



National
PKU
Alliance

RESEARCH UPDATE



The National PKU Alliance is pleased to announce its 2015 Research and Post-doctoral Fellowship Awards! These awards are made possible by our member organizations and individuals who raise funds each year on the local level for research. Thank you for helping make these awards possible as we work towards improving treatment options for PKU and accelerating the timeline for a cure.



Katherine Durrer-Deming, MS at the University of North Texas was awarded a grant with Dr. Michael Allen to continue their work in developing a genetically engineered probiotic to treat PKU. Previous efforts have demonstrated the efficacy of a genetically modified *Lactobacillus reuteri* bacterium to lower blood

phenylalanine (phe) levels in a mouse model of PKU when administered as a probiotic with food. Their proposal builds on those findings to develop a humanized variant suitable for probiotic-based treatment of PKU appropriate for use in human clinical trials. This grant follows two years of funding of Ms. Durrer-Deming's research where she was able to get promising and preliminary data for the mouse version of a PKU probiotic treatment. This new grant will continue to fund Ms. Durrer-Deming's work so she can take her research to the next level - the development of a human variant appropriate for clinical trials.

view of the safety and efficacy issues related to the therapeutic application of hPAH, Paulo will refine this approach using targeting and co-encapsulation strategies for further *in vitro* validation in relevant cell lines. The success of the proposed research work may constitute a potential enzyme reposition therapy targeting the full spectrum of PKU patients.



Dr. Nicholls is Professor of Pediatrics in the Division of Medical Genetics at Children's Hospital of Pittsburgh of UPMC. Dr. Nicholls proposes to develop an improved, clinically relevant animal model, a swine, for PKU in order to study and understand the biomedical bases and to develop therapies for the

metabolic disease. He has tremendous experience in the development of animal models especially for Prader-Willi syndrome in both mouse and swine models and over the years has developed outstanding collaboration with other animal model experts like Dr. Randall Prather at the University of Missouri. They will be using CRISPR/Cas9 technology to develop a pig model for PKU. They have assembled the entire pig PAH gene containing 13 exons and encoding a 452 amino acid enzyme. They also have confirmed high expression of PAH in pig liver, moderate expression in kidney, and low expression in brain. Therefore, they have the tools in place to produce a porcine model of PKU. In addition to producing minipigs with the PKU genotypes, they will characterize the biochemical and preliminary neurobiological phenotype of the pigs with PKU. This swine model will take us closer to the human and will be necessary for regulatory approval as we approach new genetic and stem cell solutions for a cure.



Dr. Paulo Roque Lino at iMed.Ulisboa (Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, Portugal) was awarded a fellowship to focus on the development of an Enzyme Reposition Therapy approach to PKU. This novel strategy intends to administer the functional

human Phenylalanine Hydroxylase (hPAH), which is deficient in PKU patients. His previous results proved it is possible to formulate the hPAH, thus overcoming the limited impact of currently available treatments Dr. Lino has already designed a strategy to form nanoparticles containing hPAH, which fully preserved the enzyme's integrity and functionality. Hence, in



Dr. Eddy van der Zee at the University of Groningen and at the University Medical Center of Groningen in The Netherlands and his team are studying the effects of Large Neutral Amino Acids (LNAA) on phe levels and neurotransmitters in the brains of PKU mice. They have switched to the BTBR

Pah-enu2 PKU mouse model from the C57Bl/6 PKU mouse because the BTBR PKU mouse shows a clearer behavioral phenotype than the C57Bl/6 PKU mouse. The study is being performed in the BTBR Pah-enu2 PKU mouse model with wild-type mice as controls. The aim for their second year of funding will be twofold: Firstly, they will perform the biochemical and molecular analyses in blood and brain of the mice that have received one of the different LNAA treatment regimens or one of the control diets during the first year of this study. Secondly, they aim to perform a new mouse experiment to investigate behavioral outcome (in the domains of cognition, mood and motor performance) in relation to biochemical and molecular treatment effects of two optimized LNAA treatment regimens in comparison to a Phe-restricted diet. This study will be the first to investigate behavioral outcome parameters in relation to biochemical treatment effects of LNAA treatment in PKU mice. Behavioral tests to be performed will assess learning and memory, mood, and motor control. Thereby, the effect of LNAA treatment on all areas of PKU symptomatology will be investigated. The combination of investigating both biochemical and behavioral effects of treatment offers the possibility to assess the relevance of the observed biochemical effects. Moreover, this study will be the first to compare LNAA treatment with a Phe-restricted diet. This will enable assessment whether LNAA treatment might offer a possible alternative treatment strategy for the burdensome Phe-restricted diet. In both ways, the results of this study will stimulate the development of optimal LNAA treatment for PKU patients and show the clinical relevance of its effects on a biochemical level.



Dr. Roberto Gramignoli was the first NPKUA post-doctoral fellow at UPMC under the supervision of Dr. Stephen Strom working on the hepatocyte transplant project with the PKU mouse model. In 2012, both Drs. Strom and Gramignoli moved to Karolinska Institutet

in Stockholm, Sweden, to continue their studies on human hepatocytes generated by stem cell sources and provide help to establish the Scandinavian Hepatocyte Transplant program. Dr. Gramignoli is now Assistant Professor at Department of Laboratory Medicine (Pathology Division) at the Karolinska Institutet. The research team in Sweden is pursuing the use of human amnion epithelial (hAE) cells transplantation for curing PKU and several additional

liver-based metabolic diseases. These studies have been performed in the past, and are still ongoing, in collaboration with Dr. Kristen Skvorak (at UPMC), supporting her hAE cell studies that the NPKUA is also funding. Dr Gramignoli/Strom team is starting placenta stem cell (hAE cell) banking for clinical use in the first half 2015 and funds will support the clinical Good Manufacturing Practices (cGMP) that are required for harvesting, storing, and preserving hAE cell suspensions for transplantation in PKU patients and more. The funds will support a trained technician to work in the cGMP facility and GMP supplies needed for the banking processes for the PKU related research and for technical personnel using that facility. Finally, encouraged by the well-known immune-modulatory and anti-inflammatory effects characteristic of hAE cells, Dr. Gramignoli proposes to test the hypothesis that hAE cell transplants could correct metabolic liver diseases, hopefully avoiding immunosuppressant therapy.



Dr. Skvorak at the University of Pittsburgh Medical Center will continue with her study of transplanting human amnion epithelial (hAE) stem cells in the PKU mouse model. Dr. Skvorak continues to work collaboratively with the Karolinska Institutet team under Dr. Steve Strom's guidance and Dr.

Jerry Vockley at Children's Hospital in Pittsburgh. Dr. Strom's laboratory is providing the human AE cells that Dr. Skvorak is using in these studies at UPMC. In the first year of support she injected hAE cells directly into the livers of young PKU mice and found that both the brain and blood levels of phe become significantly lower, which agreed with results she found previously with hepatocyte transplant studies in these animals. She also found a significant sex difference in the PKU mice. Untreated female PKU mice have higher blood phe levels (1.6 fold) than their male counterparts, though no sex difference was found in brain phe levels. This appears to be unique to the mouse model as this has not been described in patients. For the second year renewal, Dr. Skvorak has 2 aims: to study the impact of adding BH4 to the transplant recipients; and secondly, to evaluate the need for immunosuppressive treatment of the transplanted mice.

The NPKUA's Scientific Advisory Board is made up of eminently qualified physicians, researchers, and clinicians who are leaders in their fields to evaluate the proposals the NPKUA receives each year.

Members include: Thomas Franklin, PhD; Harvey Levy, MD; Kathryn Moseley, MS, RD; Ray Stevens, PhD; Bryan Hainline, MD, PhD; and Uta Lichter-Konecki, MD, PhD.; Cary Harding, MD, FACMG; Desiree White, PhD; Jessica Cohen, MD; Francjan J. van Spronsen, MD, PhD.

Each year this board goes through a rigorous evaluation process to select those proposals that will improve treatment options and accelerate the timeline for a cure.