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 II. Validation of Zebrafish Models for Preclinical Research

Tuesday, November 24, 2020
Virtual Meeting

Workshop Report

Table of Contents

Executive Summary

The second of 10 sessions of the Virtual Workshop on Validation of Animal Models and Tools for Biomedical Research was held on November 24, 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 II focused on the development of tools and technologies to enable the use of zebrafish model organisms for preclinical research. The participants emphasized the unique value of zebrafish models for disease study and drug discovery. Topics of discussion included genetic epilepsies, undiagnosed human diseases, scoliosis, cellular barcoding, infectious diseases, and rare disorders. Several participants noted that the challenge of imaging adult zebrafish should be addressed. During the session, the following needs were identified: (1) consideration of genetic heterogeneity in the cause of common and uncommon human diseases and the potential to develop zebrafish models of the many mutations involved, (2) additional cell type–specific promoters and strategies for conditional modification of gene function, (3) drug screens using validated zebrafish models of human disease, and (4) specific antibodies and nanobodies to detect and modulate the function of zebrafish proteins in specific cell types. Additionally, genome- and gene-editing technologies (e.g., CRISPR, single-cell genome editing of synthetic target arrays for lineage tracing [scGESTALT], CRISPR-mediated integration cassette [CRIMIC]) were highlighted for their promise as high-priority areas of research. Last, the need to support technology development and implementation, research centers, consortia, databases, and screening libraries also was emphasized. Several participants expressed their desire for the Office of Research Infrastructure Programs (ORIP) to further support preliminary model development, collaborative interactions with investigators who study other model organisms, and engagement with new investigators in the field.

Session Co-Chairs
Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University

Presenters
Gerald Downes, Ph.D., University of Massachusetts Amherst
Lalita Ramakrishnan, Ph.D., University of Cambridge
Lilianna Solnica-Krezel, Ph.D., Washington University in St. Louis
Monte Westerfield, Ph.D., University of Oregon
Leonard Zon, M.D., Boston Children's Hospital

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, 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
Franziska B. Grieder, D.V.M., Ph.D., Director, ORIP
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

Drs. Franziska B. Grieder, Director, 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. They explained that the meeting is the second in a series of 10 sessions. Drs. Grieder 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. Grieder 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.

Zebrafish Models of Neurological Disease
William Talbot, Ph.D., Stanford University

Dr. William Talbot, Co-Chair, provided an overview of zebrafish as a vertebrate model system of neurologic disease. He explained that zebrafish are powerful model organisms with well-developed forward and reverse genetic methods and large, transparent embryos, which allow microinjection, live imaging, and exquisite cellular studies. To illustrate that the combination of genetic and cellular studies can be a powerful tool to understand vertebrate gene function, Dr. Talbot shared images of green fluorescent protein (GFP)-labeled neutrophils in living mutant embryos with a hyperinflammatory phenotype. Most preclinical studies focus only on a limited number of models, and a disconnect exists between the wide array of mutations that contribute to disease susceptibility in patient populations and the smaller number of genetic models that are available for preclinical studies. He commented that future investments in resources and technologies to advance and validate zebrafish models for disease must be mindful of this genetic heterogeneity and the corresponding need to greatly increase the number of disease models that are generated and investigated. For example, multiple high-confidence risk loci are associated with autism, Alzheimer's disease, and amyotrophic lateral sclerosis, but models for these susceptibility genes are not widely available. In many cases, it remains unclear which gene in a locus predisposes to disease. Genome-editing approaches in zebrafish offer the possibility of rapidly creating zebrafish with specific patient mutations that would serve as validated disease models of many clinically relevant sequence variants. Dr. Talbot remarked that CRIMIC is a promising approach developed in *Drosophila* that is presently being piloted in zebrafish. CRIMIC promotes efficient integration of a synthetic exon to inactivate a gene of interest and express GAL4 in its place. The GAL4 transcription factor and corresponding upstream activation sequence (UAS) transgenes can be used to rescue the mutants by expressing the wild-type human gene or a tagged variant. Additionally, the GAL4-UAS system can be used to express and test the function of a human disease variant, thus representing a validated disease model. In one of many possible applications, CRIMIC could be used to determine whether missense variants contribute to disease in patients with various neurological phenotypes.
Dr. Talbot summarized that better strategies are needed to humanize the zebrafish genome to investigate functional consequences of human sequence variation. Cell-type specific promoters are needed for conditional approaches to modify gene function and reporter studies. The Zebrafish Information Network (ZFIN) database and Zebrafish International Resource Center (ZIRC) are essential for the study of vertebrate gene function. Support for these resources must be ensured and expanded as new types of data (e.g., single-cell RNA sequencing, whole-animal imaging) and new collections of transgenic and mutant strains are developed.

Zebrafish Models of Genetic Epilepsies: Challenges and Opportunities
Gerald Downes, Ph.D., University of Massachusetts Amherst

Dr. Gerald Downes presented on zebrafish models for genetic epilepsies, opportunities to study genetics and drug development, and some suggested areas for NIH investment. Epilepsy is the fourth most common human neurologic disorder, and 30% of cases become refractory or drug resistant. Most epilepsy cases are thought to be polygenic, but a minority of cases are caused by single gene mutations. The genetic background of the patient can modify monogenic or polygenic disease severity, and genetic interactions have potential as therapeutic targets. Larval zebrafish exhibit several similarities to mammalian epilepsy models: (1) Convulsants (e.g., pentylenetetrazole) trigger seizure-like hyperactive swimming that can be counteracted by anticonvulsants, (2) extracellular field recordings and calcium imaging show "ictal-like" discharges, (3) seizure-like activity upregulates the proto-oncogene c-Fos, and (4) some epilepsy genes result in seizure-like activity. Zebrafish could be used for analysis of genes linked to epilepsy, for large-scale small-molecule drug screens, for delivery of personalized medicine by modeling precise genetic mutations, and for investigation of neuronal network effects. Most epilepsy genes are present in zebrafish, and monogenic zebrafish mutants are easier and less expensive to create than mice. Zebrafish also are an excellent system for investigating genetic interactions. Dr. Downes shared data to illustrate that CRISPR could be used to screen for interactions among gene variants and described published work from Dr. Scott Baraban (University of California, San Francisco) showing that small-molecule screens validated zebrafish epilepsy models and can be used to identify novel therapeutics. NIH investment in zebrafish models for epilepsy could be directed to phenotyping; establishing centers to efficiently conduct small-molecule screens; supporting genetic interaction studies, particularly enhancer/suppressor forward genetic screens; developing genome-editing and precise gene-editing technologies; and continuing to support ZFIN, ZIRC, and cross-species databases.

Zebrafish Models Validate Undiagnosed Human Diseases
Monte Westerfield, Ph.D., University of Oregon

Dr. Monte Westerfield spoke on zebrafish models for undiagnosed human diseases and noted that his work was supported by the NIH Undiagnosed Diseases Network—a nationwide collaboration of clinical sites and core facilities that includes a Model Organisms Screening Center (MOSC) using worms, flies, and zebrafish. *YPEL3* was identified as a potential disease-causing gene in a child who presented with congenital symptoms (e.g., hypertrophic peripheral nerves, central hypomyelination, peripheral hypomyelination), which resulted in hypotonia and areflexia. *YPEL3* is a highly conserved gene that had been thought to function in cell signaling but had no known disease associations. CRISPR was used to generate zebrafish mutants based on the patient genotype. No phenotypic effects were observed in heterozygous or homozygous offspring, but investigators observed hypertrophic nerves in maternal-zygotic zebrafish mutants. The affected animals had abnormal locomotion reflecting the patient's hypotonia and areflexia. The researchers demonstrated that *YPEL3* is expressed in the central nervous system and cells adjacent to the spinal cord. Hypomyelination was observed in mutants because of a reduction in myelinating oligodendrocytes. Zebrafish with a mutation in this gene were found to have elevated phosphatidic acids and ceramides. Previous studies have shown that phosphatidic acids are negative regulators of Schwann cell differentiation, and ceramides are negative regulators of oligodendrocyte differentiation. This example illustrates how zebrafish were used to discover that *YPEL3* is a negative regulator of lipid signaling, which affects glial cell differentiation. Mutation results in decreased maturation of oligodendrocytes and defects in Schwann cells and perineurial cells. This work validated *YPEL3* as a disease-causing gene. Dr. Westerfield noted that zebrafish had been used by MOSC collaborators to analyze 41 genes that led to 13 new diagnoses. Zebrafish models for human disease can provide powerful genotypic and phenotypic validation. However, generating precise variants still is problematic, and studies of human diseases are performed one gene at a time. Zebrafish studies require

specific antibodies and nanobodies, smooth access to collaborations with clinicians, vertical integration, and support for ZFIN and ZIRC.

Forward and Reverse Genetic Approaches in Zebrafish to Understanding Scoliosis
Lilianna Solnica-Krezel, Ph.D., Washington University in St. Louis

Dr. Lilianna Solnica-Krezel discussed genetic approaches to zebrafish scoliosis models. She noted that the experiments in her presentation combined her work with that of Dr. Ryan Gray at the University of Texas at Austin. Adolescent idiopathic scoliosis (AIS) affects up to 3% of the pediatric population, but the genetic basis of the disease is poorly understood. The fundamental questions to be answered with zebrafish models are which genetic mutations cause AIS and what tissues are involved. Zebrafish with mutations in the *stat3* gene undergo normal embryogenesis and larval development but begin to show signs of scoliosis and failure to thrive by 22 days post-fertilization. The mutants have reduced bone mineral density and exhibit an increase in inflammatory markers. Dominant mutations in the human gene are associated with scoliosis and hyper-immunoglobulin E syndrome. Dr. Solnica-Krezel commented on her collaboration with Dr. Gray on forward genetic screens for recessive adult zebrafish scoliosis mutants. Thirty-six recessive mutations were found that could be divided into four phenotypic classes: (1) whole-body scoliosis with a normal animal size, (2) dwarf animals with scoliosis and some vertebral fusion, (3) thoracic scoliosis, and (4) scoliosis confined to the caudal spine. Whole-genome and whole-exome sequencing with genetic complementation were used to find mutations in many genes. Hypomorphic missense mutations of the *SSPO* loci are involved in whole-body scoliosis phenotypes. The SCO-spondin protein is the main component of the Reissner fiber; mutation causes gradual loss of the Reissner fiber associated with the onset of body curvature. Dr. Solnica-Krezel noted that the translatability of these findings is unclear; the Reissner fiber has not been documented in humans, and *SSPO* is annotated as a pseudogene. Hypomorphic mutations in the *ADAMTS9* gene that encodes matrix metalloprotease have been associated with scoliosis confined to the caudal spine. Studies of zebrafish with scoliosis phenotypes have helped to identify several other genes of interest that are investigated as part of a P01 project with Drs. Carol Wise (The University of Texas Southwestern Medical Center), Nadav Ahituv (University of California, San Francisco), and Chris Gurnett (Washington University School of Medicine in St. Louis). Zebrafish studies link scoliosis to defects in the extracellular matrix, postural control, and inflammation. These forward genetic screens can identify phenotypes caused by hypomorphic mutations in essential genes that would be missed by generating null alleles. NIH support is needed to develop cell type–specific promoters, methods to characterize adult phenotypes, and for continued support of ZFIN and ZIRC.

Cellular Barcoding in Zebrafish Models of Disease
Leonard Zon, M.D., Boston Children's Hospital

Dr. Leonard Zon presented on the use of cellular barcoding to study blood diseases and cancer. Mutations accumulate in the peripheral blood with age, leading to risks of myeloid dysplasia and acute myeloid leukemia. Barcoding strategies help scientists better understand the onset and progression of these diseases. Dr. Zon illustrated a technique to assess the number of stem cells using color barcode analysis by confocal microscopy or flow cytometry just before stem cell birth. A peripheral blood smear depicts the number of hematopoietic stem cells (HSPCs) during development. Another technique, tissue editing with inducible stem cell tagging via recombination (TWISTR), was developed by Dr. Zon's group for sorting of dominant clones by fluorescence-activated cell sorting. Using single-cell RNA sequencing, they determined that the inflammatory state in mutant marrow is driven by mature myeloid cells. They knocked out a dominant gene of interest, *axl1*, to evaluate its phenotype. They found that mosaic co-mutation of *nr4a1* with *asxl1* prevents establishment of clonal dominance. The TWISTR approach enables successful multiplex mutagenesis in zebrafish to study clonal competition. Elevated inflammatory modulators in mutant HSPCs lead to clonal selection in the context of inflammation driven by mutant

mature cells. The technology also can be used to study oligoclonal hematopoiesis in *runx1*^{KO} zebrafish as a model for congenital thrombocytopenia and leukemia. Dr. Zon also spoke on the scGESTALT barcoding system, which was recognized as the *Science* 2018 Breakthrough of the Year. Using heat shock activation in a guide fish, embryogenesis can be tracked. Barcoding then is induced following development. With this technique, different clones with gene expression signatures can be identified readily. Lineage trees reveal long-lived lineage-biased progenitors barcoded during early embryogenesis. Dr. Zon's group found that all the unbiased clones were barcoded from the lineage tree. The long-lived lymphoid- and myeloid-based progenitors, however, were linked to the early barcode. Consequently, the early progenitor gives rise to biased clones, and the stem cells give rise to unbiased clones. The barcodes can help researchers study the origin of pediatric leukemia and therapeutically target clones to prevent cancer progression. These therapeutics could be developed in fish models using this technology. Dr. Zon stated that his group has identified five small molecules in zebrafish that are being investigated in clinical trials.

Zebrafish as Models for Infectious Diseases
Lalita Ramakrishnan, Ph.D., University of Cambridge

Dr. Lalita Ramakrishnan spoke on the use of zebrafish models for the study of infectious diseases. Infectious disease models involve both the infectious agent and genetic differences affecting susceptibility. Investigators must understand the disease of interest well to use zebrafish appropriately to test predictions from known data. For example, researchers used zebrafish to demonstrate that a specific lipid in the leprosy bacterium induces macrophages to release nitric oxide, which causes peripheral neuropathy. New findings often are expected to be validated in a different cell line or animal model, and stronger correlations between zebrafish and other models are needed. Researchers need a deeper understanding of zebrafish physiology and immunology and better ways to connect with one another to address specific cross-validation experiments. Tuberculous meningitis (TBM) remains a deadly disease, even with antibiotic treatment. A zebrafish mutant that was hypersusceptible to tuberculosis (TB) was identified and used to demonstrate that deficiency or hyperactivity of the leukotriene A4 hydrolase (LTA4H) pathway drives susceptibility to mycobacterial infection. Human TBM patients with homozygous alterations in the LTA4H pathway died of the disease, but heterozygous patients survived the infection. Tumor necrosis factor activates a complicated intraorganellar circuit, leading to death of infected macrophages in TBM patients. The details of the pathway led to the identification of several U.S. Food and Drug Administration–approved drugs that could be used as host-targeting drugs for TBM and other forms of TB. The zebrafish model excels in experiments requiring detailed investigations of genetics and pharmacology. Translating findings into human clinical use, however, is complex. Researchers need formalized access to (1) experts in pharmacology, pharmacokinetics, and studies in other animals, (2) studies on drug concentrations in relevant human tissues from people who take the drug for other conditions, and (3) access to human cohorts.

A New Era for Rare Disorders
Rebecca Burdine, Ph.D., Princeton University

Dr. Rebecca Burdine, Co-Chair, discussed the challenges and dynamics of validation in relation to her group's research. Three criteria for animal model validation, as defined by Dr. Paul Willner, are (1) face validity (i.e., how well the model replicates the disease phenotype in humans), (2) construct validity (i.e., how similar the mechanism is by which phenotypes are induced in animals and humans), and (3) predictive validity (i.e., how well the model can predict currently unknown aspects of disease in humans, including therapeutic outcomes). Dr. Burdine described the validation of RASopathy mutations, which lead to the activation of extracellular signal-regulated kinase. In humans, RASopathies result in craniofacial anomalies, heart defects, and short stature. Zebrafish models for RASopathy express craniofacial anomalies (mild to severe), changes in heart surface area, and short stature. Dr. Burdine

expressed, however, that the manifestation of these phenotypes in zebrafish likely is different than it is in humans. Thus, the strength of animal models lies in their construct and predictive validities. For example, embryonic cell elongation serves as a phenotype for quantifying the RASopthahy mutation effect. This scale also has been used in a *Drosophila* model. Dr. Burdine explained that she is characterizing the property of the disease molecule, rather than the disease process. Additionally, new molecules can be identified as potential causative factors. Ranking is predictive of minimal mitogen-activated protein kinase inhibitor dose. This assay is well suited to large-scale drug screens for potential inhibitors. Dr. Burdine explained that this approach is ideal for genetic rare diseases. She concluded by raising the following points about funding in the rare diseases community: (1) Multiple animal models lead to a better predictive outcome, (2) academic science can be leveraged at comparatively low costs for large outcomes, (3) working groups must focus and communicate to accelerate discovery and paths to therapeutics, and (4) researchers must recruit all willing minds and hands to maximize creativity and productivity. She proposed (1) funding mechanisms to enable the development of models without extensive preliminary data, (2) funding mechanisms to allow the discovery of models and "fishing expeditions" to validate the predictive value of models (e.g., drug screens, CRISPR screens, learning new techniques), (3) continued support of databases and resources, (4) a change in attitude toward alternative model organisms, and (5) NIH "hubs" to bring together models in different organisms to focus on one rare disease of interest.

Group Discussion
Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University

Drs. Burdine and Talbot reviewed comments submitted through the Zoom chat and encouraged participants to contribute additional comments for discussion. Dr. Talbot explained that the speakers' feedback will be used for the development of a 2- to 3-page summary describing the outcomes of the workshop. Themes will include support for databases, genome editing, chemical screening, antibodies, cross-fertilization with other model organisms, and interactions across scientific fields and with physicians.

Dr. Hugo Bellen asked whether the thymine and adenine–rich intron regions in zebrafish affect the protospacer adjacent motif sites for CRISPR. He noted that this issue has represented a rate-limiting step reported by Chinese and Japanese research teams. Dr. Talbot responded that he has not encountered this issue in his research. They agreed that improved algorithms are likely to ameliorate the issue.

Dr. Bellen commented on strategies to facilitate vertical integration within the animal model research community. He noted that collaboration adds value to research and commented that zebrafish are an ideal model for drug testing. Dr. Burdine agreed, noting that mice are valuable models but carry limitations for predictive modeling that often can be addressed in other organisms.

Dr. Bellen suggested collaborating with bioinformaticians for systematic screening of data. Dr. Burdine noted that this approach would be particularly impactful for smaller laboratories. Several participants agreed that databases (e.g., ZFIN) will be crucial for this effort.

Dr. Zon noted that zebrafish embryos are relatively simple to study, but many researchers are interested in imaging adult zebrafish and screening for adult phenotypes. Dr. Burdine emphasized the need for funding mechanisms to support this type of work. Dr. Solnica-Krezel noted that she has performed mutant adult scoliosis screens using NIH funds. In response to a question from Dr. Zon, she stated that the mechanisms of scoliosis must be understood for the development of therapeutics for prevention and treatment and added that early diagnosis would be powerful.

Dr. Talbot asked about areas of investment to advance chemical screening. Dr. Zon suggested developing and organizing inclusive libraries. Common methodologies should be developed. Funding opportunities for chemical screens are limited, but the need for this support is clear. Dr. Downes noted that centers (e.g., ZIRC) play a crucial role in this area. He also suggested screening later in development. Dr. Zon stated that this technology exists but requires further development. Currently, most of this work is performed without automation.

Dr. Teresa Nicolson voiced her agreement on the importance of the construct and predictive validities of animal models. She commented that many investigators are facing pushback from the NIH on this issue. Drs. Burdine and Solnica-Krezel agreed and noted that interdisciplinary communication and collaboration are critical.

Dr. David Raible commented that some programs (e.g., Therapeutics for Rare and Neglected Diseases [TRND]) have allowed investigators to work beyond drug screening (e.g., pharmacokinetics safety) and suggested that these programs should be made more widely available. Dr. Bellen emphasized the importance of communication and engagement surrounding this issue. He also noted that other animal models (e.g., invertebrates) face similar challenges within the research community. Dr. Burdine suggested engaging with both physicians and families affected by the diseases of interest.

Dr. Westerfield recommended that ORIP develop a novel funding mechanism to support collaborative efforts among investigators studying different models for a disease of interest. Dr. Burdine agreed and voiced her support for enabling interactions between and among smaller laboratories.

Dr. Ramakrishnan commented that the zebrafish community should strive to bring in new investigators. She noted that misconceptions exist about the difficulty of working with zebrafish and added that education will be critical for further efforts in this area. She also noted that physicians often are enthusiastic about zebrafish models.

Additional Comments

In the Zoom chat, Dr. Keith Cheng wondered whether investigators who hold medical degrees—and thus possess an increased familiarity with clinical details—can provide a unique perspective on validation of animal models. Dr. Cheng also stated that the elongated embryo is a phenologue of the *RAS* mutant phenotype.

Dr. David Langenau commented that the National Cancer Institute maintains Requests for Applications (RFAs) specifically for mouse models and suggested that these opportunities should be inclusive to all model organisms. He added that the NIH previously offered RFAs for models and tool building in zebrafish.

Dr. Cressida Madigan stated that interesting research has been conducted in adult *Danionella translucida*. Dr. Mary Mullins noted that she has observed more hypomorphs in adult maternal effect screens than in zygotic screens; only hypomorphs allow survival to adulthood. Dr. Mullins also stated that forward screens allow for discoveries that cannot be detected by CRISPR screens. Dr. Solnica-Krezel commented that she has observed fewer nonsense mutations in maternal screening, which makes tracking difficult. Dr. Gray wondered when CRIMIC plasmids will be available for distribution, noting that modeling *de novo* mutations in a haploinsufficient gene using CRISPR has been challenging.

Dr. Randy Peterson highlighted the NIH TRND program for its support of drug screening and development. He noted that zebrafish laboratories have limited experience in and resources for clinical

translation. Dr. Burdine noted that Dr. Peterson is presenting a seminar on small-molecule screening through the International Zebrafish Society in December 2020.

Dr. Raible shared an example database: umgear.org. Dr. Elisabeth Busch suggested cross-species databases, such as the European Bioinformatics Institute Expression Atlas: ebi.ac.uk/gxa/sc/home. Dr. Busch commented that the database is easy to use and raises the profile of the zebrafish model.

Summary and Suggestions

Zebrafish are powerful model organisms with well-developed forward and reverse genetic methods that offer novel applications for disease study and drug discovery. The participants discussed and provided the following areas that require new or continued support from ORIP and the NIH:

- ZFIN and ZIRC, which are vital resources for the community.
- Technologies to humanize the zebrafish genome and knock versatile tags into zebrafish genes.
- Centers to exploit fully the potential of chemical screens, genome editing, and imaging in zebrafish to generate disease models and pursue therapies. There would be merit in a "hub-and-spoke" structure, in which the "hub" developed specific expertise (e.g., chemical screening, genome editing, imaging) that was available to support specific "spoke" projects.
- Genetic screens and new, high-throughput phenotyping technologies.
- New mechanisms to encourage collaborations among clinicians and scientists with expertise in zebrafish and other model organisms.
- Nanobodies to many zebrafish proteins implicated in human disease

Appendix A: Meeting Agenda

Session II. Validation of Zebrafish Models for Preclinical Research
2:00–4:00 p.m. EST
November 24, 2020

Chairs
Rebecca Burdine, Ph.D., Princeton University
William Talbot, Ph.D., Stanford University

2:00–2:05 p.m. **Opening Remarks**

Franziska B. Grieder, D.V.M., Ph.D., Director, Office of Research Infrastructure Programs (ORIP)
Sige Zou, Ph.D., Coordinator, Program Official, ORIP

2:05–3:30 p.m. **Presentations**

William Talbot, Ph.D., Stanford University
Zebrafish Models of Neurological Disease

Gerald Downes, Ph.D., University of Massachusetts Amherst
Zebrafish Models of Genetic Epilepsies: Challenges and Opportunities

Monte Westerfield, Ph.D., University of Oregon
Zebrafish Models Validate Undiagnosed Human Diseases

Lilianna Solnica-Krezel, Ph.D., Washington University in St. Louis
Forward and Reverse Genetic Approaches in Zebrafish to Understanding Scoliosis

Leonard Zon, M.D., Boston Children's Hospital
Cellular Barcoding in Zebrafish Models of Disease

Lalita Ramakrishnan, Ph.D., University of Cambridge
Zebrafish as Models for Infectious Diseases

Rebecca Burdine, Ph.D., Princeton University
A New Era for Rare Disorders

3:30–4:00 p.m. **Group Discussion**

Appendix B: Discussants List

Session II. Validation of Zebrafish Models for Preclinical Research
2:00–4:00 p.m. EST
November 24, 2020

Lola Ajayi, Office of Research Infrastructure Programs (ORIP)
Zhirong Bao, Ph.D., Memorial Sloan Kettering Cancer Center
Hugo Bellen, Ph.D., Baylor College of Medicine
Rebecca Burdine, Ph.D., Princeton University
Elisabeth Busch, Ph.D., University of Cambridge
Michael Chang, Ph.D., ORIP
Marc Charette, Ph.D., National Heart, Lung, and Blood Institute (NHLBI)
Keith Cheng, M.D., Ph.D., Penn State College of Medicine
Miguel Contreras, Ph.D., ORIP
Gerald Downes, Ph.D., University of Massachusetts Amherst
Bruce Fuchs, Ph.D., ORIP
Ryan Gray, Ph.D., The University of Texas at Austin
Franziska B. Grieder, D.V.M., Ph.D., ORIP
Marko Horb, Ph.D., Marine Biological Laboratory
David Langenau, Ph.D., Harvard Stem Cell Institute
Lisa Schwartz Longacre, Ph.D., NHLBI
Calum MacRae, M.D., Ph.D., Brigham and Women's Hospital
Cressida Madigan, Ph.D., University of California, San Diego
Cecilia Moens, Ph.D., University of Washington
Mayssa Mokalled, Ph.D., Washington University in St. Louis
Mary Mullins, Ph.D., University of Pennsylvania
Stephanie Murphy, V.M.D., Ph.D., ORIP
Teresa Nicolson, Ph.D., Stanford University
Antonio Pagán, Ph.D., University of Cambridge
Norbert Perrimon, Ph.D., Harvard Medical School/Howard Hughes Medical Institute
Randy Peterson, Ph.D., The University of Utah
John Postlethwait, Ph.D., University of Oregon
David Raible, Ph.D., University of Washington
Lalita Ramakrishnan, Ph.D., University of Cambridge
Francisco Roca, Ph.D., University of Cambridge
Crystal Rogers, Ph.D., University of California, Davis
Alvaro Sagasti, Ph.D., University of California, Los Angeles
Susan Sanchez, Ph.D., The University of Georgia
Lilianna Solnica-Krezel, Ph.D., Washington University in St. Louis
William Talbot, Ph.D., Stanford University
David Tobin, Ph.D., Duke University School of Medicine
Zoltan Varga, Ph.D., University of Oregon
Jesus Torres Vazquez, Ph.D., New York University Grossman School of Medicine
Douglas Wallace, Ph.D., Children's Hospital of Philadelphia
Jill Weimer, Ph.D., Sanford Research
Monte Westerfield, Ph.D., University of Oregon
Debbie Yelon, Ph.D., University of California, San Diego
Xiaoli Zhao, Ph.D., National Institute of General Medical Sciences
Leonard Zon, M.D., Boston Children's Hospital
Sige Zou, Ph.D., ORIP