"FROM TANK TO BEDSIDE: ZEBRAFISH AND TRANSLATIONAL RESEARCH"

Division of Comparative Medicine

Office of Research Infrastructure Programs

Division of Program Coordination, Planning, and Strategic Initiatives

Office of the Director, National Institutes of Health

Bethesda, MD 20892

October 29-30, 2013

Meeting Summary

OVERVIEW

The Division of Comparative Medicine (DCM), within the Office of Research Infrastructure Programs, Division of Program Coordination, Planning and Strategic Initiatives, Office of the NIH Director, convened the workshop entitled, "From Tank to Bedside: Zebrafish and Translational Research" on October 29 – 30, 2013 at the Natcher Conference Center on the NIH Campus. The objectives of the Workshop were: 1) to provide input to the NIH on the current status of projects and technologies that directly inform studies related to human health using the zebrafish as an animal model; and 2) to provide advice to the NIH on initiatives that can enhance the use of zebrafish in translational research. For the purpose of the workshop, translational research was defined broadly as investigations that are directly related to aspects of human disease, with the eventual goal of finding the cause of illness and treating it in the clinic.

Potential new uses of zebrafish for translational research would be built on a base of existing resources and knowledge. The zebrafish is used extensively by NIH funded investigators to examine many aspects of the basic biology of vertebrates, and has been an important model organism for basic research for many years. A survey of funded NIH grants using the NIH RePORT system (http://www.report.nih.gov/) performed by the DCM for fiscal year 2012 indicated approximately 600 funded grants using zebrafish, totaling approximately $233 million in total costs. Grants were funded by 20 of the NIH Institutes and Centers and included approximately 300 R01 awards. In addition to research grants, the NIH funds the Zebrafish International Resource Center (ZIRC), which archives and distributes mutant zebrafish lines and other reagents, and the Zebrafish Model Organism Database (ZFIN), which provides a wide range of information to researchers. Zebrafish-related activities funded through the various extramural programs, including specific Funding Opportunity Announcements, are coordinated by the Trans NIH Zebrafish Coordinating Committee. The NIH also funds a large intramural research effort, including one of the world's largest facilities for husbandry of zebrafish. Based on this strong foundation, it has become apparent, both to investigators and NIH staff, that the zebrafish is entering a new phase of biomedical utility for translational studies. New opportunities for the translational use of zebrafish were the subject of this workshop.

The Organizing Committee for the workshop comprised extramural and NIH intramural researchers performing cutting edge translational research using the zebrafish, the co-chairs of the Trans-NIH Zebrafish Coordinating Committee, and DCM staff (Appendix 1). The Organizing Committee suggested four subject areas that are particularly pertinent to translational uses of zebrafish and which are being pursued actively by various laboratories. These are: 1) Exome Screening and Correlation with Human Studies; 2) Elucidating Genome Wide Association Study (GWAS) "hits;" 3) High Throughput Screening; and 4) From Tank to Bedside: Direct linkage of zebrafish with human studies. The workshop comprised presentations and discussions based on these four topics (see Appendix 2). Each topic was addressed using two case studies, a panel discussion and active participation by the entire audience, which comprised approximately 180 attendees, including extramural and NIH intramural researchers and NIH program staff. The final output of the workshop was a list of recommendations aimed at improving the utility of zebrafish for translational research.

SUMMARY OF PRESENTATIONS AND PANEL DISCUSSIONS

The Keynote Address, entitled, "Modeling and Curing Disease Using the Zebrafish" was presented by Dr. Leonard Zon (Harvard Medicine School, Howard Hughes Medical Institute). The keynote address emphasized the current utility of zebrafish for facilitating many aspects of translational research, including analysis of genotype-phenotype correlations suggested by human studies, as verified in the zebrafish and high throughput screening of chemicals that affect specific phenotypes and developmental pathways.

The talk began with a discussion of the general features of zebrafish that make it a useful model for translational work, including: a) Embryos are transparent, can be readily manipulated and the effects of perturbations of gene expression visualized; b) large numbers of embryos are available - an adult female can provide 100 – 200 progeny per week; c) various genetic screens, including knockdown of gene expression using morpholino oligonucleotides (MOs), can be accomplished and chemical screening can also be performed; d) most human genes have orthologs in the zebrafish; and, e) developmental programs, such as that for hematopoiesis, are conserved between zebrafish and humans. Dr. Zon then discussed several specific examples of translational research using the zebrafish including: a) Functional studies in zebrafish that verify the effects of genes that have been initially identified in human genome wide association studies as potentially effecting a human phenotype or disease; b) Studies of cancer in the zebrafish that, for example, have identified mutations that can suppress melanoma, and experiments that have identified enhancer genes that promote the disease; c) Large scale mutagenesis screens that identify gene knockouts associated with specific phenotypes; d) Use of transcription activator-like effector nucleases (TALENS) and a system based on clustered regularly interspaced short palindromic repeats (the CRISPR/Cas system) for targeted genome editing of the zebrafish, for example, to inactivate (knock out) or activate (knock in) specific genes; e) Use of zebrafish to test for chemical suppressors of specific disease genes; and, f) Stem-cell based studies in zebrafish coupled with high throughput chemical screening to identify compounds that either promote or inhibit various stages of development. There have been two chemicals discovered in zebrafish that have made it to phase II clinical trials: 16,16-dimethyl prostaglandin E2 for enhancing engraftment in cord blood transplantation, and leflunomide treatment of metastatic melanoma.

Session 1: Exome screening and correlation with human studies.

Case Study 1, entitled "Zebrafish models of patient genotypes validate new disease genes and reveal incorrect diagnoses," was presented by Dr. Monte Westerfield (University of Oregon). Dr. Westerfield described the use of zebrafish to study the genetic basis of Usher Syndrome, a disease that causes deafness and visual impairment in infants and children. Usher syndrome in humans is associated with at least 10 genes. Exome sequencing of human patients indicated the potential involvement of the gene PDZD7, a gene of unknown function, in Usher syndrome. Zebrafish in which the gene function has been knocked down using a MO are impaired in regard to vision, hearing and balance. In zebrafish the protein co-localizes with other proteins associated with Usher Syndrome in both the eye and hearing apparatus. Therefore, these studies validated the candidate gene status of PDZD7 gene as being involved in the genetic etiology of Usher Syndrome. Dr. Westerfield also presented an example in which a gene candidate associated with a different form of deafness was not verified in zebrafish, suggesting

that the particular gene in question does not contribute to the phenotype. Dr. Westerfield pointed out that two questions raised by these case studies are: a) Are phenotypic effects using the knock down strategy limited to robust phenotypes, and b) Can studies of this type be scaled up for higher throughput?

Case Study 2 , entitled "Modeling the Morbid Pediatric Genome," was presented by Dr. Nicholas Katsanis (Duke University). Dr. Katsanis first discussed examples of several zebrafish models of human pathologies, related to neuroanatomy, craniofacial development, vascular integrity, cardiac malformations and muscular dystrophy, respectively. The remainder of the presentation summarized investigations in which zebrafish have been used to verify the functional effects of mutations first discovered in human patients by exome sequencing, including examples of gene epistasis and compensatory mutations. For example, whole-exome sequencing of DNA from patients with a syndrome characterized by ataxia, dementia and hypogonadotrophic hypogonadism identified two candidate human genes, both of which are involved in ubiquitin metabolism. Parallel functional studies in zebrafish, in which gene expression was knocked down using MOs, showed that the genes affected development of the eye, optic tectum and cerebellum. These results parallel aspects of the phenotypes of the patients, which exhibit neuronal loss in the cerebellum and hippocampus. Dr. Katsanis also reported that his laboratory currently can test about 30 human alleles per week for functional effects in zebrafish, with a projected throughput of 300 alleles per week in 2014.

Panel and General Discussion: Drs. Zon and Katsanis were joined by Dr. Allen Beggs (Boston Children's Hospital) and Dr. William Gahl (National Human Genome Research Institute). Dr. Beggs described the work in his laboratory aimed at characterizing the genetic and physiological basis of congenital myopathies. For example, these investigators have performed whole-exome or whole-genome sequencing on 60 unrelated human probands affected with nemaline myopathy, a rare congenital disease that affects function of the skeletal muscles. These investigators identified several different mutations in the KLHL41 gene associated with the disease. Zebrafish were used to examine the function of KLHL41 in skeletal muscle development. Whole mount in situ hybridization was used to study the spatio-temporal expression of the zebrafish KLHL41 genes during development and knockdown studies were performed using MOs. Taken together, these studies on zebrafish verified the hypothesis based on human sequencing that defects in this gene can cause the disease in certain individuals. Dr. Beggs also pointed out that there can be an issue regarding the specificity of knock downs in zebrafish and that the specificity of phenotypes can be an issue.

Dr. Gahl described the NIH Undiagnosed Diseases Program, which is organized by the National Human Genome Research Institute, the NIH Office of Rare Diseases Research and the NIH Clinical Center. Patients in this program (approximately 700 at present) are rigorously phenotyped and characterized genetically either by analysis using single nucleotide polymorphisms or by whole-exome sequencing. The program has identified 50 – 70 genes that are strong candidates for specific undiagnosed diseases. The challenge is now to show that these genes are causally related to a given disease and Dr. Gahl suggested that zebrafish could be very useful for these investigations.

The general discussion related to this session highlighted many of the issues that were brought up throughout the workshop. These included: 1) Potential use of higher throughput systems (e.g. 96 well

plates) to speed up analysis; 2) How can MO and CRISPR/Cas based approaches be adapted to high throughput systems, and which approach may be superior? 3) Are genetic approaches potentially superior to MO-based approaches, particularly in regard to potential off-target effects of MOs? 4) Behavioral assays need to be standardized, as an aid to using zebrafish to study neurological diseases found in humans. 5) There is a need to obtain mutants more quickly and there is an issue regarding maintaining mutant stocks. An alternative to keeping mutant stocks is to derive mutants de novo for each investigation. 6) In general, antibody resources for zebrafish are seriously deficient; there was consensus among the attendees on this point. 7) There is a need for a list of common resources. 8) There is a need for a Center (perhaps on a hub and spoke model, where the hub is a coordinating center and the spokes are individual laboratories with specific expertise) to facilitate studies using zebrafish to correlate with studies in humans.

Session 2: Elucidating GWAS "Hits"

Case Study 1, entitled "Genetic determinants of hematologic traits," was presented by Dr. Santhi Ganesh, University of Michigan. Dr. Ganesh provided an overview of large scale gene discovery projects in humans using genome wide association studies, such as the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, the National Heart Lung and Blood Institute's candidate-gene associated resource (NHLBI CARe) and the Framingham SNP Health Association Resource (SHARe). These large collaborative projects have identified many candidate genes and genetic loci that are involved in the production and physiology of red blood cells, white blood cells and platelets, respectively. Dr. Ganesh then discussed specific examples in which orthologous genes in zebrafish were knocked down using MOs and the effects on physiology observed. These studies provided a functional correlation that demonstrated the validity of the human GWAS studies, for example identifying several genes as novel regulators of blood cell formation.

Case Study 2, entitled "Functional validation of GWAS gene candidates for abnormal liver and kidney function during zebrafish development," was presented by Dr. Wolfram Goessling, Harvard Medical School. The first part of this case study involved a discussion of GWAS hits related to tests showing abnormal liver function, which can, for example, predict the presence of fatty liver disease. Major questions include how to make a long list of disease-associated genes approachable for detailed study, and how to translate a quantitative adult phenotype analyzed by GWAS into a qualitative developmental assay, as is provided by the zebrafish. Therefore, the goal of the studies in Dr. Goessling's laboratory is not to provide definitive proof of a phenotype-contributing gene, but, rather, to provide an initial assessment of the biological role of genes to allow prioritization for in-depth biological studies. Further characterization of gene function and impact using the zebrafish system can provide insight into disease pathogenesis and suggest potential therapeutic approaches. One example discussed by Dr. Goessling was the initial assessment of 69 genetic loci associated with elevated human liver enzyme levels suggested 14 gene candidates for knockdown experiments using MOs. The results of these experiments demonstrated that these various gene candidates affected such developmental aspects as the formation or hepatic progenitors and the size distribution of mature hepatocytes. These studies were extended to show that the gene candidates generally increase susceptibility to acetaminophen toxicity.

A second example related to analysis of novel candidate genes for human chronic kidney disease. Knockdown of some of these gene candidates in zebrafish resulted in glomerular abnormalities. Another example is the knockdown of the gene casp9, which results in glomerular dysfunction and causes zebrafish kidneys to be more susceptible to toxic injury, as assessed by the injection of gentamicin into the zebrafish kidney.

Overall, these studies indicate that the zebrafish can be an important tool for understanding the effects of genes identified in human GWAS studies on the developmental functions of genes implicated in chronic disease in humans. The experiments are currently limited because they model loss of function only, and do not yet address allele-specific polymorphisms or multi-gene effects associate with polygenic diseases. Finally, Dr. Goessling pointed out future needs that will both facilitate and extend the knockdown approach, including: a) a fully annotated zebrafish genome to facilitate rapid initial screening; b) complementation of knockdown studies with overexpression studies, which require the availability of a zebrafish ORFeome (complete list of open reading frames); c) better zebrafish models of chronic diseases; d) genome editing that will facilitate direct testing of disease –specific SNPs identified in human GWAS studies, and e) collaboration with population geneticists to improve the development of impactful quantitative phenotypes.

Panel and General Discussion. Dr. Zon joined Drs. Ganesh and Goessling and the topic was opened to the audience for a more general discussion. Comments from this discussion included: a) Investigators need better methods for producing knockout fish that are adaptable to both small and large laboratories. CRISPR/Cas, and perhaps TALEN technologies, in particular were cited as holding great promise; b) There is a problem with converting qualitative phenotypes to quantitative phenotypes. A suite of fluorescent fish lines would be helpful for this. c) There is a need to interface zebrafish with other model systems, such as mice or induced pluripotent stem cells that are examining the same GWAS "hit," or pathway. This should occur early in an investigation. The ability to perform zebrafish assays relatively rapidly versus some of these other systems is of particular utility.

Session 3: High Throughput Screening.

Case Study 1, entitled "Rapid in vivo screening for cell migration inhibitors," was presented by Dr. Shawn Burgess, National Human Genome Research Institute. Dr. Burgess described two systems, migration of the posterior lateral line (PLL) and chemically induced inflammation, respectively, to identify genes involved in cellular migration. Using high throughput approaches, this laboratory has identified several compounds from chemical libraries that affect migration, for example, by inhibiting migration of the PLL cells. Taken together, these studies show the feasibility and utility of using high throughput assays based on the zebrafish to understand the genetic basis of cell migration, an important point for understanding cancer metastasis in humans, and many other developmental and disease-related phenomena.

Dr. Burgess concluded with the following observations: 1) Zebrafish is perhaps the best available model for phenotype screening on a large scale; 2) The evolutionary distance between zebrafish is both a problem and an opportunity; 3) There are still significant needs in regard to automation, embryo handling and imaging; 4) Zebrafish should also be considered as an economical pre-screening system as

an alternative to rodents; 5) New mutagenesis and transgenesis technologies (such as CRISPR/Cas) will reduced barriers to using the zebrafish as a model.

Case Study 2, entitled "Small molecule screening in zebrafish" was presented by Dr. Randall Peterson, Harvard Medical School. He began the presentation by noting that academic researchers can complement, rather than replicate, industrial approaches to drug discovery by targeting important, tractable problems (rather than the large markets often required for success by a company) and by using unconventional approaches that may not be available to industrial scientists. These somewhat different academic and industry approaches can synergize for discovery of new drugs. Many features of the zebrafish system fit well with the academic approach, particularly for developing new, unconventional approaches.

Dr. Peterson discussed the investigations in his laboratory that used high throughput screening of compounds that affect the activity of bone morphogenetic protein (BMP) in zebrafish embryos. BMP is involved in establishing the dorsal-ventral axis during zebrafish development, perturbation of which can be observed readily by the morphology of the embryo. Defects in BMP-based signaling, caused by a mutation in one of the BMP receptors, is involved in the rare disease Fibrodysplasia ossificans progressiva (FOP), a subset of more common heterotopic ossification diseases. A modified, more potent, version of a molecule identified in a zebrafish screen that affected dorsalization was injected into a mouse model of FOP and found to limit disease progression. This example illustrates the utility of zebrafish screens and the progressive use of different animal models in drug discovery.

The second part of this presentation involved high throughput screening for behavioral phenotypes, such as acoustic startle, simple learning, visual response and sleep in zebrafish. The investigators have developed an optical system that allows visualization of an entire 96 well plate of fish, as opposed to most systems in which each well must be visualized sequentially. This system and the resulting behavioral assays are well suited to studying the effects of various compounds that affect the nervous system.

Panel and General Discussion. Drs. Burgess and Peterson were joined by Dr. James Chen (Stanford University) and Dr. Daniel Curtis (Novartis). Dr. Chen's laboratory has developed caged MOs that enable light-controlled gene silencing in zebrafish embryos. These investigators have integrated caged MOs, photoactivatable fluorophores, florescence-activated cell sorting and RNA profiling to investigate factors that affect patterning of the zebrafish embryo. Dr. Chen pointed out that many small molecule screens can be performed using cells, rather than whole animals. However, the details of how various biochemical pathways regulate such complex processes as embryonic patterning or tumorigenesis require the whole animal. Zebrafish are very useful for these investigations, but their full potential will only be realized by the development of new technologies for manipulating and visualizing the genetic programs that regulate complex developmental programs.

Dr. Curtis noted the necessity of understanding the scientific basis of a physiological process before moving studies into humans. A number of different systems, including yeast, Drosophila and zebrafish should be used. Dr. Curtis stated that zebrafish are perhaps best used for questions for which there is no other good model. His laboratory uses cell-based systems when possible. The laboratory emphasizes phenotypic screens. There is a particular advantage for the use of zebrafish in neurobiology, since

anatomy can be examined. Dr. Curtis cited a need for conditional knockouts for examining neurobiological development and phenotypes.

The general discussion noted the following: 1) Chemical screening is not limited by the number of embryos. However, automated methods to aliquot embryos into plates are needed. 2) Another issue is the expensive instrumentation required for fluorescent imaging. 3) A Center for chemical screening is necessary. Existing chemical screening centers (for example, the NIH Chemical Genomics Center) could potentially be used, if adapted to zebrafish. Alternatively, it may be necessary to develop a new screening Center that concentrates on zebrafish.

Session 4: From Tank to Bedside.

Case Study 1, entitled "The zebrafish and TB: three insights and two 'case studies'," was presented by Dr. Lalita Ramakrishnan, University of Washington. Dr. Ramakrishnan began by noting the significant global burden of tuberculosis, exacerbated by widespread multidrug resistance. Zebrafish infected by *Mycobacterium marinum* (*Mm*) form tuberculous granulomas very similar to those caused by *Mycobacterium tuberculosis* in humans. Studies of *Mm* infection of zebrafish have provided many insights into human tuberculosis, including the fact that the granuloma is dynamic and promotes infection and that bacterial tolerance is mediated by efflux pumps in growing bacteria present in the granuloma.

Dr. Ramakrishnan presented two case studies in which zebrafish were used to suggest potential therapies for human tuberculosis. First, studies starting with the zebrafish and moving into cultured human macrophages demonstrated that the efflux pump inhibitor, verapamil, can reduce tolerance to multidrug therapy. These studies were extended to mice and are the subject of clinical trials in India and possibly in South Africa. A second series of studies examined the role of inflammation in tuberculosis. The pathways by which tumor necrosis factor (TNF) mediates tuberculosis pathogenesis in the presence of proinflammatory genotypes were elucidated using zebrafish infected by *Mm*. These investigations identified two oral drugs, alisporivir and desipramine, that, based studies in zebrafish, may be used as potential therapeutic interventions in humans. Equally important, they suggest that adjuvant glucocorticoid therapy that is currently used for all cases of TB meningitis should only be used for patients with the high inflammatory genotype, as they may actually harm those with the low inflammatory genotype.

Case Study 2, entitled "From tank to bedside: case studies on kidney diseases" was presented by Dr. Friedhelm Hildebrandt, Boston Children's Hospital. Dr. Hildebrandt pointed out that many chronic kidney diseases are single-gene disorders, although different mutant genes may cause the same disorder in different patients. For example, cystic kidney disease is largely a single gene disorder with more than 78 genes causing the condition in various patients. Many of these mutations affect the activity of genes involved in the structure of cilia in cells and are therefore termed "ciliopathies." These investigators often begin analysis by using whole exome capture coupled with massively parallel sequencing. This method yields hundreds of variants from the normal human reference sequence, only one of which is the true cause of the disease. To filter the data these investigators have used homozygosity mapping of affected siblings followed by MO knockdown in zebrafish. In general, these investigators find the knockdown of gene expression in zebrafish using MOs recapitulates renal and

extra renal phenotypes of recessive human diseases. Furthermore, multiple (intermediate) pathogeneses can be observed simultaneously in zebrafish knockdown models of disease. Dr. Hildebrandt detailed several examples of this approach including linkage of genes in the DNA damage response pathway to nephronophthisis-related ciliopathies and the linkage of the ARHGDIA gene to nephrotic syndrome. The extensive series of investigations performed by this laboratory illustrate the power of knockdown of genes in zebrafish coupled with human genetic mapping and analysis to determine the causes of many ciliopathies.

Panel and General Discussion. Drs. Ramakrishnan and Hildebrandt were joined by Dr. Thomas Look (Dana Farber Cancer Institute) and Dr. Brant Weinstein (National Institute of Child Health and Human Development). Dr. Look described the utility of using zebrafish to understand the developmental pathways that are subverted in human cancers. He pointed out that the task is both to facilitate the application of basic research, for example from zebrafish, to clinical studies and to facilitate the use of zebrafish by clinical researchers. An important aspect of these translational interactions is training activities such as workshops that will bring together clinical researchers and zebrafish laboratories. Dr. Weinstein described activities aimed at understanding the embryonic origins of the vertebrate vascular system. Zebrafish have many advantages for these studies, including small size and short generation times, ease of manipulation of embryos and optical transparency.

RECOMMENDATIONS

The community of researchers at the Workshop provided recommendations for activities and initiatives that can facilitate the use of zebrafish for translational research. These recommendations came both from the panel discussions and an extensive general discussion that involved the entire audience. These recommendations can be segmented as follows:

Centers: There was a consensus that two types of Centers should be developed.

- A GWAS confirmation center will provide rapid facilitation, advice and training to both clinicians and zebrafish researchers. This center could also provide resources to rapidly create zebrafish mutants that are orthologous to human GWAS “hits” (for example, using CRISPR/Cas) and could provide access to MOs and genome libraries. Workshop participants suggested that a hub and spoke model might work best for this type of Center. The hub would be a central resource that can link researchers with laboratories with specific expertise (the spokes). The hub would be responsible for providing coordination and access to the spokes, which have specific expertise in screening and phenotyping, and which would likely have expertise in specific physiological systems or organs.
- A Chemical Screening Center will implement and improve screening technologies and will educate, train and advise researchers regarding screening of small molecules using zebrafish. The participants favored the idea of a single, integrated Center rather than a hub and spoke model. Industry could play an active role in this type of Center.
- A cost recovery plan for centers must be put in place that can at least partially defray operating costs.

Tools, Technologies and Conceptual Advances: The need for new or improved tools and methods was noted. These include:

- An enhanced, more completely annotated genome assembly.
- A zebrafish ORFeome.
- Improvements in MO-based technologies, including:
    - MO standards for GWAS –related experiments.
    - Improved MOs that have reduced off-target effects and that can be developed more rapidly ("MOs, version 2").
- Further development of CRISPR/Cas technologies, including validated production at centralized sites. Some participants thought that the CRISPR/Cas technology is already working very well for zebrafish and may largely supplant use of MOs.
- Standardized protocols for workup of zebrafish organs, fluorescent lines, etc., that are agreed upon by the community.
- Standardized phenotyping protocols made generally available to the research community. This can be facilitated through ZFIN, which may require additional support for this and other information-related activities.
- Improved non-fluorescent methods to measure gene expression.
- Improvements in the automation of high throughput screening, including:
    - Automated imaging protocols
    - Methods for aliquoting and orienting embryos and standardizing the position of fluorescent images, particularly for chemical screening.
    - Improved software for both handling and data analysis.
- Improved behavioral screens.
- Enhanced analysis of environmental influences such as feeding and exercise.
- Enhanced ability to follow up the results of chemical screens to achieve target identification and validation.
- Rubrics for the threshold at which a GWAS "hit" should be examined.
- Fish with human alleles.

Education, Communication and Information. The participants noted the increased need for communication, information management and training as translation-based technologies are developed. Examples include:

- Improved mechanisms for informing clinicians about the value of zebrafish for validating GWAS "hits."
- Workshops and courses on zebrafish technologies.
- User group blogs, perhaps through the Zfin database.
- Methods to provide advice to researchers and clinicians regarding chemical screening.
- Centralized information regarding chemical screening, GWAS studies and improvements in technology, perhaps through the Zfin database.
- Improved methods for facilitating the participation of zebrafish researchers in clinical meetings.
- A zebrafish book aimed at clinical researchers.

APPENDIX 1: ORGANIZING COMMITTEE

Dr. Shawn Burgess
National Human Genome Research Institute
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Dr. Michael Chang
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Dr. John Harding (Chair)
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Dr. Randall Peterson
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Dr. Lalita Ramakrishnan
Department of Microbiology
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Dr. Rebekah Rasooly
National Institute of Diabetes and Digestive
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Dr. Alexander Schier
Department of Molecular and Cellular Biology
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Dr. Monte Westerfield
Institute of Neuroscience
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Dr. Leonard Zon
Division of Hematology /Oncology
Boston Children's Hospital
Howard Hughes Medical Institute, Harvard Medical School

NIH Workshop

From Tank to Bedside: Zebrafish and Translational Research

October 29 – 30, 2013

DCM/ORIP/DPCPSI/OD
National Institutes of Health
Natcher Conference Center, NIH Campus
Bethesda, MD 20892

Agenda

3:00p.m. – 3:20p.m. **NIH Welcome and Introduction**

3:20p.m. – 4:00p.m. **Keynote Address**

- **Status and Opportunities for the Use of Zebrafish in Translational Research**
  Dr. Leonard Zon, Boston Children's Hospital/Howard Hughes Medical Institute

4:00p.m. – 4:20p.m. **Break**

4:20p.m. – 5:40p.m. **Session 1**

- **Exome Screening and Correlation with Human Studies**
  (Two 20 min. case studies followed by a 40 min. panel/audience discussion)

  Chair: Dr. Monte Westerfield, University of Oregon

  Case Study 1: Dr. Monte Westerfield

  Case Study 2: Dr. Nicholas Katsanis, Duke University

  Panel: Dr. Monte Westerfield
  Dr. Nicholas Katsanis
  Dr. Alan Beggs, Boston Children's Hospital
  Dr. William Gahl, NHGRI/NIH

5:40p.m. **Day 1 Concludes**

8:30a.m. – 9:50a.m. **Session 2**

- **Elucidating GWAS "Hits"**
  (Two 20 min. case studies followed by a 40 min. panel/audience discussion)

  Chair: Dr. Santhi Ganesh, University of Michigan

  Case Study 1: Dr. Santhi Ganesh

  Case Study 2: Dr. Wolfram Goessling, Harvard Medical School

  Panel: Dr. Santhi Ganesh
  Dr. Wolfram Goessling
  Dr. Leonard Zon

9:50a.m. – 11:10a.m. **Session 3**

- **High Throughput Screening**
  (Two 20 min. case studies followed by a 40 min. panel/audience discussion)

  Chair: Dr. Shawn Burgess, NHGRI/NIH

  Case Study 1: Dr. Shawn Burgess

  Case Study 2: Dr. Randall Peterson, Massachusetts General Hospital

  Panel: Dr. Shawn Burgess
  Dr. Randall Peterson
  Dr. James Chen, Stanford University
  Dr. Daniel Curtis, Novartis

11:10a.m. – 11:40a.m. **Break**

11:40a.m. – 1:00p.m. **Session 4**

- **From Tank to Bedside**
  (Two 20 min. case studies followed by a 40 min. panel/audience discussion)

  Chair: Dr. Lalita Ramakrishnan, University of Washington

  Case Study 1: Dr. Lalita Ramakrishnan

  Case Study 2: Dr. Friedhelm Hildebrandt, Boston Children's Hospital

Panel: Dr. Lalita Ramakrishnan
Dr. Friedhelm Hildebrandt
Dr. Thomas Look, Harvard University
Dr. Brant Weinstein, NICHD/NIH

1:00p.m. – 1:20p.m. **Break**

1:20p.m. – 2:20p.m. **Final Discussion and Wrap-up, Including Recommendations for New Initiatives**

Moderator: Dr. Jack Harding, NIH

Panel: Dr. Shawn Burgess
Dr. Santhi Ganesh
Dr. Lalita Ramakrishnan
Dr. Monte Westerfield
Dr. Leonard Zon

2:20p.m. **Workshop Concludes**