U.S. Department of Health and Human Services
National Institutes of Health
Division of Program Coordination, Planning, and Strategic Initiatives
Office of Research Infrastructure Programs
Division of Comparative Medicine

Validation of Animal Models and Tools for Biomedical Research
Session IV. Validation of Large Animal Models for Preclinical Research

Tuesday, December 8, 2020
Virtual Meeting

Workshop Report

Table of Contents

Executive Summary

The fourth of 10 sessions of the Virtual Workshop on Validation of Animal Models and Tools for Biomedical Research was held on December 8, 2020. This workshop is intended as a venue to discuss the status of and needs for the validation of animal models used in biomedical research. Session IV focused on the validation of large animal models—excluding nonhuman primates (NHPs)—and tools for preclinical research. The participants discussed key needs for the validation of large animal models, which include the following: (1) standardization of language and definitions through vertical integration (i.e., collaborative projects across large and small animal models); (2) tissue banks and sample characterization for genomics and high-throughput phenotyping across disciplines and models; (3) genetic cores to provide fully typed tissue samples and expertise in developing mutant models; (4) imaging technology with improved access to high-quality facilities and resources, including magnetic resonance imaging (MRI) atlases and software; (5) standardized sequences through vertical integration efforts; (6) informatics and artificial intelligence with big data storage capabilities for storing and disseminating different types of data from a variety of sources; (7) standardized methodology and reporting across models; (8) molecular reagents for different species; (9) naturally occurring companion animal models; (10) veterinarian training; (11) facilities to house large animals used in research; and (12) networks of scientists working with similar models, including large-animal cores that would facilitate training the next generation of researchers.

Session Co-Chairs
Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research

Presenters
Duncan Lascelles, Ph.D., North Carolina State University
Peter Nghiem, D.V.M., Ph.D., Texas A&M University
Randall Prather, Ph.D., University of Missouri
Jessica Sieren, Ph.D., The University of Iowa
Franklin West, Ph.D., The University of Georgia

ORIP Staff Members
Lola Ajayi
Susan Chandran
Michael Chang, Ph.D.
Miguel Contreras, Ph.D.
Bruce Fuchs, Ph.D.
Franziska B. Grieder, D.V.M., Ph.D.
Stephanie Murphy, V.M.D., Ph.D.
Desiree von Kollmar
Sige Zou, Ph.D.

Organizing Committee
Hugo Bellen, D.V.M., Ph.D., Chair, Baylor College of Medicine
Keith Cheng, M.D., Ph.D., Co-Chair, Penn State College of Medicine
Sige Zou, Ph.D., Coordinator, Program Official, Office of Research Infrastructure Programs (ORIP)

External Experts
Alan Attie, Ph.D., University of Wisconsin–Madison
Stefania Forner, Ph.D., University of California, Irvine
Kent Lloyd, D.V.M., Ph.D., University of California, Davis
Cathleen Lutz, Ph.D., The Jackson Laboratory
John Morrison, Ph.D., University of California, Davis
Stacey Rizzo, Ph.D., University of Pittsburgh
William Talbot, Ph.D., Stanford University
Paul Territo, Ph.D., Indiana University
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia
Jill Weimer, Ph.D., Sanford Research

NIH Program Staff
Kristine Abraham, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases
Shreaya Chakroborty, Ph.D., National Institute on Aging (NIA)
Marc Charette, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)
Miguel Contreras, Ph.D., ORIP
Bruce Fuchs, Ph.D., ORIP
Amelie Gubitz, Ph.D., National Institute of Neurological Disorders and Stroke (NINDS)
Lisa Schwartz Longacre, Ph.D., NHLBI
D.P. Mohapatra, Ph.D., NINDS
Lorenzo M. Refolo, Ph.D., NIA
Rebecca Roof, Ph.D., NINDS
Xiaoli Zhao, Ph.D., National Institute of General Medical Sciences

Workshop Report

Opening Remarks
Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, ORIP
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

Drs. Stephanie Murphy, Director, Division of Comparative Medicine, ORIP, and Sige Zou, Coordinator, Program Official, ORIP, welcomed the participants and expressed appreciation to the Organizing Committee and Session Chairs for their efforts in organizing the event. Dr. Murphy explained that the meeting is the fourth in a series of 10 sessions. Drs. Murphy and Zou also acknowledged the support of several National Institutes of Health (NIH) Institutes: the National Heart, Lung, and Blood Institute (NHLBI); National Institute on Aging (NIA); National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); National Institute of General Medical Sciences (NIGMS); and National Institute of Neurological Disorders and Stroke (NINDS). Dr. Murphy reminded the participants that validation of animal models and tools is a critical part of ORIP's trans-NIH efforts. She expressed appreciation for the participants' input.

Introduction to the Validation of Large Animal Models in Research
Jill Weimer, Ph.D., Sanford Research

Dr. Jill Weimer, Co-Chair, acknowledged Dr. Susan Sanchez, Co-Chair, as a collaborator on her presentation and explained that Session IV focuses on large animal models, including cat, dog, and pig, but excluding NHPs. She stated that the goals of the session are to identify (1) advantages of and needs for using various large animal models for addressing basic scientific questions; (2) strategies for responding to validation issues for existing (i.e., naturally occurring or genetically modified) large animal models used for drug development; (3) processes and considerations for selecting large animal models with specific research goals or questions; (4) new and emerging technologies to refine extant and establish new validation criteria; and (5) approaches for selecting and applying technologies and other resources to the study of human disease. The overarching goal of this session is to identify gaps and priorities for the NIH in supporting the use of large animal models in biomedical research. Dr. Weimer discussed uses of naturally occurring canine models and genetically modified pig models. She outlined two advantages of large animal models: phenotypic accuracy for certain diseases and similarity to humans in genetics, anatomy, size, metabolism, and physiology (i.e., relative to mice and other organisms commonly used in research). Certain large animal models mirror human reproductive physiology, development, and infectious disease behavior. Large animal models also present unique translational opportunities for developing and testing diagnostic tools and therapies that can be used in humans (e.g., medical imaging, biomarker platforms). Dr. Weimer also noted disadvantages of the various large animal models—including increased requirements for time (i.e., longer life cycle), space, technical expertise, and upfront cost—which consequently affect rigor and reproducibility, because fewer animals can be obtained for a single study. In addition, public perception of research using companion or food-source animals is a concern, and historical and comparison data for these studies are lacking.

Swine as Models of Human Disease and a Source of Organs for Xenotransplantation
Randall Prather, Ph.D., University of Missouri

Dr. Randall Prather discussed several criteria to consider when selecting models: (1) availability; (2) ability to replicate the human phenotype for the condition of interest; (3) physiological similarities to humans; (4) size (i.e., in terms of similarity to humans and ability to perform certain procedures); (5) genome accuracy, adequacy, ability to be altered, and similarity to humans; (6) availability of physiologic data on the model; and (7) acceptability of the model with regard to treatment approval. The

pig model meets most of these criteria. The National Swine Resource and Research Center (NSRRC) serves as a core facility for NIH-funded investigators interested working with pig models. The NSRRC serves as a repository, supports model curation and investigator-driven creation, and provides health monitoring, research, and training services to institutions around the world. A major limitation to validation of the various pig models produced at the NSRRC is the lack of space for full-size pigs and reproductive difficulties with miniature pigs. To address this limitation, NSRRC is building additional facilities. Additionally, improved swine genome annotation is needed. The NSRRC developed a phenylalanine hydroxylase–null pig to provide an improved model for phenylketonuria (PKU) that better reflects the neurocognitive deficits experienced by humans with this condition. The model exhibits the key biochemical phenotypes of PKU, as well as hypopigmentation, growth retardation, and brain abnormalities that occur in humans with this disorder. Investigators also have made more than 40 genetic modifications to swine to overcome immune barriers to xenotransplantation. The NSRRC recently developed three gene knockouts and a transgene that can be produced for distribution to the xenotransplantation community. The NSRRC is developing another knockout and transgene to facilitate xenotransplantation of swine tissue.

The Pig Stroke Model: Evaluating Neuroprotective and Regenerative Therapies
Franklin West, Ph.D., The University of Georgia

Dr. Franklin West discussed his group's work on a pig stroke model and testing of neuroprotective and regenerative cell therapies. He explained that these therapies could be applied to traumatic brain injury and other central nervous system models. The lack of testing in animal models that are sufficiently similar to humans in anatomy and physiology is a major reason for the failure of many stroke treatments in clinical trials. Models for testing cell replacement therapies require brains similar in size to that of humans. Functional connectivity is best tested in animals with gyrencephalic brains. The proportion of white matter relative to grey matter also should be high in animal models for stroke research, because white matter and grey matter respond differently to stroke and recovery. The pig has a large, gyrencephalic brain with a proportion of white matter only slightly less than that of humans. At The University of Georgia, researchers are examining stem cell treatment after ischemic stroke using pig models. They found that neural stem cell (NSC) extracellular vesicle treatment after stroke results in decreased lesion volume and improved white matter integrity. Studies using pig models also found that induced pluripotent stem cell (iPSC)–derived NSC (iNSC) treatment leads to recovery of N-acetyl aspartate and reduces neuron loss at the lesion border. MRI allows investigators to measure brain changes in pig models that are measured clinically in humans. Current needs include improved standardization and development of MRI sequences, analysis software, and atlases for animal studies. A consortium for conducting functional MRI studies of the pig brain would improve the value of this model by identifying functional networks that could be examined for changes after brain injury. Another need is functional behavior tests for pig models, which are being developed but need to be validated. Functional behavior testing equipment must be standardized, and sensitivity must be improved. Motor function is another critical measure of stroke outcomes. Dr. West and colleagues are developing technologies to analyze gait and motor function in pigs. Their studies have found that NSC-extracellular vesicle treatment leads to improved motor function in pig models of stroke. Other needs are antibodies, enzyme-linked immunosorbent assays, and other molecular tools for pig studies.

The Mutualistic Relationship Between Medical Imaging and Large Animal Models
Jessica Sieren, Ph.D., The University of Iowa

Dr. Jessica Sieren's research focuses on approaches for collecting medical images and processing data captured through imaging. Challenges of conducting imaging studies with human subjects are as follows: (1) diversity of types, stages, treatment strategies, and comorbidities; (2) recruitment, retention, and

scheduling; (3) limitations on frequency of imaging; and (4) biospecimen access. Obtaining sufficient samples and accessing pediatric populations also are challenging in human studies. Pig models offer an opportunity to bridge the gap between small animal models and human subjects for testing translational methodologies. Pig models allow investigators to optimize acquisition parameters for diagnosis and treatment monitoring, perform cross-modality comparisons, examine disease etiology, and conduct well-controlled treatment intervention studies. Imaging in pig models allows investigators to identify internal disease phenotypes noninvasively, compare disease presentation to its presentation in humans, and obtain longitudinal data on disease progression and treatment outcomes using a smaller cohort. Dr. Sieren described a study that employed computed tomography (CT) and MRI to characterize a pig model for Li-Fraumeni syndrome and to develop protocols phenotyping this model. Medical imaging was critical in identifying solid tumors (e.g., osteosarcomas) in these models and in providing temporal and spatial guidance of tissue collection for pathology. In addition, Dr. Sieren and colleagues have used MRI to examine the volume and tissue infiltration of neurofibromas in a pig model of neurofibromatosis and demonstrated the utility of ultra-low dose, ultra-fast CT that does not require sedation in human pediatric patients. Dr. Sieren highlighted the importance of large animal models for testing and validating new imaging methodologies and optimizing existing methods for translation to clinical care. To advance medical imaging research using these models, however, researchers will require onsite access to medical imaging equipment. Dr. Sieren also noted the steep learning curve for large animal medical imaging because of regulatory, logistical, and technical requirements. This learning curve, as well as the high cost of animal care and imaging, highlights the need for core facilities for large animal imaging. Incorporating medical imaging in phenotyping of large animal models is expensive but provides benefits (e.g., temporal and spatial characterization of disease phenotypes) and facilitates comparisons to human disease presentation.

Therapeutic Development in the Canine Models for Duchenne Muscular Dystrophy
Peter Nghiem, D.V.M., Ph.D., Texas A&M University

Dr. Peter Nghiem discussed research on canine models of Duchenne muscular dystrophy (DMD) at Texas A&M University. DMD therapies are tested first in cell cultures and validated for safety in mouse models. Dr. Nghiem's laboratory conducts phenotypic measures using DMD canine models (and DMD mouse models) that parallel testing performed in humans. A key measure for the canine DMD models is activity monitoring; activity levels differ between controls and affected dogs for both adult and senior groups. The group also has tested utrophin gene therapy and homology-directed repair of the DMD genetic mutation using canine models. Dr. Nghiem's group prioritizes adherence to the "three Rs" of animal welfare in research—replacement of animal models with other models when possible, reduction of the number of animals used in studies, and refinement of molecular techniques in cell cultures. For example, they created immortalized canine myoblast cell lines to reduce the number of animals used in research. Dr. Nghiem noted that costs of canine models include maintenance and care; production and breeding; personnel; and development, testing, and validation of outcome measures in the phenotyping and molecular laboratories. He recommended a centralized, federally funded location for animal production. Because only a few laboratories perform preclinical trials in DMD dogs, a centralized location would be beneficial for performing standardized studies using canine models. Standardization of methods, equipment, functional outcome measures, and reagents would be beneficial to research on DMD in canine models; the number of therapies under development is increasing rapidly.

Companion Animal Models of Chronic Pain
Duncan Lascelles, Ph.D., North Carolina State University

Dr. Duncan Lascelles emphasized that animal models for chronic pain are important because preclinical research is not producing new analgesics. Naturally occurring painful disease models presented by

companion animals can reflect the complex genetic, environmental, temporal, and physiological influences present in humans. Depending on the specific pain disease state, these models are common and accessible at veterinary colleges and referral or primary practices. Prior to the use of a model, scientists need to determine the fidelity between diseases that cause chronic pain in companion animals with the disease counterpart in humans. For certain painful diseases, outcome measures are well established for companion animals and are based on dimensions affected by chronic pain in humans—including gait and movement, function, somatosensory processing, affective and cognitive features, sleep, and social relationships. Valid measures of gait and limb use have been developed for osteoarthritis in dogs. Measurement of activity, activity patterns, and quality of movement; other measures of function; and measures of somatosensory processing have been developed and validated in animals. In addition, measures of sleep, cognitive function, and affective domains in animals are under development. Basing outcome measures on dimensions that are impacted in humans—and meaningful to humans—maximizes the relevance of these spontaneous models. Phenotypes and subphenotypes must be defined through systematic, detailed comparative and multidisciplinary work and perhaps could be supported by ORIP in collaboration with the Clinical and Translational Science Award One Health Alliance (COHA). The high face validity of these models, validated outcome measures, and unprecedented access to tissues (e.g., postmortem) that veterinarians possess offer opportunities for discovery of relevant novel targets. To achieve this, however, researchers must access species-specific molecular reagents and expertise and improved annotation of canine and feline genome and immune systems. Funding support is needed to optimize the collection of highly phenotyped tissues and to establish tissue repositories. Funding opportunities (e.g., U01, U24) also are needed to develop, validate, and refine standardized methodology and reporting. Dr. Lascelles proposed an NIH-funded translational program to support multi-institutional companion animal clinical trials. The COHA initiative already has developed networks of institutions dedicated to research in specific disease and therapeutic areas for companion animals. Dr. Lascelles highlighted the value of collaboration with veterinarians, who are experienced in measuring pain in companion animals and have unprecedented access to biological samples. Ultimately, a "valid" animal model is one that predicts biology or response to therapeutics in humans. When used for proof-of-concept analgesic studies, companion animals demonstrate high predictability of efficacy in humans.

Validation of Large Animal Models in Research: A Summary
Susan Sanchez, Ph.D., The University of Georgia

Dr. Sanchez summarized the presentations delivered during this session. She reiterated that the NIH is interested in learning about challenges encountered in validating large animal models and needs for technologies, resources, and methods/processes to assess the value and limitations of these models. Dr. Sanchez clarified that predictive, face, and construct validity must be established for large animal models used in research. Dissemination of information about efforts to validate the models regarding their ability to accurately reflect human conditions (i.e., face validity) is crucial. Dr. Sanchez highlighted the importance of large animal models in bridging the translational gap between small animal and human studies and noted that large animal models are necessary for certain types of research (e.g., xenotransplantation).

Group Discussion
Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research

Drs. Sanchez and Weimer reviewed comments submitted through the Zoom chat and encouraged the participants to contribute additional comments for discussion.

Dr. Sanchez read a comment by one of the attendees stating that mice are phylogenetically more similar to humans than to dogs, pigs, or goats. The attendee asked why large animals should be used. Dr. Prather

referred the attendee to the paper cited during his presentation that explains why large animal models are superior to rodents. Dr. Kevin Wells noted that several species, including rats and mice, experience punctuated evolution and might not be good models for any other species. Dr. Sanchez noted another comment highlighting the value of using large animals in stroke research.

Dr. Nghiem questioned how the need to reduce sample sizes in large animal studies for animal welfare reasons can be balanced with the need for sufficient sample sizes to ensure internal validity. He clarified that samples should not be reduced to the point where power is inadequate. One option is to use approaches that do not require the use of many or any animals (e.g., testing on cells or tissue) during the proof-of-concept phase of the study. Dr. Joe Kornegay also commented that two factors to consider when determining sample size are the number of hypotheses and the associated animal groups in the original experimental design. Large animal studies need to be more focused in their design.

Dr. West noted that when developing and testing models, extensive optimization is necessary, which leads to the use of more animals. He recommended a repository for the type of information collected during this type of study or an atlas of normal animals as a control. Initially, more animals are needed to develop and validate reagents and other tools.

Dr. Lascelles asked about tissue banks for well-phenotyped animals and multicenter replication studies, which would advance the development of analgesics and other research that has relied upon patient reported outcomes. He also responded to a question regarding the possibility of placebo effects in animals with human companions who participate in studies. Dr. Lascelles added that investigators attempt to decrease this caregiver bias by decreasing caregiver expectations up front. Studies also are blinded and appropriately powered so that potential sources of bias can be examined. More research is needed on the caregiver placebo effect.

In response to Dr. Sanchez's comment about the need for training, Dr. Lauren Trepanier noted that she is co-leading a COHA Innovation Award to train veterinarians to join translational research teams. This 5-year award funds post-residency fellowships for veterinary specialists to engage in research mentored by interdisciplinary teams. Dr. Trepanier's team has conducted two workshops to train early-career veterinary faculty in writing grants and creating translational research teams. This COHA is funding 10 translational summits to discuss specific diseases. Dr. Trepanier invited participants interested in providing a fellowship opportunity to contact her at lauren.trepanier@wisc.edu.

Dr. Weimer asked participants more broadly about successful gene-editing techniques. She mentioned an *in vivo* gene editing approach for mouse models developed at the University of Nebraska. Some similar work related to COVID-19 is underway. A participant mentioned that relatively high efficiency can be achieved with microinjection.

In response to a comment asking about a comparative, normal, and pathological CT and MRI, Dr. Sieren stated that significant protocol differences can exist across scanner manufacturers, which is a major problem affecting both human and animal imaging data, particularly for MRI. These types of problems could be overcome by pooling resources and sharing acquisition protocols. A standardized data set that is useful for all applications would be ideal but might be infeasible currently. Dr. Weimer added that Dr. Sieren's comment illustrates the need for a progressive MRI and CT atlas across large animal species. A first step would be developing such an atlas across institutions for the same species. Dr. Sieren encouraged the creation of networks but noted that investigators need to examine the feasibility of creating large-atlas data.

Dr. West stated that his institution is engaged in an atlas-building project. Data on normal pathology are lacking, which is a barrier. Dr. Dhanu Shanmuganayagam expressed interest in participating in a network. His institution has substantial imaging capacity, as well as a medical physics and radiology team and a veterinary pathology team interested in participating. Other participants responded that they are interested in the veterinary radiology and pathology resources. Drs. Cheng and Matthew Gounis discussed how to collaborate on an R24 for resource-related projects.

Dr. Nghiem clarified that when carrier breeders have completed their regimen, they are spayed and adopted out. His institution has covered the costs associated with laboratory dog adoptions; funding mechanisms in this area would beneficial. Dr. Nghiem's institution works with Homes for Animal Heroes, which provides some funding for partial costs associated with adoptions.

Dr. Wells recommended a rubric for model selection. Investigators should seek the best model, rather than the most familiar model. Dr. Gounis added that a rubric for selecting the best animal models to test a particular question would be useful. Dr. Wells noted that the rubrics should be developed for an organ system or disease. Dr. Lascelles suggested a centralized repository of detailed information about animal models and their predictive utility.

Dr. Gounis stated that the predictive utility of many models of age-related conditions is reduced because these models do not have the numerous comorbidities usually experienced by humans. Dr. West explained that his group is unable to wait for the animals to become geriatric. Hypertension and some comorbidities of aging, however, can be induced. Dr. Shanmuganayagam recommended a resource where pigs could be aged. Dr. Johnson pointed out that, in aging, the microglial cells shift toward a proinflammatory phenotype and are hypersensitive to external stimuli, such as injury or peripheral infection. For this reason, the inflammatory reaction in a geriatric brain is markedly different than in a young adult brain. Dr. Wells suggested that studies make greater use of client-owned animals identified through veterinary hospitals. Dr. Gounis stated that he has used retired breeders for research.

Dr. Steven Stice stated that induced pluripotent stem cell (iPSC) models allow investigators to screen therapies *in vitro* for specific diseases. Historically, iPSCs and germline cells have been difficult to produce. Dr. Stice responded that the *in vitro* differentiation of iPSCs from animal models is repeatable. In response to a query from Dr. Wells, Dr. Stice explained that *in vitro* studies cannot be translated directly to humans; therapies must first be tested in animal models. Dr. Weimer pointed out that *in vitro* studies using both human and animal model cells might be useful.

Dr. Sanchez proposed studying the impact of the microbiome in large animal models. In mice, the microbiome has an important effect in variations of model phenotype and response to interventions. Dr. Lascelles clarified that differences in microbiomes between companion and laboratory animals represent both limitations and opportunities. He pointed out that side effects in humans cannot be fully predicted by large or small animal models. Dr. Kornegay clarified that canine models can predict side effects in humans better than rodent models. Canine models, however, cannot consistently predict the immune response in humans. Dr. Kornegay added that canine and human transgenes have been matched.

Dr. Engelhardt asked how the NIH would fund the proposed resources. Study sections are accustomed to reviewing applications for studies using cells or mouse models, and members frequently do not understand the unique challenges and opportunities associated with large animal models. Dr. Weimer asked the participants to consider the idea of a study section focused on large animal models. Dr. Weimer stated that participants should approach the CSR with suggestions regarding study sections. Dr. Wells also suggested making reviewers and NIH leadership and program staff more aware of the limitations of mouse models.

Additional Comments

Dr. Prather asked about the efficiency of creating precise gene fusions in pigs using zygote gene editing. He noted that his laboratory has not made substantial efforts with zygote injection. The few attempts were only moderately successful. That lack of efficiency may be target-specific, given that only a few targets were attempted. Dr. Prather and colleagues intend to revisit these tests. Dr. Weimer noted that a colleague has discussed performing this procedure in the pig. Direct *in vivo* injection into the oviduct lumen and electroporation would eliminate the need for *ex vivo* handling of zygotes.

Dr. Cheng asked Dr. Nghiem how histologically and ultra-structurally similar the mild and full phenotype DMD models were. Dr. Cheng also asked whether the model system was available through the institution's website. Dr. Nghiem responded that his institution does not have a centralized website detailing these pathological changes. Dr. Prather shared a reference on genetic similarity of large animals to humans:

- Wernersson R, Schierup MH, Jørgensen FG, et al. Pigs in sequence space: A 0.66 coverage pig genome survey based on shotgun sequencing. *BMC Genomics* 2005:6;70. doi.org/10.1186/1471-2164-6-70

Dr. Trepanier shared the following two links: ctsaonehealthalliance.org/resources/ctsa-translational-research-fellowship-opportunity; ctsaonehealthalliance.org/resources/2nd-translational-research-immersion-program-trip.

Dr. Cheng asked how much work has been done in large models on cross-correlation between scales of phenotyping. Dr. Cheng also asked about any history of collaboration between CTSA and ORIP to address validation. Dr. John Postlethwait commented that investigators should not describe dogs and pigs as genetically similar but as physiologically, morphologically, and functionally more similar to humans than mice. Dr. Kornegay explained that outbred dogs tend to better model the immune response to adeno-associated virus-based gene and stem cell therapies compared to inbred rodents. Dr. Stice noted that his laboratory has created pig, chicken, and quail iPSCs that differentiate into three germ layer cell types *in vitro*. He added that making germline chimeras is very difficult.

Dr. Cheng stated that shared pathological mechanisms are evident from histology, even if aspects of those changes are species- or strain-specific. Web-based atlases would be achievable with collaboration.

Dr. Cheng's colleague is planning to propose a comparative atlas using an R24 resource (bio-atlas.psu.edu). The bio-atlas was compiled more than a decade ago, but it requires data using advanced technologies (e.g., MRI, CT, fluorescence, other imaging-based omics approaches). Dr. Watson added that, in addition to development of an iPSC-derived line, the large-animal field could benefit from the development of primary cell lines and organoids.

Summary and Suggestions

Predictive, face, and construct validity must be established for large animal models used in research. Dissemination of information about efforts to validate the models regarding their ability to accurately reflect human conditions (i.e., face validity) is crucial. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- A "Rosetta Stone" of animal models (i.e., standardization of language, definitions, and required validation data) for vertical integration (e.g., collaborative projects, validation)

- Tissue banks for tissue and sample characterization (e.g., genomics, genetic manipulations)
- Phenomics for high-throughput phenotypic characterization (e.g., informatics, artificial intelligence, big data, storage availability)
- Expanded capabilities for imaging (e.g., computed tomography, MRI) for vertical integration (e.g., collaborative projects, informatics) to (1) improve the currently limited access to adequate facilities; (2) address the current limitations of MRI atlases; and (3) perform MRI software analysis that is specific for large species and standardization of sequence
- Standardization of methodology and reporting for vertical integration (e.g., collaborative projects)
- Species-specific molecular reagents for vertical integration (e.g., collaborative projects)
- Naturally occurring models for vertical integration (e.g., collaborative projects, validation)
- Training of veterinarians and future researchers in the complexities of using large animal models for research

Appendix A: Meeting Agenda

Session IV. Validation of Large Animal Models for Preclinical Research
2:00–4:00 p.m. EST
December 8, 2020

Chairs
Susan Sanchez, Ph.D., The University of Georgia
Jill Weimer, Ph.D., Sanford Research

2:00–2:05 p.m. **Opening Remarks**

Stephanie Murphy, V.M.D., Ph.D., Director, Division of Comparative Medicine, Office of Research Infrastructure Programs (ORIP)
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

2:05–3:30 p.m. **Presentations**

Jill Weimer, Ph.D., Sanford Research
Introduction to the Validation of Large Animal Models in Research

Randall Prather, Ph.D., University of Missouri
Swine as Models of Human Disease and a Source of Organs for Xenotransplantation

Franklin West, Ph.D., The University of Georgia
The Pig Stroke Model: Evaluating Neuroprotective and Regenerative Therapies

Jessica Sieren, Ph.D., The University of Iowa
The Mutualistic Relationship Between Medical Imaging and Large Animal Models

Peter Nghiem, D.V.M., Ph.D., Texas A&M University
Therapeutic Development in the Canine Models for Duchenne Muscular Dystrophy

Duncan Lascelles, Ph.D., North Carolina State University
Companion Animal Models of Chronic Pain

Susan Sanchez, Ph.D., The University of Georgia
Validation of Large Animal Models in Research, A Summary

3:30–4:00 p.m. **Group Discussion**

Appendix B: Discussants List

Session IV. Validation of Large Animal Models for Preclinical Research
2:00–4:00 p.m. EST
December 8, 2020

Kristin Abraham, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases
Lola Ajayi, Office of Research Infrastructure Programs (ORIP)
Alan Attie, Ph.D., University of California, San Diego
Hugo Bellen, Ph.D., Baylor College of Medicine
Dorothy Brown, D.V.M., Elanco Animal Health
Michael Chang, Ph.D., ORIP
Marc Charette, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)
Keith Cheng, M.D., Ph.D., Pennsylvania State University
Miguel Contreras, Ph.D., ORIP
John Engelhardt, Ph.D., The University of Iowa
Matthew Gounis, Ph.D., University of Massachusetts Medical School
Margaret Gruen, D.V.M., Ph.D., North Carolina State University
Amelie Gubitz, Ph.D., National Institute of Neurological Disorders and Stroke (NINDS)
Eric Hoffman, Ph.D., ReveraGen BioPharma
Rodney Johnson, Ph.D., University of Minnesota
Joe Kornegay, Ph.D., D.V.M., Texas A&M University
Duncan Lascelles, Ph.D., North Carolina State University
Kathryn Meurs, D.V.M., North Carolina State University
John Morrison, Ph.D., University of California, Davis
Stephanie Murphy, V.M.D., Ph.D., ORIP
Peter Nghiem, D.V.M., Ph.D., Texas A&M University
Todd O'Hara, D.V.M., Ph.D., University of Alaska Fairbanks
Natasha Olby, Ph.D., North Carolina State University
John Postlethwait, Ph.D., University of Oregon
Randall Prather, Ph.D., University of Missouri
Dawn Quelle, Ph.D., The University of Iowa
Andrew Rice, Ph.D., Baylor College of Medicine
Rebecca Roof, Ph.D., NINDS
John Rossmeisl, D.V.M., Virginia–Maryland College of Veterinary Medicine
Susan Sanchez, Ph.D., The University of Georgia
Lawrence Schook, Ph.D., University of Illinois
Lisa Schwartz Longacre, Ph.D., NHLBI
Dhanu Shanmuganayagam, Ph.D., University of Wisconsin–Madison
Jessica Sieren, Ph.D., The University of Iowa
Hansell Stedman, M.D., University of Pennsylvania
Steven Stice, Ph.D., The University of Georgia
Bhanu Telugu, Ph.D., University of Maryland, College Park
Lauren Trepanier, D.V.M., Ph.D., University of Wisconsin–Madison
Charles Vite, D.V.M., Ph.D., University of Pennsylvania
Piotr Walczak, M.D., Ph.D., University of Maryland School of Medicine
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia
Adrienne Watson, Ph.D., Recombinetics
Jill Weimer, Ph.D., Sanford Research
Kevin Wells, Ph.D., The University of Southern Mississippi

Franklin West, Ph.D., The University of Georgia
Dileep Yavagal, M.D., University of Miami
Xiaoli Zhao, Ph.D., National Institute of General Medical Sciences
Sige Zou, Ph.D., ORIP