Reduction in Plasma Phenylalanine Levels in Patients with Phenylketonuria with Live Bacterial Therapeutic SYNB1618

*Interim analysis from ongoing Phase 2 study*

Jerry Vockley, George A Diaz, Dorothy Grange, Cary O. Harding, Nicola Longo, Hope Northrup, John A Phillips III, Shawn Searle, Janet Thomas, Roberto Zori, Sharon Ernst, Nicole McWhorter, Mesaki Ndugga-Kabuye, Kristina Humphreys, Casey Woodbury, William S Denney, Caroline B Kurtz, Aoife Brennan and Marja Puurunen

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Jerry Vockley, M.D., Ph.D.

University of Pittsburgh
  Cleveland Family Endowed Chair in Pediatric Research
  Professor of Human Genetics

UPMC Children’s Hospital of Pittsburgh
  Chief of Genetic and Genomic Medicine
  Director of the Center for Rare Disease Therapy
PKU: Significant Need Remains for New Treatment Options

**US Population**

<table>
<thead>
<tr>
<th>Pediatrics</th>
<th>Adults</th>
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<tr>
<td>~5,000 U.S.</td>
<td>~12,300 U.S.</td>
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</tbody>
</table>

25% out of Phe control² | 65% out of Phe control²

**Challenges**

- Significant risk for **neurocognitive impairment** if untreated
- Extremely challenging diet with **low compliance**
- **Low response** to current oral therapies: 80% fail to respond³
- Most adult patients **out of Phe control** and difficulties in **executive function**
- Substantial need for increased intake of **natural protein**

¹ Epi from National PKU Alliance npkua.org
Mechanism of Action for SYNB1618, a Live Bacterial Biotherapeutic

Phe Metabolism
The Target for PKU Biotherapeutic

Healthy $\rightarrow$ Phe $\rightarrow$ PKU $\rightarrow$ Impaired PAH

Phenylalanine Hydroxylase (PAH) converts Phe into tyrosine

Accumulation of Phe to toxic levels

SYNB1618

Manage Phe levels

Conversion of Phe into non-toxic metabolites

- PAL3 enzyme converts Phe to $trans$-cinnamic acid
- LAAD enzyme converts Phe to phenylpyruvate

Safety

- $\Delta$ dap: Auxotrophy – requires diaminopimelic acid (DAP) to grow
## SynPheny-1: Phase 2 Proof-of-Concept Study for SYNB1618

### Population
- Adults with **classic PKU**
- Plasma **Phe levels ≥ 600 µmol/L**
- Not currently on sapropterin or pegvaliase-pqpz
- Stable diet history

### Efficacy Endpoints
- **Fasting plasma Phe levels** after low dose and high dose of SYNB1618
- **Labeled plasma D5-Phe AUC**, after a meal challenge

### Strict diet control
- Stable **study diet**
- **Individualized diet plan** to match baseline Phe intake
- **6-day diet run** in prior to baseline to achieve steady state
- Continued **diet control for 2 weeks** after last dose
**SynPheny-1 for SYNB1618: Phase 2 Study Design**

**Dosing**
- Oral, 3 times/day with meals
- Days 1-3: 1e11 live cells TID
- Days 4-6: 3e11 live cells TID
- Days 7-13: 1e12 live cells TID
- On Days 14 & 15 a single dose of 2e12 live cells

**Measurements**
- Plasma D5-Phe AUC_{0-24hr} at baseline and on Day 14
- Fasting Phe at baseline, after low dose (Day 7), after high dose (Day 14), and 2 weeks after cessation of dosing (Day 29)
Interim Analysis (n=9) for Synpheny-1: Baseline Characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Age</td>
<td>31.7 (10.8; 20-50) (mean, SD, range)</td>
</tr>
<tr>
<td>Gender</td>
<td>5 Female, 4 Male (55.6% fem)</td>
</tr>
<tr>
<td>Baseline Phe level</td>
<td>969 (435.5) umol/L (mean, SD)</td>
</tr>
<tr>
<td></td>
<td>507 -1925 umol/L (range)</td>
</tr>
<tr>
<td>Baseline Phe intake</td>
<td>1889 (2393) mg (mean, SD)</td>
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<td>595-8200 mg (range)</td>
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**Meal challenge**

- Protein shake / meal (20 g)
- D5-Phe (1 g)
- SYNB1618

**Measures over 24 hours**

- **Plasma concentration:**
  - Phe/D5-Phe
  - TCA/D5-TCA
- **Urinary amount excreted:**
  - HA/D5-HA
Interim Analysis: D5-Phe Absorption is Reduced by Treatment

**Interim Analysis: D5 Phe Tracer Study (2e12 dose, N = 8)**

- Meal challenge at Day -1 and Day 14 at high dose 2e12
- Phe load as D5-labeled and protein-bound unlabeled Phe

**Clear evidence of strain Phe metabolism from GI Tract**

- Mean (upper CI, Lower CI) reduction in D5-Plasma Phe AUC of -39.99% (2.7% - 64.95%)*
- 4 of 8 patients experienced >40% D5-Phe lowering after meal challenge
- Corresponding plasma D5-TCA and urinary D5-HA biomarker signal confirms strain activity
- Similar reductions in labeled and unlabeled Phe levels post meal

*Percent change from baseline +/- 95% confidence interval
Interim Analysis: Mean “All-Comers” Results for Phe Reductions

% Mean Change in Plasma Phe (1e12 dose N=8)

- Rapid reduction of fasting Plasma Phe at 3e11 dose
- Mean “all-comers” fasting plasma Phe lowering at 1e12 dose met 20%
- Elevation of plasma Phe upon cessation of treatment
Interim Analysis: Phe Reduction for >20% Responders

Response defined as >20% reduction in Phe at either day 7 or day 14.

Four subjects met this responder criterion in interim analysis.

254 μM mean reduction in Phe in responder population (N = 4).
### Safety and tolerability summary from interim analysis

<table>
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<tr>
<th>Tolerability summary</th>
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<tbody>
<tr>
<td>No SAEs or systemic safety issues identified</td>
</tr>
<tr>
<td>Tolerability profile <strong>consistent with experience</strong> in healthy volunteers</td>
</tr>
<tr>
<td><strong>Mild to Moderate GI AEs</strong></td>
</tr>
<tr>
<td><strong>1 discontinued</strong> (anxiety due to PKU)</td>
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</table>

Efficacy response and tolerability suggest **individualized dosing and titration** may be available to meet patient needs.

This will be **evaluated in future studies**.
Conclusions from the Interim Analysis of Synpheny-1, Phase 2 for SYNB1618

- SYNB1618 has demonstrated ability to access Phe from within the GI tract
- 40% reduction in D5-Phe absorption after a meal challenge
- 20% reduction in fasting plasma Phe across interim analysis population
- 254 μM mean reduction in fasting plasma Phe among responders (>20% reduction)
- SYNB1618 was generally well tolerated, with profile consistent with Phase 1 study
- An optimized version of SYNB1618, SYNB1934 with improved Phe conversion potential has demonstrated Phe metabolism in healthy volunteers and will be evaluated in SynPheny-1 (abstract #569)

Development of live bacterial biotherapeutics as novel modality for treatment of PKU warrants further study in late-stage trials
Thank you to study patients and investigators!