



National
PKU
Alliance

RESEARCH UPDATE



The National PKU Alliance is pleased to announce its 2016 Research and Post-doctoral Fellowship Awards! These awards are made possible by our member organizations, *Lifting the Limits for PKU national events* 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.



Dr. Juan Cabrera-Luque is Research Assistant Professor at George Washington University. His research aims to establish a novel *in vitro* model to study the effects that high blood phenylalanine concentrations have at the human blood brain barrier level. To reach this goal, Dr.

Cabrera-Luque will develop a cell culture model that more realistically represents the human blood brain barrier by growing in layers the different cell types found on this tissue in combination with the HemoShear Vascular System technology (developed by HemoShear Therapeutics). Once established, this model will be used to study the effects of high blood phenylalanine concentrations at the human blood brain barrier level. Among other results, he expects to identify potential new targets to develop new, or improve current, therapeutic approaches to PKU (such as large neutral amino acids treatment). This project represents the initial steps towards an invaluable screening tool to test different strategies for the treatment of PKU at the blood brain barrier level.

a Phenylalanine Ammonia Lyase (PAL) gene. Once produced, PAL enzyme activity will be verified in cell culture extracts. To confirm efficacy and safety, a large group of mice will be fed the treatment probiotic for several months. Animals will be monitored for three months post treatment to determine how long the treatment probiotic remains in the gut. The data generated from these experiments will assist us in working towards FDA approval for human clinical trials.



Dr. Cary O. Harding at the Oregon Health & Science University, is researching gene therapy as a promising new approach to treating metabolic diseases including phenylketonuria (PKU). His laboratory has had success using novel adeno-associated virus (AAV)

vectors to add a copy of the normal phenylalanine hydroxylase (PAH) gene into livers of PKU mice. This treatment restores liver activity of the missing PAH enzyme and lowers blood phenylalanine in the mice. However, he has found that the treatment is only temporary. CRISPR-Cas9 gene editing is a very new technology that is capable of permanently correcting mutations in a disease gene. In this project, he will design the necessary CRISPR-Cas9 reagents to directly correct the Pah gene in PKU mice. This should lead to long term restoration of liver PAH activity and normal blood phenylalanine concentrations in treated mice. Dr. Harding is very excited to evaluate this new technology as a potential treatment for PKU and other metabolic diseases.



Dr. Katherine Durrer-Deming at the University of North Texas is researching the ability of a genetically engineered probiotic to lower blood Phe levels in PKU mice. In the current project, she will construct a human safe version of a genetically engineered probiotic

with the hope of eventual clinical trials. The human version will be a *Lactobacillus* strain engineered to carry



Dr. Roberto Gramignoli at the Karolinska Institutet in Stockholm, Sweden has described the importance and peculiar characteristics of amnion epithelial cells (AEC) in the human placenta. Recently they have differentiated these stem cells into liver cells and successfully

tested the efficacy in preclinical models of inborn error of metabolism, including PKU. His studies have motivated translation to clinical grade isolation and banking of human AECs at Karolinska Institute (Stockholm, Sweden), and obtained approval to include PKU patients in a new promising clinical trial. With the innovative regenerative therapy proposed, people with PKU might repair and regenerate the native liver and avoid life-long immunosuppressive regiment. Human AECs are NOT immortal, NOT tumorigenic, and have important immune-modulatory and anti-inflammatory characteristics. During the next year, he will investigate these immune-modulatory properties that may result in the first cellular therapy for PKU, without immunosuppression.



Dr. Dong Yizhou is Assistant Professor at Ohio State University. Dr. Yizhou hypothesizes that gene corrections to PAH will produce a functional PAH protein and recover the metabolic process, resulting in a cure for PKU. In this study, he will develop a gene-engineering

platform in order to induce gene editing as well as correct the mutations of the PAH gene. This strategy would provide a new avenue for the treatment of PKU and such strategies could potentially be applied to other therapeutic applications.



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

would administer the functional PAH enzyme which is deficient in PKU patients. His previous results proved it is possible to formulate the functional human PAH, thus overcoming the limited impact of currently available treatments. Dr. Lino has designed a strategy to form nanoparticles containing PAH which fully preserved the enzyme's integrity and functionality. Hence, in view of the safety and efficacy issues related to the therapeutic application of PAH, Dr. Lino is refining this approach and implementing the first *in vivo* studies in the PKU animal model. The success of the proposed research work may constitute a potential novel 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. In the first year of NPKUA funding, Dr. Nicholls and colleagues (including Dr. Randall Prather at the University of Missouri) developed reagents for genome editing of PAH

in pig embryos to produce a miniature pig model of PKU. Due to the similar physiology as well as brain size and development of the pig and human brain, they predict that this will represent a greatly improved, pre-clinical large animal model for PKU. Studies on a mini-pig model will provide a better understanding of the biomedical basis of PKU and for testing new therapies for the disease. For the new year of funding, Dr. Nicholls and colleagues will obtain the first PKU piglets, will characterize the biochemical and clinical/neurological features of the animals, and with Dr. Kristen Skvorak will use the pig model to further improve and develop hepatocyte transplant as a potential clinical therapy for PKU.

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: Cary Harding, MD, FACMG; Thomas Franklin, PhD; Harvey Levy, MD; Kathryn Moseley, MS, RD; Ray Stevens, PhD; Bryan Hainline, MD, PhD; Uta Lichter-Konecki, MD, PhD.; Rddney Howell, MD; Denise Ney, PhD, RD; Erin MacLeod, PhD, RD, LD; Desiree White, PhD; Jessica Cohen, MD; Ira Fox, MD and 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.