Panel: Report of Scientific Advisory Board – Research Advancements
July 30, 8:15am-9:15am

Dr. Cary Harding
National PKU Alliance (NPKUA)
July 28-31, 2016, Indianapolis, IN
Disclosures

• Cary Harding has received compensation for consultation services and for sponsored research from:

  • BioMarin Pharmaceutical Corp.

  • Cydan Development, Inc.

  • Dimension Therapeutics

  • Horizon Pharma, Inc.

  • Synlogic
Agenda

• Brief review of the Scientific Advisory Board accomplishments

• Update on Pegvaliase trials

• Update on Genetically Engineered Probiotics
  • Marc Tewey, Trayer Therapeutics

• Update on progress toward a gene therapy clinical trial

• Update on The Home Phe Monitor Challenge
  • Tom Franklin
2016 Funded projects:

- Dr. Juan Cabrera-Luque, Children’s National Medical Center: establish an in vitro model to study the effects of high blood Phe at the human BBB

- Dr. Katherine Durrer-Deming, University of North Texas: genetically engineered probiotic

- Dr. Cary Harding, Oregon Health & Science University: gene therapy

- Dr. Roberto Gramignoli, Karolinska Institutet: cellular therapy

- Dr. Robert Nicholls, UPMC: PKU swine model and cellular therapy

- Dr. Dong Yizhou, Ohio State University: gene therapy

- Dr. Paulo Roque Lino, University of Lisbon: enzyme reposition therapy
SAB meeting – July 28, 2016

- Roberto Gramignoli - Isolation of Human Amnion Epithelial (hAE) Cells According to Good Manufacturing Procedures
- Kristen Skvorak - Cell Transplantation for PKU: moving forward from mouse to pig model
- Katherine Deming - AvPAL Probiotic Therapy for PKU
- Cary Harding - CRISPR/Cas9 mediated gene editing
- Tom Franklin - Phe-monitor challenge
- Eddy van der Zee - Effects of LNAA supplementation upon brain neurochemistry in $Pah^{enu2}$ mice
- Cary Harding - Therapeutic effects upon brain neurochemistry in $Pah^{enu2}$ mice
- Shawn Christ - New insights into PKU and the Brain
PKU: Phenylalanine Hydroxylase Deficiency

- PKU is caused by phenylalanine hydroxylase deficiency which leads to elevated phenylalanine (Phe) levels in the blood and brain.
- High levels of Phe are harmful to the brain and can cause symptoms
  - Mood disorders
  - Attention problems
  - Executive function deficits

PKU pathophysiology

- Liver: Phenylalanine (Phe) enters the liver.
- Blood: Phenylalanine (Phe) in the blood stream.
- Brain: Phenylalanine (Phe) crosses the blood-brain barrier (BBB).

Brain: Phenylalanine (Phe)

Reduces:
- Glutamatergic synaptic transmission
- Pyruvate kinase activity
- HMG-CoA activity

Damasages:
- Myelin (white matter lesions)

Inhibits:
- Tyr and Trp hydroxylase

Decreased:
- Protein synthesis
- Neurotransmitter synthesis
  - Trp ➔ Serotonin
  - Trp ➔ Dopamine

Cognitive deficits, neurophysiological and neuropsychological dysfunction

Feillet et al. 2010
Potential therapies for PKU

Pegvaliase: enzyme substitution treatment to lower blood Phe levels

- Pegvaliase is being developed as a enzyme substitution treatment to lower blood Phe levels in adults with PKU

**Rapid conversion and excretion.**
The effectiveness and safety of pegvaliase is currently being evaluated in clinical trials.

**Phase 1**
- **PAL-001** Single dose
  - **PAL-002** Weekly weight-based dosing
  - **PAL-004** Dosing 5 days/week
  - **165-205** Induction, titration & maintenance dosing

**Phase 2**
- **PAL-003** Long Term, Open Label

**Phase 3**
- **PRISM-1** Treatment naïve & open-label
  - Induction, titration and maintenance dosing
- **PRISM-2** Placebo-controlled, Randomized Discontinuation Trial & Open-label, long-term extension study
  - Part 1: Phe Eligibility
  - Part 2: RDT
  - Part 3: PK/PD
  - Part 4: OLE
Phase 2, dose-finding study to evaluate the safety, effectiveness, and tolerability of injected pegvaliase in adults with PKU.

Pegvaliase Phase 2 165-205: Induction, Titration and Maintenance Dosing Regimen

**Induction**
- 4-8 weeks
- Start pegvaliase at 2.5 mg per week

**Titration**
- At least 4 weeks
- Adjust dose to achieve blood Phe ≤ 600 µmol/L

**Maintenance**
- 8-15 weeks

**Extension**
- Up to 98 weeks
- Adjust dose to maintain blood Phe 60-600 µmol/L

This study helped design the Phase 3 PRISM Program.
Phase 3 Studies: Patient Criteria

• PRISM study patient criteria:
  • An adult with PKU diagnosis
  • Blood Phe > 600 µmol/L
  • Willing and able to maintain a consistent diet (Phe-restricted diet was not required)

• Patients taking any medication to treat PKU (for example, sapropterin) had to stop taking the PKU medication for at least 14 days before starting pegvaliase treatment
Pegvaliase Phase 3 Program

**PRISM-1: Treatment Introduction Study**
- Patients randomly assigned to treatment groups
- **Induction** 4 weeks
  - 2.5 mg weekly
  - 2.5 mg weekly
- **Titration & Maintenance** 5–37 weeks
  - 20 mg daily
  - 40 mg daily

**PRISM-2: Pivotal Study with RDT**
- **Randomized Discontinuation Trial (RDT)**
  - Randomized 2:1
  - 13 weeks
  - ≥20% blood Phe reduction from entry into PRISM-1
- **PK and PD**
  - 6 weeks
  - 20 mg daily
  - 40 mg daily
  - PLACEBO
- **Long-term open-label extension**
  - Dosing to individual efficacy targets
  - 212 weeks
  - 5–60 mg daily

PRISM-2 Randomized Discontinuation Trial

- Patients taking pegvaliase were randomly assigned to continue pegvaliase or start placebo treatment
  - Patients and doctors did not know which treatment group patients entered
- Clinical trial was designed to study the effectiveness of pegvaliase without the side effects that occur when first starting pegvaliase treatment
  - This study design minimized time patients were without pegvaliase treatment

PRISM-2: Pivotal Study with Randomized Discontinuation Trial

- 20 mg pegvaliase daily
  - Placebo
- 40 mg pegvaliase daily
  - Placebo

8 treatment weeks
## PRISM-2 Study Outcomes for the Randomized Discontinuation Trial

### PRISM-2: Pivotal Study with Randomized Discontinuation Trial

<table>
<thead>
<tr>
<th>Endpoint Type</th>
<th>Outcome Description</th>
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<tbody>
<tr>
<td><strong>Primary Endpoint:</strong></td>
<td>Change in blood Phe</td>
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<tr>
<td><strong>Secondary Endpoints:</strong></td>
<td>Change in attention and mood</td>
</tr>
<tr>
<td><strong>Exploratory Endpoint:</strong></td>
<td>Change in neurocognitive performance (i.e., Cambridge Neuropsychological Test Automated Battery)</td>
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For some patient with PKU, high Phe levels may have negative effect on mood.

The POMS questionnaire is a simple tool to evaluate mood.

POMS can be used by patients themselves or an observer (for example, a parent or caregiver).

Attention Deficit Hyperactivity Disorder Rating Scales (ADHD-RS)

• The ADHD-RS was designed to evaluate ADHD disease symptoms
  • ADHD-RS has two 9-question subscales that measure inattention and hyperactivity/impulsivity symptoms
• The ADHD-RS inattention subscale has been used in patients with PKU
• Long-term data will be evaluated for patients enrolled in the PRISM program

Cambridge and Amsterdam Neuropsychological Test Automated Batteries (CANTAB)

- CANTAB (Cambridge Cognition Ltd) and ANT (Amsterdam Neuropsychological Tasks) Batteries
  - Computer-based test
  - Measures neurofunctional skills such as
    - Attention
    - Processing speed
    - Working memory
- In a PKU study, there was a significant difference in CANTAB scores for patients on a Phe-restricted diet compared to patients not on a Phe-restricted diet

Bik-Multanowski et al., (2010)
• Clinical trial designed to study the effectiveness and safety of pegvaliase treatment with long-term use
  • Blood Phe
  • Side effects
  • Symptoms related to mood and inattention
• Dose adjusted to minimize side effects
Patient Characteristics in PRISM-1

- At the start of PRISM-1 (before pegvaliase treatment), patients had high blood Phe levels and symptoms of inattention.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment Groups</th>
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<tr>
<td></td>
<td>20 mg daily (n=131)</td>
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<tr>
<td></td>
<td>40 mg daily (n=130)</td>
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<tr>
<td>Age at enrollment, mean (SD), years</td>
<td>30 (9)</td>
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<tr>
<td></td>
<td>28 (9)</td>
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<tr>
<td>Female, n (%), n (%)</td>
<td>62 (47%)</td>
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<td></td>
<td>68 (52%)</td>
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<tr>
<td>Race, white, n (%)</td>
<td>130 (99%)</td>
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<td></td>
<td>124 (95%)</td>
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<td>Daily protein intake from intact food, grams, Mean (SD)</td>
<td>39 (27)</td>
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<td></td>
<td>38 (28)</td>
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<td>Baseline blood Phe (μmol/L), mean (SD)</td>
<td>1241 (390)</td>
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<tr>
<td></td>
<td>1224 (384)</td>
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<tr>
<td>Baseline ADHD-RS Inattention score, Mean (SD)</td>
<td>n=129</td>
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<tr>
<td></td>
<td>10 (7)</td>
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<tr>
<td>Baseline ADHD-RS Inattention score in subjects with baseline score &gt; 9 points, Mean (SD)</td>
<td>n=59</td>
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<tr>
<td></td>
<td>16 (5)</td>
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<td></td>
<td>n=57</td>
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<td>14 (3)</td>
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ADHD-RS IA score >9 points is considered symptomatic for inattention.

ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale; SD, Standard deviation.
More information

• BioMarin Pharmaceutical Inc.
  http://www.biomarin.com/
• Clinicaltrials.gov
**Acknowledgements**

BioMarin expresses our deep gratitude to all of the site staff and patients who contributed to this study.

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<th>Name</th>
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<tr>
<td>Darius Adams</td>
<td>Dorothy K. Grange</td>
<td>C. Ronald Scott</td>
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<tr>
<td>Stephen Amato</td>
<td>Cary O. Harding</td>
<td>Natasha Shur</td>
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<td>Jirair Bedoyan</td>
<td>Paul Harmatz</td>
<td>Mary Stuy</td>
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<td>Shawn McCandless</td>
<td>Harvey Levy</td>
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<td>Olaf A. Bodamer</td>
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<td>Barbara Burton</td>
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