Phenylalanine Ammonia Lyase (PAL) Enzyme substitution therapy for PKU and clinical trials

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Objectives

- Describe the impact of elevated Phe on long-term patient outcomes
- Discuss current therapeutic guidance in the treatment of PKU
- Discuss preliminary results of injectable phenylalanine ammonia lyase in patients with phenylketonuria
Phenylketonuria (OMIM 261600)

Autosomal recessive disorder of amino acid metabolism. Results in the accumulation of phenylalanine in all body fluids.

Frequency in Caucasians 1:10,000

Cause:
1. Mutations PAH gene (98%) (12q, 13 exons, > 500 mutations). R408W causes a complete impairment of enzyme activity and requires stringent dietary restriction of phenylalanine. I65T is leaky and associated with a milder form

2. BH₄ biosynthesis/recycling defect (2%)

Phenylalanine Metabolism

PKU
- Phenylalanine
  - **X** Phenylalanine hydroxylase
- Tyrosine
- Tyrosine aminotransferase
- p-OH-phenylpyruvate
- 4-OH-phenylpyruvate dioxygenase
- Homogentisic acid oxidase
- Homogentisic acid
- Maleylacetoacetate
- Maleylacetoacetate isomerase
- Fumarylacetoacetate
- Fumarylacetoacetate hydrolase
- Fumarate + Acetoacetate

Tyr type 2
- Tyrosine

Tyr type 3
- p-OH-phenylpyruvate

Alkaptonuria
- Homogentisic acid oxidase
- Maleylacetoacetate
- Maleylacetoacetate isomerase
- Fumarylacetoacetate
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Tyr type 1
- Succinylacetone

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Phenylketonuria (OMIM 261600)

**Presentation:** No symptoms at birth, eczema, fair skin and hairs, develop microcephaly, delays noted at 6 m, MR. Older patients can have epilepsy, spastic paraparesis, occasional parkinsonism.

**Diagnosis:** Usually identified by newborn screening. Plasma amino acids: elevated Phe levels; urine organic acids: phenylketones; normal urine neopterin profile and blood DHPR. Enzyme expressed only in liver. DNA testing is available.

There is genotype-phenotype correlation:

- Hyperphenylalaninemia
- Mild PKU
- Classic PKU

Phe 120-360 360-1200 > 1,200 uM

Newborn screening mandatory in all States

**Therapy:** Low protein/low Phe diet, Supplemental Tyr, BH₄

**Monitoring:** Plasma amino acids, Phe/Tyr

**Outcome:** IQ 5-10 points below normal sibs in old studies

PKU

TREATED

UNTREATED
PKU OUTCOME

• Early diagnosis and treatment prevent mental retardation.
• Even with treatment, there can be a reduction of IQ and impaired executive functioning.
• There is less adherence to dietary therapy as patients get older.
Compliance With Dietary Treatment Decreases With Age

Plasma phenylalanine levels increase with age as children have a more varied diet and start having a social life.
PKU THERAPY

• Diet low in phenylalanine and proteins.
• Large neutral amino acids.
• Sapropterin
• Enzyme substitution therapy (Investigational): Phenylalanine ammonia lyase.
Enzyme Replacement Therapy (ERT) IN PKU

- Why not use phenylalanine hydroxylase (PAH)?
- PAH is unstable and requires an essential cofactor (tetrahydrobiopterin). It has inherent protease sensitivity and potential immunogenicity, rendering isolation and purification complex and therapy potentially unsafe.
- Pegylation and truncation of the natural PAH can improve enzyme stability and activity, but it remain a formidable task to obtain large scale production of stable, active molecules.

Phenylalanine Ammonia Lyase (PAL)

- Phenylalanine-metabolizing enzyme found in many plants, several fungi, and bacteria
- Requires no exogenous cofactor

Phenylalanine $\rightarrow$ Cinnamic acid

$\text{Phenylalanine}$

$\text{Cinnamic acid}$
Cinnamic acid is a compound of low toxicity which is converted in the mammalian body primarily to hippuric acid and excreted in urine.

The plant enzyme phenylalanine ammonia lyase (PAL) will survive in the gut for long enough to deplete the phenylalanine derived from food protein and so reduce the rise in blood phenylalanine that otherwise occurs after a protein meal.

When the enzyme was given to an untreated PKU patient for 12 consecutive days (three doses a day after food), blood phenylalanine levels were reduced on average by 25%.

Intraperitoneal injection (left) and oral (administered in E. coli) recombinant phenylalanine ammonia lyase (PAL) reduced phenylalanine levels in the Enu/Enu mice, a model for phenylketonuria.

Reduction was dose-dependent

Injectable PAL was used to decrease Phe levels in the mouse model of PKU.

Comparison of therapy with PEGylated phenylalanine ammonia lyase (PEG-PAL) from 4 different species indicated that the most effective was PAL from *Anabaena variabilis* with two substitutions that decreased aggregation and enhanced stability (p.C503S/p.C565S).

• Subcutaneous injection of WT- PAL had only transient effects that disappeared with repeated injections (A).
• PEG-PAL caused a sustained decline in Phe levels (B).
• Antibody production was responsible for disappearance of activity in WT-PAL.

Long-term therapy with PEG-PAL with weekly injections maintained Phe levels within the therapeutic range after 4 weeks in which the effect seemed to disappear.

Lower maintenance doses were more effective in achieving adequate Phe levels.

• PAL-PEG (PEGylated recombinant phenylalanine ammonia lyase) is an investigational enzyme substitution therapy for the treatment of PKU.
• Pharmacology studies conducted in the PKU mouse model demonstrated that weekly subcutaneous administrations of PAL-PEG resulted in a significant and stable decrease of plasma phenylalanine.
Phase I study – rAvPAL-PEG

• The primary objective of the study was to assess the safety and tolerability of single, subcutaneous injections of PAL-PEG in subjects with PKU.

• The secondary objectives of the study were to evaluate the pharmacokinetics of single, subcutaneous injections of PAL-PEG administered at escalating doses and to evaluate the effect of PAL-PEG on blood Phe concentrations in subjects with PKU.
Phase I study – rAvPAL-PEG

- The Phase 1 clinical trial was an open-label, multi-center study conducted in 25 PKU patients in a series of five dose-escalating cohorts:
  - 0.001 mg/kg
  - 0.003 mg/kg
  - 0.01 mg/kg
  - 0.03 mg/kg
  - 0.1 mg/kg
- Each cohort received a single dose
- 6-week follow-up period.
Seven out of 25 patients developed late mild to moderate injection-site reactions, of which two also developed skin rashes without other symptoms.

Two patients developed reactions to medroxyprogesterone acetate.

Three subjects experienced generalized skin reactions. None of these events were considered serious or severe, all 3 events resolved, and the 3 subjects completed the study.
Injection site reactions
Body rashes
Antibody test results

- **PEG**
  - Low IgM and IgG titers for PEG were observed in all subjects
    - Only 1 subject did not have anti-PEG IgM antibody
- **rAvPAL**
  - No subjects had detectable IgM titers for rAvPAL
  - ~½ of the subjects develop low IgG titers for rAvPAL
- **Neutralizing antibodies**
  - No Subject had rAvPAL-PEG neutralizing antibodies
- No correlations between antibody titers and presumed immunological reactions or blood Phe response were observed
Effect of rAvPAL-PEG on serum ammonia

No significant changes in plasma ammonia with rAvPAL-PEG
Phase 1 – rAvPAL-PEG - RESULTS

- Substantial blood Phe level reductions in the range of 36% to 97% (mean of 62%) were observed in all patients in the fifth dosing cohort (0.1 mg/kg) with a mean baseline blood Phe level of 1,113 umol/L.

- No notable blood Phe level reductions were observed in the first four dosing cohorts (0.001 to 0.03 mg/kg).
PEG-PAL: Phenylalanine levels decrease at 0.1mg/kg

![Graph showing phenylalanine levels over time for different cohorts.]

- Cohort 1 (0.001 mg/kg)
- Cohort 2 (0.003 mg/kg)
- Cohort 3 (0.01 mg/kg)
- Cohort 4 (0.03 mg/kg)
- Cohort 5 (0.1 mg/kg)
Effect of rAvPAL-PEG on Phe levels
Significant correlation between levels of PAL-PEG and phenylalanine levels in all patients as a group and in individual patients.
Effect of rAvPAL-PEG on Tyr levels

No significant correlation between levels of PAL-PEG and tyrosine levels in 2 patients as a group or in individual patients.
Phase 2 study – rAvPAL-PEG

• The Phase 2 clinical trial is an open-label, multi-center study to be conducted in 35-50 patients in a series of dose-escalating cohorts from 0.001 mg/kg.

• The primary treatment period of eight once weekly injections at a fixed dose will be followed by eight weeks of dose and frequency optimization and an extension period where doses can be increased up to 2 mg/kg/week.
Phase 2 study – rAvPAL-PEG

- The primary objective is to evaluate the effect of PAL-PEG on blood Phe concentrations in subjects with PKU.
- The secondary objectives are to evaluate the safety and tolerability, immune response and steady state PK of subcutaneous injections of multiple dose levels of PAL-PEG.
- Phase 2 trial started in September 2009.
Overview of Current Plan for Phase 1 and 2 Studies

PAL-001 Single Dose
- Each subject received 1 dose
- Up to 7 dose cohorts planned, 5 cohorts dosed
- N=5 per cohort

PAL-002 part 1 Multiple Dose
- Weekly fixed dose x 8 wks
- Up to 50 subjects
- N=7-10 per cohort

PAL-002 part 2/PAL-003 Extension Study
- Dose adjustment is permitted x 8 wks then 24 mos (-003)
- Maximum dose is 2 mg/kg/week
Summary

• Phenylketonuria is characterized by increased phenylalanine levels that can cause mental retardation and/or impaired executive functioning.

• Recombinant *Anabaena variabilis* Phenylalanine ammonia lyase can lower phenylalanine levels in a mouse model of PKU.

• rAvPAL-PEG is well tolerated by human subjects, with a minority having local or generalized reactions to the drug.

• rAvPAL-PEG at 0.1 mg/kg per dose was effective in reducing phenylalanine levels in patients with PKU.

• Ongoing trials (Phase 2) are assessing the safety and efficacy of repeated doses of rAvPAL-PEG in patients with PKU.
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