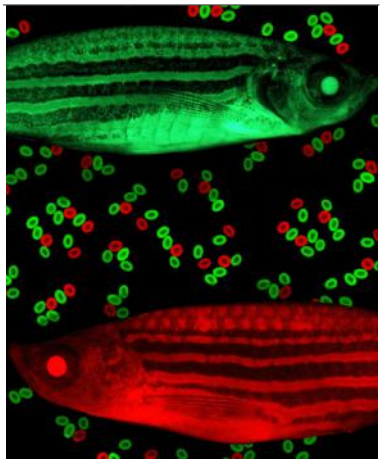
The *RUNX1* Research Program Quarterly Newsletter

Dear Friends,

Our first scientific conference is fast approaching on November 13th in Philadelphia as part of the 21st International RUNX 2017 Conference held at the University of Pennsylvania. Our RUNX1 day is November 13, when recipients of our two grant programs (in partnership with Alex's Lemonade Foundation for Childhood Cancer and the Leukemia & Lymphoma Society) will be featured in talks and breakout sessions. We are grateful to everyone who has made this event possible including generous sponsorships from the Abramson Cancer Center, Alex's Lemonade Stand Foundation, the Leukemia & Lymphoma Society, the Eunice and Irving Leopold Annual Scientific Symposium and Retreat, and our own RUNX1 Research Program. The Editors of the forthcoming book, to be published by Springer Press, "RUNX Proteins in Development and Cancer" have also generously donated their royalties to help support the meeting. Finally, thank you to the head of our Scientific Advisory Board, Dr. Nancy Speck, and the Executive Assistant and Faculty Affairs Coordinator of the Department of Cell and Development Biology at UPenn's Perelman School of Medicine, Venetia Baldwin, who both have worked tirelessly to make the program happen. For session topics and more information, please visit http://runx1.com/philly-2017/program.

This autumn newsletter will include a question-and-answer between Dr. Speck and one of our esteemed grant recipients, Dr. Leonard I. Zon, and highlight some updates to our website, introducing the secure online platform we have selected to facilitate patient connection and dialogue: RareConnect. We have our eyes set on hosting our first patient conference for the end of next year, and are beginning a big 'push' to ensure that all individuals with the germline *RUNX1* mutation know about us. Please help get the word out. If you are a clinician or a genetic testing company, we have a one-page document about us and our efforts that you can share with interested parties: http://www.runx1.com/sites/default/files/RUNX1OnePager.pdf.

Image courtesy of J. Menninger & V. Binder



A Q & A with Dr. Leonard I. Zon

Dr. Leonard I. Zon is the Grousbeck Professor of Pediatric Medicine at Harvard Medical School, an Investigator with the Howard Hughes Medical Institute, and Director of the Stem Cell Program at Children's Hospital Boston. Dr. Zon presides over the International Society for Stem Cell Research, the American Society for Clinical Investigation, is Head of the external investigators of the Zebrafish Genome Institution and Chairman of the Harvard Stem Cell Institute's Executive Committee. He is internationally recognized for his pioneering research in the new fields of stem cell biology and cancer genetics, and is a recipient of one of our grants, in partnership with Alex's Lemonade Stand Foundation for Childhood Cancer (ALSF).

Dr. Nancy Speck: What do you think are some of the important scientific problems to address in FPD/AML?

Dr. Leonard Zon: FPD/AML is a disorder that can cause significant problems. Patients have two major issues. There is thrombocytopenia that can be very difficult to manage and patients can transform to leukemia. The disease was discovered decades ago. Yet we have a poor understanding of how it causes these phenotypes. The platelet deficiency is most likely due to the action of the DNA-binding protein, runx1, and a deficiency of target gene expression of the platelets and their precursors. One major question is simply how many target genes in the platelet lineage are responsible for the thrombocytopenia. Is it possible that there are a few targets and that somehow stimulating the target genes could rescue the thrombocytopenia. For the transformation to leukemia there is even less known. It is possible that there is a clone of stem cells that becomes transformed based on a mutational event or perhaps an event that does not involve mutation (called epigenetic). Defining the mechanism by which the transformation occurs will have an impact on therapies.

Dr. Nancy Speck: Why is zebrafish a good model for addressing some of these problems? What can you do using zebrafish that would hard to do by studying individuals with FPD/AML directly?

Dr. Leonard Zon: The zebrafish is a great model to study FPD and AML. It is possible that the disease initiates during embryogenesis when the thrombocyte lineage and stem cell lineage are first formed. This initiation event is best studied in the zebrafish because of its optical clarity and the ability to use transgenic fluorescent reporters to elucidate stem cells. Fluorescent stem cells are easy to track in the zebrafish embryo. The zebrafish allows for genetics to be done and it is possible to use CRISPR technology in a tissue-specific manner to knockout genes responsible for the disease and also that could rescue the disease. Zebrafish are also seen as a premier model for understanding clonal decisions. Clones of stem cells emerge normally during embryogenesis and this diversity of clones has an impact on the disease. It is possible that as additional mutations occur, there is stress upon certain clones and they transform and become malignant. The zebrafish has a technology to mark each stem cell in a different color. By tracking clones of stem cells or clones of colors, it is possible to define genetic or epigenetic differences that lead to clonal expansion particularly in the background of runx1 mutations. The chemical biology in the zebrafish allows for screening to be done of small molecules for potential cures to the disease, perhaps even preventing the disease from happening.

Dr. Nancy Speck

Dr. Leonard I. Zon

Dr. Nancy Speck: What do you love best about working with zebrafish?

Dr. Leonard Zon: The zebrafish is a fantastic model system. We have been studying it for over 25 years. Zebrafish are transparent as embryos and one can see all of the organs in the circulating blood within 24 hours. We have made adult zebrafish transparent by introducing pigmentation mutations, and called this line, Casper. Use this type of fish for transplantation experiments fluorescent marrow can be placed into the Casper fish and imaged for migrating cells to the marrow. Each zebrafish mother has 100 to 200 babies per week. This makes it facile as a

genetics system. We have found mutants that have blood diseases in the zebrafish and found humans later have mutations in the same genes as our fish. We also can add chemicals to the water and see interesting events occur such as an expansion of stem cells or clones.

Dr. Nancy Speck: How many zebrafish and fish tanks do you have in your lab, and how many people do you need to take care of them?

Dr. Leonard Zon: We currently have 300,000 fish in our facility at Boston Children's Hospital and at Harvard. We also have roughly 3,000 tanks. We have developed the fish husbandry in the zebrafish system. We currently have a Fish manager and two technicians who manage the entire facility, particularly focusing on feeding the fish. The embryos are now fed by a robot. We also perfected a mating chamber. The iSPAWN allows for the production of 10,000 embryos in 10 minutes. This facilitates our chemical screening processes.

Dr. Nancy Speck: What are you studying in your lab using funding from the RUNX1 Research Program?

Dr. Leonard Zon: We are using the zebrafish to study FPD/AML. Using the Zebrabow system, we have developed a color system to mark each stem cell with a different color. We have shown using this system that there are 20 stem cells that originate in the developing embryo. Using CRISPR technology we can inactivate the genes that are mutated in human FPD and AML and establish an excellent model of the disease. Clones marked by different colors will allow us to witness single colors that are transformed to MDS, and the clones can be studied because they can be sorted on a cell sorter. By studying clones and seeing how they transform to leukemia, we should be able to drill down on the mechanism by which the disease occurs. Chemical screening is very facile with the zebrafish system, and we plan to undertake a screen to find small molecules that would affect clone size in our model, thereby having an impact on the development of the disease.

RUNX1 **Research Program Website Updates**

Over the last month we have made some changes and updates to the website. We have added a search bar to assist with navigation, more content and detail have been added to the Frequently Asked Questions section, http://www.runx1.com/faq, and we have created a section called Clinical Care, http://www.runx1.com/learn/clinical-care, which will be continually updated as we are made aware of new and best practices in terms of clinical management of the disorder. We would like to give special thanks to one of our Scientific Advisors, Dr. Lucy Godley, for her time and efforts spent reviewing and making edits to our content. Under Learn > Additional Resources, we have provided links to other relevant websites, and have now added a simple one-page pdf document about our organization for both clinician-researchers to share with patients and for media and press inquiries. This can be found in our About > Newsroom section or directly at http://www.runx1.com/sites/default/files/RUNX1OnePager.pdf. When details of our family conference become available, we will add a registration page through our website and forward the link. We will use our Facebook page as a way to keep followers updated on our news and progress, and to spread word of our organization.

RareConnect

The *RUNX1* Research Program has chosen to partner with RareConnect to facilitate patient dialogue in a safe, secure online space. The online community aims to not only support patients' needs by fostering dialogue, but also to help identify and understand the challenges faced by people living with *RUNX1* FPD/AML. Should broad, recurring issues arise, these can be brought to the attention of researchers for further exploration. Through this community, patients and those who care for them can communicate and share experiences and information in a safe, moderated online forum. We hope and encourage you to register and join the conversation on our site through the following link: https://www.rareconnect.org/en/community/runx1-fpd-aml/members. Your story is an important part of finding the solution.

Our upcoming winter newsletter will highlight some of the scientific updates gleaned from the November Philadelphia conference, and announce the grant recipients for our second joint grant cycle with Alex's Lemonade Stand Foundation. Thanks for your continued interest and support.

Tim and Monica Babich