ICIEM 2021

14TH INTERNATIONAL CONGRESS OF INBORN ERRORS OF METABOLISM

21-23 NOVEMBER 2021, SYDNEY, AUSTRALIA

Enhanced potential for Phe lowering with SYNB1934, a live biotherapeutic with an improved PAL variant

Marja Puurunen[1], William S Denney[2], Mesaki K. Ndugga-Kabuye[1] , Melanie Fein[3], Kristina Humphreys[1], Casey Woodbury[1], Aoife Brennan[1], and Caroline Kurtz[1]

[1]Synlogic Inc

[2]Human Predictions LLC

[3]High Point Clinical Trials Center

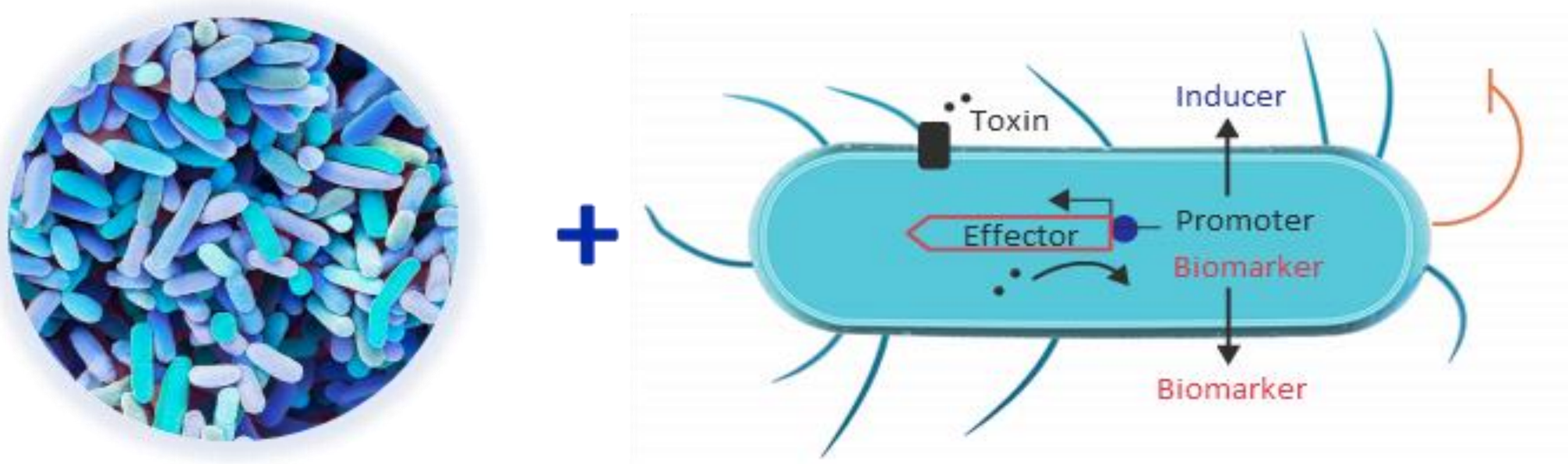
Introduction

Phenylketonuria (PKU) is a human metabolic disease characterized by an inability to degrade phenyl-alanine (Phe) causing neurotoxicity. Dietary control of phenylalanine (Phe) intake is a primary method of management of PKU, but due to its highly restrictive nature is difficult to follow. Despite recommendations supporting life-long control of Phe levels and existing treatments, some children and most adults continue to have Phe levels above the recommended range, which puts them at risk of cognitive and psychiatric disease.

Synthetic Biotic medicines, live modified strains of the probiotic bacterium E. coli Nissle, have been engineered to consume phenylalanine (Phe) in the gastrointestinal tract (GI) as a novel treatment approach with the goal of providing safe, oral, and reversible treatment options for patients living with PKU. SYNB1618 is a Synthetic Biotic investigational drug for the treatment of PKU. Synlogic has initiated a Phase 2 study in PKU patients (NCT04534842). SYNB1618 has demonstrated the ability to access Phe from within the gut in interim results of this study (see late-breaking #568 presentation by Dr. Jerry Vockley).

An optimized version of SYNB1618, SYNB1934 with increased Phe conversion potential was developed (see abstract #278) and studied in healthy volunteers.

Synthetic Biotic™ Medicines platform



Non-pathogenic bacterial chassis Programable, engineering

Fig 1. The Synthetic Biotic platform combines a reproducible, modular approach to microbial engineering with a well characterized chassis organism, E. coli Nissle 1917, using synthetic biology tools to introduce effector functions into the organism which consume toxic metabolites or exert other therapeutic effects *in vivo*.

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SynPheny-1 Proof-of-concept study in PKU

SYNB1618 demonstrated Phe lowering in PKU patients

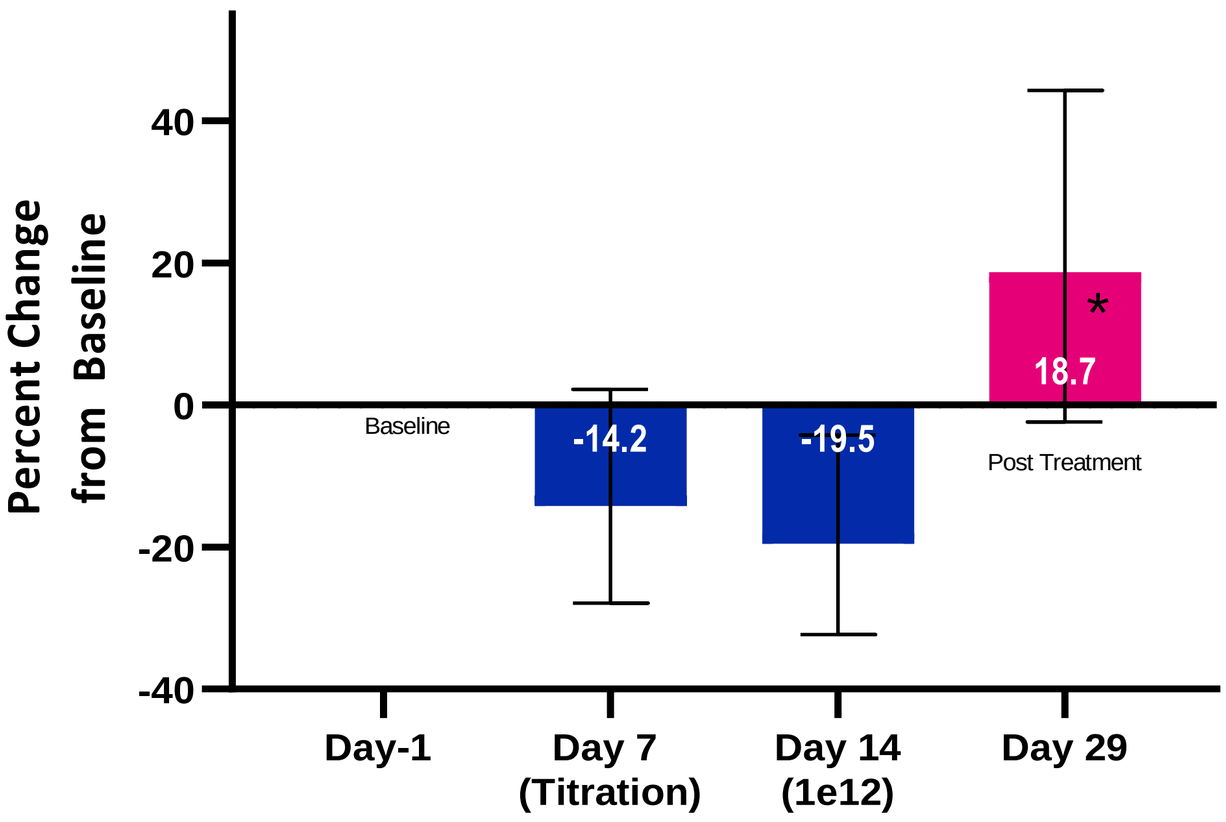

Fig 2. SYNB1618, the first generation strain, has shown reduction of fasting plasma Phe in PKU patients in the SynPheny-1 study. Mean all comers Phe reduction was 20%.

SYNB1618 led to reduction of D5-Phe absorption in PKU patients

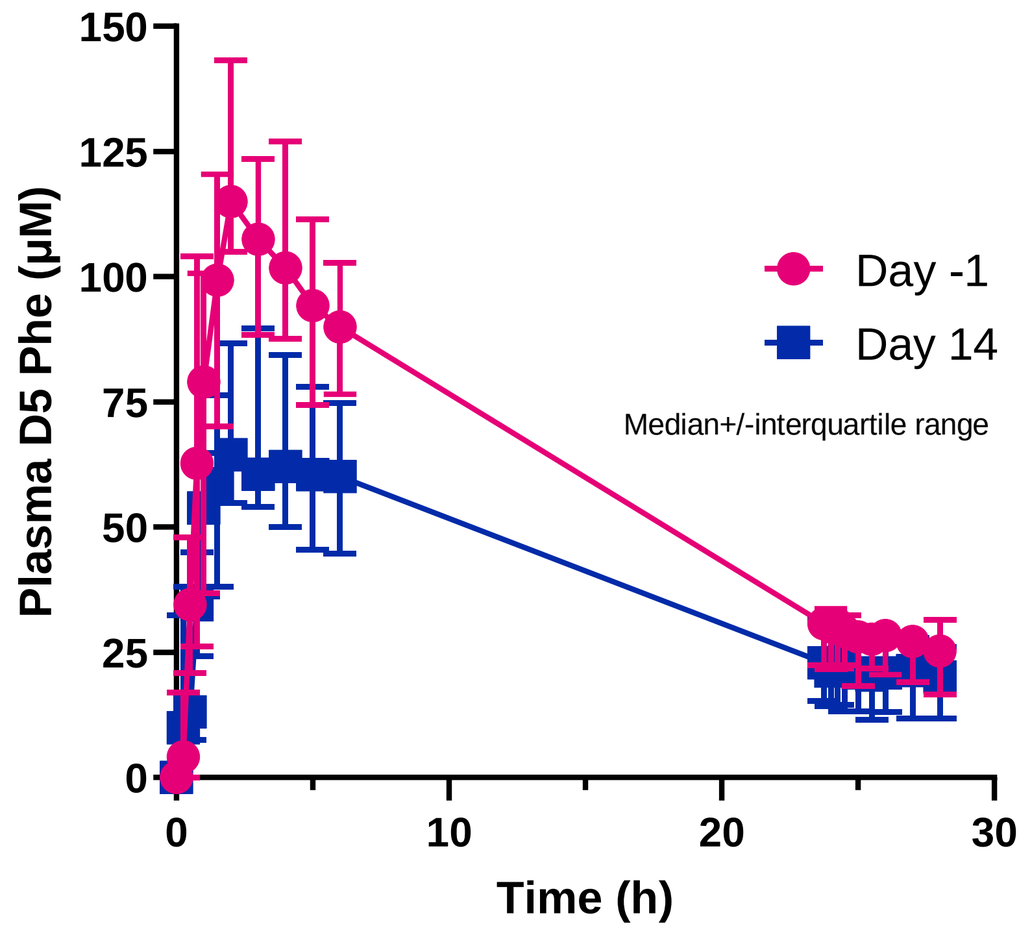

Fig 3. PKU patients underwent a meal challenge with a protein shake (20g) and D5-Phe (1g). Day 14 40% reduction of D5-Phe AUC^{0-24h} relative to Day -1 demonstrated strain access to Phe within the GI tract.

Optimized strain SYNB193: Key engineered genetic elements

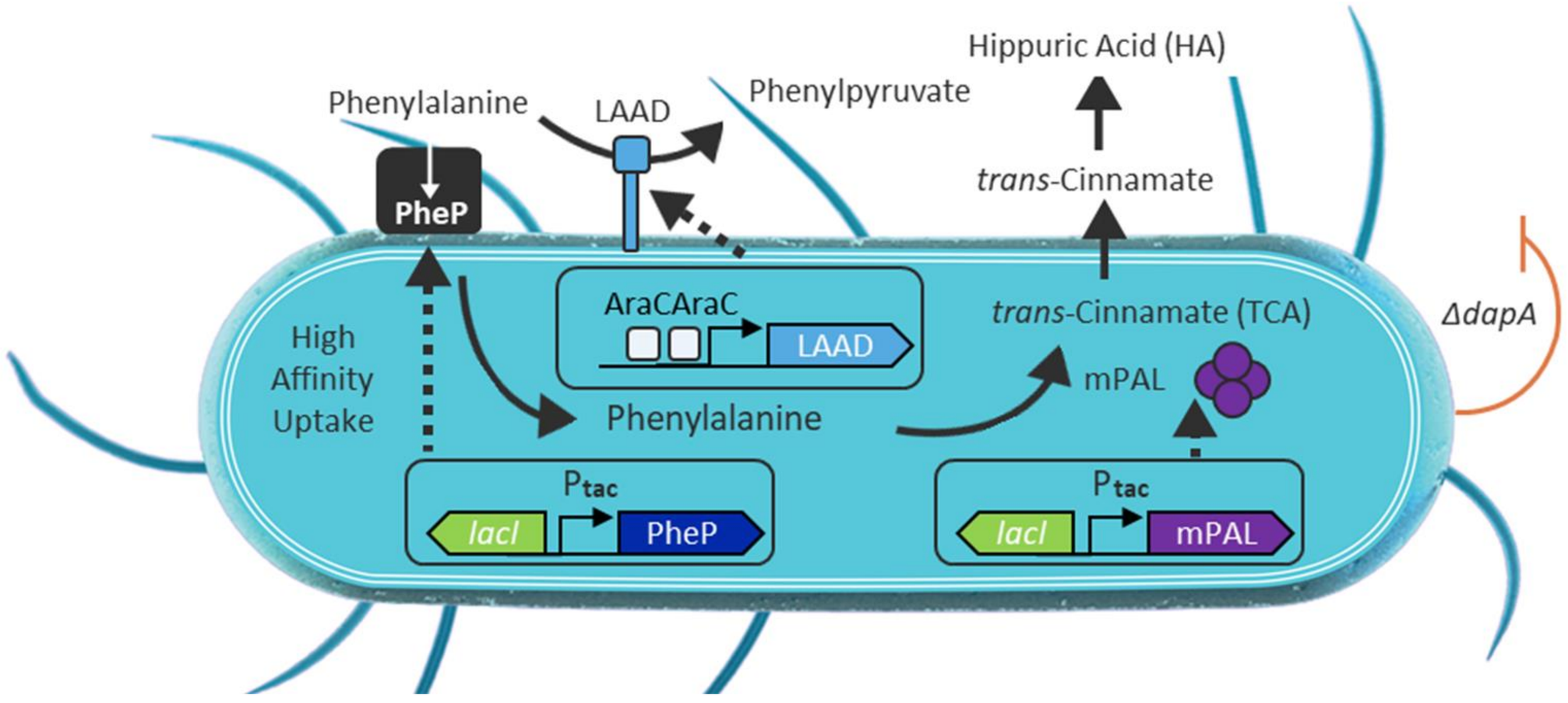

Fig 4. SYNB1934 contains chromosomally integrated genes encoding PheP, a high affinity Phe transporter, mPAL, phenylalanine ammonia lyase from *Photorhabdus luminescens* with the following engineered mutations: S92G, H133M, I167K, L432I, V470A which converts Phe to TCA, and LAAD, L-amino acid deaminase, which converts Phe to phenylpyruvate (PP). Regulation of PheP and mPAL is carried out by IPTG inducible promoters and LAAD is L-arabinose inducible promoter. For biocontainment, the strain is a diaminopimelate (DAP) auxotroph. **The key difference compared to SYNB1618 is the modified PAL enzyme leading to improved Phe conversion capacity.**

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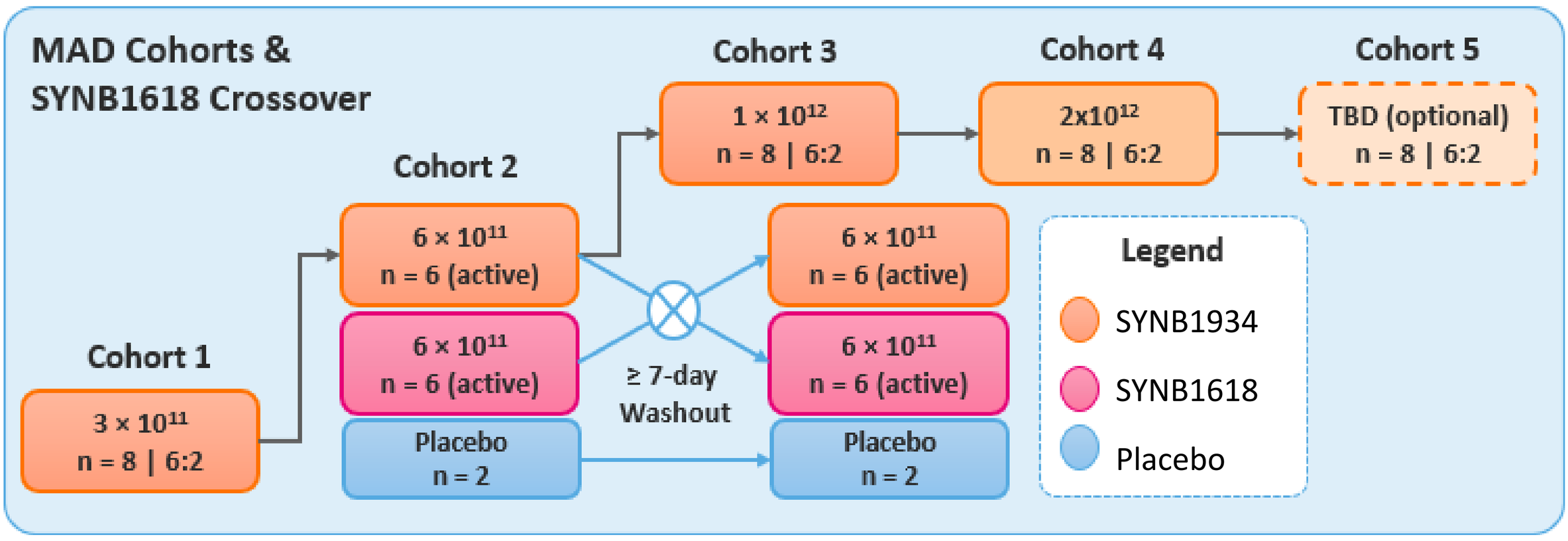
A Phase 1, Dose-escalation, Placebo- and Active-Controlled Crossover Study to Assess the Safety, Tolerability, and Pharmacodynamics of SYNB1934 in Healthy Volunteers (NCT04984525)

Study Design and Methods:

- This Phase 1 study is an ongoing inpatient, double-blind, placebo-controlled, multiple-ascending dose (MAD) study, in healthy subjects.
- Study dosing in Cohorts 1 through 3 were randomly assigned according to a MAD design (Figure 5), with all subjects completing a treatment period with SYNB1934 or placebo (randomized 6:2).
- Additionally, in Cohort 2 subjects entered a second treatment period after a ≥ 7-day washout period and received SYNB1618 (treatment sequence was randomized).
- On Day –1 once daily PPI (esomeprazole 40 mg) was initiated.
- A D5-Phe Tracer Study was performed on Day –1 and last day of dosing.
- Subjects were dosed, after meals, 3 times per day on Day 1 and once at breakfast on Day 2.
- A Safety Follow-up Visit occurred 28 days after the last dose.
- Interim results from the ongoing study are reported here.

Phase 1 study schematic



Tracer study

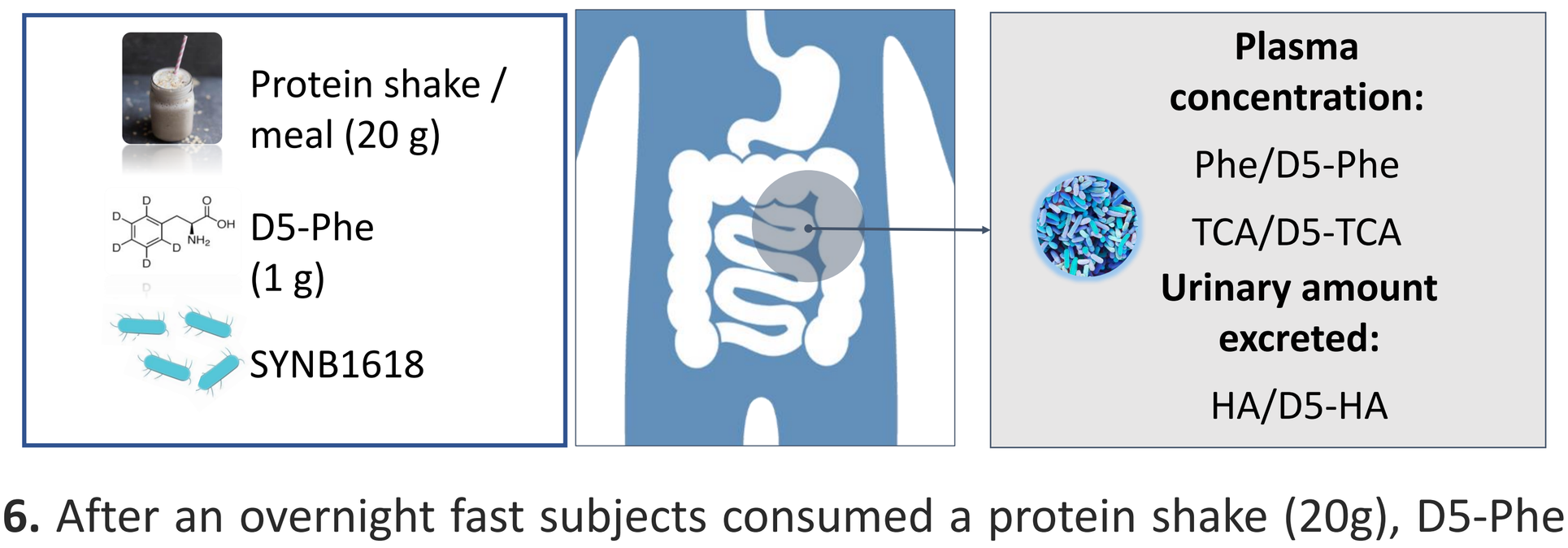


Fig 6. After an overnight fast subjects consumed a protein shake (20g), D5-Phe (1g) diluted in water and SYNB1934 with 15 minutes. Plasma Phe/D5-Phe and TCA/D5-TCA and urine HA/D5-HA were measured over 6 hrs.

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Safety and Tolerability (Cohorts 1-4):

- SYNB1934 was well tolerated at dose levels up to 2e12 live cells
- A protocol-defined maximum tolerated dose has not been achieved
- There were no SAEs, and no clinically relevant changes in laboratory parameters
- AEs were mild to moderate and GI related (most common nausea, vomiting, diarrhea)
- 4 subjects discontinued due to AEs at 6e11: no discontinuations were observed at higher doses (up to 2e12)
- SYNB1618 and SYNB1934 showed a similar tolerability profile (Cohort 2)
- There was no evidence of colonization and SYNB1934 cleared from the GI tract following cessation of dosing as expected

Pharmacodynamic Effects

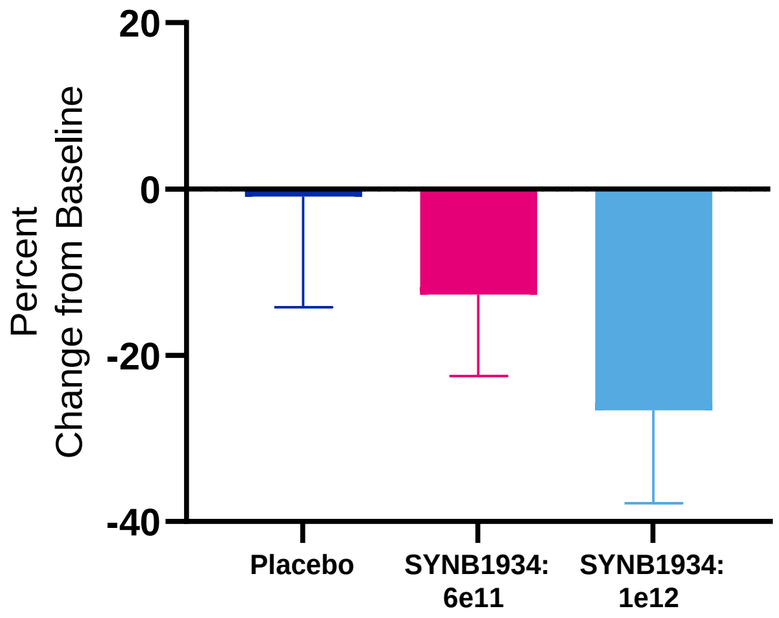

Fig 8: SYNB1934 demonstrated dose dependent reduction in plasma D5-Phe $AUC^{0\text{-}6hr}$

(Cohorts 1-3 data presented)

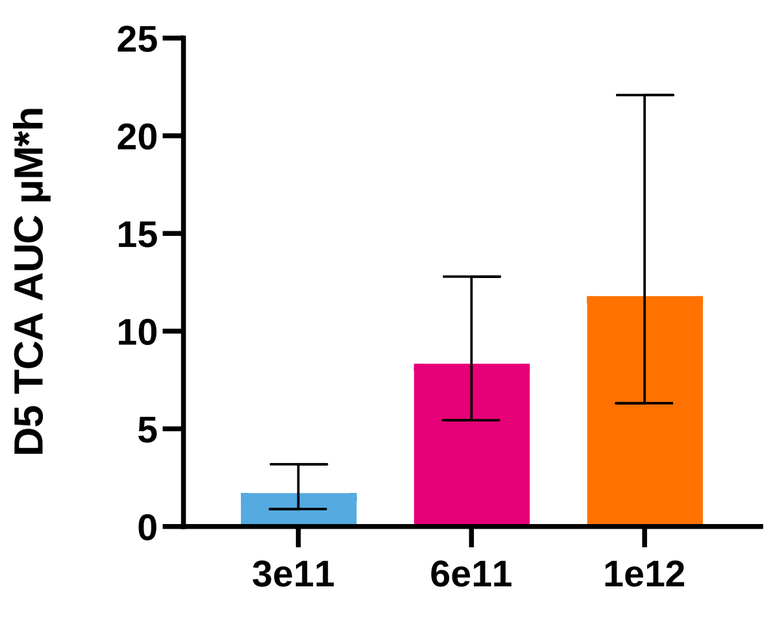

Fig 9: SYNB1934 dose dependent production of strain biomarker D5-TCA $AUC^{0\text{-}6hr}$

(Cohorts 1-3 data presented)

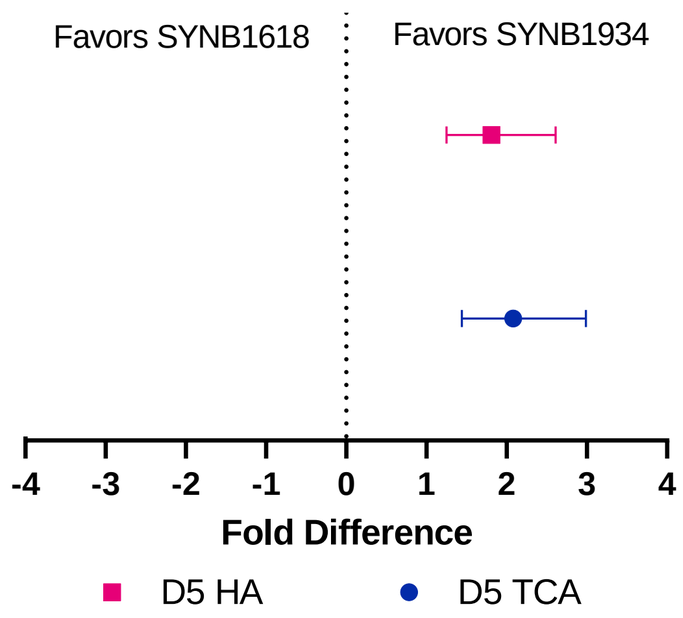

Fig 10: Strain biomarkersD5-and D5-HA^{aet} demonstrate 2-fold activity of SYNB1934 relative to SYNB1618

(Crossover Cohort 2 presented)

Conclusions

- SYNB1934 was generally well tolerated at doses up to 2e12 live cells
- SYNB1934 demonstrated dose-dependent reduction of D5-Phe absorption in plasma with corresponding strain-specific biomarker production (plasma D5-TCA and urine D5-HA)
- SYNB1934 showed improved potential for conversion of Phe to nontoxic metabolites as demonstrated with 2-fold production of strain-specific biomarkers compared to SYNB1618
- SYNB1934 will be evaluated in SynPheny-1 study in PKU patients