a number of papers related to ICH M7), three books as well as five book chapters.

Kevin E. Healy, PhD, University of California, Berkeley

Dr Healy is the Jan Fandrianto and Selfia Halim Distinguished Professor in Engineering at the University of California at Berkeley in the Departments of Bioengineering, and Materials Science and Engineering. He served as Chair of the Department of Bioengineering from 2011 to 2015. He received a B.Sc. in Chemical Engineering from the University of Rochester in 1983. He obtained graduate degrees in Bioengineering from the University of Pennsylvania (M.Eng.: 1985; Ph.D.: 1990). He is a thought leader and innovator working at the interface between stem cells and materials science to develop dynamic engineered systems to explore both fundamental biological phenomena and new applications in translational medicine. His group currently conducts research in the areas of: bioinspired stem cell microenvironments to control stem cell lineage specification and self-organization into microtissues or organs; bioinspired systems for regenerative medicine; biological interfaces; and, microphysiological systems for drug toxicity screening. Major discoveries from his laboratory have centered on the control of cell fate and tissue formation in contract with materials that are tunable in both their biological content and mechanical properties. These materials find applications in medicine, dentistry, and biotechnology. Prof. Healy has authored or co-authored more than 350 published articles, abstracts, or book chapters. He recently co-edited a multi-volume scholarly reference work on the biomaterials field, containing an all-encompassing comprehensive treatise that accurately captures the diversity, breadth, and dimensions of the field. He is an elected Fellow of the American Institute of Medical and Biological Engineering (AIMBE), American Association for the Advancement of Science (AAAS), a Fellow in Biomaterials Science and Engineering (FBSE), and a Alexander von Humboldt Foundation Fellow. He has chaired the Gordon Research Conference on Biomaterials and Biocompatibility, and has been honored with the 2011 Clemson award for outstanding contributions to basic biomaterials science. He is a named inventor on numerous issued United States and international patents relating to biomaterials, therapeutics, stem cells, and medical devices, and has founded several companies to develop these systems for applications in biotechnology and regenerative medicine. He is currently an Associate Editor

of the Journal of Biomedical Materials Research. He has served on numerous panels and grant review study sections for N.I.H. and international scientific agencies. He has given more than 300 invited lectures in the fields of Biomedical Engineering and Biomaterials.