

Exchange Award

Conference Title: Third AIMBE/NIH Workshop on Validation and Qualification of New *In Vitro* Tools and Models for the Pre-clinical Drug Discovery Process

Principal Investigator: William Bentley, PhD

Conference Date: March 14 -15, 2013

The third AIMBE/NIH workshop on the validation and qualification of new *in vitro* tools and models for the pre-clinical drug discovery process was held on March 14th and 15th at the NIH Campus in Bethesda, MD. There were over 200 attendees with representatives from government agencies (NIH, FDA, HSS), academia and the private sector. The two day workshop allowed for the discussion of how to validate and qualify *in vitro* technology to reduce and replace the use of animal models. The workshop was broken up into four sections, three sessions of presentations and one breakout session. The three sessions covered the perspectives of the current *in vitro* models from three different viewpoints: regulatory (governmental views), industrial products and research, as well as the state of current research in academics. The breakout sessions were held on the second day to allow for the discussion of the different technologies that were showcased during the presentations. These breakout sessions specifically discussed the validation and qualification of the *in vitro* models. The breakout session was structured to allow for direct interaction between the presenters and the audience in a dynamic environment. The workshop concluded with a discussion of the findings from the breakout sessions as well as feedback regarding this and future workshops.

Day 1 – March 14th

Session 1: Current Government Perspectives on validation and Qualification of New In Vitro Tools and Models for the Pre-Clinical Drug Discovery Process

Session 1, “Current Government Perspectives on validation and Qualification of New *In Vitro* Tools and Models for the Pre-Clinical Drug Discovery Process,” highlighted the viewpoints the Belgian Federal Agency for Medicines and Health Products (FAMHP) and the U.S. Food and Drug Administration (FDA) (current and past employees). The presenters included: Federico Goodsaid, Ph.D., Sonja Beken, Ph.D. and Thomas Colasky, Ph.D. Christine Kelley, Ph.D., was the moderator for this session. Dr. Kelly started the session by focusing on the current need to implement the 3Rs (replacement, reduction and refinement of animal models) through implementing lab on a chip models. These models show great promise; however, the requirements for validation and qualification are unclear for the adoption of this technology. Therefore we look to this session’s speakers to provide some insight into the potential requirements.

Federico Goodsaid, Ph.D., currently the Vice President for Strategic Regulatory Intelligence at Vertex Pharmaceuticals, previously the Associate Director for Operations in Genomics and Biomarker Qualification Coordinator at the Office of Clinical Pharmacology at the FDA, was the first speaker. Dr. Goodsaid highlighted the possible paths of how new technologies are accepted in regulatory agencies. He stressed that there is significant need for clearly defining the context of use for the qualification of this technology. It must fully and clearly describe the manner and purpose of the use of the drug development. However for a tool to be used for drug development the boundaries must be defined within the specific data to justify use. It also must include a discussion of the potential value for data that is outside of these set boundaries.

Sonja Beken presented an update on the EMA implementation of 3R’s policy. Dr. Beken is the Chair of the European CVMP/ Committee on Human Medicinal Products (CHMP) Joint Ad Hoc Expert

Group on 3R's (JEG 3r's) at the EMA. She spoke about the role of the joint ad hoc committee to identify opportunities to implement 3Rs in the regulatory arena and to provide a concept paper for the replacement of animal studies for *in vitro* models. She discussed the difference between validated (reliable, reproducible, predictable; regulatory recognized) and valid (scientific data, reliability is proven; not formally recognized). She then talked about the desire to move away from an *ad hoc* revision of each guideline through the role of the ICH "Safety Topic Recommendation Working Group (STRWG)." The STRWG's role is to identify, monitor and evaluate new scientific and technological developments. The STRWG can then recommend to the ICH SH how to implement the testing paradigms that are considered relevant. This process occurs through three stages: 1) identify new test paradigms (no commercial proposals), 2) evaluate then 3) Recommend to the ICH SC. The evaluation stage will consider many factors including if the testing paradigm is: well defined, known to have reproducible test characteristics, able to identify false negatives and false positive rates, used to fill a potential gap, as well as contributes to the 3Rs or to a reduction in drug development timelines. During the recommendation stage it will be determined if the new technology would fit within a current guidance and would allow for data compilation during a safe harbor time period. Dr. Beken concluded that although drug development and discovery methods are not subject to regulation they still must comply with the minimal criteria for qualification and validation.

Tom Colatsky, Ph.D. followed Dr. Beken's presentation with a discussion on the FDA's point of view. Dr. Colatsky is the Director of the Division of Drug Safety Research in Center for Drug Evaluation and Research (CDER). He spoke about how the use of pharmacology and toxicity testing for drug development is not governed by any specific regulatory requirements. He investigated the link between failures of drug development during Phase II, III and submission failures, compared to the failure reason, either safety or efficacy. He identified that the efficacy of *in vitro* and *in vivo* studies may not reliably predict clinical efficacy. Particularly that most issues with toxicity stem from cardiovascular and liver concerns. Therefore for a move to *in vitro* models to predict human response there may be problems modeling subject/species metabolic and biological variability, repeated vs. single exposure to chemicals as well as modeling the different solubility, concentration, protein binding and relationship to plasma levels seen in different chemicals. Some critical issues include that: cells don't get disease, not all compounds can be screened *in vitro*, many compounds experience metabolism, and chemical exposure may trigger acute and chronic responses from different organs which may compound other organ responses. Currently three programs for drug development tool qualifications have been identified: biomarkers, clinical outcome assessments and animal models. However these three programs do not apply to assays or computational models. Overall Dr. Colatsky concluded that the FDA is trying to reduce animal testing. Although the drug development qualifications do not apply to assays the qualification concepts may be useful when evaluating new drugs to help to reduce clinical trial failures due to safety and efficacy.

Session 2: In Vitro Technologies for Draft Validation Guidelines

Session 2 discussed draft validation guidelines for *in vitro* technologies and was moderated by Rosemarie Hunziker, Ph.D. Margaret Sutherland, Ph.D., NINDS, NIH co-Director of the MPS program, was the first presenter of the session. She spoke about the current progress of the NIH Microphysiological Systems Programs (MPS). Dr. Sutherland emphasized the need for advancing regulatory science to improve the current system for drug and vaccine development. The current system is characterized by high attrition at every stage. With the addition of *in vitro* drug development tools this system could be improved. The NIH approach to developing a model of 3D tissues is to develop a microsystem that is physiologically accurate, genetically diverse and is pathologically representative. Current NIH awards for Microsystems are UH2/UH3 awards. MPS cell sources are working to identify a minimum standard of criteria for each organ system. For each primary tissue the sex, ethnicity, age, disease (or control)

phenotype is identified as well as the same for all isolated cells. This initial step will help to define an organ system to test across all of the awarded projects.

Kyle Kolaja, the Vice President, Business Development at Cellular Dynamics, International, (CDI) was the second speaker of the session. Dr. Kolaja discussed the work of CDI to develop and identify the potential of iPS cells. Currently cardiomyocytes, neurons, hepatocytes and endothelial cells are available and under the CDI warrantee; however, many other cell types are available for collaborators. Some current questions in the manufacturing of iPS cells include: donor consent and intellectual property, and how to identifying cell populations from donors with known adverse effects to drugs vs. using cells from patients without adverse responses. CDI iPS hepatocytes have been shown to exhibit more of a fetal phenotype than an adult phenotype; however, the cytotoxicity profile is similar. With the desire to better understand the impact of human diversity iPS based tissues may be used as a tool.

The third speaker of the session was Jonathan Himmelfarb, M.D. Dr. Himmelfarb, the Director of Kidney Research Institute at the University of Washington, presented "A Tissue Engineered Human Kidney Microphysiological System." He spoke about the need for developing a kidney on a chip due to the increased incidence and prevalence of kidney disease, which leads to high levels of morbidity, mortality and increasing costs. This is compounded by the lack of innovation in therapies and the lack of success in clinical trials that were shown to be efficacious in animal models. Currently their research focuses on using the nephron as a Kidney on a Chip (KOAC) with specifically using the proximal tubule to model a KOAC. Current KOAC technology is the Nortis Chip Technology (NortisBio.com), which can control shear force and mechanical stimuli on a disposable, chip-like PDMS device. The KOAC technology investigated by Dr. Himmelfarb includes the addition of decellularized human kidney tissues which can be used as a location to harvest extracellular matrix for the isolated kidney resections. Some challenges that the KOAC encounter are questions of: cell source, seeding and adhesion; flow dynamics and how to implement analytical chemistry on analytes that are $\sim 50 \mu L$ in volume. Potential for a KOAC include: improved drug dosing, tools to understand uremia, improving kidney transplantation, improved drug development and a step toward a wearable kidney device.

Brett Blackman, Ph.D., Chief Scientific Officer of HemoShear, LLC, was the fourth speaker of the session. Dr. Blackman spoke about the validation of new technology from a developer's viewpoint. Dr. Blackman spoke about the need to develop new technologies to improve the drug development process. Currently 92% of all drugs fail, such that a successful, FDA approved, drug costs about \$1.3 billion to develop. In some cases drugs that pass animal trials fail human trials only to be sent back for more animal trials. But the question is, why test on animals again if the results were not indicative of results in human trials? Therefore HemoShear, LLC looks to use two human system models, vasculature and liver, as a tool for drug development. Cells are sourced from primary cell sources and each study is performed using different donors to promote integration of natural heterogeneity and prove robustness. Currently phase one trials are completed with expectation of phase three trials to be completed soon.

Day 2 - March 15th

Session 3: Development of Draft Validation Guidelines

Session 3 consisted of a discussion of current research for *in vitro* technologies and the development of validation methods for these technologies. Session three was moderated by Dr. Warren Grundfest. Dr. Grundfest empathized that workshops, like these, are where standards should be discussed and developed within the group of individuals from different aspects of the *in vitro* modeling systems. The first speaker of the session was Tom Hartung, Ph.D. Dr. Hartung is the Directory for the Centers for Alternatives to Animal Testing at the Johns Hopkins University Bloomberg School of Public Health. He was previously the head of the European Center for the Validation of Alternative Methods (ECVAM). His presentation focused on discussing a "3D Model of Human Brain Development for

Studying Gene/Environment Interactions.” A 3D model of brain development is thought to provide an improved resource for developing drugs (and counter terrorism measures) compared to animal and clinical trials. iPSCs are used to create brain cell cultures. After characterizing the iPSCs, they have to evaluate the best platform for a 3D model. 3D cultures are known to be more complex than traditional 2D cultures for multiple reasons. However, how does one evaluate toxicity to improve the current methods? Also Dr. Hartung discussed the questions that surround the move away from the “gold standard” of animal testing to *in vitro* testing. Particularly he discussed how to validate these *in vitro* systems. He suggested that one could implement evidence-based toxicology. He also concluded that this is a particular area of interest and that there are multiple conferences and committees which look to address using evidence-based toxicology (EBT).

The second speaker of session 3 was George Truskey, Ph.D. Dr. Truskey is the R. Eugene and Susie E. Goodson Professor of Biomedical Engineering at Duke University. Dr. Truskey spoke about the development and use of a “Circulatory System and Integrated Muscle Tissue for Drug and Tissue Toxicity.” The goal of the project is to develop a circulatory system that consists of a high-pressure arterial system interfaced with 3D skeletal muscle microcirculatory test beds. Skeletal muscle accounts for 40% of the body’s mass and plays critical metabolic and energy usage roles. Diseases that have significant impact (diabetes, muscular dystrophy, sarcopenia) and that are increasing in frequency are involved with the skeleton or with skeletal muscle metabolism. Investigating the interaction of endothelial cell and skeletal muscles is a significant step in developing MPS unit. This work spans four milestones over two years. The MPS unit consists of small diameter arteries and 3D engineered human skeletal muscle. These two components must be evaluated with assays to evaluate their metabolite responses. The MPS unit uses a magnetically-active polymer sponge to regulate flow to create a pulsatile perfusion system. Cells are derived from multiple sources. Endothelial cells are from endothelial progenitor cells (adult and umbilical cord blood). Fibroblasts are from neonatal and adult dermal fibroblasts. Skeletal muscle myoblasts are from biopsies from healthy middle-aged volunteers.

Kevin E. Healy, Ph.D. was the fourth speaker of the session. Dr. Healy is the Jan Fandrianto Distinguished Professor in Engineering at University of California at Berkeley. His talk discussed the development of “Disease Specific Integrated Microphysiological Human Tissue Models.” Dr. Healy discussed how patient specific iMAPs can use somatic cells to reprogram to patient hiPSCs. These hiPSCs can then be differentiated into multiple tissue types for evaluation. iMAPs can be used for real time sampling including ELISAs, mAb assays, Raman microscopy, mass spectrometry, metabolism and electrophysiology. However, before implementing this platform one must understand the minimal organ or organoid size needed for drug discovery. In addition, microenvironments of each of these minimally sized organs/organoids must be physiologically relevant. One suggestion that Dr. Healy made was that the simplest organoid should be used to see if it can be replicated and evaluated first, to determine if the method is efficacious. First milestone for this iMAPs project was to generate a population of iPSC derived hepatocytes that mimic the metabolism of human primary hepatocytes during drug processing. Additionally the liver sinusoids must be modeled with respect to the microfluidic environment and continuous mass transfer. Milestone 2 is to develop the platform of healthy and diseased iPSC derived cardiomyocytes for a 3D *in vitro* model. Diseased iPSCs should model long QT syndrome. Dr. Healy concluded with a discussion of the validation the iMAPs liver and heart systems that will be required. He suggested that the validation will look to model normal physiological activity and responses to different drug and disease models.

Karen Hirschi, Ph.D., was the next speaker of the session. Dr. Hirschi is a Professor of Medicine (Cardiology) and Internal Medicine at Yale University. Her presentation was titled “Integrated Heart-Liver-Vascular Systems for Drug Testing in Human Health and Disease.” Dr. Hirschi’s work is collaboration between three research groups which look to develop a HeLiVa (heart – liver- vasculature) Chip iMAP. The chip looks to recapitulate the microenvironment as well as provide pulsatile flow in the

chip. The integration of multiple organ systems will allow for disease modeling that may impact multiple organ systems concurrently. The team uses iPS cells to generate each of the different tissue types. Endothelial cells (Hirschi lab), cardiomyocytes (Vunjak-Novakovic lab) and hepatocytes (Bhatia lab) have all been generated with mature phenotype and function. The plug and play bioreactor has long term goals of a modular platform, ability for perfusion as well as electrical and mechanical stimulation, portable, allow for real time imaging, and allow for the long term culture of these organ systems.

Lansing D. Taylor, Ph.D. is the Director of the Drug Discovery Institute and Professor at the University of Pittsburgh, was the fifth speaker of session 3. Dr. Taylor presented research on "3D Biomimetic Liver Construct for Predicting Physiology and Toxicity." Dr. Taylor initiated his talk with a discussion of why human 3D tissue models are valuable. He stated that animal studies have been shown to be expensive and not very predicative of many human disease states, and they occur late in the discovery process. With an earlier discovery of drug safety through prescreening using 3D tissue models would reduce the number of animal trials and costs for the development of new drugs. The design of a 3D biomimetic liver device includes many factors which are based on the liver acinus. Device should be able to measure of albumin, urea, LDH leakage and glucose production, drug metabolism and bile production. The device design is inspired by liver channels and includes hepatocytes, stellate, endothelial cells and Kupffer cells. Validation of the components and the complete system is paramount with the appropriate responses to positive control drugs, the ability to zone oxygen and pH, greater than 85% viability, ROC curves and bile production after one month.

Breakout Sessions: Parallel Discussions of Technology Platforms

After session three, the workshop broke out into three groups to discuss the validation and implementation of three different technology platforms, the HemoShear, Berkeley and Pittsburgh technology platforms. After the breakout sessions the workshop reconvened to discuss the groups' findings. The HemoShear discussion was led by Luke Lee, Ph.D. His group discussed that there was no current business argument for the qualification of new drug development technologies. However the validation of such technology may be difficult since the technology is privatized and not for general use. Instead it is a better business model to address the customer (pharmaceutical companies) needs for these tools. Industrial validation is accomplished through testing a known set of compounds to see how the technology performs. The discussion of the Berkeley Technology Platform was led by Warren Grundfest, Ph.D. along with James Hickman, Ph.D., and Kevin Healy, Ph.D. Their session agreed that the move from animal testing in one step will not work. Alternately the community must work in multiple methods using reference compounds to investigate *in vitro* models. The breakout session also discussed how to address chronic issues, the need for multiple organ systems. The session concluded by discussing whether stem cells can be accurately used to predict the response of an aging patient. The discussion of Pittsburgh Technology platform was led by Rosemarie Hunziker, Ph.D., and Michael Schuler, Ph.D. Their breakout group concluded that pharmaceutical companies are not sufficiently involved; that to be successful the design of *in vitro* technologies must include the consumer. Also the initial steps of technology development are integral to success including standardization and quality control before validation and qualification. They must first test well-known and characterized reference drugs before continuing to validation and qualification.

The workshop concluded with a final discussion and some feedback from the audience. Some comments were that the format was well-liked, that future meetings should focus on toxicity, efficacy, and biologics. The inclusion of CBER (FDA Center for Biologics Evaluation and Research) would be helpful for a discussion on biologics. Otherwise the inclusion of regulatory agencies is not as helpful compared to other presenters.