"Animal Models and Personalized Medicine"

Division of Comparative Medicine,

Office of Research Infrastructure Programs,

Division of Program Coordination, Planning, and Strategic Initiatives,

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Meeting Summary

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A. Overview

On October 28 and 29, 2013, the NIH convened a workshop entitled, “Animal Models and Personalized Medicine.” The goals of the workshop were: 1. to discuss the status of human personalized genomics and the use of comparative functional genomics in other organisms to interpret patient information for clinical use; 2. to review the current status of the development and use of the personalized animal models based upon a variety of animal species; 3. to evaluate the potential use of personalized animal models for translational medicine applications and; 4. to develop the consensus and provide recommendations to the NIH regarding the potential strategic initiatives which would make a valuable contribution to the field.

Dr. David Valle, M.D. (Johns Hopkins University School of Medicine, Baltimore, Maryland) began the event with a keynote presentation on general principles of the Individualized Medicine concept and role of animal models.
The remainder of the 2-day workshop consisted of topical sessions related to the creation and use of the precision animal models for Personalized Medicine and their use for preclinical studies. Each session included four to five individual presentations, followed by a round table discussion and the opportunity for audience participation. Topics of the sessions were:

1. The Use of Comparative and Functional Genomics to Build Animal Models of Human Diseases
2. Technological Advances and Available Resources for Building Predictive Animal Models
3. Using Personalized Animals for Drug Discovery and Biomarker Development
4. Which Human Disease Conditions Are the Best Candidates for Use of Personalized Animal Models?
5. How Can Personalized Animal Models Guide Clinical Trials?
6. Closing Remarks/Recommendations: Current Challenges and How to Accelerate the Progress

B. Introduction

Recent advances in diverse areas of biomedical science and breakthroughs in technology such as affordable whole genome sequencing and molecular profiling (epigenomic, transcriptomic, proteomic and metabolomic) provide a unique opportunity to study the genetics and pathogenesis of a wide variety of human diseases with the eventual goal of using this information to inform clinical practice. Heterogeneity of patient populations and the absence of effective means to interpret patient genetic/omic information for clinical use are significant obstacles toward achieving this goal. Creating optimally informative animal models to generate reliable preclinical data for human studies is a fundamental aspect of this challenge.
The Division of Comparative Medicine at ORIP/DPCPSI/OD, convened a small brainstorming meeting on September 6, 2012 in Bethesda, Maryland to discuss the current status of and requirements to develop and use animal models for personalized pre-clinical studies, with the eventual goal of wide application of this practice in clinics (http://dpcpsi.nih.gov/orip/documents/The_Next_Generation_Animal_Models.pdf). One of the major recommendations of the meeting participants was to organize a large symposium inviting US and international investigators and medical professionals, representatives of the NIH extramural and intramural communities as well as pharmaceutical company representatives for a two-day meeting devoted to the in-depth discussion of the current status, future developments as well as the most urgent needs for the application of advanced animal models for targeting personalized disease phenotypes. Based on those recommendations, The Division of

Comparative Medicine at ORIP/DPCPSI/OD, organized a large symposium on October 28 and 29, 2013 in Bethesda, Maryland.

C. Summary of Presentations

Introduction and Welcome

Oleg Mirochnitchenko - Oleg Mirochnitchenko, Ph.D. from DCM convened the meeting. He explained that DCM organized the gathering to address the issues of using animal models in personalized medicine because of DCM's continuing interest in supporting medical research via animal model repositories and related research activities. Dr. Mirochnitchenko briefly reviewed emergent opportunities and challenges in the field. He noted the meeting provided an occasion to review the current development status and use of personalized animal models from a variety of animal species, evaluate the potential use of personalized animal models for translational medicine applications, and develop a consensus to provide recommendations for potential NIH initiatives. He described the small workshop that occurred in September 2012 to discuss the status of and requirements to use animal models for personalized pre-clinical studies and indicated the current meeting responded in part to recommendations that emerged from the earlier gathering. Dr. Mirochnitchenko asked participants to identify during the symposium the general unmet medical needs for precision animal models, potential beneficiaries of animal models in the biomedical and clinical fields, and the model systems likely to provide immediate clinical benefits. He asked for suggestions about which model systems and disease categories will yield the greatest advances in the future. Finally, he suggested the need to understand how to build informative and predictive preclinical pipelines for using this new generation of animal models.

James Anderson - James Anderson, M.D., Ph.D., Director of DPCPSI, thanked the participants for attending and staff from DCM for organizing the event. Dr. Anderson explained the NIH's mission and its relevance to the initiative to develop precision animal models. He noted that the NIH sought this meeting to identify potential needs and funding opportunities for animal models and personalized medicine. He highlighted the general topics of the meeting, including the need for collection and exchange of omics data to enhance the precision of models, the redesign of animal models to more precisely model human conditions, and the adaptation of models for higher throughput screening.

Keynote Presentation

David Valle's presentation was introduced by Harold Watson, Ph.D. (ORIP).

David Valle - David Valle, M.D., Ph.D., Director of the Institute of Genetic Medicine and a Professor at The Johns Hopkins University School of Medicine, presented on the challenges and opportunities of individualized medicine in the genomic era. Dr. Valle commenced by quoting Dr. Francis Collins's observation that "personalized medicine refers to using information about a person's genetic makeup to tailor strategies for the detection, treatment, or prevention of disease." Dr. Valle agreed with the definition but suggested that "individualized medicine" serves as a better denotation since medicine now seeks to address the distinctive presentation of illness in individuals. He described approaches to medicine in the 20th century that sought to treat the average patient and that offered possible treatments through trial and error. With individualized medicine in the genomic era, Dr. Valle indicated that experienced physicians need to appreciate that each patient has his or her own unique set of clinical characteristics;

features that set them apart from all other patients, even those with the same diagnosis. He indicated these differences include variables such as age of onset, severity, complications, family history, socio-cultural differences, response to treatment and long-term outcomes. Such variables combine to make unique individuals. Dr. Valle described the reasons for the rise of individualized medicine at this point in time. With the recent, rapid, and ongoing developments in genomic technologies and knowledge, he indicated that we are becoming more sophisticated in our ability to identify and understand the genetic contributions to individuality in the presentation of human diseases and conditions. He pointed to five advances that have permitted this shift, including the (1) success of the Human Genome Project, sequencing technology, and a growing appreciation of sequence variation; (2) prominence of Whole Genome Sequence biology; (3) increase of evolutionary thinking in medicine; (4) understanding of individual genome sequences; and (5) identification of disease genes. Dr. Valle proposed that these advances increasingly hold the promise of providing medicine with the ability to individualize the approach to each patient and improve the capability to diagnose, treat, and even prevent illness.

Dr. Valle observed that animal models promise to play an important role in individualized medicine, and he outlined several areas where model organisms can contribute to biomedical research. The strategies that he outlined for the use of the model organisms (MOs) included variant-screens in the model to identify candidate genes for human phenotypes, human candidate gene evaluation in the context of MO studies, direct functional testing of candidate disease genes in experimentally malleable systems, directed expansion of knowledge about a particular biological system, and drug development and testing. To reach the goal of individualized medicine, however, much work remains to be done. Dr. Valle summed up the consequences of the “science of the individual” for medicine. He said it exposes the pitfalls of typological thinking, confirms the physiologic view that each individual has his or her own disease, emphasizes the importance of understanding why a given patient has a given illness at a given time, and asks what prevention or treatment is best for this individual. Dr. Valle called for rigorous basic, translational, and clinical research and efficient use of new technology.

Session 1: The Use of Comparative and Functional Genomics to Build Animal Models of Human Diseases

Michael P. Snyder - Michael P. Snyder, Ph.D., Chair of Genetics and the Director of the Center of Genomics and Personalized Medicine at Stanford University, presented on genome sequencing and personalized medicine. He also spoke about comparative mouse and human genomics in the mouse Encyclopedia of DNA Elements project (ENCODE). Dr. Snyder addressed the impact of genomics on medicine in understanding and treating diseases, pharmacogenomics, and managing healthcare in healthy individuals. He discussed cancer genome sequencing noting that cancer is both an inherited and somatic genetic disease with different numbers of driver mutations based on the type of cancer. He described the application of sequencing cancer genomes to compare them with normal genomes in order to suggest possible therapies. He provided several examples of the cancer cases with single as well as diverse potential therapeutic targets. Genome sequencing presents challenges to solving “mystery” diseases because of the number of candidate variant genes that require investigation. To date, of the 30 families with genetic health issues enrolled in his genome sequencing initiative, his team has solved five cases. In each case they study, they identify previously unknown gene mutations and worked through identification of the casual mutation. Dr. Snyder noted the animal model is essential to identify risk, because drugging candidate mutations in a human is not always possible; he emphasized the current process is a challenge because it is both labor intensive and time-consuming. In discussing the mouse model, he described issues

related to specific alleles, the relevance of the mouse in specific circumstances, and the need to study phenotypes linked to human disease genes. He described the mouse ENCODE consortium which is applying the same experimental pipelines developed for human ENCODE to annotate the mouse genome. This project seeks to understand the similarities and differences of human and mouse genetic blueprints and access cell types, tissues, and developmental time points not addressable in humans; and inform and accelerate ongoing efforts in mouse genomics and disease modeling with human translational potential.

Jeffrey Rogers - Jeffrey Rogers, Ph.D., Associate Professor of Molecular and Human Genetics at the Baylor College of Medicine and a Core Scientist at the Wisconsin National Primate Research Center, presented on nonhuman primates and modelling the genetics of risk factors in human diseases. Dr. Rogers stressed that nonhuman primates are unique models of human biology and disease because of their genetic and physiological similarity to humans. He noted that because genome sequencing is revealing substantial amounts of functionally significant variation in primate colonies, genetic surveys of primate research colonies can efficiently and inexpensively identify variants through targeted follow-up phenotyping. The rhesus macaque has a special role in this research: as examples, he described a rhesus model of behavioral inhibition and anxiety, the Mauritian cynomolgus macaque model for HIV/AIDS research, a rhesus pharmacogenetic model of naltrexone-induced attenuation of alcohol consumption, and SNP density in rhesus macaques. Dr. Rogers discussed the large amount of genetic variation among rhesus macaques in research colonies and among other primates. He noted the significant fraction of low-frequency variation that may have functional effects and the potential of these functional variants for new genetic models to understand the genetics of human disease and suggest therapies. Given the capacity to consider functional variation in every gene in a primate species, using these animals will inform and improve biomedical research.

Mark Ellisman - Mark Ellisman, Ph.D., Professor of Neurosciences and Bioengineering at the University of California at San Diego School of Medicine and Director of the National Center for Microscopy and Imaging Research, presented on connecting humans to models by linking and analyzing distributed data to uncover disease mechanisms and propel biomedical research. Dr. Ellisman indicated that to make effective use of the rich and complex information about human disease that emerges in part from advances of modern genomics and proteomics, we rely on numerous software tools rising from the parallel revolution occurring in informatics. He summarized several of the ongoing software development strategies and allied laboratory studies which link individual differences in complex human diseases to practical biomedical research tools represented by animal models and cell culture systems. He noted information frameworks in development to connect rapidly accruing and diverse biomedical data, discussing gene orthologues, semantics, ontology, spatial frameworks ranging from atlases of organs to subcellular molecular anatomy and protein structure, and the network views of modern Systems Biology. Dr. Ellisman treated these navigation and exploration frameworks, which help bind elements of knowledge together to facilitate a generation of hypotheses, in the context of certain applications. The cases he discussed represent current biomedical research challenges requiring researchers to connect the results of human genetics in neurodegenerative disorders like Parkinson's disease to mouse and cell culture systems. He indicated that investigators now more precisely model multiple forms of this disease in patients and can target new opportunities for prevention or intervention. He concluded that the exploitation of new information about humans by the development of open and scalable information systems to connect researchers with appropriate model systems or strategies will enable more effective biomedical research.

Paul N. Schofield - Paul N. Schofield, Ph.D., Reader in Biomedical Informatics in the Department of Physiology, Development and Neuroscience at the University of Cambridge,

presented on the use of model organism phenotype data for rare diseases and personalized medicine. Dr. Schofield indicated that fully understanding the function of genes and their role in human diseases requires the characterization of the phenotypic effects of variation or functional inactivation in whole organisms. The most important sources of knowledge for genotype/phenotype relationships in humans are the Mendelian monogenic diseases, but based on OMIM data, we currently have phenotypes for only 2,800 genes. Model organisms promise a rich source of phenotype and gene function information. For example, there are currently more than 5,000 genes about which we know nothing phenotypically in humans but for which detailed phenotypic information is available for their mouse and/or zebrafish orthologs. There are 17,500 genes with mutant alleles available in mice and, from the International Knockout Mouse Consortium alone, 18,000 targeted mutations or gene traps available as ES cells. Exploitation of these resources requires association of phenotypic information between species, which has to date, been a difficult conceptual and computational task. Schofield's group has developed a semantic strategy, based on the computational definition of phenotype ontology terms, which makes use of species-agnostic foundational ontologies to relate phenotypes between species using the phenotype and trait ontology, (PATO). This now permits the seamless integration and analysis of animal and human phenotypes. Dr. Schofield discussed the computational use of model organism data to identify genes likely to cause phenotype in recurrent human copy number variation disorders, and the use of animal phenotype data to provide candidates for rare disease genes, improve gene function annotation, and potentially provide diagnostic support for individuals with rare diseases.

Nicholas Katsanis - Nicholas Katsanis, Ph.D., Director of the Center for Human Disease Modeling and a Professor at Duke University, presented on modeling the pediatric morbid genome. Dr. Katsanis observed that recent advances in genetics and genomics have enabled the rapid and inexpensive sequencing of exomes and genomes of patients with a variety of clinical presentations, with the anticipation that implementing these tools will potentiate discovery, accelerate diagnoses, and improve the efficiency of health care delivery. He also observed that at the same time, our ability to interpret the functional consequences of genetic variation in humans is almost exclusively dependent on prior discovery of disease-causing alleles and on population-based genomic data. He indicated the Center for Human Disease Modeling at Duke University has initiated an ambitious project to functionalize a significant fraction of the morbid pediatric genome through the use of complementation *in vitro* and *in vivo* assays. To date, his team has generated robust *in vivo* models for approximately 400 human disease genes and 1,200 non-synonymous alleles, with an experimental success rate of ~70% and specificity and sensitivity estimates of 99% and 86%, respectively. As they continue to ramp up this process, fuelled by the combination of community-driven gene discovery and focused efforts at Duke through the systematic sequencing of neonates with anatomical defects (www.dukegenes.org), Dr. Katsanis anticipates that they will saturate a significant fraction of the pediatric disease space in the next few years. The speaker concluded that understanding germline genetic disease architecture will facilitate the transition of genomic data from a descriptive to a predictive tool.

Round Table Discussion - David Valle, Jeffrey Rogers, Mark Ellisman, Paul Schofield, and Nicholas Katsanis participated in a round table discussion. Michael Snyder led the discussion on the use of comparative and functional genomics to build animal models of human diseases.

Dr. Snyder asked presenters what one thing they would do to speed up the application of animal models for personalized medicine. Dr. Valle indicated that though there are many bottlenecks, the one that most concerned him was functional testing misidentifications. Dr. Ellisman stressed the necessity of connecting all the omics information to cell biology, which means

eventually to uncover how different systems actually work together in what he calls *spatial systems biology*. To achieve this goal, he proposed more effective phenotyping with widely-distributed multiscan microscopy.

Dr. Snyder asked Dr. Schofield how to get the databases he needs. Dr. Schofield replied he wants a database that stores all the known information on the variation of human phenotypes. He observed scientists have had a huge problem systematically annotating all the information which is currently scattered in different repositories. Among the problems included in the development of such a database is structured phenotyping and adequate incentives to investigators for contribution of their data.

Dr. Snyder asked participants in the audience for comments and questions. One participant followed up on Dr. Schofield's comments with the observation that identification of human phenotypes is a very subjective matter. For this reason, he called for a new phenotyping strategy comprised of a completely agnostic set of data. Other members of the audience concurred and elaborated the problem in light of the different dimensions of phenotype data. Dr. Ellisman, drawing on a prior experience with standardizing data, indicated what the participants were discussing is possible and often will require very simple solutions to encode data points; he emphasized location is key. Dr. Schofield drew on his many years of experience in attempting to standardize phenotyping in mice to indicate that one problem to solve is the specificity of the data. Other problems which need solutions are data usability and practicality. An additional issue among ontologists is time variability, including the onset and progression of diseases and the timing of the therapeutic response.

Other panelists added their comments on phenotyping. Dr. Rogers noted that phenotyping is an important ongoing discussion among investigators who employ primate models because of the lack of a standardized collection regime. Among the challenges is the need to draw on expertise across disciplines, such as neurology and cardiology. Dr. Valle commented that timing is critical in developmental biology/pediatrics, but also these considerations should be taken into account for phenotyping approaches over the lifespan. Dr. Valle added medical schools offer prospective physicians some commonalities in phenotyping when they instruct in procedures to take patient medical histories, so research investigators potentially can receive and use this information. He suggested that the use of the cohorts of patients to whom investigators can return over and over again to conduct iterative phenotyping will be very important. Dr. Ellisman concurred in the notion of the importance of defining the "*when"* for phenotyping. Dr. Snyder elaborated that capturing differential time frames is similarly critical.

A member of the audience indicated that patients have anxiety to know outcomes of testing; he recommended NIH invest in efforts to expedite iterative processes in employing animal models to develop diagnoses tools for personalized medicine. Dr. Katsanis concurred with the speaker's point adding that it was something his laboratory struggles to address; he called for general knockdowns to facilitate the timeliness of his work. A participant in the audience also indicated that discussions of powerful quick tools also need to consider the means to facilitate collaborations, perhaps through a center dedicated to answering questions that clinicians pose. Dr. Katsanis indicated he and his colleagues have built such a center but they require investigators and clinicians to build the alleles in model organisms before they submit requests for analysis.

Dr. Ellisman commented that many investigators fail to include metadata on their procedures. Researchers, he said, make assumptions about general knowledge methods, but these gaps in

their descriptions reduce the reproducibility of their work. He emphasized that one scientist's metadata may be another's data and called for standardized protocols for reporting methods.

Session 2: Technological Advances and Available Resources for Building Predictive Animal Models

K.C. Kent Lloyd - K.C. Kent Lloyd, D.V.M., Ph.D., the Director of the Mouse Biology Program at the University of California, Davis, presented on *in vivo* modeling in translation from the Knockout Mouse Project (KOMP) to next-generation precision animal models of disease. The efforts of KOMP and aligned international projects will allow researchers to create a comprehensive, genome-wide mouse library of knockout alleles and associated broad-based, unbiased phenotyping data bank. Though these efforts lead to discoveries about the biological and pathological roles of genes, there is limited correlation to the precise genomic alterations associated with the diversity of human diseases, developmental and behavioral phenotypes. For instance, the KOMP program delivers innovative tools and technologies, generates basic science data on individual gene knockouts, but cannot be considered a translational science effort. However, without analysis of the properties of new knockout models, creation of precision disease models will be very difficult. His laboratory is addressing new gene editing technologies, including TALENs and CRISPR/Cas, to enable high-throughput and targeted genetic manipulation customized to mimic allele-specific disease-associated mutations in human populations. These studies seek to improve development of precision mouse models relevant to human disease for pre- and co-clinical diagnostic, therapeutic, and prognostic testing in potentially real-time fashion. To ensure the validity, reliability, and relevance of phenotyping results for applications in humans, newly-derived precision mouse models will undergo rigorous testing with adequate sample size, randomization, concealed allocation, and blinded outcome assessment. Faster yet cheaper genome sequencing both in clinical and direct-to-consumer settings promotes opportunities to combine next-gen gene targeting technologies with specialized, challenge-type phenotyping pipelines in translational studies to predict pathophysiological impact and inform preventative strategies. The development and application of precision animal models will engage not only the research community but citizen scientists and the general public in an effort to improve human health.

Hazel L. Sive - Hazel L. Sive, Ph.D., a Professor at the Massachusetts Institute of Technology, presented on the zebrafish as a tool to understand mental health disorders. The zebrafish cannot develop behaviors characteristic of autism spectrum disorders (ASD), schizophrenia, or other human mental health disorders. However, conservation of genes and pathways between fish and mammals indicates the fish can serve as a tool to analyze these human diseases. The fish permitted identification of key genes from the human 16p11.2 copy number variant region, where deletion or duplication is associated with intellectual disability, ASD, or schizophrenia. Changes in gene dosage and expression of one or more 16p11.2 genes are presumed pivotal for disease etiology. Since no single gene variants in 16p11.2 have emerged after extensive patient analysis, her team hypothesizes that two or more genes from the 25 core genes in this interval interact to confer dosage sensitivity. They already have defined full loss of function phenotypes for 22 zebrafish 16p11.2 homologs. They have analyzed pair-wise interactions among these genes after partial loss of function in each using antisense oligonucleotides or RNAi. Fifteen pairs have emerged as synergistic in a dosage-sensitive manner in embryos or larvae and are undergoing testing in older fish using TALEN or CRISPR/Cas mutants. Physical interactions are being assessed in human cell lines. All interactions are novel, not predicted by current databases. These approaches and those using the zebrafish to analyze activity of human gene variants can contribute significantly in addressing human disease gene function and therapeutics.

Leonard D. Shultz - Leonard D. Shultz, Ph.D., Professor at the Jackson Laboratory in the Graduate School of Biomedical Sciences at the University of Maine, presented on humanized SCID mouse models for cancer research. Studies on the growth of human tumors and responses to experimental therapy in small animal models commenced after the discovery of the nude mutation in 1962. Improvements in animal models for human tumor growth followed the discovery of the *scid* mutation and targeting of the *Rag1* and *Rag2* genes. Homozygosity for the *scid, Rag1*^{null}, or *Rag2*^{null} mutations eliminates adaptive immune function. Additional genetic crosses with mice bearing targeted mutations in the *IL2r*γ gene completely prevented the development of functional mouse natural killer cells and induced additional deficiencies in innate immunity. These severely immunodeficient mouse models, including the NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/Sz (NSG) strain, the NODShi.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug} (NOG) strain, and the C;129S4-*Rag2*$^{tm1.Flv}$ (BRG) strain, are in use worldwide as hosts for human tumor engraftment and experimentation. His laboratory focuses on the NSG strain. They have used genetic alterations designed to further weaken innate immune components in NSG mice to enhance primary human tumor xenograft growth. Additional modifications of NSG mice have used human transgenes that encode HLA class I and class II antigens, human cytokines, and other human species-specific molecules selected to increase levels of engraftment and function of human hematopoietic and immune cell populations as well as supporting the growth of primary human tumors. NSG mice support engraftment with almost all types of primary human solid tumors and hematological malignancies. Responses to tumor therapy are dependent on the molecular fingerprint of individual tumors. Multiple laboratories have initiated programs to optimize patient-specific therapy using genomic characterization to identify treatment protocols for pre-clinical evaluation of patient-derived xenograft (PDX) tumor growth in NSG mice. Use of humanized mice as avatars provides a preclinical bridge for patient-specific therapy and offers the potential to optimize clinical outcomes. The ability of NSG mice to support engraftment with functional human hematopoietic stem cells and peripheral blood leukocytes supports the potential study of primary human tumors *in vivo* in the presence of a human immune system. Advances in the derivation of human hematopoietic stem cells and thymic epithelial cells from human iPS cells should facilitate the future *in vivo* study of patient-derived hematopoietic and immune cell interactions with autologous primary tumor cells. This should potentiate the power of immunotherapy of human cancer.

Wolfgang Wurst - Wolfgang Wurst, Ph.D., Director of the Institute of Developmental Genetics at the German Research Center for Environmental Health and Professor at Technische Universität München, presented on efficient targeted mutagenesis in mice using TALENs. He observed that targeted mouse mutants are instrumental for the analysis of gene function in health and disease. His team recently provided proof-of-principle for the fast track mutagenesis of the mouse genome using TALENs in one-cell embryos. Dr. Wurst reported a routine procedure for the efficient production of disease-related knockin and knockout mutants using improved TALEN mRNAs. To knockout the *C9orf72* gene as a model of frontotemporal lobar degeneration, TALEN mutagenesis induced sequence deletion in 41% of pups derived from microinjected embryos. Using TALENs together with mutagenic oligodeoxynucleotides he and his team introduced amyotrophic lateral sclerosis patient-derived missense mutations in fused sarcoma (*Fus*) gene at a rate of 6.8%. For the simple identification of TALEN-induced mutants and their progeny, they validate high-resolution melt analysis (HRMA) of PCR products as a sensitive and universal genotyping tool. The combination of improved TALEN mRNAs for enhanced mutagenesis and of HRMA for simplified genotyping enables the accelerated, routine production of new mouse models for the study of genetic disease mechanisms in personalized medicine applications.

Andrew P. Feinberg - Andrew P. Feinberg, M.D., M.P.H., Director of the Center for Epigenetics at the Institute of Basic Biomedical Sciences and a Professor in the Bloomberg School of Public Health at The Johns Hopkins University, was ill and unable to attend. His graduate student Michael Multhaup presented on Dr. Feinberg's cross-species analysis to identify differentially methylated regions across species relevant to type 2 diabetes. Dr. Feinberg and his team sought to increase the analytical power of epigenetic analysis by comparing mouse models and human data, largely ameliorating the issue of population heterogeneity and tissue access that complicates human epigenetic epidemiology, while retaining the translational relevance of human studies. They examined white adipose tissue, liver, skeletal muscle, hypothalamus, and pancreatic islets in 30 male C57BL/6 mice on a high fat diet, and a similar number on a low fat diet. They performed comprehensive genome-scale methylation analysis with custom CHARM arrays, assaying several million CpG sites for DNA methylation. They also compared these data to a sample set of adipose tissue biopsies from obese and lean patients and matched patients from the former group who had undergone bariatric surgery. Dr. Feinberg's team identified a large number of differentially-methylated regions between the two groups of mice and also related to measures, such as the glucose tolerance test. They observed with high statistical significance many of the same DMRs seen in human samples. They suggest a new paradigm for cross-species epigenetic studies of common human disease.

Round Table Discussion - Hazel L. Sive, Leonard Schultz, Wolfgang Wurst, and Michael Multhaup participated in the round table discussion. Dr. Lloyd led the discussion on technological advances and available resources for building predictive animal models. Dr. Lloyd invited comments on major obstacles, challenges, and opportunities for a potential NIH initiative. Hazel Sive encouraged the NIH to contemplate zebrafish use to model complex genetic diseases such as mental health disorders.

A participant in the audience asked panelists to address the need and difficulty to study heterogeneous populations of cells affected in a particular disease state. Dr. Wurst responded and agreed that it was a challenging question. He said he could envisage the use of viral vectors for precision delivery of gene modification tools with subsequent tracking of the particular cell behavior. Another participant in the audience suggested the need for development of aged animal models in a short period of time. Dr. Wurst expressed hesitation, because it would require the introduction of a confounding variable if the aging process was sped up. Dr. Sive suggested that short-circuiting approaches in animals with short life spans might provide a path to decrease periods of time to conduct experiments.

Members of the audience addressed issues related to TALEN and CRISPR applications. One attendee asked how efficiently one can add traceable markers for these models. Dr. Wurst responded that the issue is to have extensive information about the target locus. The participant who asked the question added that efforts to create next generation microscopies combined with such markers would be very powerful, and Dr. Wurst agreed.

Another participant from the audience asked about the lack of interaction between investigators to build understanding of the functionality of specific genes across different species. Dr. Wurst indicated he believed these comparisons will eventually occur but given the current pace of research and technologies, it may require 10 years. The audience member observed that at the moment if he needs information about a mouse, he must access Jackson Lab, or if he requires information about a zebrafish, he must consult data from the Sanger Center. At present, he has no single location to acquire all the information he needs about the same gene in both species. Another member of the audience mentioned Phenonet as a site to obtain integrated phenotype information, though he noted the site is neither graphically appealing nor fast. Dr. Sive

concurred this topic is important given the difficulties of cross-referencing species information; she also added that many conditions are multi-genic, and this fact adds another layer of complexity. She indicated any effort to bring them to a database ultimately ought to interface not just single gene knockouts but also multi-genic mutant models across species. Another speaker noted the importance of creating egalitarian resources that are serviceable to both small and large laboratories.

In response to a question from the audience, Dr. Sive expressed interest in iterative processes using diverse species to answer different aspects of questions about what is occurring during disease development at different times within an organ, such as the brain. She noted the need to determine the right mutation in the right animal species and compare it to humans to address different aspects of a question. This process requires conversations between clinicians, and investigators. She believes it is complex and long-term, but requires interactions. A member of the audience noted the need to include not just lower species in such a program but also primates.

A member of the audience asked whether a technology exists to measure the magnitude of the methylation changes in the genome. Michael Multhaup responded it is, of course, possible to acquire a sense of magnitude of the methylation changeover at a specific gene, but at this point, it is very difficult to tell how that change might affect a transcription factor that influences other gene activity. He alluded to recent research that suggested such analysis will be possible in the future. Dr. Lloyd concluded that during the course of the round table, he heard disease-agnostic discussion about bioinformatics, genetics, general medicine, infrastructure and resources, clinical science, and biotechnologies as multiple scientific disciplines to achieve the goals in the subject matter of the round table.

Session 3: Using Personalized Animals for Drug Discovery and Biomarker Development

Calum MacRae - Calum MacRae, M.D., Ph.D., Associate Physician at Brigham and Women's Hospital and Associate Professor of Medicine at Harvard Medical School, presented on scalable models of complex adult onset disorders in larval zebrafish. Dr. MacRae described a system-level understanding of gene, organism, and small molecule interactions to gain insight into disease biology and promote drug discovery. He observed that many chronic diseases result from perturbations of homeostatic responses that cross multiple physiologic systems. His laboratory employs zebrafish for its research since this model organism is a great tool for scalable *in vivo* genomics and chemical biology. He and his colleagues have built genetic models of several morbid cardiac and vascular diseases with adult onset and used these to explore the developmental basis of diseases. This work has facilitated the generation of automated assays that enable direct screening of small molecule libraries for chemical probes and potential therapeutic leads. He also developed a series of toxicology reporter strains and other quantitative assays to facilitate formal SAR studies and is using these in conjunction with computational approaches to annotate chemical libraries. Combining disease models with these toxicology systems, he indicated, will allow us to optimize the balance between efficacy and risk *in vivo*. Dr. MacRae observed that he and his colleagues have begun to explore the transition from zebrafish to mammalian models in an extended academic pipeline with collaborators in chemistry, physiology, and pharmacology. In conclusion, Dr. MacRae suggested that phenotypic innovation will speed up discovery and eventually will affect translation, clinical care, and medical costs.

Ross L. Cagan - Ross L. Cagan, Ph.D., Professor in the Department of Developmental and Regenerative Biology and Associate Dean of the Graduate School of Biological Sciences of the Icahn School of Medicine at Mount Sinai, presented on a drosophila approach to personalized cancer therapeutics. Dr. Cagan observed that cancer's complexity is a key difficulty in identifying useful therapeutics. For this reason, a key question researchers have yet to fully answer in the cancer field is how to model this complexity to develop useful therapeutics. Dr. Cagan discussed two approaches to explore the answer to this question. First, he described his and his colleagues' efforts to build multigenic cancer drosophila models that represent the complexity observed in colorectal patients using human tumor sequencing data. He described how tumor properties and drug response can differ on different genetic backgrounds. Subsequently, he discussed his efforts at the new Center for Personalized Cancer Therapeutics, which Mount Sinai launched in July 2013 to build personalized fly models of individual patients based on the patient's personal cancer genome. They use these individualized models to identify personalized therapeutic cocktails that are appropriate to address a given patient's tumor. In addition to providing novel therapeutic avenues for patients, his team's efforts seek to understand the necessary information required to build useful models. Dr. Cagan identified a number of challenges, including the coordination of all aspects of the work; issues related to FDA approval of novel drug combination; the relative speed of sequencing flies, mice, and pigs; issues related to other and new technologies; and raising funds to support the work.

Tilo Grosser- -Tilo Grosser, M.D., Ph.D., Research Assistant Professor in the Institute for Translational Medicine and Therapeutics at the University of Pennsylvania, presented on the encouragement and challenges in the translational therapeutics of the prostaglandin pathway. His presentation addressed the personalization of drug therapy for chronic disease, mouse models to study the molecular mechanisms of adverse drug reactions, and the consequences of variability in the prostaglandin pathway. Dr. Grosser observed that chronic pain affects about 100 million American adults and costs the nation up to $635 billion each year in medical treatment and lost productivity. He observed the prostaglandin pathway plays an important role in inflammation and pain. Cyclooxygenase (COX) inhibitors, the nonsteroidal anti-inflammatory drugs (NSAIDs), depress prostaglandin formation and are widely used to treat inflammatory pain. About 25% of the U.S. population, roughly 60 million people, regularly consumes NSAIDs which include both traditional NSAIDs, such as naproxen and ibuprofen, and NSAIDs selective for COX-2, including rofecoxib, valdecoxib, etoricoxib, lumiracoxib, celecoxib. However, the therapeutic benefit of NSAIDs is offset by serious side-effects, primarily gastrointestinal and cardiovascular complications such as heart failure, myocardial infarction, and stroke. Evidence from human pharmacology and genetics, genetically manipulated rodents and other animal models and randomized trials indicates that the cardiovascular adverse events are consequent to suppression of COX-2 dependent cardioprotective prostagladins, particularly, prostacyclin. He indicated the challenge is to integrate such diversified forms of information in order to pursue a more personalized approach to study drug efficacy and risk.

Geoffrey M. Duyk - Geoffrey M. Duyk, M.D., Ph.D., partner and managing Director of the private equity firm, TPG Biotechnology presented on lost and found in translation. Dr. Duyk pointed out that though advances with the pharmacology industry drove drug innovation in most of the last century, a number of factors caused changes in the industry. He pointed to the high cost of healthcare and the reduction of profits as high-value drug patents expire faster than the industry can replace them with new high-value patented products. Another change in recent years is the retirement of highly experienced staff that possessed the industry's institutional memory. Dr. Duyk called for studies of drug predictability, drug metabolism, and issues such as the passage of drugs through the blood-brain barrier. He stressed that research over the last 20

years has gotten faster, cheaper, and smarter. Since there is a high rate of failure, an important lesson for pre-clinical and clinical development of new therapeutics is to "fail fast, fail early, fail well." The notion of failing well, he indicated, implies the capacity to derive insight from work that constructively informs future decisions. Current development paradigms are linear and do not leverage the opportunities to iterate between the clinical and pre-clinical settings as well as to directly address the gaps that emerge between the physiology and the molecular biology/genomics. Any design of precision models, Dr. Duyk suggested, must accommodate iterative design. Areas he proposed the need to address included investment in basic research, streamlining of drug approval, the division of public and individual health, the changing dynamics of transparency and privacy, big data, the availability of sensors, crowdsourcing, the power of community, and alternative research models.

Round Table Discussion - Ross L. Cagan, Tilo Grosser, and Geoffrey Duyk participated in the round table discussion on using personalized animals for drug discovery and biomarker development. Dr. MacRae led the discussion. He opened the discussion by noting that he had heard again the message that arose throughout the day, that is, the need for a clear anchor between genotype and phenotype across multiple species and to bring those two components together in a systematic way. He also had heard a call for an environment where investigators can collaborate and share information and resources with one another.

Dr. MacRae then asked what the major outcomes and impact of new programs in this area would be. Dr. Duyk responded that if investigators could improve predictability by even a small increment, that outcome will represent a major contribution. Dr. Cagan added that efforts to address the problems inherent in the ubiquity of big data also will contribute significantly to the field. He suggested the need to consider both the content of big data and the processes to manage and analyze it. A tension in big data, Dr. Cagan also noted, is the focus on commonalities; when researchers do not identify them in a data set, they set out to search for larger data sets. The danger in this procedure, he suggested, is the potential loss of insight into what is happening in the individual patient. The audience members discussed issues such as the size of big data, the heterogeneity of data, computational issues, and the role of key data in big data. Using NASA's mission model, Dr. Duyk suggested that rather than a project to address issues in handling big data, the NIH should engage in several focused projects that require big data to answer questions; he conjectured that these efforts will drive infrastructure that addresses the questions raised in the round table discussion. He recommended the creation of such efforts to formulate the right questions and recruit personnel with appropriate skills to answer them.

Session 4: Which Human Diseases Conditions Are the Best Candidates for Use of Personalized Animal Models?

Leonard I. Zon - Leonard Zon, M.D., a Howard Hughes Medical Institute Investigator at Children's Hospital in Boston and Professor of Pediatrics at Harvard Medical School, presented on modelling diseases and developing therapeutics using the zebrafish. Dr. Zon laid out issues for animal modelling, including accuracy, speed, availability of drug screens, methods for validation, and rapid translation to the patient. He praised the zebrafish as animal to disease modeling that makes the development of new therapies possible to treat human disease effectively and in a short period of time. His laboratory recently created a zebrafish model of Diamond Blackfan anemia that recapitulates many aspects of the human disease. A chemical suppressor screen was done on this rps29 zebrafish mutant that resembles Diamond Blackfan anemia. Calmodulin antagonists rescued the defect in hematopoiesis. These antagonists also

rescued the hematopoietic block found in human CD34 cells treated with an shRNA to rps19. Dr. Zon indicated this suggests that calmodulin antagonists can potentially be used to treat patients with Diamond Blackfan anemia. Additionally, a screen for blood stem cells in the developing aorta led to the discovery of PGE2 as a regulator of stem cell engraftment. After studies with mouse and human hematopoietic cells, a clinical trial proceeded to test PGE2 in cord blood transplantation. In 12 leukemic patients receiving 2 cord blood units, one was treated with dmPGE2, and 10 patients were engrafted with the treated cord blood. The neutrophils and platelets recovered more quickly than the untreated cord blood. Another chemical, leflunomide, was found to block neural crest development in zebrafish. Leflunomide also suppressed neural crest gene transcription in human melanomas, and reduced the rate of human tumor formation in mouse xenografts. The effect was stronger in combination with a BRAF inhibitor known to block cell proliferation. This has led to a clinical trial in which 43 patients will be treated for metastatic melanoma. To date, three patients have been treated. Speaker concluded that zebrafish models offer an excellent opportunity to screen for new therapeutics.

Maria Karayiorgou - Maria Karayiorgou, M.D., Professor of Psychiatry in Genetics and Physiology at Columbia University, presented on schizophrenia: from genes to mechanisms and novel treatments. Dr. Karayiorgou noted the complexity of the genetics of schizophrenia. The past five years, she observed, have seen enormous advances in genomic technology; development of new paradigms for gene discovery, including genome-wide studies to discover common and rare variants that predispose to disease; and initial steps to apply these findings in clinical settings. These advances have led to the identification of several disease genes and pathways that play a role in the disease development. They also clarified the role that rare variants play in schizophrenia, where individual rare alleles have a large effect on increased susceptibility. One of the most intriguing types of rare variation is copy-number variants (CNVs), that is, deletions or duplications. The general prevalence of large (>1 kb) and rare (<1%) CNVs are higher in schizophrenia patients than in population controls. 22q11.2 microdeletions and other CNVs seen recurrently in multiple patients carry higher likelihood of being pathogenic and offer a very strong entry point for therapeutic and translational studies. In particular, recurrent CNVs affecting single genes can be particularly useful in designing new routes for treatment. One such CNV is the microduplication encompassing the vasoactive intestinal peptide receptor 2 (*VIPR2*) gene and increasing its expression levels, which is strongly associated with schizophrenia. Much work remains for animal models to connect genomic alterations to altered pathways and acquired cellular vulnerabilities, and to use this information to guide the development and application of therapies.

Dale L. Greiner - Dale L. Greiner, Ph.D., Co-Director of the Diabetes Center of Excellence and Professor at the University of Massachusetts Medical School, presented on humanized mice in the study of diabetes. Rodent models have contributed to understanding the causes and identification of diabetes treatments; however, mouse and human islets, as well as their immune systems, differ in cell composition, function, and gene expression. Moreover, the inaccessibility of the pancreas for study and the inability to analyze *in vivo* the interaction of immune cells with islets have impeded understanding the pathogenesis of human diabetes. Existing animal models were not helpful so far. For example, decades of studies with rodent models of type 1 diabetes (T1D) have not identified therapies to prevent or cure the disease in humans. Dr. Greiner and his colleagues have sought to develop humanized mice to study diabetes, including the transplantation of human islets and engraftment of human hematopoietic and immune systems. Researchers need to investigate human-specific therapies on human cells and tissues *in vivo*. They have developed models of hyperglycemic/immunodeficient mice to engraft functional human islets and human immune systems. These models develop a spontaneous genetically determined hyperglycemia, can be induced at will to develop hyperglycemia, or can

be regulated to become hyperglycemic and revert to normoglycemia by addition or removal of doxycycline. They have also developed mouse models of insulin resistance and obesity to investigate the response of human islets and human immune systems to these metabolic states of diabetes. These models allow the placement of islets from a single donor (or generated *in vitro*) into different *in vivo* environments (euglycemic or hyperglycemic) for direct study of those effects in a controlled setting. These models also permit the study of the effect of an immune-beta cell interaction *in vivo* over time and correlation of *in vivo* functional studies of islets with immunohistological and gene expression studies: analyses not possible in the clinical setting. Dr. Greiner and his colleagues are currently using these humanized mouse models as a pre-clinical bridge to facilitate identification and translation to clinical settings of novel discoveries in T1D and type 2 diabetes.

Richard S. Blumberg - Richard S. Blumberg, M.D., Professor in the Harvard Medical School, presented on animal models of inflammatory bowel disease (IBD) and insights from studies with X box binding protein 1. Dr. Blumberg noted IBD is a complex disease with a multifaceted etiology that emerges from an abnormal environmental response of mucosal tissues associated with intestines in a genetically-susceptible host that is dependent on and regulated by the commensal microbiota. Most contemporary insights into the pathogenesis of this disease, he observed, have come from advances in genetics, microbiology, and immunology and their investigation in animal models. Most animal model studies depend upon the mouse system and include spontaneous models as well as induced by exposure to exogenous stimuli models (e.g., dextran sodium sulfate), caused by the genetic manipulation of the host, or the adoptive transfer of T cells into immune-deficient animals. Examining the role of specific cell types or pathways in the pathogenesis of disease brought new insights into the immunogenetic and microbial basis for these disorders. Dr. Blumberg noted significantly expanded knowledge of the mucosal immune system and the derangements associated with IBD. He observed that the remaining major challenges are to use these models to advance the understanding of the genetic mutations associated with these diseases and further leverage the development of targeted therapeutic agents. Dr. Blumberg concluded his presentation with a discussion of interactions between the unfolded protein response and autophagy in the intestinal epithelium.

Round Table Discussion - Maria Karayiorgou, Dale L. Greiner, and Richard Blumberg participated in the round table discussion that Leonard Zon led on the best disease candidate for application of personalized animal models.

Dr. Zon reflected that the investigators in the room are encountering a large number of genes that are affecting pathological processes. He alluded to the tension between reductionist models and a larger number of models that describe multiple phenotypes. Dr. Karayiorgou called for robust animal models to provide clarity and focus for specific genes that underlie the question under investigation. Richard Blumberg observed models of diseases can facilitate the process of parsing out pathways and noted we need to develop and use as many tools as possible. Dr. Zon noted that some studies require a large number of cases to determine a smaller number of implicated pathways. Dr. Karayiorgou, in response to a question from the audience about targeting different aspects or traits of autism spectrum disorder, noted that some are distinguishable, but it becomes fuzzy when researchers examine hundreds of thousands of cases. She said adhering to the diagnosis in a recognizable and reductionist process, starting with what drives phenotypes, and examining penetrants, provides better focus.

Presenters and audience members alike noted the need for more resources to support their work. One audience member particularly noted the need for a repository of human tissues and products to promote translational work. Dr. Blumberg noted that in microbiome research a large

question is the specific molecular pathways that link a microbial community, a specific microbe, its components, or its metabolite to the epithelial function or immune system of the host. He said this project is only commencing, but its significance lies in the large number of therapeutic opportunities for the microbiome. Another related question is how the phenotypic compositions or imputed metabolic functions of these populations are associated with disease and what kinds of interventions will repair it through potential interventions such as a microbiome transplant.

A participant in the audience, reflecting on Dr. Karayiorgou's presentation, suggested the need for a pipeline of different animal models, such as the zebrafish, mice, and primates, to serve as a circular discovery tool. She pointed at zebrafish, which exhibit robust behavioral phenotypes, as a possible natural point of departure to investigators.

Another participant in the audience invited discussion about trans-disciplinary infrastructure and resources that will support investigators, regardless of their specific focuses. Dr. Blumberg reiterated the earlier comment about access, even for hospitals, to human tissues through dedicated banks. He also suggested the need for government funding in consortium grants that promote collaboration among communities of researchers. Several participants remarked that a consortium could more easily work across several animal models to answer a given question. One member of the audience observed that a specialized panel of NIH reviewers is necessary to permit these collaborations across disciplines, since traditional R01 reviewers will level the criticism that all the co-investigators must share similar trans-disciplinary skills to make a project work. Dr. Zon also commented on the need for a pipeline across specialties to breakdown silos and to facilitate collaborations. A participant noted that the NIH may need to lengthen traditional periods of funding to permit time for teams to develop and address issues. Another participant suggested the possibility in developing these consortia of core teams with responsibility to develop new approaches that also will have to reach out to other investigators if required. Another member of the audience volunteered that these collaborative efforts are the model that European investigators employ. Suggestions for NIH contributions to the consortia included communication such as videoconferencing.

Session 5: How Can Personalized Animal Models Guide Clinical Trials?

Pier Paolo Pandolfi - Pier Paolo Pandolfi, M.D., Ph.D., Scientific Director at Beth Israel Deaconess Cancer Center and a Professor at Harvard Medical School, presented on the mouse hospital and the co-clinical trial project. Dr. Pandolfi observed that advances in technology have provided powerful insights into the molecular and genetic mechanisms of cancer, but translation of this knowledge into effective therapeutics has often proven to be difficult. To facilitate this process, Dr. Pandolfi and his colleagues have launched a new initiative, both nationally and internationally, described as the Co-Clinical Project. Its major goal is to accelerate the stratification of patients based on molecular and genetic criteria; to identify mechanisms of acquired resistance to specific treatments and develop novel therapies to overcome this resistance. To achieve this goal, they integrated data from preclinical trials and performed the preclinical trials in the mouse hospital using genetically engineered mouse models of human cancer and transplanted primary human tumor tissues. They run these experiments simultaneously with experimental clinical trials according to existing standard-of-care treatment protocols. Required parts and their goals and function in these complex projects were discussed in Dr. Pandolfi's presentation.

Megan Sykes - Megan Sykes, M.D., Professor of Microbiology and Immunology at Columbia University, presented on a humanized mouse model for personalized assessment of

immunopathogenesis and immunotherapy. Studies of human immune diseases are generally limited to the analysis of peripheral blood lymphocytes in heterogeneous patient population. Dr. Sykes described the need for improved models to analyze fundamental immunologic abnormalities and assess immunotherapies. Immunodeficient mice receiving human fetal thymus grafts and fetal CD34+ cells i.v. produce robust human immune systems, allowing analysis of human T cell development and function. However, the use of humanized mice to study human immune-mediated disorders requires the generation of immune systems from adult hematopoietic cells of patients with established diseases. Dr. Sykes and her colleagues have achieved robust immune reconstitution in immunodeficient NSG mice receiving CD34+ hematopoietic stem cells (HSCs) aspirated from bone marrow of adults with T1D and healthy control volunteers. Cryopreservation of HLA allele-matched fetal thymic tissue permits engraftment of the HSCs and allows high levels of T cell reconstitution without graft-vs.-host reaction. Newly generated T cells, which include regulatory T cells (Tregs), are functional, self-tolerant, and have a diverse repertoire. The immune recognition of these mice mimics that of the adult CD34+ cell donor, but the T cell phenotypes are more predominantly naïve than those of the adult donors. HSCs from T1D and control donors generate similar proportions of natural Tregs intrathymically and the function of peripheral Tregs is similar in both groups. However, investigators have obtained evidence for HSC-intrinsic differences in T cell homeostasis in T1D-derived immune systems. This personalized immune (PI) mouse provides a new model for individualized analysis of human immune responses that may provide new insights into the immunopathogenesis not only of T1D but also of other immune-mediated diseases. Additionally, it provides an opportunity to assess and compare responses to immunotherapies in a personalized manner in the cohorts of PI mice, of which 15-35 can be generated from a rapidly performed bedside bone marrow aspirate.

Dennis A. Steindler - Dennis A. Steindler, Ph.D., Professor of Medical Research in the Department of Neurosurgery at the University of Florida College of Medicine, presented by phone on patient-specific neurological disease avatars for monitoring response to treatment. Dr. Steindler noted NSG mice engraft human stem/progenitor cells better than other humanized mouse models. Since their introduction, he and his colleagues have used NSG mice with transplanted human immune and nervous systems to attempt to better predict disease course and response to therapy for diseases which include Parkinson's Disease and cancer. NSG, and possibly other immunocompromised mice, can create better disease- and patient-specific surrogate, or avatar, mice. He noted these avatar mice with patient-matched immune system cells and at-risk neurons or their precursor cells represent a valuable model to better screen for new molecular therapeutics within a large patient population. Earlier diagnosis, and better screening of new therapeutics and monitoring of their response will be possible by using significant advances of animal models, stem cell biology and regenerative medicine. Dr. Steindler discussed the most pressing obstacles in clinical trial design and implementation using disease- and patient-specific avatars. These obstacles included absence of optimal *in vitro* and *in vivo* bioassays and standardized operating protocols with GLP/GMP reagents, avatar mouse shortcomings, and scale issues in mouse vs. human for testing therapeutics. Dr. Steindler cited challenges working with mice, such as avatar mice availability, survivability, and husbandry issues; and correct/synchronous disease or remission stage of the particular patient. He called for multidisciplinary collaboration between basic science and clinical investigators open to state-of-the-art applications, and IRB protocols for compassionate-use regenerative medicine.

David A. Tuveson - David A. Tuveson, M.D., Ph.D., Professor and Deputy Director of the Cancer Centre at the Cold Spring Harbor Laboratory, presented on developing therapies in pancreatic cancer. Dr. Tuveson observed that with an estimated incidence of 43,000 new cases and 37,000 deaths in the U.S. each year, pancreatic cancer is a deadly disease that lacks

effective therapies. Problems in pancreatic cancer medicine include the lack of KRAS inhibitors. Tuveson's laboratory have generated a mouse model of advanced pancreatic cancer and used it to investigate the response to conventional and investigational agents. Recently, they showed that drug delivery is hampered by poor perfusion and diffusion of agents into pancreatic tumors and devised a variety of strategies to circumvent this circumstance. More recently, Dr. Tuveson found that extracellular proteins provide survival cues that suppress therapeutic responses. Animal models, therefore, represent a means to understand and correct both the biophysical and biochemical resistance mechanisms that are important in therapy of pancreatic cancers and other malignancies.

Round Table Discussion - Megan Sykes and David Tuveson participated in a round table discussion on how personalized animal models can guide clinical trials. Pier Paulo Pandolfi led the conversation.

Dr. Pandolfi asked about potential opportunities for the NIH to assist basic science and translational models. Dr. Tuveson reflected that over the last two decades, scientists have developed and employed sophisticated cancer models but the landscape in cancer research is not yet changed. He recommended an early focus in the investigatory process with animal models on the genetics and epigenetics of the disease along with consideration of immunotherapies. Dr. Pandolfi discussed the need to integrate physicians and scientists in the development of clinical trials. Dr. Sykes noted that though the personalized immune mouse possesses significant potential in many diseases, successful application requires close coordination between investigators and clinicians. She noted the need for a bank of human thymus tissues to create personalized immune mice for wide use. She identified a need for human thymic tissue from stem cells. Dr. Tuveson suggested the utility of a pre-clinical development unit in the background of some trials with several locations across the states. Dr. Sykes, noting regulatory and infectious disease issues, suggested the possibility of developing human T cells in mice that clinicians can infuse back into humans.

D. Recommendations

Session 6: Current Challenges and How to Accelerate Progress

Closing Remarks-Recommendations—Kent Lloyd, Mark Ellisman, and Pier Paolo Pandolfi joined together to lead the closing session. The discussion resulted in the following remarks and recommendations related to each of the five questions that Dr. Mirochnitchenko raised at the start of the meeting:

1. **What are the major obstacles/challenges/opportunities that a potential NIH initiative should address?**

    - *Impediments and limitations to communication and sharing experimental capabilities, expertise, and models between disparate research groups using diverse animal model systems*
    - *Capacities and resources for rapidly generating and testing scalable libraries of next-generation animal models reflecting personalized –omics profiles*
    - *Need for knowledge-based connections between individual –omics, other attributes of phenotypes, and predictive animal models*

- *Lack of coordinated translational pipelines using animal model systems with appropriate standardization and harmonization*

2. Are multiple scientific disciplines needed to achieve these goals?

Yes: bioinformatics, genetics, basic and applied sciences (e.g., chemistry, engineering), medicine and disease areas (e.g., cancer, infectious/inflammatory, immunologic, metabolic, neurological, genetic, etc.), infrastructure and resources, clinical sciences, biotechnologies, information sciences, high-throughput multiscale multimodal imaging etc.

3. What initiatives might form the strategic plan for this topic?

- *Develop new and refining extant scalable animal modeling technologies (molecular, transplant, etc) and capacities that enable rapid and highly flexible genomic editing, epigenetic analysis, diagnostic profiling, drug testing, pharmacologic/pharmacodynamics modeling, intervention strategies, integrated biorepositories, and full-length human genomic libraries (cDNA, non-coding, etc.) informed by individual patient sequencing data*
- *Create frameworks to federate accruing databases of human -omics data and other forms of biomedically-relevant information about subjects and experimental methods*
- *Develop integrated analysis platforms for efficient transfer and analysis of results between and within preclinical and clinical settings*
- *Advance preclinical trials and target drug testing studies using animal model systems through systematic integrated "phenotype correlation"*
- *Optimize processes for drug repositioning and combinatorial treatments*
- *Facilitate education and opportunities for cross-training/interdisciplinary training to maximize and sustain the workforce necessary to ensure scientific utilization of animal model systems*

4. What would be the goals of the program?

- *Functional and physical links bridging basic and clinical studies*
- *Integrative training in clinical medicine for scientists, and in basic science for physicians*
- *High-throughput, high-fidelity, high-capacity targeted genomic editing capacity*
- *Highly-controlled, preclinical and challenge phenotyping capabilities that reflect the human condition*
- *Tools and resources for validation, quality control verification, archiving, dissemination, and distribution to ensure broad availability and accessibility of predictive animal models to the translational science community*
- *Demonstration projects using integrated and complementary animal model systems in multiple disease areas*
- *Highly-organized, highly-annotated, user-friendly federated databases of human and animal genomic sequence data to inform the creation and use of predictive animal models*

- *Novel and evolving paradigms and consortia of scientific and clinical experts and trainees with access to animal model expertise, systems, resources enabling open, synergistic and egalitarian interactions*

5. What would be the major outcomes and impact of a new program in this area?

- *Traditional bottlenecks preventing translation of basic science discoveries into clinically relevant diagnostics, therapeutics, and preventative strategies are overcome*
- *Focused, targeted, and effective therapies resulting in improved health care and enhanced longevity*
- *Acceleration of the scientific development, implementation, and application of clinical interventions*
- *Recognition of the value of collaborative "team science" on par with that of a principal investigator-driven project*
- *Greater public and private (e.g., pharmaceutical industry) participation and cooperative investment in practices that promote healthy living*
- *Credentialed interdisciplinary pipelines that enable translation of scientific knowledge to the practice of individualized medicine*
- *Reassessment of training programs emphasizing science and expertise necessary to sustain advancements in individualized medicine*

E. Conclusion

The symposium gathered a diverse group of biomedical experts to evaluate the status of animal production using specific genetic modifications and replacement of specific cells and tissues in a variety of species to create animal phenotypes closely analogous to that of human patients (precision models). Meeting participants came to the conclusion that there is an urgent need to apply and expand upon successful examples of the creation and use of this next generation of animal models. Support of specialized research projects that facilitate broad use and sharing of knowledge and unique expertise will optimize the utilization of the limited resources available to address the requirements of individual patients or group of patients. Understanding the high level of biological complexity will require the involvement of computational biologists, geneticists, veterinarians, experimental biologists and clinicians in an integrated fashion. This approach recognizes the need for centralized services to collect and process genetic and omics information, improve phenotype-disease ontologies and create genetically modified animals of different species as well as interspecies somatic hybrids. Specific recommendations were given by the speakers as well as by the audience during the discussion sessions which are being evaluated and will be used to plan new NIH initiatives.

Appendix A. Symposium Agenda

Day 1 - Monday, October 28, 2013

8:30 – 9:00 **Introduction and welcome**

Symposium Introduction: Oleg Mirochnitchenko (OD/NIH)

Welcome: James M. Anderson, DPCPSI Director (OD/NIH), Harold Watson (OD/NIH)

9:00 – 9:45 **Keynote Presentation**

Individualized Medicine in the Genomic Era
David Valle (Johns Hopkins, MD)

Session 1: The Use of Comparative and Functional Genomics to Build Animal Models of Human Diseases ***(Moderator Michael P. Snyder)***

9:45 – 10:05 Personalized Medicine: Personal Omics Profiling of Healthy and Disease States, **Michael P. Snyder** (Stanford University, CA)

10:05 – 10:25 Nonhuman Primates: Modeling the Genetics of Risk Factors in Human Disease**, Jeffrey Rogers** (Baylor College of Medicine, TX)

10:25 – 10:45 Connecting Humans to Models: Linking and Analyzing Distributed Data to Uncover Disease Mechanisms and Propel Biomedical Research, **Mark Ellisman** (UC San Diego, CA)

10:45 – 11:05 Mobilizing Model Organism Phenotype Data for Rare Diseases and Personalized Medicine, **Paul N. Schofield** (University of Cambridge, UK)

11:05 – 11:25 Modeling the Pediatric Morbid Genome, **Nicholas Katsanis** (Duke University, NC)

11:25 – 11:55 **Round Table Discussion**

11:55 – 12:55 **LUNCH**

Session 2: Technological Advances and Available Resources for Building Predictive Animal Models (*Moderator K.C. Kent Lloyd*)

12:55 – 13:15 In Vivo Modeling in Transition: From KOMP to Next-generation Precision Animal Models of Disease, **K.C. Kent Lloyd** (UC Davis, CA)

13:15 – 13:35 The Zebrafish As a Tool for Understanding Mental Health Disorders, **Hazel L. Sive** (MIT, MA)

13:35 – 13:55 Humanized SCID Mouse Models for Cancer Research, **Leonard D. Shultz** (Jackson Lab., ME)

13:55 – 14:15 Efficient Targeted Mutagenesis in Mice Using TALENs, **Wolfgang Wurst** (Institute of Developmental Genetics, Munchen, Germany)

14:15 – 14:35 Cross-species Analysis Identifies Differentially Methylated Regions Across Species Relevant to Type 2 Diabetes, **Andrew P. Feinberg** (Johns Hopkins University, MD)

14:35 – 15:05 **Round Table Discussion**

15:05 – 15:25 **BREAK**

Session 3: Using Personalized Animals for Drug Discovery and Biomarker Development (*Moderator Calum A. MacRae)*

15:25 – 15:45 Scalable Models of Complex Adult Onset Disorders in Larval Zebrafish, **Calum A. MacRae** (Harvard Medical School, MA)

15:45 – 16:05 A Drosophila Approach to Personalized Cancer Therapeutics, **Ross L. Cagan** (Mount Sinai Hospital, NY)

16:05 – 16:25 Encouragement and Challenge in the Translational Therapeutics of the Prostaglandin Pathway, **Tilo Grosser** (University of Pennsylvania, PA)

16:25 – 16:45 Lost and Found in Translation, **Geoffrey M. Duyk** (TPG Biotech, CA)

16:45 – 17:15 **Round Table Discussion**

Day 2 – Tuesday, October 29, 2013

Session 4: Which Human Disease Conditions Are the Best Candidates for Use of Personalized Animal Models? (*Leonard I. Zon*)

8:30 – 8:50 Modeling Disease and Developing Therapeutics Using the Zebrafish, **Leonard I. Zon** (Harvard Medical School, MA)

8:50 – 9:10 Schizophrenia: From Genes to Mechanisms to Novel Treatments, **Maria Karayiorgou** (Columbia University, NY)

9:10 – 9:30 Humanized Mice for the Study of Diabetes, **Dale L. Greiner** (UMass, MA)

9:30 – 9:50 Animal Models and Understanding the Pathogenesis and Treatment of Inflammatory Bowel Disease, **Richard S. Blumberg** (Harvard Medical School, MA)

9:50 – 10:20 **Round Table Discussion**

10:40 – 11:40 **LUNCH**

Session 5: How Can Personalized Animal Models Guide Clinical Trials? (*Moderator Pier P. Pandolfi*)

11:40 – 12:00 The Mouse Hospital and the Co-Clinical Trial Project, **Pier P. Pandolfi** (Harvard Medical School, MA)

12:00 – 12:20 A Humanized Mouse Model for Personalized Assessment of Immunopathogenesis and Immunotherapy, **Megan Sykes** (Columbia University, NY)

12:20 – 12:40 Patient-Specific Neurological Disease Avatars for Monitoring Response to Treatment, **Dennis A. Steindler** (University of Florida, FL)

12:40 – 13:00 Developing Therapies in Pancreatic Cancer**, David A. Tuveson** (CSH, NY)

13:00 – 13:30 **Round Table Discussion**

Session 6: Current Challenges and How to Accelerate Progress

13:30 – 14:00 Closing Remarks/Recommendations: **(*Moderators Pier P. Pandolfi , Marc Ellisman, Kent Lloyd*)**

Appendix B. Participant List

Revised to reflect final attendance.

	Last Name	First Name	Affiliation
1.	Abraham	Kristin	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
2.	Akolkar	Beena	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
3.	Alamri	Ahmad	Georgetown University
4.	Alcoser	Sergio	Biological Testing Branch, National Cancer Institute
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8.	Arias	Jonathan	CSR/National Institutes of Health
9.	Arreaza	Guillermo	National Institutes of Health
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11.	Bang	Hyeeun	LCID/National Institute of Allergy and Infectious Diseases, NIH
12.	Beckel-Mitchener	Andrea	National Institute of Mental Health, NIH
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21.	Coulombe	James	National Institute of Child Health and Human Development, NIH
22.	Cymerblit-Sabba	Adi	National Institute of Mental Health, NIH
23.	Davis	E. Ann	National Institutes of Health-Fogarty International Center
24.	Davis	Cheryl	SAIC Frederick
25.	Dirami	Ghenima	Lung Injury, Repair and Remodeling Study Section, NIH
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27.	Dunty	Bill	National Institute on Alcohol and Alcoholism, NIH
28.	Duverger	Olivier	National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
29.	**Duyk**	**Geoffrey M.**	**TPG Biotechnology**
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31.	Feigenbaum	Lionel	SAIC-Frederick, Inc., Frederick National Laboratory
32.	Fuchs	Bruce	NIH/ORIP/OD
33.	Furth	Priscilla A.	Georgetown University
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35.	Gandolfi	Barbara	University of Missouri - Columbia
36.	Gelderman	Monique	Food and Drug Administration
37.	Gould	Todd	University of Maryland School of Medicine
38.	**Greiner**	**Dale L.**	**University of Massachusetts Medical School**
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47.	Haynes	Susan	National Institute of General Medical Sciences, NIH
48.	*Heckler*	*John*	*NIH/ORIP/DPCPSI/OD*
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50.	Helm	Jeannine	National Institute of Dental and Craniofacial Research, NIH
51.	Henken	Deborah	National Institute of Child Health and Human Development, NIH
52.	Hernandez	Lidia	CCR, National Cancer Institute, NIH
53.	Hewitt	A. Tyl	National Institute of Child Health and Human Development, NIH
54.	Hollingshead	Melinda	National Cancer Institute – Frederick
55.	Hong	Sogun	National Heart, Blood, and Lung Institute, National Institutes of Health
56.	Hornbeak	Hortencia	National Institute of Allergy and Infectious Diseases/DEA/SRP, NIH
57.	Hunziker	Rosemarie	National Institute of Biological Imaging and Bioengineering, NIH
58.	Javois	Lorette	National Institute of Child Health and Human Development/DBSVB, NIH
59.	Jett	Marti	U.S. Army Center for Environmental Health Research
60.	Jung	Moonjung	National Institutes of Health
61.	**Karayiorgou**	**Maria**	**Columbia University**
62.	Karp	Robert	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
63.	**Katsanis**	**Nicholas**	**Duke University**
64.	Koduri	Sailaja	National Center for Advancing Translational Sciences, NIH
65.	Kovtunovych	Gennadiy	National Institute of Child Health and Human Development, NIH
66.	Kozlov	Serguei	SAIC-Frederick
67.	Lee	Jaeho	National Institutes of Health

68.	Li	Hongzhen	MCHB, National Heart, Blood, and Lung Institute, National Institutes of Health
69.	Li	Rui	National Institute of Diabetes and Digestive and Kidney Diseases, NIH
70.	Lin	Ti	National Institutes of Health/OD
71.	Lin	Qing	National Heart, Lung and Blood Institute, NIH
72.	Lipsky	Robert	Inova Health System
73.	Liu	Chengyu	National Institutes of Health
74.	**Lloyd**	**Kent**	**University California, Davis**
75.	Lossie	Amy	Office of Behavioral and Social Sciences Research, NIH
76.	Lundberg	Martha	National Heart, Lung and Blood Institute, NIH
77.	Lyons	Leslie	University of Missouri - Columbia
78.	**MacRae**	**Calum A.**	**Brigham and Women's Hospital**
79.	Marks	Cheryl	National Cancer Institute, NIH
80.	Martin	George R.	National Institute of Dental and Craniofacial Research, NIH
81.	Mashima	Ted Y.	Association of American Veterinary Medical Colleges
82.	*Meyer*	*Michael*	*Lockheed Martin*
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84.	Mohassel	Payam	National Institute of Neurological Disorders and Stroke, NIH
85.	Mojsiak	Jurij	National Institute on Drug Abuse, NIH
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89.	Okita	Richard	National Institute of General Medical Sciences, NIH
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93.	Pawlyk	Aaron	National Institute of Diabetes and Digestive and Kidney

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126.	Vinson	Charles	National Cancer Institute, NIH
127.	Vivian	Jay	University of Kansas Medical Center
128.	Wan	Qin	National Eye Institute, NIH
129.	Wang	Xiaoming	National Institute of Allergy and Infectious Diseases, NIH
130.	Wang	Qian	National Institute of Neurological Disorders and Stroke, NIH
131.	Watanabe	Rira	National Cancer Institute, NIH
132.	Watson	Harold	Division of Program Coordination, Planning, and Strategic Initiatives, NIH
133.	Wei	Bih	National Cancer Institute, NIH
134.	Wong	Renee	National Heart, Blood, and Lung Institute, NIH
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136.	Yang	Yu-an	LCBG/National Cancer Institute, NIH
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