Comparison of Phenylalanine Absorption in Healthy Volunteers and PKU Patients in the Synpheny-1 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 and SYNB1934 are genetically engineered probiotic bacteria designed to metabolize Phe in the GI tract and lower blood Phe levels *(figure 1).* The key difference between the strains is that SYNB1934 has a modified PAL variant designed to have higher Phe metabolizing activity.
- To characterize the activity of these bacteria in the GI tract, we developed a method to measure the post-prandial rise of blood Phe levels in both healthy volunteers (HVs) and in patients with PKU.

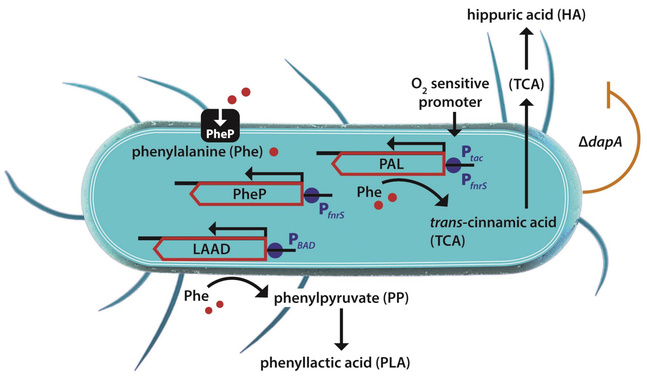


Figure 1: SYNB1618 and SYNB1934 are non-colonizing strains genetically engineered to contain genes encoding phenylalanine ammonia lyase (PAL), which converts Phe to trans-cinnamic acid (TCA), and ammonia. TCA is further converted to hippuric acid (HA) by the host and excreted in urine. A second Phe degradation pathway in the strains is through the enzyme L- amino acid deaminase (LAAD), which converts Phe to phenylpyruvate. Phenylpyruvate is further degraded by multiple pathways in the host, including conversion to phenyllactate, which is excreted in urine.

Methods

Patients

- SYNB1618 and SYNB1934 were tested in multiple-ascending dose cohorts in two HV studies. Data for the 2 x 10^{12} live cells dose is shown for each.
- SYNB1618 was studied in PKU patients in a Phase 2 study: Synpheny-1, NCT04534842 *(figure 2)*.
- Synpheny-1 is ongoing with interim analysis data from N=8 PKU patients in Arm 1 with SYNB1618 dosing available.

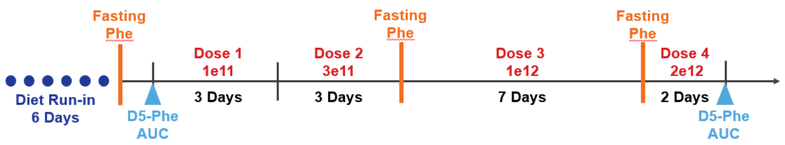


Figure 2: Synpheny-1 phase 2 study design [NCT04534842]**.** PKU patients with uncontrolled plasma Phe level (>600 µmol/L) are eligible. Participants follow a study diet based on their baseline protein and Phe intake for the duration of the study. Fasting plasma Phe is measured at baseline, after the dose-escalation period on Day 7, at the end of the dosing period on Day 14, and 2 weeks after the last dose. D5-Phe meal challenge is conducted at baseline prior to study drug start and on Day 14 at the 2 x 10^{12} live cells dose.

Phe meal test (D5-Phe tracer)

- A Phe meal test was performed in each cohort at baseline and following dosing with either 2 x 10^{12} live cells of engineered strain (SYNB1618 or SYNB1934) or placebo.
- After an overnight fast subjects received a protein shake (20 g protein) and an oral dose of D5-Phe (1g or 15 mg/kg)*; figure 3.*
- Blood and urine samples were collected for up to 24 hrs. Plasma D5-Phe and its strain-specific metabolites plasma D5-TCA and urine D5-HA were measured *(table 1).*

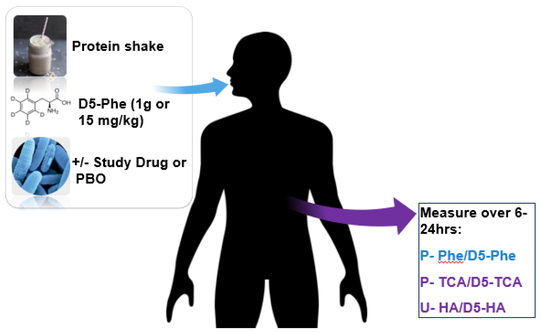


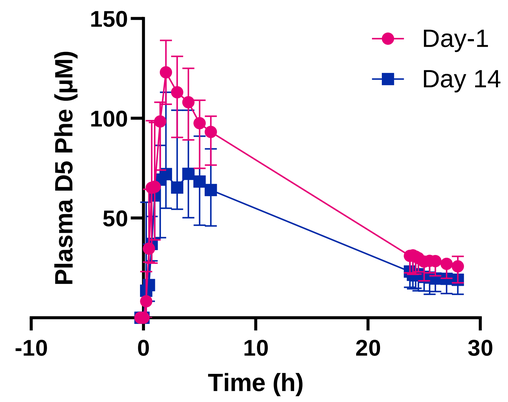
Figure 3: Graphic representation of the D5-Phe meal test.

Tracer Study	At baseline and on-treatment												
Time relative to tracer administration (min)	-15	0	15	30	45	60	90	120	180	240 (4 h)	300 (5 h)	360 (6 h)	1440 (24 h)
Blood for Phe tracer	•		•	•	•	•	•	•	•	•	•	•	•
Spot urine collection	•												
Ensure Enlive protein shake		•											
Phe tracer administration (1g or 15 mg/kg of D5-Phe)		•											
Administer study drug (or placebo)		•											
Urine collection	0 to 4 h										4 to 6 h		6 to 24 h

Table 1: Conduct of the Phe meal test with sampling schedule.

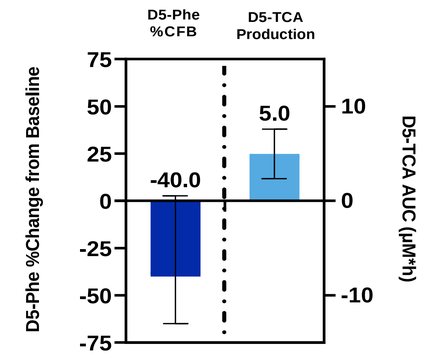
Results

- Compared to HVs, patients with PKU had a greater increase in peak plasma D5-Phe (approximately 120 µmol/L versus 60 µmol/L).
- Peak plasma D5-Phe returned to fasting levels more slowly in PKU patients compared to HVs (>24 hours for PKU patients and approximately 6 hours for HVs).
- Administration of an engineered strain reduced post meal plasma D5-Phe levels in HVs and PKU patients compared to baseline **(Figure 4, Table 2)** and led to a corresponding increase in strain-specific biomarkers in plasma (D5-TCA, **Figure 5**) and urine (D5-HA, data not shown).



median+/-interquartile range (n=8)

Figure 4: D5-Phe AUC was decreased by 39% as compared to baseline in PKU patients , when dosed with 2 x 10^{12} live cells of SYNB1618 , demonstrating decreased absorption of D5-Phe from the GI tract.



mean+/- 95% CI

Figure 5: The decrease in D5-Phe AUC in the Synpheny-1 study (N=8) was accompanied by a corresponding increase in D5-TCA AUC, a strain-specific biomarker, confirming that the reduction in plasma D5-Phe is a result of strain activity within the gut.

Group	Strain	D5-Phe AUC (hr×µmol/L) Baseline / On-Study	D5-Phe AUC % change from baseline
Healthy	SYNB1618	95.8 [82.4, 111] 74.3 [63.9, 86.4]	-20 [-26, -14]
	SYNB1934	80.7 [68.2, 95.4] 48.8 [41.3, 57.7]	-40 [-49, -29]
PKU	SYNB1618	1472 [1002, 2162] 883.3 [601.5, 1297]	-40 [-65, 3]

Table 2: Cross-study comparison of least-squares means of D5-Phe AUC following administration of 2×10^{12} live cells. Values are mean [90% confidence interval].

Conclusions

- The D5-labeled Phe meal test is a promising method to evaluate the activity of gut restricted therapies in the treatment of PKU.
- Likely due to intact PAH, HVs have lower post-meal excursion of D5-Phe and a lower percent reduction in response to SYNB1618 compared to PKU patients.
- Genetically engineered bacteria SYNB1618 and its optimized version SYNB1934 have demonstrated an ability to metabolize Phe within the GI tract and reduce post-meal plasma Phe levels significantly.
- Increase in strain-specific biomarker D5-TCA demonstrates Phe consumption through the PAL enzyme.
- SYNB1934 appears more active in HVs compared to SYNB1618 and is currently being evaluated in PKU patients in Arm 2 of Synpheny-1.