Background
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, putting them at risk of poor cognitive outcomes. SYNB1618, a live modified strain of the probiotic bacterial E. coli Nissle was engineered to consume phenylalanine (Phe) in the gastrointestinal tract (GI) as a novel treatment approach. The safety, tolerability and pharmacodynamics (PD) of multiple doses of a solid oral formulation of SYNB1618 were evaluated in healthy volunteers (HV).

Methods
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 aromatic 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. For biocontainment the strain is a diaminopimelate (dap) auxotroph. A schematic of the strain design is shown below.

Results

Study Population
A total of 88 healthy volunteers were enrolled; 72 subjects in 9 MAD cohorts and 16 subjects in Part 2 in 2 dose escalation cohorts; majority were Caucasian (86%), age range 18-63 y, 39% female. The study dose ranged from 1 x 10^11 to 4 x 10^12 live cells TID.

Safety
The solid oral formulation of SYNB1618 was well tolerated. The MTD was 2 x 10^12 live cells. The most commonly occurring TEAEs were GI-related events and headache; the majority of these events were mild or moderate. Nausea and vomiting were the dose-limiting symptoms. Adding a dose-ramp in Part 2 Cohort 2 improved the tolerability of the 2 x 10^11 dose and decreased the incidence of AEs. Prophylactic use of ondansetron did not prevent nausea and vomiting. No deaths or SAEs occurred during the study. One subject withdrew from the study due to vomiting. A temporary dose-related increase in CRP was observed in some subjects; the clinical significance of this finding is unclear. All subjects cleared SYNB1618 from their stool within 7 days after the last dose.

Tracer Study: PD effects
Dose-dependent reductions in D5-Phe AUC were observed in subjects administered SYNB1618 compared with placebo subjects after the protein load in the tracer study, but not in unlabelled Phe AUC. A corresponding increase in strain-specific biomarkers D5-TCA and D5-HA was clearly demonstrating strain consumption of Phe within the GI tract. Figure 1.

Activity of SYNB1618 without dietary protein load
Biomarker Study (fasted state)
In a placebo-controlled cohort after an overnight fast on Day 1, subjects were given 2 x 10^11 live cells of SYNB1618. They remained fasted until blood and urine sample collection was completed after 6 hrs. The production of TCA in the fasted state was similar in magnitude to the production after a protein load in the tracer study on Day 2 in the SYNB1618 treated group. Figure 3 No TCA production was observed in the placebo group as expected. Similarly, in an uncontrolled SYNB1618 cohort in Part 2 dosed with 2 x 10^11 live cells of SYNB1618 after an overnight fast, plasma Phe level measured 2hrs later tended to decrease slightly in the fasted state compared to an increase of a 20g protein load. Figure 4. These data indicate presence of resident Phe in the gut even in the fasted state enabling strain activity independent of dietary Phe intake.

Conclusions
• The solid oral formulation of SYNB1618 was well tolerated and metabolically active in the human GI tract.
• SYNB1618 reduced the increase of plasma D5-Phe in the tracer study in a dose-dependent manner in healthy volunteers.
• SYNB1618 demonstrated evidence of activity even without protein intake (fasted state), suggesting residual Phe in the GI tract.
• These data support the further clinical development of this live biotherapeutic therapy for the treatment of PKU. A Phase 2 proof-of-concept study SynPheny-1 (NCT04534842) is ongoing.