NorCal SOT Fall Symposium:

Beyond Small Molecules: Stem Cells and New Frontiers in Large Molecule Development

October 21, 2014

Venue: Genentech Inc., Building 42, 475 E Grand Ave., South San Francisco

Morning Session	
8:00 am – 9:00 am	Registration
9:00 am – 9:15 am	Opening message from The President Pamela J. Lein, Ph.D., Department of Molecular Biosciences, UC Davis
9:15 am – 10:00 am	Stem Cells and Small Molecules, Linking Manganese Biology and Neurodegenerative Disease Aaron Bowman, PhD, Department of Neurology, Vanderbilt University
10:10 am - 10:45 am	Predictive High-Throughput Assays for Cardiac Physiology and Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Cell Models Oksana Sirenko, PhD, Research Scientist, Molecular Devices, LLC
10:45am – 11:00 am	Coffee Break
11:00 am – 11:45 am	Strategies for Development of Pharmacodynamic and In Vitro Functional Immunoassays in Support of Preclinical and Clinical Oncology Biopharmaceutical Programs Christina Satterwhite, PhD, Director, Laboratory Sciences, Charles RiverNonclinical
11:45 pm – 1:45 pm	Lunch Break & Meet with a Mentor

Afternoon Session	
1:45 pm-2:00 pm	Chapter announcements and acknowledgements
2:00 pm- 2:45 pm	Nonclinical Safety Assessment of ADC's: Challenges of the Next Generation Melissa Schutten, DVM, PhD, DACVP, Pathologist-Scientist, Genentech, Inc.
2:45 pm – 3:30 pm	Development of Antibody-Drug Conjugates: Brentuximab Vedotin Tae Han, PhD, Director and Head, Clinical Pharmacology, Stem CentRx, Inc.
3:30 pm – 4:15 pm	Genome Engineering at the Dawn of the Golden Age David Segal, PhD, Professor, UC Davis
4:15 pm- 4:30 pm	Closing Remarks
4:30 pm-6:00 pm	Wine Tasting and Networking

Aaron B. Bowman, Ph.D.
Assistant Professor, Neurology, Vanderbilt University
"Stem Cells and Small Molecules, Linking Manganese Biology and Neurodegenerative Disease"

The essential micronutrient manganese is enriched in brain, especially the basal ganglia. This brain region is also highly vulnerable to Mn neurotoxicity. We sought to identify neuronal signaling pathways responsive to neurologically relevant manganese levels, as previous data suggested alterations in neuronal handling of metal occur in Huntington's disease (HD). We found that p53 phosphorylation is highly responsive to manganese levels in both a mouse striatal cell line and human stem cell derived striatal-like neuroprogenitors. The Ataxia Telangiectasia Mutated (ATM) kinase is responsible for this manganese-dependent phosphorylation of p53. Activation of ATM-p53 by manganese was severely blunted by pathogenic alleles of *Huntingtin*. HD neuroprogenitors exhibited a highly manganese selective deficit in ATM kinase activation, since DNA damage and oxidative injury, canonical activators of ATM, did not show similar deficits. Pharmacological manipulation to equalize manganese transport between HD and control neuroprogenitors rescued the ATM-p53 signaling deficit. This work demonstrates that substantial alterations in neuronal Mn biology occur in Huntington's disease. Mn exposure is also an environmental risk factor in parkinsonism. We have performed a high throughput screen to identify novel small molecules that modify neuronal Mn handling. Mesencephalic dopaminergic neurons are especially vulnerable to Mn exposure and degeneration or dysfunction of this neuronal population occurs in parkinsonism. We tested the activity of validated small molecules from our screen in developing human mesencephalic dopaminergic neurons. We report differential Mn accumulation between developmental stages and stage-specific differences in the Mn-altering activity of individual small molecules. This work demonstrates differential regulation of cellular Mn-handling mechanisms in developing neuronal populations that are vulnerable in Parkinson's disease and parkinsonism.

Dr. Aaron B. Bowman, Ph.D. is an Assistant Professor in the Department of Neurology at Vanderbilt University. Dr. Bowman received his PhD in Biomedical Sciences in 2000 from the University of California San Diego. He did postdoctoral fellowship training at Princeton University and Baylor College of Medicine. Dr. Bowman is a 2008 recipient of the Outstanding New Environmental Scientist (ONES) RO1 award from the National Institute of Environmental Health Sciences. In 2012, Dr. Bowman was elected Vice-president Elect of the Neurotoxicology Specialty Section (NTSS) of the Society of Toxicology (SOT) in 2012, a four-year term and currently serves as President of NTSS. He also served as the Senior Councilor of the Stem Cells Specialty Section of SOT in 2012. Dr. Bowman also serves on the editorial boards of NeuroToxicology, BMC Pharmacology and Toxicology, as well as performing other *ad hoc* editorial and peer review services. The goal of Dr. Bowman's research is to define mechanisms of neuronal dysfunction and understand the basis of selective neuropathology, by characterizing the molecular function of disease genes and their interaction with environmental risk factors, especially metals. His work is supported by two RO1 grants focused on the

influence of manganese exposure in Huntington's disease (ES016931, PI) and parkinsonism (ES010563, MPI). Furthermore, he is a co-recipient of a NIEHS ViCTER RO1 (ES010563-13S1, MPI) examining a role for manganese in restless legs syndrome. His lab utilizes patient-specific induced pluripotent stem cells, high throughput screening, mouse models and biochemical approaches to examine gene-environment interactions in neurological disease.

Oksana Sirenko, PhD
Research Scientist, Molecular Devices, LLC
"Predictive High-Throughput Assays for Cardiac Physiology and Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Cell Models"

Development of highly predictive in vitro assays is extremely important for improving the drug development process and reducing drug attrition due to toxicity. iPSC-derived cell models are rapidly being adopted by the pharmaceutical industry for pre-clinical toxicity studies. Human iPSC-derived hepatocytes, cardiomyocytes or neurons show great promise with respect to primary tissue-like phenotype, unlimited availability, and a potential to establish cells from different individuals. To enable the full potential of iPSC-derived cell models, it is necessary to develop highly predictive in vitro assays that can be performed in a high throughput manner. We have developed several assays to assess drug-induced cardiotoxicity and hepatotoxicity. First, we have developed a physiological assay for measuring the impact of pharmacological compounds on the beating rate of human iPSC-derived cardiomyocytes with fast kinetic fluorescence imaging systems. Cardiomyocyte contraction rate and pattern was characterized by monitoring changes in intracellular Ca^{2+} measured using calcium sensitive dyes. The assay allows automated characterization of deviations from normal beating rate, peak width, or waveform irregularities. We have validated the assay system with a library of cardiotoxic compounds consisting of 131 compounds representing different classes of anti-arrhythmia drugs, receptor antagonists, ion channel blockers, and kinase inhibitors. The estimated predictive value of the multi-parametric assay was greater than 80% (with 86% sensitivity and 93% specificity). The multi-parametric analysis has also demonstrated great value in predicting long QT syndrome, and other types of arrhythmic and non-arrhythmic cardiotoxicity.
Second, we used high content imaging and iPSC-derived hepatocytes to assess general and mechanism-specific toxicity. A library of 240 known hepatotoxic compounds was analyzed over a range of concentrations using automated multi-parametric image analysis on a cell-by-cell basis. The endpoints assessed were cell viability, nuclear shape, average and integrated cell area, mitochondrial membrane potential, accumulation of phospholipids, cytoskeleton integrity, and apoptosis. We found that multi-parametric automated image analysis greatly increases assay sensitivity while also providing important information about toxicity mechanisms. Specifically, we found that multi-parameter assessment increased sensitivity of the assay to 60% (with 92% specificity). In addition, the assay also demonstrated high sensitivity (70%) for selected classes of compounds such as neuroleptic, cardiac, anti-fungal, anti-cancer drugs.
We conclude that these high-throughput, high-content automated screening assays using iPSC-derived hepatocytes or cardiomyocytes are feasible, reproducible, have high predictive value

and correlation with known *in vivo* toxicity, and can facilitate safety assessment or drugs and chemicals.

Received B.S. in Biology from Kiev State University, Ukraine
PhD in Biochemistry from the Biochemistry Inst. Academy of Sciences in Ukraine
Post -doctoral training in Immunology at UNC Chapel Hill, USA.
More than 12 years of experience as a Research Scientist in the Bay Area at Biotech: Bayer, BioSeek, Fibrogen. Expertise in the Assay Development, Drug discovery and Development, Immunology, Stem Cell Biology. Currently is a Project Scientist at Molecular Devices.

Tae H Han, PhD
Director and Head, Clinical Pharmacology, Stem CentRx, Inc.
"Nonclinical Development of Antibody-Drug Conjugates: Brentuximab Vedotin"

Antibody-drug conjugates (ADCs) are a class of therapeutics that are designed to deliver potent small molecule drugs selectively to cells that express a specific target antigen while limiting systemic exposure to the drug. This is accomplished by conjugating a potent drug onto an antibody-based therapeutic with a linker that is exquisitely stable in plasma. Brentuximab vedotin is a CD30 directed antibody-drug conjugate that represents the promise ADCs have toward the treatment of cancer. Key aspects of the nonclinical development associated with brentuximab vedotin will be presented.

Tae is currently Director and Head of Clinical Pharmacology at Stem CentRx where he is responsible for the nonclinical development (pharmacology, pharmacokinetics, and toxicology) and clinical pharmacology strategy and execution for all compounds in development. He received his PhD in Chemical and Biomolecular Engineering from UCLA where he also completed an NIH postdoctoral fellowship in pharmacology and cardiology. He then joined Merck & Co, Inc. in the Drug Metabolism and Pharmacokinetics department where he supported clinical development. He then joined Seattle Genetics to build and head the Clinical Pharmacology department. Tae led the clinical pharmacology and preclinical pharmacokinetic evaluation of brentuximab vedotin while managing the Seattle Genetics' pipeline and a team of clinical pharmacologists.

Melissa Schutten, DVM, PhD, DACVP
Pathologist-Scientist, Genentech, Inc.
"Nonclinical Safety Assessment of ADC's: Challenges of the Next Generation"

Antibody-drug conjugates (ADCs) represent a class of targeted therapies that hold great promise for oncology patients as they are purposefully designed to minimize systemic toxicity while delivering a highly potent, cytotoxic payload to a target cell population. ADCs are being developed as novel cancer therapeutics to offer a potentially widened therapeutic index over standard cytotoxic chemotherapies. In recent years, there has been significant expansion of the number of ADCs in preclinical and clinical development. Many of the current ADCs in the clinic, while offering robust proof of concept for targeted cancer therapy, have been met with several

challenges including linker instability, immunogenicity, and insufficient potency. “Next generation” ADCs are being designed with a wider array of antibody targets, improved linker-drug design, more potent cytotoxins, and site-directed conjugations. The ever-evolving complexity of these conjugates poses unique safety assessment challenges in preclinical drug development. This presentation will review the general determinants of ADC toxicity and provide case examples highlighting the impact of antibody format, target antigen biology, linker stability and drug potency on toxicity and efficacy.

Melissa Schutten is currently a Pathologist-Scientist in the Safety Assessment Pathology group at Genentech. In this capacity, she supports oncology programs in late stage research through development and is involved with the preclinical safety assessment of a variety of novel antibody-drug conjugate therapeutic candidates. Prior to her tenure at Genentech, she held a clinical-track faculty appointment at the University of Minnesota, College of Veterinary Medicine and Masonic Comprehensive Cancer Center. Dr. Schutten received her DVM and PhD at the University of Wisconsin-Madison and is an American College of Veterinary Pathology board- certified anatomic pathologist. Her research interests include the pathology of genetically engineered models of human cancers, in particular pancreatic cancer, and the development of novel targeted cancer therapies.

Christina M. Satterwhite, PhD
Director, Laboratory Sciences, Charles River
“Strategies for Development of Pharmacodynamic and In Vitro Functional Immunoassays in Support of Preclinical and Clinical Oncology Biopharmaceutical Programs”

Successful preclinical and clinical development of biopharmaceutical programs in support of oncology indications require development, qualification and validation of pharmacodynamic and in vitro function immunoassays. The overall strategy should consider a multi-assay scientifically driven approach in determining the key assays that will provide relevant data to support the program. This presentation will investigate the critical factors to consider in assay development with key examples of case studies.

Christina Satterwhite has over twelve years of experience in biologic drug development. The breadth and number of compounds she has worked on is extensive as she has worked for Charles River Laboratories in the areas of pathology, toxicology (emphasis in immunotoxicology studies), analytical chemistry and immunobiology. Her experience in toxicology and preclinical and clinical assay support has given rise to a comprehensive understanding of biologic drug development. She joined Charles River Laboratories as a Study Director in 2002 as an Associate Research Scientist. Prior to joining Charles River Laboratories, Christina received her PhD in Cellular and Molecular Pharmacology and Physiology at the University of Nevada, Reno, School of Medicine in 2002. Christina’s area of scientific expertise was in the study of ion channels and their role in cardiovascular disease. Christina’s focus in her career at Charles River has allowed her to work with multiple types of compounds such as monoclonal antibodies, bispecific antibodies, antibody drug conjugates, enzymes, peptides, and oligonucleotides. Christina has direct experience in study directing general toxicology and immunotoxicology studies in non-

human primates. In addition to study directing a variety of toxicology studies, Dr. Satterwhite through her career had direct oversight of study design and assay development/validation in the areas of Special Pathology (tissue cross reactivity and immunohistochemistry), analytical chemistry, ligand binding assays, immunogenicity assays including neutralizing antibody cell based assays, flow cytometry (e.g., immunophenotyping, receptor occupancy, phagocytosis/oxidative burst, intracellular cytokines), bioassays (ADCC/CDC/binding assays), immune biomarkers (e.g., cytokines) and molecular technologies. She has had overall responsibility for the successful interactions with the regulatory authorities and Sponsor's alike, interpretation and reporting of study data, as well as the conduct, management and regulatory compliance of assigned nonclinical and clinical studies. Follow-on biologics, which include biosimilars and biobetters, are approved subsequent versions of innovator biopharmaceutical products, typically made by a different sponsor following or nearing expiration of patent protection of the innovator product. The patent expiration dates that are quickly approaching for biotherapeutics has led to many companies developing biosimilar products to obtain market share. Christina has been working on biosimilar and biosuperior biotherapeutics for the last 10 years in her areas of expertise and is the co-Chair of the AAPS Biosimilar Focus Group subcommittee on nonclinical and clinical assays.

David J. Segal
University of California – Davis
"Genome Engineering at the Dawn of the Golden Age"

Genome engineering – the ability to precisely alter the DNA information in living cells – is beginning to transform human genetics and genomics. Advances in tools and methods have enabled genetic modifications ranging from the "scarless" correction of a single base pair to the deletion of entire chromosomes. Targetable nucleases and transcription factors based on zinc fingers, TALEs and CRISPR/Cas are leading the advances in this field, providing the tools to precisely modify the sequence or expression of any gene in seemingly any organism with high efficiency. Our efforts to reactivate an epigenetically silenced gene in adult mice show one path to disease treatment. A nuclease now in clinical trials further signals the beginning of genome engineering as therapy. The dramatic increase in the number of investigators using these tools signifies a transition away from methods development toward a new age of exciting applications.

David Segal is Professor of Biochemistry and Molecular Medicine, the Department of Pharmacology, and the MIND Institute, and is Associate Director of the UC Davis Genome Center. He received his Ph.D. from the University of Utah in 1996, where he studied the stimulation of recombination by double strand breaks in DNA. Seeking better methods, he pursued post-doctoral studies with Dr. Carlos F. Barbas III at The Scripps Research Institute, where he helped develop one of the most widely used methods for engineering zinc finger DNA-binding proteins. He joined the University of Arizona in 2002 and UC Davis in 2005. Dr. Segal's research continues to focus on targetable nucleases and transcription factors for gene therapy and genomic research. With over 70 publications in this field, his work has been

supported by the NIH, DOD, the Foundation for Angelman Syndrome Therapeutics, the W.M. Keck Foundation, and the Bill and Melinda Gates Foundation.