

Development of an Optimized and Improved Phenylalanine Degrading Live Bacterial Therapeutic, SYN1934, for the Treatment of Phenylketonuria

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Introduction

Phenylketonuria is a human metabolic disease characterized by an inability to degrade phenylalanine (Phe) causing neurotoxicity. Dietary control of phenylalanine (Phe) intake is a primary method of management of phenylketonuria (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.

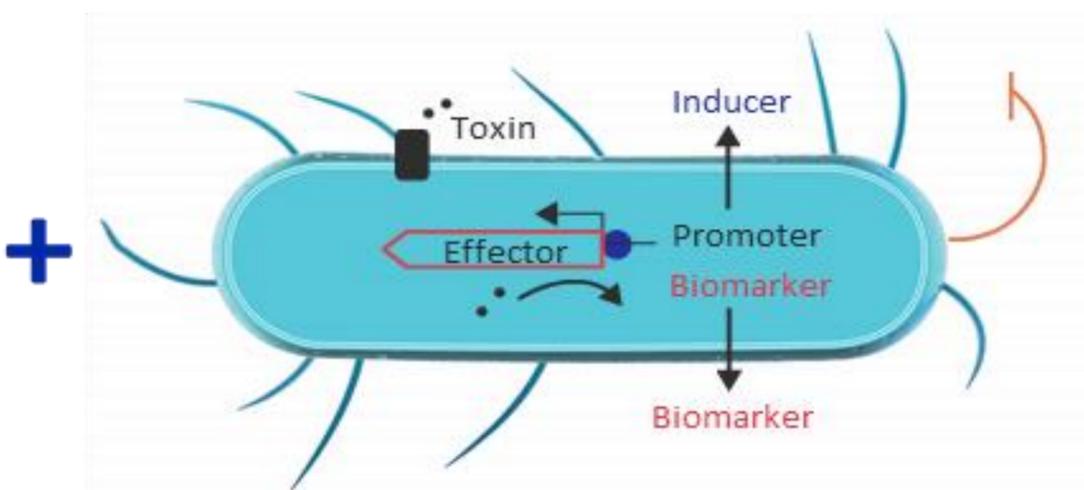
SYNB1618 is a Synthetic Biotic investigational drug for the treatment of PKU. A solid oral lyophilized formulation of SYNB1618 was found to be safe and well-tolerated and consumes Phe in the GI tract. Synlogic has initiated a Phase 2 study in PKU patients (NCT04534842).

SYNB1934 is an optimized live bacterial therapeutic based on SYNB1618 and was 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*.

SYNB1618 strain design

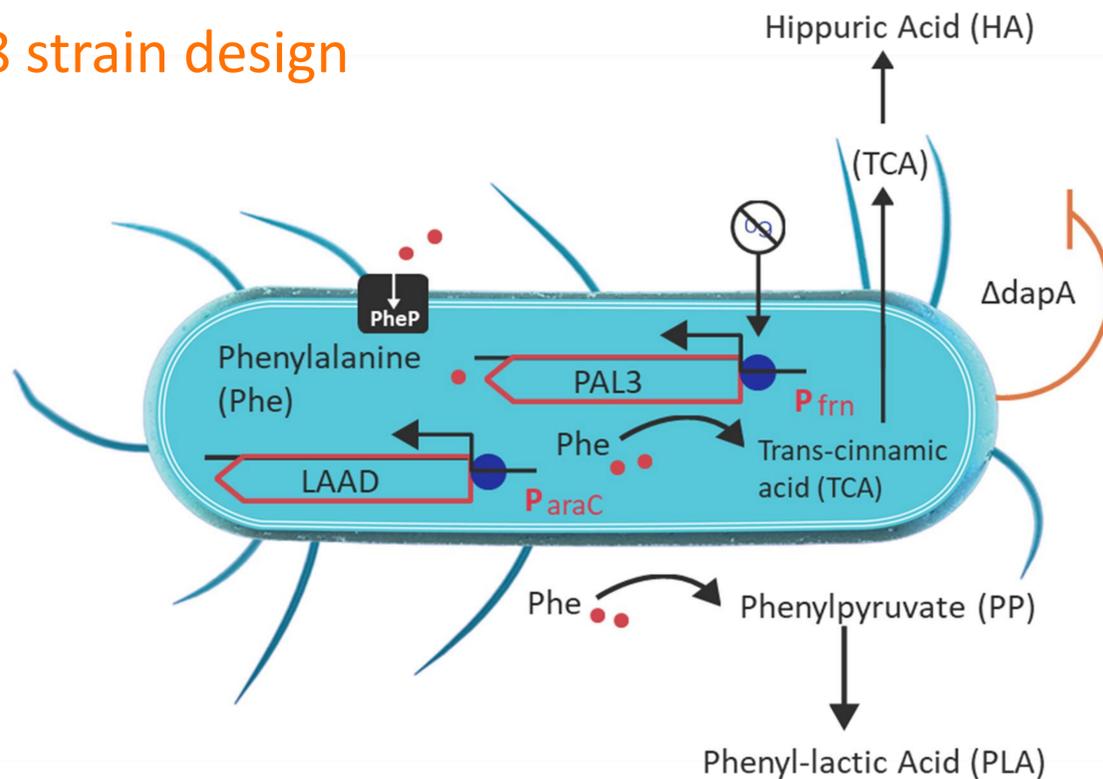


Fig 2. SYNB1618 is a non-colonizing strain genetically engineered to contain genes encoding phenylalanine ammonia lyase (PAL), which converts Phe to trans-cinnamic acid (TCA), and ammonia. TCA is further converted to hippuric acid (HA) by the host and excreted in urine. A second Phe degradation pathway in the strain 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.

SYNB1618 metabolized d5-Phe in healthy volunteers

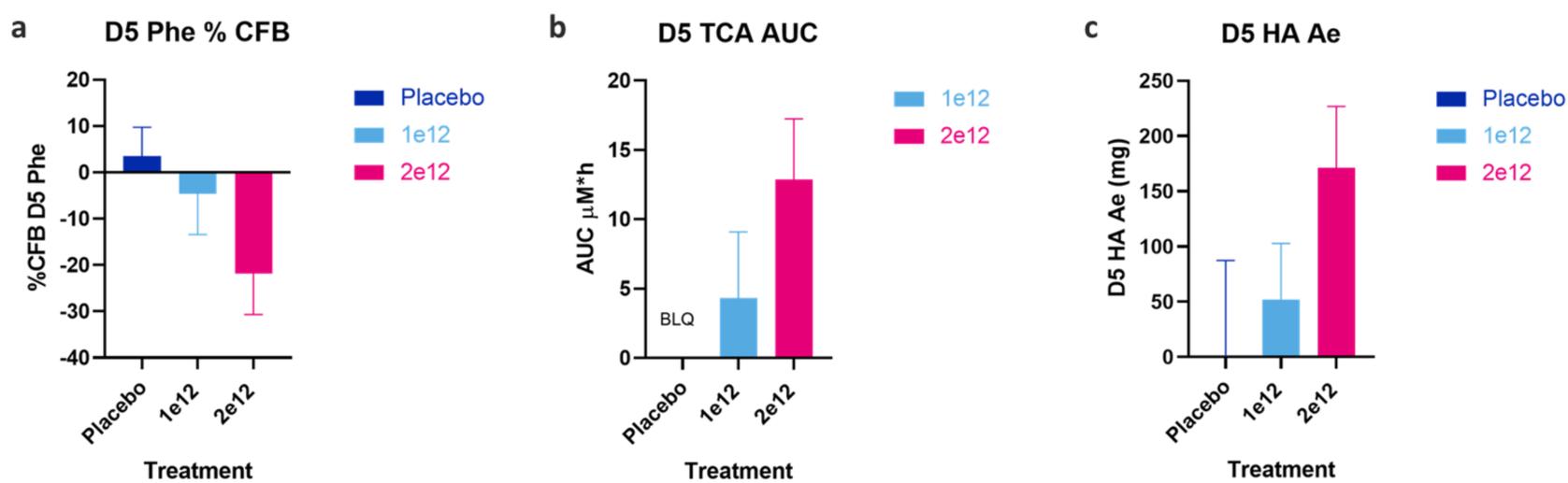


Fig 3: Healthy volunteers were enrolled in *multiple ascending dose (MAD)*, randomized, double-blind, placebo-controlled study. Subjects received 15/mg/kg of D5-Phe and a 20 g protein load (meal replacement shake) with blood and urine collection over 6hrs and plasma D5 Phe and its metabolites D5-TCA and D5 HA were assessed over 24 hours in plasma and urine. A lowering of plasma D5-Phe AUC (a) was observed at the 1e12 and 2e12 live cell doses. D5-TCA AUC (b) was elevated in serum and D5-HA (c) amount excreted (Ae) was elevated in urine as a result of strain-specific metabolism of D5-Phe and host metabolism of D5-TCA.

Safety of SYNB1618 in HV: SYNB1618 was well tolerated. The MTD was 2×10^{12} live cells. The most commonly occurring TEAEs were GI-related events and headache; the majority of these events were mild or moderate. SYNB1618 cleared from the GI tract within 9 days following the last dose, as indicated by fecal quantitative polymerase chain reaction (PCR) analyses. There was no evidence of colonization, and no subject required antibiotic treatment.

SYNB1618 is capable of consuming Phe from the GI tract and blunting Phe elevation in healthy volunteers.

Can the strain be further optimized to increase Phe lowering?

Zymergen developed TCA-responsive biosensor and HTS campaign

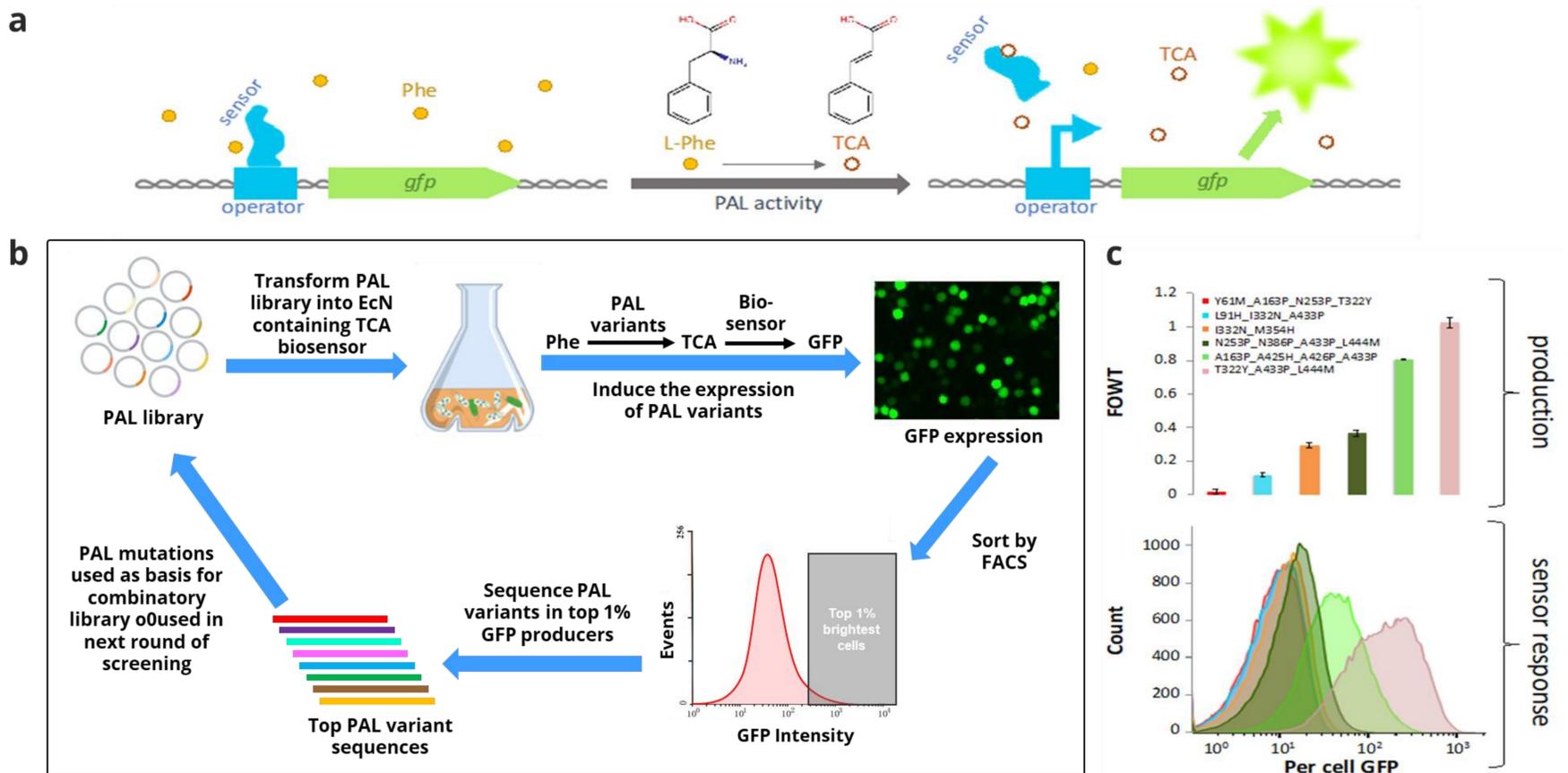


Fig. 4. (a) specific TCA-responsive allosteric transcription factor biosensor represses expression of the fluorescent reporter gene *gfp* in the absence of TCA; when TCA is introduced to the system, the repression is relieved and *gfp* is expressed (b) Directed evolution method where library of PAL variants (on low copy plasmids) were transformed into EcN with biosensor (on high copy plasmid), grown as a pool, washed, encapsulated by microfluidics in water-in-oil droplets containing Phe and inducer of PAL expression (aTc) at ~1 cell/droplet, incubated, and sorted by FACS for highest GFP producers. Sequences of top 1% PAL variants used to library design and construction for next round of screening. This ultra-high-throughput screening approach allowed screening of >1M-member combinatorial libraries. (c) Demonstration of rank-order GFP to production correlation. PAL variants with different levels production (upper panel) from Round 1 (Fig. 3) correspond to correlated levels of GFP expression (lower panel). Colors are consistent between graphs.

In vitro gastric simulation model show increased activity of top PALs

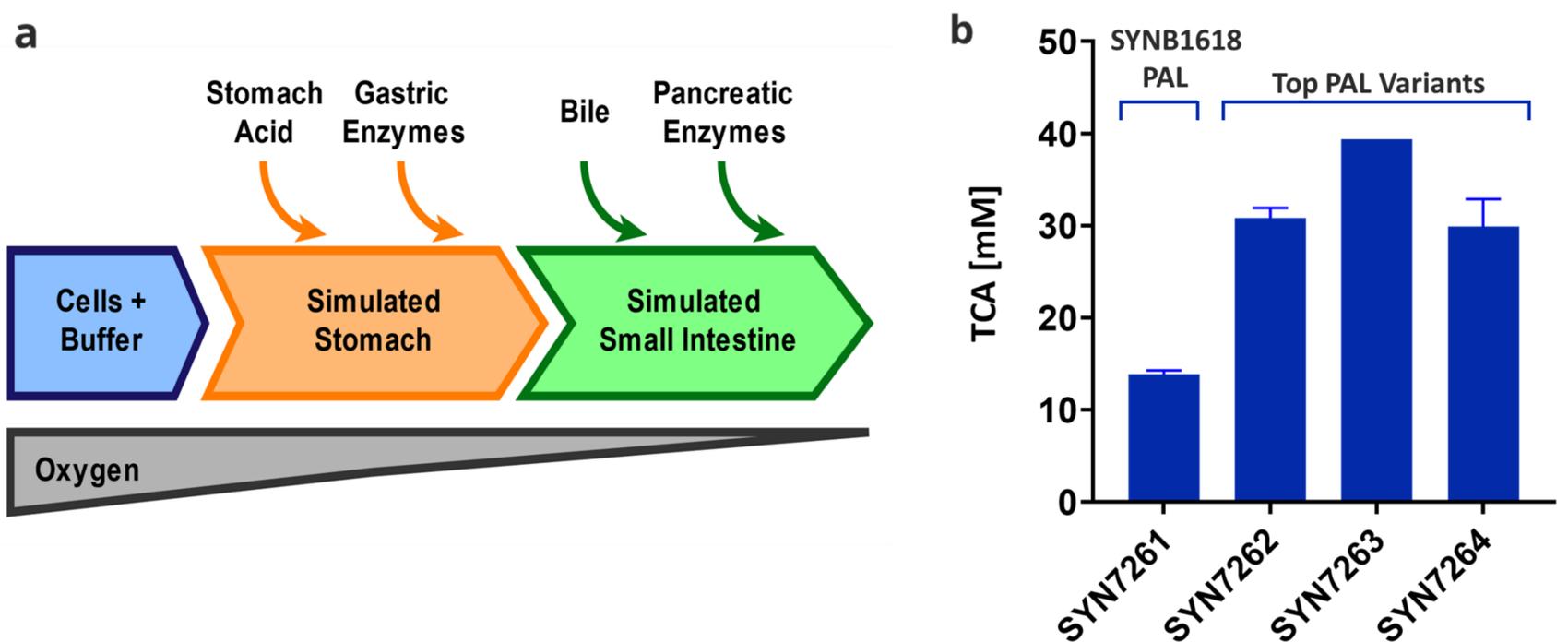


Fig 5: (a) Model of the *in vitro* gastric simulation (IVS) model (b) Fermenter produced cells were tested in IVS model. Cells (2.5×10^9) were incubated under microaerobic conditions for 2 h at 37°C in simulated gastric fluid containing 20mM Phe. TCA was quantified by LC-MS/MS. The increase in TCA production of PAL variants over rounds of enrichments was seen in IVS assay.

Key engineered genetic elements of SYN1934

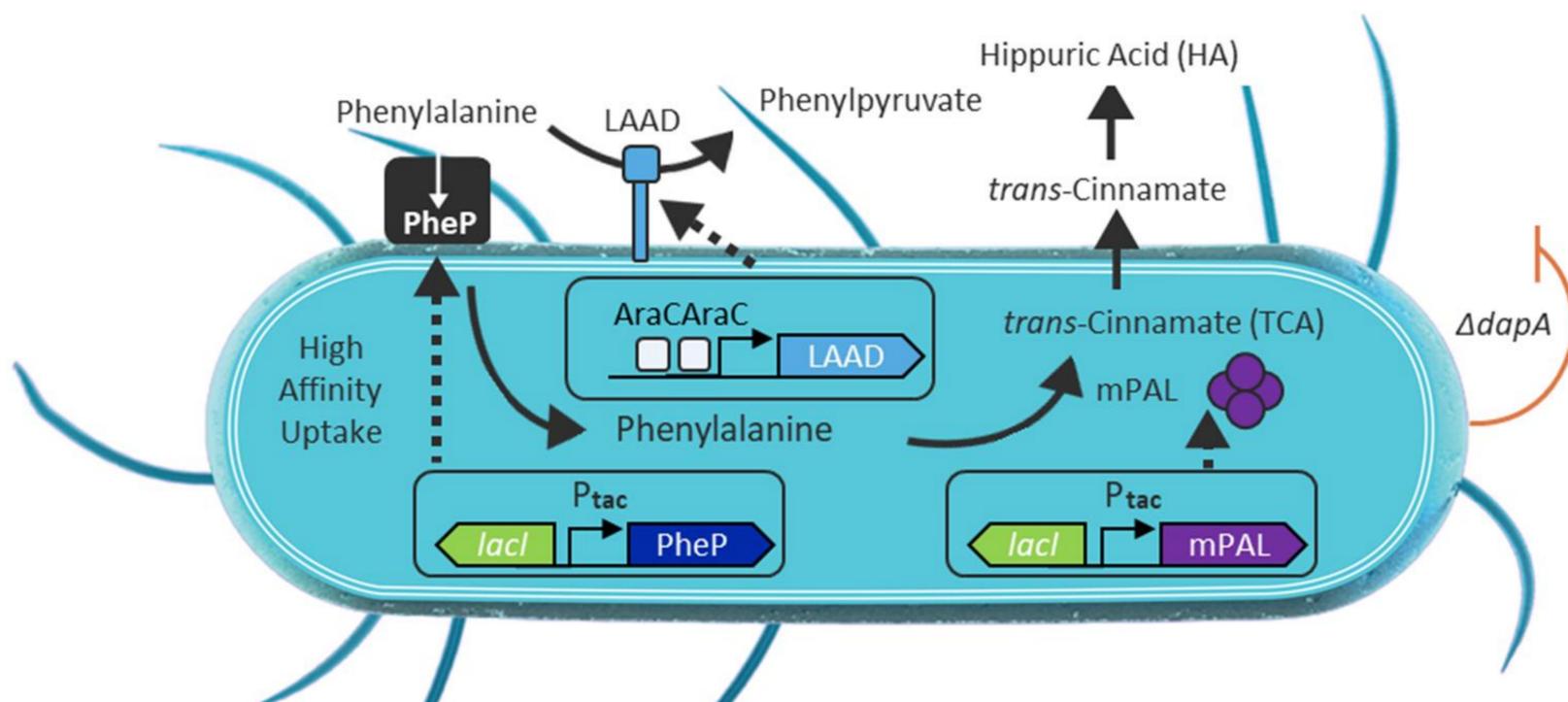


Fig. 6. SYN1934 contains chromosomally integrated genes encoding PheP, a high affinity Phe transporter, mPAL, phenylalanine ammonia lyase from *Photobacterium luminescens* with the following engineered mutations: S92G, H133M, I167K, L432I, V470A which converts Phe to TCA, and LAAD, L-amino acid deaminase, 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.

SYN1934 demonstrated higher activity *in vitro*

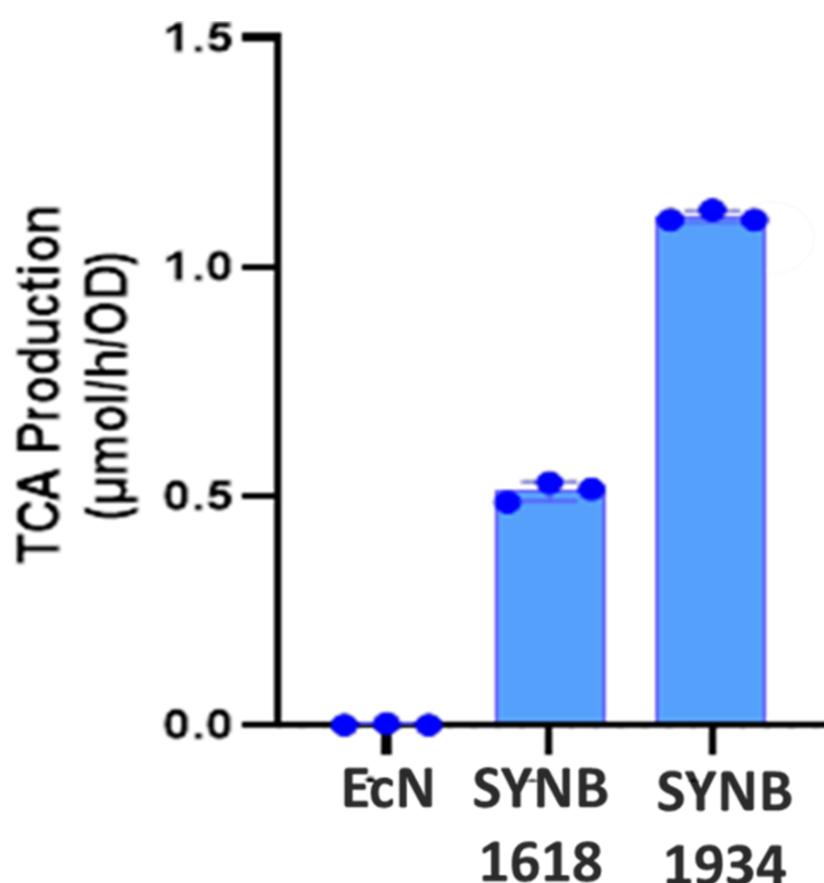


Fig 7. Fermenter produced cells were incubated statically under reduced oxygen conditions for 90 min at 37°C in M9 media containing 4mM Phe. The concentration of TCA was determined by LC-MS/MS. The data demonstrate that SYN1934 is capable of consuming higher levels of Phe compared to SYN1618 *in vitro* and the increase is due to expression of the evolved mPAL enzyme.

SYNB1934 demonstrated increased biomarker *in vivo*

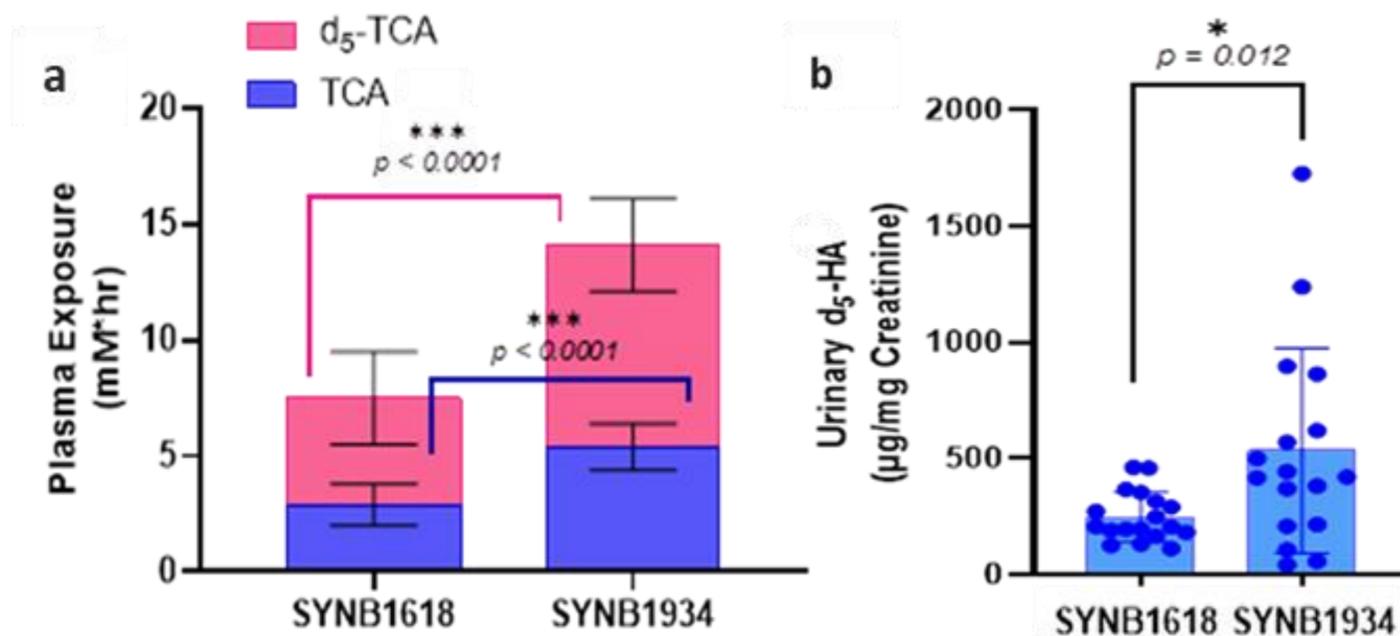


Fig. 8. Non-human primates (NHPs) were dosed orally with a 5 g peptide and 0.25 g d₅-Phe bolus followed by dosing with 1×10^{11} resuspended lyophilized SYNB1618 or SYNB1934 cells. Plasma areas under the curve (AUCs) for strain-specific biomarkers TCA and d₅-TCA (a) and urinary d₅-HA concentration normalized to creatinine (b) are shown. NHPs that received SYNB1934 demonstrated a significant increase in plasma exposure to both TCA and d₅-TCA as well as a urinary d₅-HA concentration 2-fold higher than NHPs that received SYNB1618.

Conclusions

- A specific TCA-responsive biosensor as well as large library of PAL variants were developed for initial HTS in whole cell format using microfluidics and FACS sorting.
- Using the directed evolution platform to screen >1M-member combinatorial library, PAL variants were identified with increased activity; top PAL variant (mPAL) with mutations: S92G, H133M, I167K, L432I, V470A.
- SYNB1934 was developed by integrating four copies of mPAL in *E. coli* Nissle containing PheP and LAAD.
- SYNB1934 demonstrated 2-fold higher TCA production *in vitro* compared to SYNB1618 and increased strain specific biomarkers in non-human primates compared to SYNB1618.
- SYNB1934 was included in Phase 1, dose-escalation, placebo- and active-controlled crossover study to assess the safety, tolerability, and pharmacodynamics in healthy volunteers (NCT04984525).