# Reduction in Plasma Phenylalanine Levels in Patients with Phenylketonuria with Live Biotherapeutic SYNB1618: Interim Analysis from an Ongoing Phase 2 Study

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## Background

- Phenylketonuria (PKU) is characterized by the inability to metabolize dietary phenylalanine (Phe) resulting in sustained elevation of plasma Phe levels following a protein meal.
- SYNB1618, a live, modified strain of the probiotic bacterium *E. coli* Nissle was engineered to consume Phe in the gastrointestinal (GI) tract through expression of the enzymes phenylalanine ammonia lyase (PAL) and L-amino acid deaminase (LAAD).
- SYNB1618 metabolizes Phe to harmless compounds trans-cinnamic acid (TCA) which is excreted as hippuric acid (HA) in urine, and phenylpyruvate.
- The potential of SYNB1618 to lower blood Phe level in PKU patients was studied in SynPheny-1. [NCT04534842]

### *Conversion of Phe into non-toxic metabolites*

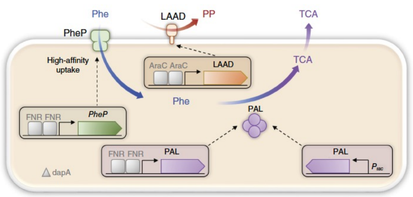



**Figure 1.** Mechanism of Action for SYNB1618, a Live Bacterial Biotherapeutic

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

### *Safety*

- Δ dap: Auxotrophy – requires diaminopimelic acid (DAP) to grow

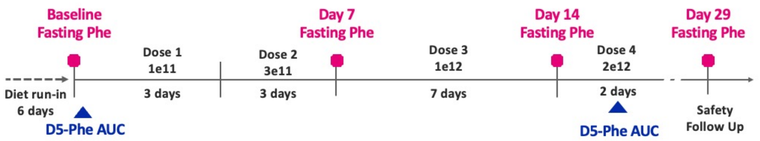
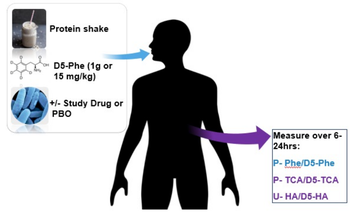



**Figure 2.** Synpheny-1 Study Design

## Methods

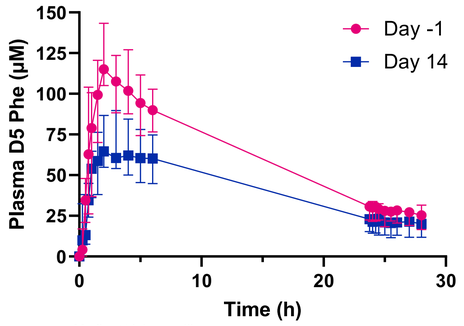
- SynPheny-1 is an open-label study in adult PKU patients with Phe ≥600 micromol/L *(Figure 2).*
- Patients followed individualized study diets reflecting baseline Phe intake from 7 days prior to dosing to 2 weeks after the last dose.
- The dose of SYNB1618 was gradually increased: $1x10^{11}$ live cells for the first 3 days, $3x10^{11}$ on days 4-6, then $1x10^{12}$ TID on days 7-13.
- A D5-Phe tracer study was conducted at baseline and on Day 14 at the $2x10^{12}$ dose.
- Key outcomes: change from baseline in D5-Phe $AUC_{0\text{-}24h}$ and fasting blood Phe.




## Results

- Data from an interim analysis of 9 PKU patients are presented.
- SYNB1618 was generally well-tolerated. The most common AEs were mild to moderate GI symptoms. No SAEs or deaths were observed. 1 patient discontinued the study due to anxiety.
- D5-Phe absorption from the gut was reduced by treatment (*Figure 3*).
- Plasma Phe was reduced by treatment on Day 7 and Day 14 (*Figure 4*).
- 4 of 8 patients had at least 20% blood Phe lowering at either Day 7 or Day 14 (*Figure 5*).

**Figure 3.** D5-Phe absorption from the gut was reduced. D5-Phe $AUC_{0\text{-}24h}$ showed a 40% (95% CI 64.9, -2.7) decrease compared to baseline.




Median+/-interquartile range

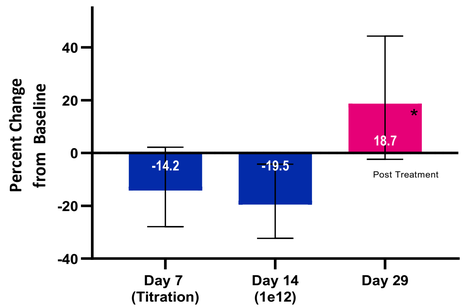
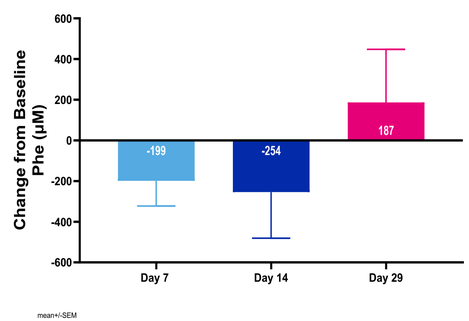



**Figure 4.**
Change from baseline in fasting blood Phe level was -14.5% (CI -27.9, 2.2) on Day 7 after the dose-ramp and at the $3x10^{11}$ dose, -19.5% (CI -32.3, -4.3) on Day 14 at the $1x10^{12}$ dose, and +18.7% (CI -2.4, 44.3) after cessation of dosing.




mean+/-SEM

**Figure 5.**
Four subjects (50%) met the responder criterion of at least 20% Phe lowering at either Day 7 or Day 14. The mean reduction of Phe level was 254 μM in the responder population.

## Conclusions

- SYNB1618 has demonstrated ability to access Phe from within the GI tract.
- Treatment with SYNB1618 led to a clinically meaningful decrease in blood Phe level.
- An optimized version of SYNB1618, SYNB1934 with improved Phe conversion potential has demonstrated Phe metabolism in healthy volunteers and is currently being evaluated in SynPheny-1.
- Development of live bacterial biotherapeutics as novel modality for treatment of PKU warrants further study in late-stage trials.