Summary of the "Animal Models Impacting Human Disease", the Tenth Comparative Medicine Resource Directors Meeting

August 12-13, 2014

Introduction

"Animal Models Impacting Human Disease," the Tenth Comparative Medicine Resource Directors Meeting, was held August 12-13, 2014, in Bethesda, Maryland. All Resource Directors funded by the Office of Research Infrastructure Programs (ORIP)/Division of Comparative Medicine (DCM) were invited to attend. This biannual meeting provides a forum to (1) Exchange new information, advances, and ideas among grantees and NIH staff members from several Institutes and Centers (ICs), (2) Increase collaborations and sharing among DCM-funded Resources and between these Resources and various NIH ICs, (3) Inform Resources and NIH staff about accomplishments and challenges, and (4) Identify Resource-related scientific advances on evolving animal-human correlations, emerging technologies, and reproducibility in animal models of human disease. There were 61 Resource Directors and personnel from 46 DCM-funded resources from 19 states and Puerto Rico as well as 30 NIH staff representing 9 ICs and the Office of the Director (OD) at the meeting. The attendees included the Principal Investigators of DCM-supported centers funded by contracts, P40, U24 or U42 grant mechanisms, as well as some grantees that have Resource-related projects funded via the R24 mechanism. There were six sessions with 26 presentations and 56 posters that covered aspects of "Optimizing Strategies for DCM-Supported Resources," "Promoting a Resource's Animals and Services," "Resource-Related Scientific Advances: Evolving Animal-Human Correlations and CRISPR Technology," " Resource - Related Scientific Advances: Impact of the "Reproducibility" of Animal Preclinical Studies on Animal Model Resources," "Growing a Resource via Alternative Support," and "Administrative Practices at NIH-Supported Resources."

Meeting Agenda

Dr. Manuel Moro, DCM, welcomed the attendees and Dr. Stephanie Murphy, Director of DCM, provided opening remarks and introduced the keynote speaker, Dr. Norbert Perrimon, Harvard Medical School, whose lecture was devoted to Drosophila Resources for functional genomics and understanding communication between cells and organs in this model organism. Dr. Perrimon highlighted the Drosophila RNAi Screening Center, the Transgenic RNAi Project (TRIP) and the RNAi Stock Validation & Phenotypes (RSVP) database, developed in collaboration with the Berkeley Drosophila Genome project, Bloomington Drosophila Stock Center, FlyBase, and the Drosophila Genomics Resource Center. Dr. Perrimon uses combinations of genome-wide RNAi and large-scale mass spectrometry to identify components of signaling networks and to characterize their activities using readouts such as phosphorylation changes, transcriptional changes, transcriptional outputs, and cellular phenotypes. Dr. Perrimon's laboratory has developed COMPLEAT (Protein Complex Enrichment Analysis Tool), an online tool for analysis of high-through datasets (or small-scale datasets) using protein complex enrichment analysis as the backend annotation data instead of conventional Gene Ontology (or pathway) based

annotations. Recently, Dr. Perrimon has begun using clustered regularly interspaced short palindromic repeats (CRISPR)-based knockout strategies in combination with RNAi to probe the functional redundancies within networks. He also described the efforts to extend signaling network studies beyond cell lines to complex tissues and implementing a proximity labelling approach, based on the engineered peroxidase enzyme ascorbate peroxidase (APEX) allowing characterization of subcellular proteomes in live tissues. Results of in vivo validation of insulin signaling network were presented. Details of the online resources and their potential use were described. TRIP currently has 9,624 stocks (RNAi and toolbox) that have been sent to the Bloomington Drosophila Stock Center for their distribution. The RSVP database is an online tool to evaluate the performance of existing and new TRIP stocks; it currently holds 5,963 data entries.

Session 1 of the meeting, **"Optimizing Strategies for DCM-Supported Resources,"** was moderated by Dr. Jack Harding, DCM, and Dr. James Coulombe, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Several aspects of DCM supported Resources were highlighted, including (a) Usage – continue relevance of models to a given research community; (b) Service – are research community needs being met?; (c) Innovation -- making models more useful and accessible to researchers and making new models available; (d) Sustainability – operational costs and recovery; and (e) "Sensing" – contribution of Resources toward understanding changes in the field based on requests and conversations with users and the research community.

Technologies that may impact DCM Resources include both "disruptive" and non-animal based technologies ("alternatives"). Examples of "disruptive" technologies that were mentioned included CRISPRs, inexpensive whole genome sequencing, RNAseq, iPS cells, and other areas (i.e., imaging, phenotyping, archiving). Examples given for non-animal based technologies included procedures first done in humans, tissues on a chip, cultured cells, and in silico approaches.

"Breaking News" at NIH included the new National Primate Research Center Research and Capabilities Inventory Website (http://nprcresearch.org) which was highlighted as a potential web-site model for access to multiple resources. This is a publically available website that provides investigators and program officers with an on-line resource to facilitate collaborations. The website was shown and several features highlighted such as what species are available from what centers, service/expertise available at each site, etc. The website also provides links to all National Primate Research Centers. A similar approach could be used for smaller Resources supporting nontraditional/nonmammalian models.

Interactive Topic 1, which focused on "Challenges and Opportunities for Creating New Animal Disease Models," was initiated by Dr. Coulombe who reminded the audience about effective

ways to communicate with the NIH about new or specific research community needs. The importance of white papers was emphasized as a mechanism of "sensing" from the scientific community to provide feedback to the NIH. DCM-supported Resources were strongly encouraged to involve the NIH as early as possible in the process of developing workshops and resulting white papers.

Other questions and items discussed by audience members included:

- How can DCM Resources help in specific, critical, "real time" situations? The role of some Resource Centers in the ongoing epidemic of Ebola was cited as an example.
- How could there be better collaborative interactions across the different Resources? Having better integrative and informatics tools across the Resources will likely have a greater and more innovative impact on biomedical research.
- Need to continue identifying and funding technologies that will work for precision modeling.
- Problems with licensing the models that are being created and consequent restriction in accessibility to these models. Restricting licensing in RFAs was proposed.
- Need to identify natural models and follow-up with whole genome sequencing.

Interactive Topic 2, on "Informatics Challenges and Opportunities," initiated a discussion led by Dr. Harding on the disconnect between genotypes and collection of disease-related phenotypes. Specific items discussed by the audience included:

- Concerns about how little shared phenotypic data exists in contrast with genomic data.
- A Phenome project was proposed where one could align phenotypes from several known animal model species with shared genome end points.
- The goal of the Monarch Initiative is to look at strategies to structure the data and to query access to many phenotypes.
- A key challenge is that different communities work with different ontologies.
- It was strongly recommended to have a "Phenomics" meeting to address all of the relevant points discussed at the Tenth Comparative Medicine Resource Directors Meeting.

Interactive Topic 3, on "New Initiatives and Directions for Resources," was introduced by Dr. Harding and included the following points of discussion and recommendations:

- Need to look at genotype-environment interactions and personalized medicine.
- Consensus on best practices in reporting results.
- Consideration of variables that affect phenotypes (e. g., microbiome, diet, environment).

- Advantages of using marmosets vs. rhesus macaques.
- Centralization of steps for the process of making transgenic NHPs.
- Need for clinical therapeutic platforms matched to genetic mutations.
- Best use and promotion of the available Resources – examples of Resource maximization included short courses, You Tube videos, and the NPRC Research and Capabilities Inventory Website.
- Alternative approaches to organize Centers: concept specific rather than species specific. There is a need to have different models in the same place (Centers of Comparative Medicine). Geographic barriers could be overcome with evolving communication technology (virtual universities).
- Centralized website with links to all the Resource websites - a unified online Resource. Resources could offer training such as online modules, courses, technical concepts, etc. It was recommended to use human diseases as the link.

Interactive Topic 4, "Correlating Knowledge of Animal Models with Human Biology and Disease," included remarks by Dr. Cheryl Marks, National Cancer Institute (NCI), who discussed NCI's mouse consortium strategy to reach out to other disciplines/clinicians working in cancer by (a) Getting together clinicians and the cancer modelling community at a specially designated "Oncology Models Forum" and (b) Utilizing the "Hope Zero" program which allows collaborations in real-time and in cyber space. NCI is interested in how to model cancer and use models more effectively. FOAs on Oncology Models Forum [PAR-14-239], Collaborative Research Projects Related to Mouse Models for Translational Research [PAR-14-240] and Research Projects to Enhance Applicability of Mouse Models for Translational Research [PAR-14-241] were mentioned.

Additional points of discussions included:

- How much information should be reported for animal models to ensure reproducibility?
- Historically, the role of DCM Resources has been: (a) Preservation, archive of models, (b) Hypothesis generation and (c) Hypothesis testing. Do Resources need to consider changing (evolving) from an emphasis on animal/disease to molecular pathways?
- There is an imperative need to make Resources better known to clinicians as well to engage them in projects. Resources should explore partnerships with Clinical and Translational Science Awards funded Institutions.

Session 2, titled "**Promoting a Resource's Animals and Services,**" was moderated by Dr. Moro and consisted of ten examples of three to five minute "Elevator Speeches" or "Promotional Messages" about a Resource via oral or video formats. The speakers defined the Resource, described services, summarized customer benefits, identified challenges, and stressed solutions

to overcome these challenges. The goals of these presentations were to promote the Resource to NIH staff and to other Resources as well as to encourage collaborations among Resources and the various NIH ICs.

Elevator speeches and promotional messages from newly DCM-funded Resources, new attendees, and established Resources were presented by: Dr. Jeffrey Wall, University of California, San Francisco, "Development of a Pedigreed Baboon Genome Resource for Biomedical Research;" Dr. Calum MacRae, Brigham & Women's Hospital/Boston Children's Hospital/Harvard Medical School, "A Community Zebrafish Resource for Modeling GWAS Biology;" Dr. Melween Martinez, University of Puerto Rico, "Caribbean Primate Research Center;" Dr. Craig Hodges, Case Western Reserve University, "Animal Model Resources for Cystic Fibrosis;" Dr. Cynthia Bethea, Oregon Health and Science University, "Postmenopausal Monkey Resource;" Dr. David Langenau, Massachusetts General Hospital, "Immune Compromised Zebrafish for Cell Transplantation;" Dr. Larisa Poluektova, University of Nebraska Medical Center, "Center for Humanized Mice Development;" Dr. Roman Wolf, University of Oklahoma Health Sciences Center "University of Oklahoma Health Sciences Center Specific Pathogen Free Baboon Research Resource;" Dr. Michael Brehm, University of Massachusetts Medical School "Novel NSG Mouse models for Human Stem Cell Therapy;" and Dr. Keith Cheng, Penn State University College of Medicine, "Pancellular Tissue Tomography."

Session 3, titled "**Resource-Related Scientific Advances:** Evolving Animal-Human Correlations and CRISPR Technology" included 2 segments moderated by Dr. Oleg Mirochnitchenko, DCM, and Dr. Lorette Javois, NICHD, on (a) Evolving Animal-Human Correlations-The Undiagnosed Diseases Program and (b) Impact of CRISPR/Cas9 Mutagenesis on Resources of Various Species [KOMP^2 Common Fund Supported Supplements for CRISPR Pilots]. The first segment was presented by Dr. William Gahl, Director of the Undiagnosed Diseases Program, a trans-NIH program. He described several examples of rare, undiagnosed diseases and the criteria used to select potential patients for participation in the program. Most of these individuals have unique genotypes, many of which have not yet been characterized relative to gene function.

The second segment of this session was presented by a panel of speakers which included Dr. Steve Murray, The Jackson Laboratory, Dr. Art Beaudet, Baylor College of Medicine, and Dr. Kent Lloyd, University of California, Davis. An overview of the technology and a general introduction to genome editing tools were provided. Advantages and disadvantages of the system were discussed, CRISPRs are simple to design, can target any spot on the genome, etc., as well as differences in cost with other technologies. Examples of using CRISPRs in several laboratory animals were provided. The overall conclusion was that CRISPR techniques save time and money.

The first day of the meeting ended with a **Poster Session.** All resources were invited to present Resource- and research-related posters. The Poster Session provided an opportunity for the attendees to have individual and detailed discussions with Resource directors from other scientific fields.

On the second day of the meeting, Dr. James Anderson, Director of the Division of Program Coordination, Planning, and Strategic Initiatives, welcomed the attendees and talked about enhancing efficiency of core facilities.

Session 4, titled **"Resource-Related Scientific Advances: Impact of the Reproducibility of Animal Studies on Animal Model Resources,"** was moderated by Drs. Moro and Marks and consisted of six presentations addressing the topic of reproducibility of animal studies in biomedical research. Dr. Lloyd started the session by introducing the definition of "Reproducibility" as it applies to research. He stressed the dramatic increase in the number of research publications that has been accompanied with a significant number of researchers expressing their concern regarding their inability to duplicate results of the original publications. Dr. Lloyd described the efforts made by members of the KOMP^2 to ensure reproducibility of the results reported by this project.

Dr. Stuart Zola, Emory University, presented results of a recent discussion of reproducibility issues at the Institute for Laboratory Animal Research meeting in Washington DC on June 4-5, 2014. One of the main concerns of the biomedical community is that current efforts to reduce numbers of animal used in research might contribute to the problem of the inability of several investigators to obtain similar results as those reported in earlier scientific articles. Possible solutions to address the high number of false positive results in studies using animals include ensuring randomization, quality control, and appropriate statistical analysis; providing sufficient details for the methodology description; and having adequate expertise with experimental design and selection of suitable animal models.

Dr. Cathy Lutz, The Jackson Laboratory, discussed specific challenges that animal resources are experiencing regarding reproducibility such as contamination, genetic background/phenotypic reproducibility and extent of education of the user community. A recommendation was made that public repositories should have multiple levels of quality assurance to ensure reproducibility of experimental results obtained using animals from resource facilities.

Dr. Melissa Haendel, Oregon Health and Science University, reported on current efforts of her group to improve complex data integrations for better prediction of animal model phenotypes and human disease ontologies, consequently improving the reproducibility of scientific results. A common problem is poor phenotype coverage of the human genes. Animal models can help to improve this situation and now can be linked to more than 75% of human genes. Joint efforts

are required to evaluate diverse models together to inform disease discovery and promote reproducible data capture.

Dr. Craig Franklin, University of Missouri, presentation was devoted to the effect of the microbiome on the reproducibility of animal studies. There are numerous examples of the significant effects of microbiota on disease phenotypes, including gastrointestinal diseases as well as viral or neurodegenerative diseases. Microbiota is constantly affected by the environment, age of the host, and time of the day among many other factors in humans and experimental animals. He stated that is critical to understand the microbiota differences and their effects on the host.

Dr. Murphy addressed the issue of sex and gender in animal disease models. She emphasized that sex and gender are not the same characteristic but are distinct terms that should not be used interchangeably. Sex is a biological quality that can be applied to both animals and humans whereas gender is a socio-cultural process that is generally applied to humans. The predominant use of a single sex (mostly male) instead of both sexes in animal studies is most likely contributing to issues of reproducibility in research with animal models. NIH is currently developing new policies to balance sex in cell and animal studies. These efforts include the formation of a trans-NIH Sex Differences In Research Working Group, which is actively developing ways for the scientific community to provide input to NIH regarding the issues related to consideration of sex in preclinical research.

Session 5, entitled **"Growing a Resource via Alternative Support,"** was moderated by Dr. Miguel Contreras, DCM, and Dr. Mathew Portnoy, NIH Office of Extramural Research. Dr. Portnoy presented an overview of the NIH Small Business Innovation Research (SBIR) /Small Business Technology Transfer (STTR) program, which included a discussion of the differences between SBIR and the STTR programs, ICs individual budgets for 2014, and success rates for these types of applications. Dr. James Hawkins, FOCUS Investment Bank, Washington, DC, presented a talk about his personal career evolution from academician/scientist to managing director/owner small business. He highlighted the roles and responsibilities that are associated with the business/management aspects of a small business company.

Session 6, entitled **"Administrative Practices at NIH-supported Resources,"** was moderated by Dr. Murphy and consisted of a presentation about Program income for certain types of Resource grants by Mrs. Stacia Fleisher, National Center for Advancing Translational Sciences. Dr. Mirochnitchenko and Dr. Harding provided updates of recent DCM sponsored workshops on zebrafish, regenerative medicine, and personalized medicine as well as new research initiatives/funding announcements based upon findings and recommendations from these workshops.

Dr. Murphy provided concluding remarks which included the following key questions and themes that had emerged and had been discussed during the meeting:

- Finding the most effective ways to communicate and promote DCM resources to the biomedical research and clinical communities.
- Exploring ways to better integrate concepts, disease models, tools and services across Resources.
- How best to use naturally occurring disease models along with genetically engineered models.
- Phenomics – How best to organize and leverage phenotypic information.
- How will centers be defined in the future? Will centers be organized around concepts, approaches or pathways rather than by species and diseases?
- Ensuring reproducibility of animal models and resources.
- What kinds of training can DCM supported Resource scientists offer the biomedical research community?

Based on speaker presentations and attendee discussions at the Tenth Comparative Medicine Resource Directors Meeting, the following next steps were suggested:

- Explore further the concept of "Phenomics" through a workshop evaluating challenges and opportunities related to phenomics; consideration of systems for integrating large amounts of phenotypic data related to disease models; and examination of ontologies that work across species, including humans
- Improve data sharing across Resources and with the research community through development of a web site similar to the National Primate Research Center Research and Capabilities Inventory Website which would integrate concepts, disease models, tools and services across Resources
- Consider future directions that include organizing future centers around concepts, approaches or pathways rather than species and diseases as well as leveraging naturally occurring diseases with genetically engineered disease models

Evaluation forms were provided to the participants, and feedback will be considered in planning the 2016 meeting. Participants were told that additional feedback should be communicated to Dr. Murphy, Director of DCM.