PRE-MEETING REPORT

Pre-meeting to the Workshop on Validation of Animal Models and Tools for Biomedical Research

May 29, 2020

Office of Research Infrastructure Programs, NIH

Virtual Meeting

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Background

A workshop titled "Validation of Animal Models and Tools for Biomedical Research" that had been organized by the Office of Research Infrastructure Programs (ORIP) at the National Institutes of Health (NIH) was postponed to fiscal year 2021 as a result of the COVID-19 pandemic. The workshop will serve as a forum for discussing the status and need for validation of animal models in preclinical research. The organizing committee for the workshop—comprising experts from the biomedical research community and program staff from six NIH Institutes, Centers, and Offices (ICOs)—has drafted a preliminary agenda for the full event. The organizing committee decided to hold a virtual pre-meeting on May 29, 2020 to discuss the session topics proposed for the workshop in greater detail and to finalize a list of potential speakers. Members of the organizing committee from the biomedical research community delivered a short presentation related to their area of interest, which was followed by a brief discussion. These presenters considered issues related to the validation of animal models, described their own areas of research, and outlined the technologies and resources needed to advance their fields.

Executive Summary

NIH's ORIP convened a virtual pre-workshop meeting of scientific experts in the field to discuss validation of animal models and the development of tools and resources to advance biomedical research. This virtual pre-workshop meeting, held on May 29, 2020, brought together NIH ICO representatives and investigators who use animal models, tools, and resources for their research. The agenda included 12 presentations by experts in the field summarizing key perspectives with time for follow-up question sessions. The speakers provided both background and up-to-date perspectives to better understand the status of existing and emerging issues with validation of animal models. Participants identified gaps in knowledge and resources that limit animal model use in research, outlined needed validation tools and resources for various model organisms, shared concerns about sustainability issues related to resources among researchers using different model organisms, and stressed the importance of information flow between preclinical researchers and clinicians.

The organizing committee suggested session topics and speakers for the planned workshop that will be held in person in 2021 based on the discussions held in this pre-workshop meeting and previous committee meetings. The proposed speakers are expected to address topics critical for validation of animal models and tools and discuss required resources and technologies in their fields of expertise, including but not limited to—

- Strategies for addressing validation issues in existing animal models used for drug development.
- Processes and considerations for selection of animal models best suited for specific research goals or questions.
- New and emerging technologies to refine extant and establish new validation criteria.
- Selection and application of new technologies and resources of animal models for studying human diseases.
- Approaches of utilizing a combination of different species and assays when addressing biological questions or pursuing drug development.

National Institutes of Health (NIH)
Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs (ORIP)

Pre-meeting to the Workshop on Validation of Animal Models and Tools for Biomedical Research
May 29, 2020
12:00 p.m. – 3:00 p.m.
Virtual Meeting

Introduction and Welcome

Sige Zou, Ph.D., ORIP, NIH; Franziska Grieder, D.V.M., Ph.D., Director, ORIP, NIH; Meeting Chair Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine; Meeting Co-Chair Keith Cheng, M.D., Ph.D., The Pennsylvania State University

Dr. Sige Zou welcomed the participants and thanked them for sharing their expertise. Dr. Franziska Grieder, the Director of ORIP, explained that this pre-meeting will inform the full workshop planned for 2021, emphasizing that validation of animal models is an integral part of ORIP's mission to support infrastructure for innovation. She expressed appreciation for attendees' contributions to planning and guiding the full workshop. Dr. Hugo Bellen, Chair of the Workshop Organizing Committee, noted that the full meeting would focus on tools and resources for validation; the presentations at this meeting were planned to allow the committee members to present ideas and data related to the tools and resources they develop and use and to allow them to convey what members of individual communities feel is most needed in their respective fields. Meeting Co-Chair Dr. Keith Cheng reminded attendees to note new insights they may wish to incorporate into revised abstracts that would be used to summarize the meeting. He then introduced the guest speaker, Dr. Glenn Gerhard, who is active in medicine as a clinical pathologist, in personalized medicine—including analysis of human tissue specimens—, and in basic research with mouse and zebrafish as model systems.

Keynote Presentation: The Multiple Facets of Validation of Animal Models

Glenn Gerhard, M.D., Temple University

Dr. Gerhard commented that because he works in both medicine and research, he can consider the challenges of external validity from both perspectives. Some researchers have proposed that differences between species make full validation impossible, but Dr. Gerhard indicated that the precise taxonomy of human disease needs to be better defined at the molecular and cellular level. The issue is not so much validation of animal models for drug development but rather the appropriate use of animal models for specific forms of human disease. To illustrate the importance of the flow of information between preclinical researchers and clinicians, he described what has happened in drug development for amyotrophic lateral sclerosis (ALS). All but one high-profile clinical trial (29 of 30) failed because ALS is genetically heterogeneous in origin. Inclusion criteria for most open ALS trials do not subdivide patients by the specific genes likely involved in their disease. A phase III trial at Biogen was the first gene-specific treatment developed for ALS and has been effective in animal models; this success demonstrates how a well-defined clinical phenotype can support the validation of translation from animal models to humans. In summary, the selection criteria for clinical trials must focus on the cellular pathway specific to each individual, and drug discovery should acknowledge that some molecules will be relevant only to a subset of the population; in other words, personalized medicine is needed.

Discussion

- Participants commented on the concerns about rigor in some preclinical studies in the superoxide dismutase 1 (SOD1) mouse model, many of which have been underpowered and not sufficiently

accounting for the high variability within the model. Additionally, the predictive validity of the model is unproven for sporadic ALS. Dr. Gerhard commented that although familial ALS can involve 15 to 20 genes, the number of subgroups for sporadic ALS has not been defined.

- Attendees pointed out that animal models often are driven by a single mutation, despite the heterogeneity of disease, and they noted that, similar to ALS, familial Alzheimer's disease (AD) is studied much more frequently, despite the fact that almost 90 percent of AD cases are sporadic.

Invertebrate Models and Validation

Flies Facilitate Rare Disease Diagnosis and Therapeutic Avenues

Hugo Bellen, D.V.M., Ph.D., Baylor College of Medicine

Dr. Bellen encouraged vertical integration between genetically defined model organisms, rather than validation based on a single model organism. Modeling genes in a variety of organisms can contribute to greater understanding. For example, in humans, whole-genome and whole-exome sequencing technologies can now sequence samples from a single individual and find variants that lead to disease, which can be particularly useful for rare diseases. The human genes can be inserted into model organisms to determine whether the phenotype is rescued. This humanization of model organisms helps refine the processes of diagnosis, mechanism identification, and drug screening. Dr. Bellen explained that millions of patients in the United States have conditions that are undiagnosed; these patients—many of whom are children—can apply to the Undiagnosed Disease Network (UDN), which will sequence the genomes of the patients, as well as their parents and siblings, and identify candidate genes for rare diseases. These candidate genes are selected and sent to the UDN's Model Organism Screening Center (MOSC), which performs bioinformatics studies and mines existing data available on model organisms to identify which variants to pursue in which model organism. About half of the genes submitted are studied through the MOSC's *Drosophila* core; the others are assigned to the fish and worm cores. Humanization is the primary method used in flies to test the function of genes and variants identified in individuals. The drugs approved by the U.S. Food and Drug Administration (FDA) that may work on the identified mechanism also can be assessed. Dr. Bellen explained the process through which an artificial GAL4 exon is inserted, which then can be used to drive exploration of expression of the human gene and the variants to assess whether the fly phenotypes are rescued. Tools and resources that Dr. Bellen suggested for future studies include the generation of knockout libraries, documentation of expression of each gene in specific cells, systematic documentation of phenotypes associated with mutations in genes at all levels, robotic assays for high-throughput behavioral assays, documentation of biochemical phenotypes associated with mutants, documentation of systematic change in biomarkers to provide druggable targets, better and simpler dataset mining approaches, artificial intelligence strategies, promotion of vertical integration, and training in best practices and the strengths and weaknesses of various model organisms.

Discussion

- In response to a question, Dr. Bellen elaborated on the process of prioritization when related genes are found. One to four variants typically are identified by clinicians, and data related to the homologs of the human genes are assessed in the main model organisms mentioned above by mining available human and model organism data through MARRVEL.org and PubMed. Dr. Bellen emphasized that data integration is key to allowing his team to compare phenotypes. Ideally, this process could eventually be done via artificial intelligence.
- When asked whether a fly model correlating to a human model always exists, Dr. Bellen clarified the process of assessing homologues, which starts by reviewing available model organism data and communicating with experts. He noted that about 15 to 25 percent of human genes that are submitted to the Model Organism Screening Center have no homologs in flies and worms. These genes are studied in zebrafish. Dr. Bellen noted that, at the current success rate, two out of three genes that are studied are shown to be the cause of disease and the data are disseminated in publications.

Fundamentals of Mouse Biology and Genetics to Optimize Model Validation

General Comments and "Macro-Genetics"
Kent Lloyd, D.V.M., Ph.D., University of California, Davis

Dr. Kent Lloyd explained that validation criteria are important to consider when using the mouse as a model of human disease. Researchers should assess the level of phenotypic and mechanistic homology; the presence of paralogs can influence the interpretation of the phenotype. Dr. Lloyd presented examples from his research areas. In the first area, *in vivo* analysis is used to illuminate gene function when researchers have information about a gene but lack a model to correlate to that gene or have a model without a functionally annotated genome. As part of the Knockout Mouse Project in the International Mouse Phenotyping Consortium, models are made using CRISPR/Cas9, and a series of analyses are used to define the phenotype. The mouse is moved to the Mutant Mouse Resource and Research Centers, a public repository for mouse strains created by individual laboratories, which adds scientific value through quality control and standard operating procedures. The Mouse Metabolic Phenotyping Consortium then can further analyze the phenotypes. Protocols across the consortium are fully validated, and the standard operating procedures are fully harmonized, allowing data to be compared. In Dr. Lloyd's second research area, *in vivo* modeling is used to inform precision medicine via targeted phenotyping. An *in vivo* model is created of a variant of unknown importance but likely pathogenicity to confirm causation. These studies involve a clinical component with patient evaluations; bioinformatics analysis to evaluate the whole genome or whole exome; and the creation, testing, analysis, and improvement of the animal models. Dr. Lloyd recommended that granting offices require direct and meta information on all mice and stressed the importance of bioinformatic confirmation and humanization of all membrane receptors in new models.

Metabolic Diseases
Alan Attie, Ph.D., University of Wisconsin–Madison

Dr. Alan Attie described his research on how mouse models contribute to the understanding of human metabolic disease, noting that obesity and diabetes are an unprecedented worldwide epidemic. Key syndromes include diabetes, dyslipidemia, and hepatic steatosis. Researchers in metabolic disease are interested in islet biology, adipocyte biology—encompassing endocrinology, thermogenesis, and inflammation—hepatic metabolism, and genetics and genomics. In islet biology, humans and rodents differ in architecture, development, and proliferative capacity. Dr. Attie suggested that Seung Kim from Stanford University or Debbie Thurmond from City of Hope National Medical Center could speak on this topic. In the area of adipocyte biology, Dr. Attie suggested that Barbara Kahn from Harvard University or Phil Scherer from The University of Texas Southwestern could address the endocrine production of lipokines; Shingo Kajimura from Harvard University could speak about thermogenesis; or Alan Saltiel from the University of California, San Diego, could comment on inflammation. Dr. Attie noted that hepatic and whole-body metabolism can be studied in mice but not in humans; Richard Kibbey from Yale University could address the importance of measurement of metabolic flux, and Morris Birnbaum from Pfizer could comment on drug discovery. The speakers in the area of genetics and genomics could address many potential topics related to how mouse models inform human genetics. Speakers in this area could include Dr. Attie, Nancy Cox from Vanderbilt University, or Aldons (Jake) Lusis from the University of California, Los Angeles.

Populations and Environment
Kent Lloyd on behalf of Cathleen Lutz, Ph.D., M.B.A., The Jackson Laboratory

Dr. Lloyd used Dr. Cathleen Lutz's slides to explain that using mice to model human disease requires considerations beyond disease biology. Although most studies currently use inbred mouse strains, the use of more genetically diverse mouse models may better represent the genetic diversity of patient populations and improve translation. Dr. Lloyd outlined examples of how a more translationally relevant

phenotype could be used. In the human population, memory is a trait that varies greatly as people age, and mice also show individual variation. Studying the genetic risk score of various lines in the context of memory could provide more useful models; for example, Black 6 (C57BL/6) mice have good memories but are not the strain most used for Alzheimer's disease studies. The microbiome is relevant to mouse studies. Dr. Lloyd outlined studies showing the importance of environmental exposures and reiterated the overarching recommendation for facilities and grants that explore environmental exposures in mouse models.

Complexity of Mouse Models
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia

Dr. Douglas Wallace explained that mouse models have been inbred for more than 100 years and distributed around the world. Historically, mice originated from three sources: strains developed by Abbie Lothrop in the early 20th century, strains developed in Switzerland, and strains developed in China and Japan. Many lines with the same name have since diverged and are no longer genetically identical. As a result, inbred mouse lines maintained under the same or similar names may now harbor significant nuclear and mitochondrial DNA genetic alterations. Mice also periodically undergo retroviral activation, which can change their physiology. Dr. Wallace provided an example of a spontaneous nuclear DNA mutation in a common mouse strain from The Jackson Laboratory. This mutation inactivated the gene for the enzyme nicotinamide nucleotide transhydrogenase, which regulates the redox potential of mitochondria. The mutation impairs the adrenal gland and reduces stress, predisposes mice to metabolic syndrome, and increases sensitivity to mitochondrial oxidative stress. Dr. Wallace emphasized that using mice from this strain from The Jackson Laboratory in behavioral studies will give different results than mice with very similar names from another mouse vendor. Mitochondrial DNA mutations also have arisen in various inbred strains. Such mutations may not be anatomically obvious but can have important effects on learning and memory, physical capacity, visual and auditory function, and neuropsychiatric behavior. Dr. Wallace noted that although one mouse was a "mitochondrial DNA mouse Eve" from which multiple inbred strains were derived, the mitochondrial DNA mutation rate is higher in inbred strains, so these mice may now have functionally significant mitochondrial DNA mutations. Because strains with similar names from different sources are often assumed to be interchangeable, mice bought from different vendors have crossed, resulting in different alleles segregating in the colony and creating variability. Unfortunately, sub-strain nomenclature is complicated and may be overlooked by the more causal mice experimenter. Dr. Wallace recommended that researchers access the history of each strain they plan to study and then consistently use that strain throughout their study. He also proposed that vendors take responsibility for providing characterization of their strains, so the specifics of the genotype are known. Unfortunately, this strategy would not work for laboratories that breed their own mice.

Discussion

- When asked whether sequencing would be more cost-effective than backcrossing, Dr. Wallace explained that one strain might have many single nucleotide changes and that the extensive bioinformatics work required to identify the important mutations likely would not be possible for most investigators.
- Dr. Wallace emphasized that outcrossing has some advantages. However, it also adds genetic diversity, requiring much larger numbers of mice to generate a statistically significant result.
- Several participants recommended that the field reconsider the meaning and use of models to encourage innovative ideas but noted that this discussion is larger than the topics considered in this workshop.
- When asked about the rat model, Dr. Wallace explained that because rat models are newer, fewer useful mutations are available. However, rat models have significant advantages, such as being larger and thus making many procedures easier.

- Dr. Cheng noted that the Ekker Laboratory at the Mayo Clinic has developed genome editing of mitochondria.

Aquatic Models and Validation

Validating Zebrafish Models of Human Disease

William Talbot, Ph.D., Stanford University

Dr. William Talbot explained that zebrafish can be used to model many shared aspects of vertebrate biology. The model has a long history of genetic screens for genes of interest, and reverse genetics has become a recent strength since the advent of CRISPR/Cas9 technology. Zebrafish have transparent embryos, which make zebrafish a good model for cellular biology, and many laboratories have been able to model various rare diseases. Dr. Talbot provided examples of his own research with *gpr126,* a G protein-coupled receptor important for forming myelin in peripheral nerves, noting that myelin is vertebrate-specific. Rare mutants have been found that have a similar phenotype to that defined in Dr. Talbot's studies. Dr. Talbot commented that other researchers have suggested that nearly any cancer can be generated in zebrafish. Zebrafish models for rare diseases usually can be validated with a genomic sequence match; when this is not possible, such as when individual variants are expressed in a tissue-specific way, histology and molecular signatures can be used. In some cases, phenotypic similarity can be applied, although Dr. Talbot noted that researchers debate how close these considerations are to human phenotypes. He suggested that the most important resource for advancing zebrafish as a model of human disease is the development of better methods for humanizing the zebrafish genome. Other resources to advance the model include the development of cell type–specific promoters for reporter studies and conditional transgenic approaches and development of monoclonal antibodies. Additionally, Dr. Talbot encouraged continued investment in the ZFIN database and Zebrafish International Research Center and suggested that better phenotyping assays, especially for adult zebrafish, could be developed.

Discussion

- Participants discussed the differences in available technology between zebrafish and other models, such as *Drosophila*. Dr. Talbot emphasized the importance of developing better ways to humanize the zebrafish genome and resources to enable that.
- Dr. Cheng commented about the availability of libraries of gene trap expression lines that can be made homozygous to reveal mutant phenotypes and reverted by Cre excision or by injection with antisense morpholinos targeted to splice junctions of the expression/mutagenizing inserts. Dr. Talbot noted that zebrafish stock centers play a central role in preserving such resources for the use of zebrafish models for studying human disease.

Mouse Models and Validation

Lessons Learned of Mice (and Men): Developing the Next Generation of Alzheimer's Disease Mouse Models

Stefania Forner, Ph.D., University of California, Irvine

Dr. Stefania Forner explained that the main goal of the Model Organism Development and Evaluation for Late-onset Alzheimer's Disease (MODEL-AD) project is to develop the next generation of mouse AD models, which can not only improve the knowledge of AD but also help address issues in other fields. She listed concerns with current mouse models of AD—including differences in behavior between mice and humans; a lack of diversity in the genetic background of mice that does not reflect the human population; complications of outcrossing; legal restrictions and reproducibility and transparency concerns; a narrow focus on familial AD in current mouse models, despite the greater frequency in humans of spontaneous AD; and a lack of robust neurodegeneration in the current mouse models. Dr. Forner listed the elements of modeling AD in mice that are required, including the preferred and the desired. She

outlined a number of key challenges to modeling late-onset AD—including humanizing the mouse genes by introducing several key AD-related genes, the inability of a single mouse to present all human pathologies, the need to incorporate aging and environmental factors, and the need to account for the profound impact of genetic background on the phenotype. Dr. Forner explained the primary screening process used to prioritize new variants. Variants are selected and introduced into the mouse model via CRISPR/Cas9, and a primary screen determines whether the phenotype can be used. If the phenotype is usable, cross-sectional deep phenotyping is performed. If the mice do not present enhanced pathology, deep phenotyping is not performed, but the model becomes available through The Jackson Laboratory. Dr. Forner emphasized that all new models generated through the MODEL-AD project are made available through open science, and all data are made available publicly and without legal restrictions.

Discussion

- Dr. Forner clarified that her laboratory tests mice at 4, 12, 18, and 24 months.
- Dr. Forner also explained that her laboratory selects the variants that are most important and closer in pathology to humans.
- In response to a question about information provided via open science, Dr. Forner explained that all slides and raw images are uploaded to the AD Knowledge Portal. Those that are too large to house on the portal can be requested.

Translational Validation

Translational Challenges for Studying Human Behaviors in Animal Models

Stacey Rizzo, Ph.D., University of Pittsburgh

Dr. Stacey Rizzo pointed out that most diagnostic criteria for central nervous system disorders are behavioral; issues of reproducibility have been especially challenging in behavioral assays, so valid behavioral tools are needed to support full validation of animal models. She outlined the four types of validity used for behavioral assays: face, construct, predictive, and translational validity. An assay that is valid in one way may not necessarily be translatable, and several cognitive behavioral assays for rodents—fear conditioning, water maze, and novel object recognition—have not translated well to cognition in humans for translational studies. Concerns for assay translation include confounding factors, such as hyperactivity, visual impairments, or sedative effects; lack of consideration for responses to stimuli that vary across sex, age, and genotype; high inter-subject variability and inconsistent baseline responses in controls; limited application of the ARRIVE guidelines; and ignorance of single-dose "efficacy" in the absence of dose response and pharmacokinetic data. Central nervous system disorders manifest as a spectrum of behavioral traits, and clinical trials often use composite scores of many assessments. However, animal models frequently use a single behavioral outcome. Dr. Rizzo emphasized the need for the field to move forward from historical measures and traditional behavioral endpoints by considering composite scores that can be used in animal studies as translational outcomes. She noted that assays must account for environmental factors, validated composite scores and that greater transparency is needed. Also, models must incorporate genetic diversity, as well as pharmacokinetic data and dose-response relationships.

Discussion

- Participants commented on the need for better human to animal back-translation and identification of biochemical measures related to both disease-causative and consequential behavior in both rodents and humans.
- When asked how to identify cognitive decline, such as dementia, in animal models, Dr. Rizzo explained that dementia is a uniquely human construct and there is no clear definition of dementia in animal models, although researchers are aware that behaviors beyond cognitive decline—such as

sleep disturbance or aging-related traits—can be monitored in animals. She noted that the definition of dementia in animal models may be a knowledge gap to address.

Nonhuman Primates and Disease Model Validation

Validation of Nonhuman Primate Models of Human Disease: Neuroscience and Infectious Disease

John Morrison, Ph.D., University of California, Davis

Dr. John Morrison emphasized the importance of early intervention in preserving synaptic health and preventing cognitive decline. He outlined three nonhuman primate models of AD currently in development—an amyloid-based model of early AD with mostly synaptic pathology, a tau-based model of a later phase with neuron death, and an HIV-associated neurocognitive decline model—and provided an example of tau response studies that have been phenotyped pathologically in nonhuman primates. He added that although the studies have not yet been phenotyped clinically or behaviorally, the biomarkers are promising. Dr. Morrison explained that HIV/AIDS studies have developed the best nonhuman primate model of infectious disease, with many interactive decision points that are critically important to determining whether the challenge to the vaccine will be successful. Researchers can optimize infectious disease models by determining the best combination of host, pathogen, and delivery methods, with many variable options. Dr. Morrison emphasized that the extensive work required to develop such a nuanced model ensures that researchers now choose their variables very carefully. He noted that nonhuman primates have a higher burden of validation because of the cost of maintaining colonies and their phylogenetic closeness to humans, so development of a faithful phenotype is critical. Valid models should be able to cause disease through similar exposure and linked genetic mutations, and they should have similar pathologic and clinical disease phenotypes and similar disease progression biomarkers. The nonhuman primate model also should be useful to investigate causes, prevention, treatments, and cures that have clear translational impact on human diseases. He listed a number of needed innovations and resources in both neuroscience and infectious disease. In the infectious disease area, these included next-generation sequencing, improved methods for vaccine development, more animal biological safety level 3 capacity and multiple species ready for characterization, enhanced data analysis and sharing, and a global approach to be ready for the next pandemic. Neurology studies require CRISPR/Cas9 models of gene-linked brain disorders, additional resources to care for and distribute those new models, higher-resolution *in vivo* imaging, standardized biomarker panels, and specialized facilities for neuro-engineering and prosthetics.

Discussion

- Participants discussed the difficulty of incorporating aging in studies with nonhuman primates because of the longer life span and high expense.

Technology and Validation

Validation from Computational Organismal and Tissue Phenotyping

Keith Cheng, M.D., Ph.D., The Pennsylvania State University

Dr. Cheng pointed out that virtually all human disease is associated with three-dimensional change in cells and tissues in the micron scale. The current gold standard of tissue phenotyping—histology—anchors the study of human disease mechanisms and, as is well known among comparative pathologists, will play an important role in validation and vertical integration of animal models of human disease. Histology is commonly limited to being two-dimensional and largely descriptive, leading to a need for three-dimensional, quantitative analysis of tissue phenotypes. The value of phenome projects aimed at characterizing all the functions of each gene would be greatly enhanced by a quantitative mechanism for phenotyping whole model organisms across all tissues and cell types. Its small size, genetic manipulability (including antisense knockdowns and knockouts) and facile use in chemical screens made

the zebrafish a powerful vertebrate model for developing a new tool for three-dimensional quantitative phenotyping: a form of micro computed tomography (microCT) called X-ray histotomography. Every cell in a whole zebrafish can be studied from single three-dimensional reconstructions. Cytological changes and subtle differences in region-specific cell density can be visualized readily. Dr. Cheng indicated that a national center for organismal and tissue phenomics based on synchrotron and local microCT resources could produce public databases for phenomics, just as sequencing centers have produced databases for genomics, and be integrated with bioinformatic and chemical phenomic resources. Significant computing resources, including storage and computational power, will be needed. Machine learning-based artificial intelligence phenotyping is under development and can be expected to grow, just as bioinformatic tools grew after the public release of DNA sequences. Finally, he emphasized that human reference normal and diseased tissue data need to be made broadly available for characterizing the similarities and differences between human and disease model tissues. Quantitative computational phenomic tools for three-dimensional histopathology across phylogeny will become a strong complement to histology-based comparative analyses.

Improving Preclinical-to-Clinical Translation in Alzheimer's Disease: The MODEL-AD Preclinical Testing Pipeline
Paul Territo, Ph.D., Indiana University

Dr. Paul Territo outlined key challenges in translating between preclinical and clinical drug testing, noting that many processes must be considered, including active pharmaceutical ingredient (API) qualification, physiological impacts on disease, pharmacodynamics, and pharmacology and pharmacokinetics. Examples of API qualifications include material purity, confirmation of the compound of interest, identification of the optimal route and vehicle of administration, and compound stability. Physiological impact considerations include whether the biological impact is understood, whether impacts are differentiable between normal and disease pathology, whether the dynamic range is large enough for detection, and the stage of the disease, which affects the optimal time to detect signal readout. Pharmacology and pharmacokinetic considerations include whether receptor and enzyme levels are sufficient and whether drug kinetics are reasonable. Pharmacodynamic considerations include whether the pharmacodynamics have been mapped appropriately, whether dose frequency is sufficient to minimize peak/trough levels and off-target effects, and whether the readout has a wide dynamic range and a good signal-to-noise ratio. He commented on the need to optimize the intersection of disease, drug, and biomarker and outlined the elements of a quality drug study, including the assessment of face and construct validity, appropriate measurements and concepts, ability to test the desired question, suitability for the question of interest, and predictability. Dr. Territo emphasized the importance of standardization and quality control and noted that the standardizations developed by his laboratory are available publicly. Additionally, all studies should be conducted under the ARRIVE guidelines, animal care should be standardized across all sites, and oversight of all studies should be tight. Dr. Territo also emphasized the importance of providing open access to data gathered from these studies. He outlined the pipeline characteristics for the Preclinical Testing Core (of the Indiana University/JAX Alzheimer's Disease Precision Models Center), which include one or two compounds per year and initial pipeline validation with well-known models and compounds. He noted that validation is conducted at each step. Dr. Territo also explained how the Preclinical Testing Core matches mouse models to the compound of interest based on both disease pathology and the compound's mechanism of action, emphasizing that this process includes many go/no-go gates and well-powered numbers for both males and females at every stage.

Discussion

- When asked about the challenge of the large files associated with histotomography, Dr. Cheng noted that computer resources continue to grow, but that lower resolutions can be used for preliminary scans, just as physical exams are used before radiology in a progression toward tissue biopsies in humans. Highest resolution certainly will be needed for experimental questions that require the characterization of cells and subcellular structures.

- Participants discussed the modeling of hormone levels in male and female animals, agreeing on the importance of accounting for sex. Dr. Territo suggested that anti-inflammatory estrogen effects in relation to AD are not studied sufficiently.

Discussion of Format and Agenda of the Future Workshop

Dr. Bellen thanked the participants and noted that the workshop would support only 30 speakers. A preliminary agenda has been drafted based on discussions from previous committee meetings. Drs. Bellen and Zou pointed out that two additional speakers from outside of the Washington, D.C., area, as well as a few local speakers if needed to finalize the list, could be included. Dr. Bellen asked participants to revisit the appropriateness of the sessions and topics proposed in the agenda, as well as the suitability of the speakers and alternate speakers listed.

Dr. David Grunwald was suggested to chair an integrated genome editing session for zebrafish; Dr. Bellen pointed out that this topic could be covered in the session about genetic research for validation of models. After a brief discussion, the consensus was that the suggested session was already covered. Proposed speakers have not yet been contacted because the date and location have not been set yet, but they will be contacted to determine if they are interested in participating.

A suggestion made to strengthen the agenda was the inclusion of sessions on an additional species (rat) and sex differences. On the inclusion side, an additional woman speaker was also suggested. Participants agreed that Drs. Karen Frick, Catherine Woolley, or Art Arnold would be suitable to cover both topics—rats and sex differences.

One participant suggested including a talk on canine research, which could be addressed by Dr. Peter Nghiem, who currently is on the list of alternate speakers.

When a participant suggested the inclusion of a discussion on multicentric strategies for preclinical work in new animal models, Dr. Rizzo noted that the MODEL-AD project funded by the National Institute on Aging for developing and characterizing mouse models for studying AD operates in this way. Dr. Zou suggested that a speaker from the MODEL-AD program could outline its operations.

Dr. Cheng asked speakers to help draft a list of the most important general validation considerations for each group that presented at the pre-meeting. Dr. Bellen reminded attendees that the full workshop likely would occur in 9 months to a year because the course of the COVID-19 pandemic makes setting a date for an in-person meeting uncertain and unsafe.

The organizing committee suggested session topics and speakers, including backup speakers, for the future workshop. The proposed speakers are expected to address the topics critical for validation of animal models and tools with respect to resources and technologies, including but not limited to—

- Strategies for addressing validation issues in existing animal models used for drug development.
- Processes and considerations for selection of animal models best suited for specific research goals or questions.
- New and emerging technologies to refine extant and establish new validation criteria.
- Selection and application of new technologies and resources of animal models for studying human diseases.
- Approaches of utilizing a combination of different species and assays when addressing biological questions or pursuing drug development.

Appendix A: Pre-meeting Agenda

Pre-meeting to the
Workshop on Validation of Animal Models and Tools for Biomedical Research

Time and Date: 12-3 pm on May 29th, 2020

Venue: Virtual Meeting **(**Zoom)

Objectives: ORIP is organizing a workshop entitled "Validation of Animal Models and Tools for Biomedical Research", which has been postponed to fiscal year 2021 due to the COVID-19 Pandemic. An organizing committee has been formed with experts from the research communities and program staff from six NIH institutions and offices (ICOs). The organizing committee has met and drafted a preliminary agenda of the workshop. This pre-meeting is for the organizing committee to have more in-depth discussion on selected session topics and proposed speakers. Each subject matter expert and a guest speaker will give an approximately 10-min presentation including Q&A. Each presenter will cover key issues related to the validation of animal models, optionally describe their areas of research, and stress technologies and resources that should be developed in their field. The pre-meeting summary and a more refined workshop agenda will be drafted for the future full workshop.

Organizing Committee

Subject Matter Experts (Speakers)

Hugo Bellen, Chair	Baylor College of Medicine
Keith Cheng, Co-Chair	Penn State College of Medicine
Alan Attie	University of Wisconsin
Stefania Forner	University of California, Irvine
Kent Lloyd	University of California, Davis
Cathleen Lutz	Jackson Laboratory
John Morrison	University of California, Davis
Stacey Rizzo	University of Pittsburgh
William Talbot	Stanford University
Paul Territo	Indiana University
Douglas Wallace	Children's Hospital of Philadelphia
Jill Weimer	Sanford Research

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Sige Zou, Coordinator	ORIP/DPCPSI/OD/NIH
Kristine Abraham	NIDDK/NIH
Shreaya Chakroborty	NIA/NIH
Marc Charette	NHLBI/NIH
Miguel Contreras	ORIP/DPCPSI/OD/NIH
Bruce Fuchs	ORIP/DPCPSI/OD/NIH
Amelie Gubitz	NINDS/NIH
Lisa Schwartz Longacre	NHLBI/NIH
D.P. Mohapatra	NINDS/NIH
Lorenzo M. Refolo	NIA/NIH
Rebecca Roof	NINDS/NIH
Xiaoli Zhao	NIGMS/NIH

NIH Supporting Team

Lola Ajayi	ORIP/DPCPSI/OD/NIH
Susan Chandran	ORIP/DPCPSI/OD/NIH
Desiree von Kollmar	ORIP/DPCPSI/OD/NIH

Guest Speaker

Glenn Gerhard	Temple University (Personalized Medicine)

Science Writer

Sally Paustian	The Scientific Consulting Group, Inc.

Pre-meeting Agenda

Noon-12:10 pm Introduction and Welcome

- Sige Zou, Coordinator of the Organizing Committee, ORIP, NIH
- Franziska Grieder, Director, ORIP, NIH
- Hugo Bellen, Chair of the Organizing Committee, Baylor College of Medicine
- Keith Cheng, Co-Chair of the Organizing Committee, Penn State College of Medicine

12:10-12:25 pm Keynote Presentation

The Multiple Facets of Validation of Animal Models

- Glenn Gerhard, Temple University
- 5 min Q&A

12:25-12:35 pm Invertebrate Models and Validation

Flies Facilitate Rare Disease Diagnosis and Therapeutic Avenues

- Hugo Bellen, Baylor College of Medicine
- 5 min Q&A

12:35-1:10 pm Fundamentals of Mouse Biology and Genetics to Optimize Model Validation

General Comments and "Macro-Genetics"

- Kent Lloyd, University of California, Davis

Metabolic Diseases

- Alan Attie, University of Wisconsin

Populations and Environment

- Cathleen Lutz, Jackson Laboratory

Complexity of Mouse Models

- Douglas Wallace, Children's Hospital of Philadelphia

15 min Q&A

1:10-1:20 pm Aquatic Models and Validation

Validating Zebrafish Models of Human Disease

- William Talbot, Stanford University
- 5 min Q&A

1:20-1:30 pm Break

1:30-1:40 pm Mouse Models and Validation

Lessons Learned of Mice (and Men): Developing the Next Generation of AD Mouse Models

- Stefania Forner, University of California, Davis)
- 5 min Q&A

1:40-1:50 pm Translational Validation

Translational Challenges for Studying Human Behaviors in Animal Models

- Stacey Rizzo, University of Pittsburgh

- 5 min Q&A

1:50-2:00 pm Nonhuman Primates and Disease Model Validation

Validation of Nonhuman Primate Models of Human Disease: Neuroscience and Infectious Disease

- John Morrison, University of California, Davis
- 5 min Q&A

2:00-2:20 pm Technology and Validation

Validation from Computational Organismal and Tissue Phenotyping

- Keith Cheng, Penn State College of Medicine

Improving Preclinical to Clinical Translation in Alzheimer's Disease: The MODEL-AD Preclinical Testing Pipeline

- Paul Territo, Indiana University

10 min Q&A

2:20-3:00 pm Discussion of Format and Agenda of the Future Workshop

- *Format of sessions in the future workshop*
- *Session topics*
- *Selection of chairs and speakers for each session*

Appendix B: Pre-Meeting Attendees

Organizing Committee

Subject Matter Experts (Speakers)

Hugo Bellen, Chair	Baylor College of Medicine
Keith Cheng, Co-Chair	Penn State College of Medicine
Alan Attie	University of Wisconsin-Madison
Stefania Forner	University of California, Irvine
Kent Lloyd	University of California, Davis
John Morrison	University of California, Davis
Stacey Rizzo	University of Pittsburgh
William Talbot	Stanford University
Paul Territo	Indiana University
Douglas Wallace	Children's Hospital of Philadelphia

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NIH Attendees

Matthew Arnegard	ORIP/DPCPSI/OD/NIH
Franziska Grieder	ORIP/DPCPSI/OD/NIH
Sheri Hild	ORIP/DPCPSI/OD/NIH
Oleg Mirochnitchenko	ORIP/DPCPSI/OD/NIH
Stephanie Murphy	ORIP/DPCPSI/OD/NIH

Guest Speaker

Glenn Gerhard	Temple University

Science Writer

Sally Paustian	The Scientific Consulting Group, Inc.