Project Title: Survey of Palynziq: An Enzyme Therapy that Modifies Phenylalanine Metabolism in Patients Affected by PKU.

Project Thesis: Treatment with Palynziq, a new drug therapy, reroutes the protein metabolism pathway in patients lacking the Phenylalanine (PHE) Hydroxylase (PAH) enzyme, resulting in reduced PHE blood levels, enabling diet liberalization.

Individual Section: A survey of the research and technological breakthroughs leading to the discovery of Palynziq, the biochemical/metabolic mechanism of the drug, as well as the clinical treatment process.

Palynziq Introduction

In 2018, the Federal Drug Administration (FDA) approved the drug Palynziq™, manufactured by BioMarin Pharmaceuticals, as a treatment for PKU (1). The FDA approval came after three full phase human clinical trials, spread out over ten years, that were preceded by laboratory experiments using dogs, mice and a human PKU subject (2). Over the years of development and clinical trials, the drug name changed as well. During the clinical trials the drug was called PEG-PAL or pegvaliase, referring to the PEGylated process used to attach polyethylene glycol (PEG) molecules to phenylalanine ammonia lyase (PAL) (2, 3).

PAL is a genetically modified plant enzyme that acts as a stand-in for the missing PAH enzyme in patients affected by PKU. PAL prevents serum phenylalanine build up by creating a new metabolic pathway - decomposing PHE into cinnamic acid and ammonia, as shown in Figure 1.

The top half of the figure shows the mechanism of normal human metabolism in an individual that is not affected by PKU. With an active PAH enzyme, PHE is metabolized to tyrosine. The bottom row of the figure shows the metabolic pathway of an individual affected with PKU (no PAH, or reduced PAH activity) and how PAL provides substitution enzymatic action in place of the missing PAH, to decompose PHE to cinnamic acid and ammonia (2).

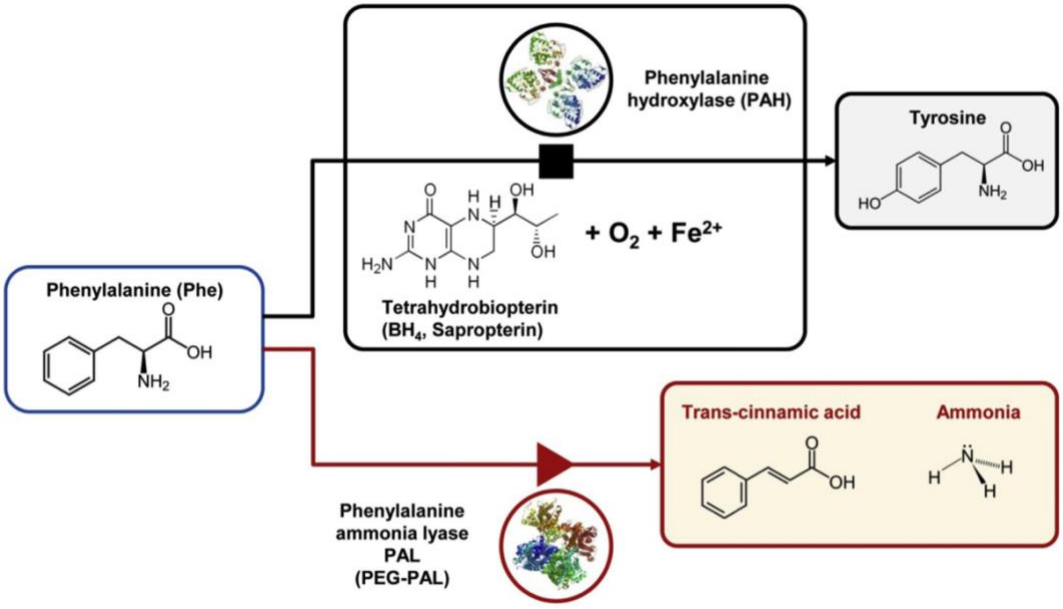


Figure 1: PHE Metabolism Enzymatic Action: PAH vs PAL

Today, Palynziq is in widespread use in the United States and is considered a miracle drug, because of the lifechanging effects that it has on patients who respond to treatment.

Palynziq Discovery: Unique History of Scientific Innovation

The process that led to the drug discovery is very unique because of the diverse fields of science and medicine involved. The drug invention can be attributed to work of botanists/plant scientists, biochemists, toxicologists, clinical practitioners, and medical researchers from the

fields of human metabolism, enzyme replacement therapy and neuropsychology. All of that body of research can be distilled down to three major watershed innovations.

The first key innovation was determining that cinnamic acid and phenylalanine are interchangeable in plant metabolism. This discovery came through research on the biochemical pathway that occurs during the synthesis of lignin, the cellular framework of plants. A Canadian plant physiologist discovered that lignin development required phenylpyruvic acid, which is a known metabolic by-product of impaired phenylalanine metabolism, as shown in Figure 2 (2).

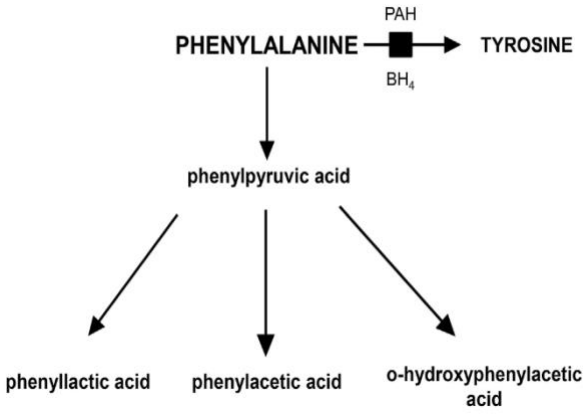


Figure 2: Impaired PHE Metabolism By-Products

Through further experimentation plant biochemists determined that either cinnamic acid or phenylalanine could be used to synthesize lignin. This "swap out" discovery, led the researchers backwards through the lignin synthesis pathway to discover PAL.

The second key innovation connected the PAL with human enzyme replacement therapy manufacturing methods. Naturally occurring plant enzymes are difficult to extract and manufacture, however enzyme replacement researchers found that the bacteria *Anabaena variabilis* was a good source of PAL. Researchers then found a way to synthetically manufacture PAL to increase its catalytic activity and improve its temperature stability (4).

The third key innovation was from research conducted by toxicologist John A. Hoskins. Working in a chemical warfare research facility during World War II, Hoskins collaborated with the public health lab which managed the newborn screening process, to conduct experiments using PAL, first on dogs then on a human subject. The team extensively researched the

biochemical action of PAL on PHE, by measuring blood plasma PHE levels before and after the intake of a high protein meal. During these experiments, PAL was administered orally. Blood test revealed lowered PHE blood plasma with PAL treatment which confirmed the results previously demonstrated in laboratory mice models. A primary outcome of this research was the confirmation that the metabolic by-product of PAL metabolism, cinnamic acid, was converted to hippuric acid, and benzoic acid which are excreted in the urine with no underling toxic effects or health risks (5).

Clinical Treatment Process

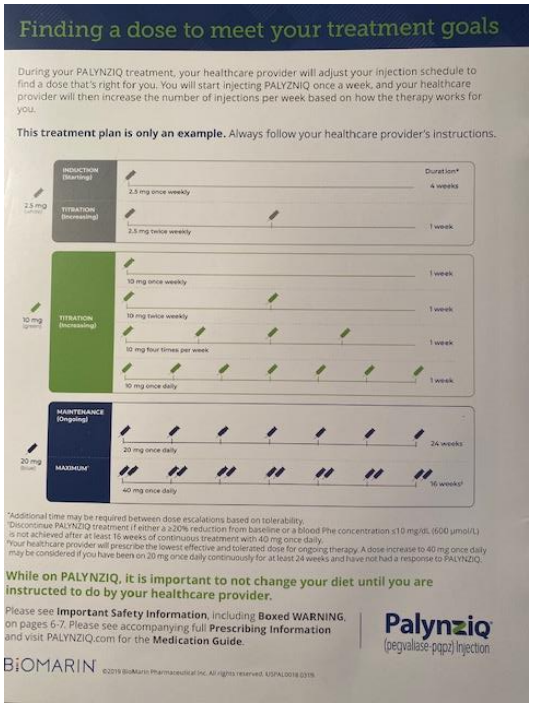
Despite the development of PAL, widespread clinical use was hindered by two things. First, because the plant enzyme is a foreign substance, it creates a negative immune response. Second, PAL is rapidly metabolized out of blood plasma and excreted thus limiting the duration of its efficacy. While neither of these barriers have yet been completely overcome – the use of PEGylated glycol together with changing from oral delivery to subcutaneous injection delivery as shown in Figure 3, reduced these hinderances. Regulated human trials begin in earnest in 2008 under the name PEG-PAL. Human subjects receiving treatment not only had reduced PHE levels, but also had marked improvement in attention, concentration, executive function, and lowered levels of self-reported anxiety and depression (6,7,8).

Figure 3: Expended Syringe of Palynziq

The clinical treatment process requires a daily injection of Palynziq using a prefilled syringe. The initial schedule is a once or twice weekly injection(s) of 2.5 mg of Palynziq, which is gradually increased until

titration is reached. In some patients a dose of up to 60 mg of Palynziq per day is required to gain treatment response (7,8).

Throughout the treatment process, regular blood PHE monitoring occurs to determine the required dose to reach titration, ie., full replacement of PAH enzymatic activity by PAL. When the patent is fully titrated, then the diet can be liberalized.



The most common side effects of treatment include anaphylactic shock, joint pain, hives, injection site rash, swelling and headaches, most of which can be reduced by antihistamines (3,7).

In order for PHE blood levels to remain low, treatment must be administered daily, for life (3).

Although PAL treatment for PKU is now widespread, access to the drug is still limited due to its cost, requirement for insurance approval, and need for regular monitoring and follow up. Unfortunately, Palynziq has limited availability outside of the United States (8). In parallel, alternative treatments for PKU are in development such as orally administered probiotics, gene editing and liver transplants (4).

Reference List

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