NPKUA
5-YEAR SCIENTIFIC STRATEGIC RESEARCH PLAN
THE NATIONAL PKU ALLIANCE (NPKUA) LAUNCHES A STRATEGIC RESEARCH PLAN TO ACCELERATE THE RESEARCH AND DEVELOPMENT OF THERAPIES FOR PKU.

While tremendous advancements in diagnosis and treatment of PKU have been made over the last 50 years, there is still significant unmet medical need. One major challenge for PKU patients is lifetime adherence to the PKU diet. With the aging of the first generation of PKU diagnosed and diet-treated patients, it is now clear that patients who cannot manage their PKU with diet suffer from a wide range of intellectual and behavioral challenges.

As a rare condition where the worst clinical outcomes can now be avoided through adherence to a strict diet, PKU is often viewed as a problem solved and struggles to attract research funding from larger funding agencies to meet the current needs of PKU patients. Through our efforts, NPKUA aims to ensure that this devastating condition continues to receive the research funding required to understand the underlying principles of the disease, and identify and develop new therapeutic approaches that will lead to an improved quality of life and ultimately to a cure.

Over the next five years, NPKUA will take an increasingly directed approach to accelerate the development of new therapies for PKU. We will work to bring new treatments to those with PKU in the most efficient manner. NPKUA will continue to fund the Research Grant and Postdoctoral Fellow Programs. The grant program will continue to fund highly innovative, early-stage studies in areas that are not being met in the pharmacological/biotech side. Also, we look to work with translational research through funding, collaboration, and networking. NPKUA will accelerate research showing translational promise to give PKU patients early access to emerging therapies via clinical trials and pilot studies. Short-term goals include: developing new tools to understand the disease process, accelerating drug development, and improving the current treatments of PKU. This includes developing additional cognitive tests and a home Phe monitor.

How can you help us make sure the plan is completed?
The 5-year NPKUA Research Plan will need funds for its success. To accelerate PKU research and complete this plan, we need your help! You can change the lives of our PKU community by contributing. Visit www.npkua.org/donate.

CHALLENGES IN THE DEVELOPMENT OF PKU TREATMENTS AND A CURE

There have been tremendous advancements in our understanding of the underlying genetics of PKU, which have created new drug development opportunities. However, these new understandings have also revealed a challenging complexity of the disease. For example, PKU is caused by mutations in the phenylalanine hydroxylase (PAH) gene that reduces PAH availability in the body. PAH is an enzyme responsible for breaking down phenylalanine (Phe) as it enters the body. This reduction in PAH results in a build up of Phe to toxic levels in the blood and brain. To date, hundreds of PAH mutations have been identified, and each mutation has a unique impact on PAH activity ranging from no measurable reduction in enzyme activity to complete absence of enzyme activity. Thus, depending on which mutation they have, each patient can have different enzyme activity, severity of disease, and respond differently to current therapies. An ideal PKU therapy would; work for all mutations, allow all affected individuals to live with a normal diet, have a low burden of administration, and work for even the most severe cases.
**THERAPEUTIC APPROACHES**

Because there is still a significant unmet need, many scientists and pharmaceutical companies are actively working on applying the latest medical technologies to PKU to develop a cure. The therapeutic approaches fall into three broad categories: 1. using chaperone therapies to restore function to an otherwise dysfunctional enzyme, 2. adding enzyme via the bloodstream or the gastrointestinal tract, or 3. using genetic or cellular therapies to enable affected individuals to produce new, functional enzyme.

1. **RESTORE ENZYME ACTIVITY**

Some PKU mutations can be corrected by using pharmacologic chaperones to restore catalytic activity to an otherwise dysfunctional enzyme. There are two known approaches to this for PKU depending on the mutations: BH4 chaperones or PAH chaperones.

**BH4 CHAPERONE**

PAH requires the presence of the naturally occurring cofactor or chaperone called tetrahydrobiopterin (BH4, also known as sapropterin and marketed as Kuvan) in order to function properly. Approximately 25% of PKU patients have mutations that cause a defect in the biosynthesis or recycling of BH4. For these individuals, cofactor can be given as treatment.

**PAH CHAPERONE**

Other PKU mutations impact the folding or conformation of PAH itself, which may reduce or inhibit its ability to break down Phe. For individuals affected by these mutations, a small-molecule chaperone could bind to PAH and correct its conformation.

2. **ADD FUNCTIONAL ENZYME**

For PKU mutations that are not amenable to restoration by chaperones, a new functional enzyme must be provided to replace the missing or dysfunctional enzyme. PAH is difficult to manufacture using current biotechnology methods, but similar enzymes can be manufactured to perform the same function as PAH. Two alternatives used in this type of “enzyme substitution therapy” are phenylalanine ammonia lyase (PAL) and phenylalanine dehydrogenase (PDH).

**ADMINISTERING ENZYME VIA THE BLOODSTREAM**

One approach developed a recombinant PAH or equivalent enzyme that can be administered via injection. PALYNZIQ is a recently approved recombinant phenylalanine ammonia lyase that is injected subcutaneously to replace defective PAH. While PALYNZIQ is beneficial to many patients, there is still a need for improvement.

**GENETICALLY ENGINEERED CELLS**

Red blood cells can be genetically engineered to produce a variety of therapeutic proteins. The FDA recently cleared a clinical trial to test red blood cells expressing phenylalanine ammonia lyase for the treatment of PKU. In this treatment, patients would receive an intravenous infusion to red blood cells engineered to produce PAL. Details are yet to be worked out in clinical trials, but it is expected that patients will have to be treated every 3 to 4 months.

Other investigators are exploring the use of other cells such as liver cells or stem cells as potential treatment of PKU. In these scenarios, cells would be engineered to express PAH and then introduced into the liver where they would be expected to grow and supply functional PAH. With one treatment, this type of approach can potentially provide a lifetime cure.

**DRUG-LOAD ERYTHROCYTES**

Erythrocytes, or red blood cells, are capable of being loaded with a variety of biologically active substances, including PAH, PAL, and PDH. The drug is entrapped into the erythrocytes which, upon injection, serve as circulation depots that can slowly release the enzyme over a few months before another treatment is needed.
ADMINISTERING ENZYME VIA THE GASTROINTESTINAL TRACT
Researchers are exploring ways to deliver enzyme via probiotic bacteria. Companies are pursuing this approach by genetically modifying gut bacteria to break down Phe. It is anticipated that similar to probiotics, these engineered probiotic bacteria would be delivered orally.

For any orally administered enzyme, there are unanswered questions about exposure time. These enzymes perform their action in the upper intestines; they are not expected to go into the blood. It is not yet known whether the enzymes will be exposed to Phe in the gut long enough to be effective.

3) PRODUCE NEW ENZYME
Instead of correcting dysfunctional enzyme or adding a new enzyme, several innovative genetic techniques are being developed to use the body’s genetic machinery to produce new, functional enzyme within its own cells. This can be accomplished through both genetic and cellular therapies.

Messenger RNA (mRNA) is a molecule that carries a portion of DNA code to other parts of the cell for processing. Therapeutics are being developed to selectively activate mRNA to increase protein levels in the body.

New DNA can be added to cells by various gene therapy techniques. The FDA recently approved the initiation of a gene therapy trial to replace the defective PAH with a fully functional PAH in the patients own cells. This treatment has the potential to cure PKU.

CRISPR/Cas9 genomic editing technology allows permanent modification of genes within organisms with a one-time administration. An appeal of CRISPER technology for PKU is that very low levels of enzyme activity (2-3%) are required to have a therapeutic effect. However, there are many PAH mutations complicating drug development.

Genes can be added to cells by engineering a healthy gene into a virus that can then be used to deliver the gene to a diseased cell. Different viral vectors can be used to achieve this: lentiviral vector, adeno-associated virus (AAV), and mini-circle DNA.

Additional research is ongoing using cellular therapies, which work by transplanting whole, healthy cells into the liver of PKU patients. Clinical trials are ongoing at this time.
RESEARCH GOALS AND PRIORITIES

FIND A CURE FOR PKU
NPKUA’s highest priority is to find a cure for PKU. We believe that we are at the confluence of the understanding of the underlying molecular causes of PKU and the emergence of powerful new therapeutic technologies that, when focused on PKU, will lead to a cure. To achieve a cure, NPKUA works with academic research and industrial partners from around the world.

FOCUS ON TRANSLATIONAL RESEARCH
Translational Research is defined as the process by which the results of research done in the laboratory are used to develop new ways to diagnose and treat disease. Through funding, collaboration, and networking, the NPKUA will accelerate research showing translational promise to give PKU patients early access to emerging therapies via clinical trials and pilot studies.

SUPPORT RESEARCH THAT CAN LEAD TO A CURE
Because PKU is caused by mutation of a single gene, PAH, PKU is particularly well suited for gene replacement therapy. Over the last decade, gene therapy has undergone rapid development with four gene therapy products now approved by the FDA. NPKUA will support gene replacement strategies that, with a single application, hold the hope of a lifetime cure.

SUPPORT RESEARCH THAT CAN LEAD TO IMPROVED TREATMENT
• NPKUA will prioritize PAH gene delivery methods that require easy and convenient repeat gene delivery such as probiotic delivery of PAH or equivalent enzymes.
• Support development of oral formulations of PAH.
• Support development of non-oral treatments that provide a significant improvement over existing treatments in route of administration, dose schedule, and tolerance.

DEVELOP TOOLS TO IMPROVE UNDERSTANDING AND MANAGEMENT OF PKU
• Develop a home Phe blood monitor to help healthcare professionals and patients manage diet and other therapies.
• Develop cognitive tests to help healthcare professionals and the patient evaluate their status to assist in the management of diet and other therapies.
• Develop new tools to understand the disease process and accelerate drug development.
• Support longterm patient studies to better understand the disease process to improve treatment.
• Continue to support the NPKUA Patient registry, a comprehensive database of individuals with PKU that will help us better understand the full spectrum of PKU including: how the disease progresses, potential comorbidities, expedite the process of clinical trials, identify gaps in current scientific knowledge, and determine future research needs. The registry accelerates the science of PKU.
• Develop gene phenotyping projects that will help researchers and healthcare professionals create precise treatments for each individual.

SUPPORT POST-DOCTORAL FELLOWSHIP TRAINING PROGRAM
Because of the rarity of PKU, accessing research funding for PKU from traditional sources is challenging. As a result of the striking success of early diagnosis and treatment by diet, PKU is often perceived as a “problem solved.” Today we know that even on a restricted diet, PKU patients may still experience significant health, intellectual, and behavioral challenges. Together, rarity and the perception of “problem solved” make it difficult to attract the best and brightest new researchers into the field. By supporting Post-Doctoral Fellowship training programs, the NPKUA will help build the next generation of PKU researchers.

ENCOURAGE INNOVATION AND COLLABORATION
Apply advances from other research areas, fund new ideas, enable international collaboration, and collaborate with other funding bodies to co-fund projects to extend PKU research.

WORK GLOBALLY WITH RESEARCH PARTNERS
NPKUA is committed to identifying and funding the best PKU research on a global scale. To achieve this goal, NPKUA has recruited internationally recognized PKU Thought Leaders to our Scientific Advisory Board. Our Research Grant program supports leading researchers at internationally recognized research institutions both in the United States and abroad.
WORK WITH INDUSTRY PARTNERS
Work with industry partners to help accelerate the development of new therapies. By highlighting the continued need of PKU patients and providing valuable resources, NPKUA can drive industry interest in the development of a cure for PKU. The NPKUA Patient Registry is a valuable resource to industry partners to help identify and recruit patients for clinical trials. The NPKUA also provides patient expertise to clinical development programs to ensure the patient voice is at the center of drug development efforts.

WORK WITH REGULATORY AGENCIES
The NPKUA has and will continue to work with regulatory agencies, such as the US Food and Drug Administration (FDA), to educate agency reviewers, to inform agency decisions, and drive policy regarding the continued need for new and improved PKU therapeutics, and ultimately a cure.

LOBBY THE FEDERAL GOVERNMENT
NPKUA is working with other patient advocacy organizations to lobby the federal government to enact legislation to ensure fair and equitable access to medical nutrition therapies, which are often excluded from health insurance coverage.

HOW ARE WE FUNDED?
Our funds are raised through grassroots fundraising activities initiated by the NPKUA, local affiliate organizations, and individual donations.

We must ensure those investments are used to fund research that has the greatest chance of having an impact on the lives of individuals affected by PKU. The funds allow NPKUA to support leading research and innovative ideas from the world’s best scientists.

Whenever possible, we will invest funds with the potential for not only a scientific return but also a commercial return, allowing us to optimize our limited resources and minimize risks associated with funding research.

NPKUA has a competitive research grant application program to determine the applicants to fund. All grant applications are assessed through a robust scientific peer review process involving our scientific advisory board. NPKUA Scientific Advisory Board is composed of scientific thought leaders with expertise in all areas of PKU research and drug development. The grants are critical for the PKU research community. NPKUA supports new Fellowships in PKU, established PKU researchers who want to explore new areas and researchers outside of the PKU area that wish to apply their expertise to PKU. NPKUA will continue to fund innovative projects each year that will continue to both advance the understanding and treatment of PKU and help discover new therapies and a cure.
RESEARCH AND FELLOWSHIP GRANT PROGRAMS
Each fall the NPKUA solicits research proposals and postdoctoral fellowship proposals to advance our objective of finding a cure. One important objective of the NPKUA Grant Program is to provide initial funding to move novel ideas from the concept stage to a data-driven hypothesis, making them eligible for larger funding from more traditional sources. This can often be accomplished with a relatively small amount of funding. We do not intend to fund research from concept to market; rather, we identify promising research that meets our objectives and provide funding to position the research to be eligible for more traditional funding sources.

Proposals are first evaluated for scientific merit by the NPKUA Scientific Advisory Board. Proposals are then prioritized based on scientific merit, alignment of the NPKUA research priorities, and budget. The NPKUA Board of Directors gives final review and approval.

Using this scientific peer review process ensures that research selected for funding is:
• Scientifically valid, relevant, and significant
• Timely and achievable
• Not duplicative of other work
• Uses appropriate methodologies
• Carried out by researchers with the right skills and facilities
• Provides value for money

Application instructions and deadlines for research grant and fellowship applications can be found at www.npkua.org.

In the last decade, the NPKUA has:
• Invested close to $4 million in research that has led to new scientific knowledge about PKU and accelerated the development of new treatments and a cure
• Connected the PKU community through a biennial patient conference that attracts more than 600 attendees each year
• Launched the PKU Patient Registry to accelerate research and understanding of PKU
• Provided support to adults wanting to return to treatment
• Assisted women with PKU with the Maternal PKU Mentoring Program and Emergency Assistance Program
• Advocated successfully with the FDA and NIH on the unmet medical needs in PKU and the importance of new treatments and a cure
• Advanced technological innovation in the development of a home Phe monitor to improve treatment

The NPKUA and its partners are 100% committed to improving the lives of PKU patients and their families. We will not stop until all those living with PKU have a wide range of treatments and curative therapies to choose from.