Development and Preclinical Characterization of a Solid Oral Formulation of a Synthetic Biotic for the Treatment of PKU

William Querbes, Vasu Sethuraman, Vincent Isabella, Eugene Antipov, Philippa Reeder, Mylene Perreault, Lok Heng Wong, Mary Castillo, Mark Charbonneau, Pasquale Cantarella, Marja Puurunen, Larry Blankstein, Ron Shmueli, Mary Daza, Mrinalini Nikrad, Alex Taylor, Alex Wong, Paul F Miller, Caroline Kurtz

Synlogic Inc., Cambridge, Massachusetts, USA

BACKGROUND

Abstract

Phenylketonuria (PKU) is an inherited metabolic disorder resulting from the inability of the liver to break down Phenylalanine (Phe) leading to the dysregulation of metabolites in the brain and neurotoxicity. To develop new alternative approaches to a strict low-protein diet we have designed a genetically engineered strain of *Escherichia coli* Nissle that can metabolize Phe in the mammalian gut. The engineered stain, SYNB1618, has been designed to convert Phe into trans-cinnamate (TCA) with the phenylalanine ammonia lysase (PAL) enzyme or to phenylpyruvate (PP) via the L-amino acid deaminase (LAAD) enzyme.

Previously we have shown that oral dosing of SYNB1618 can mediate dose dependent decreases in circulating Phe levels and increases in TCA (or its downstream metabolite, Hippurate, HA) or PP in animal models or in healthy human volunteers. Initial studies have utilized a frozen liquid cell suspension, however, methods to develop a solid dosage form are desirable to improve stability, enable outpatient studies, and improve ease of use for patients. To that end, we have developed a process for lyophilization of SYNB1618 cells which does not impact cell morphology or significantly compromise viability or biologic activity. Lyophilized cells retain the ability to consume Phe and produce TCA or PP in vitro or produce TCA in an *in vitro*, intestinal simulation model. Furthermore, the lyophilized SYNB1618 demonstrated similar HA production to a liquid cell suspension in both a mouse model of PKU and in healthy non-human primates. We conclude that lyophilization of SYNB1618 does not result in significant loss of activity and there is a clear path forward for a solid oral formulation in the development of SYNB1618 for the potential treatment of PKU.

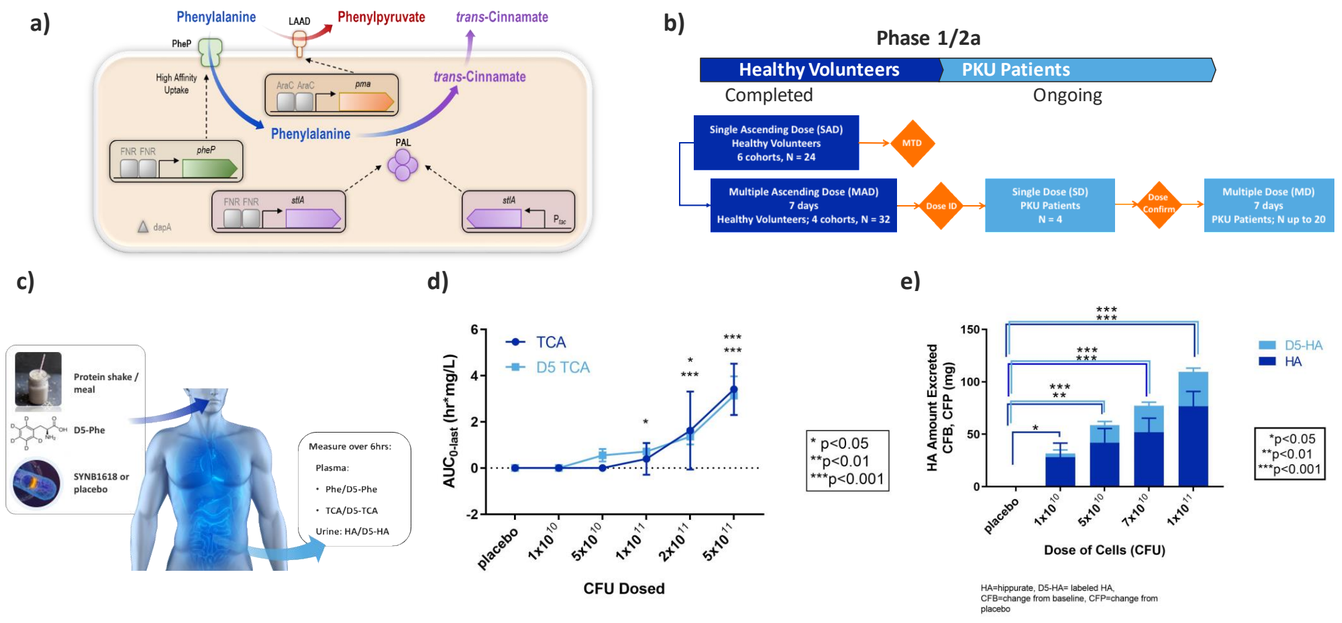
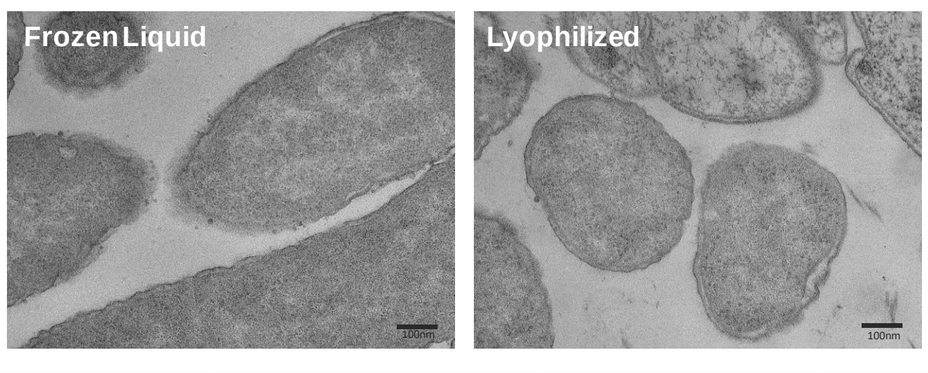


Figure 1. Design of SYNB1618 strain and activity in healthy human volunteers. a) The genome of *E. coli* Nissle has been modified to include additional copies of the high affinity Phe transporter (PheP) to increase Phe uptake, copies of the PAL enzyme to covert Phe to trans-cinnamate (TCA) and the LAAD enzyme to convert Phe to Phenylpyruvate. The dapA gene has been deleted to make the strain a diaminopimelate (DAP) auxotroph which limits replication *in vivo* and acts as a biocontainment strategy after excretion without exogenous DAP present. b) Overview of SYNB1618 Phase 1/2a study in healthy volunteers and PKU patients. c) Dosing paradigm including use of D5-Phe tracer to monitor SYNB1618 activity. d) Plasma TCA levels following a single dose of SYNB1618 in healthy volunteers. e) Urinary HA and D5-HA levels following multiple doses of SYNB1618 in healthy volunteers.

METHODS

Figure 2. Process to develop a solid SYNB1618 formulation

RESULTS



Presentation	Total Cells/mL Mean (SD)	Live Cells/mL Mean (SD)	CFU/mL Mean (SD)
Frozen Liquid	2.36e11 (9.5e9)	2.13e11 (8.4e9)	8.27e10 (2e10)
Lyophilized	1.65e11 (1.6e10)	1.33e11 (1.6e10)	1.09e10 (1.5e9)

Figure 3. Morphology and viability of solid formulation a) Electron microscopy images of SYNB1618 cells prepared as frozen liquid or lyophilized. b) Total cell, live cell, or CFU measurements of each presentation

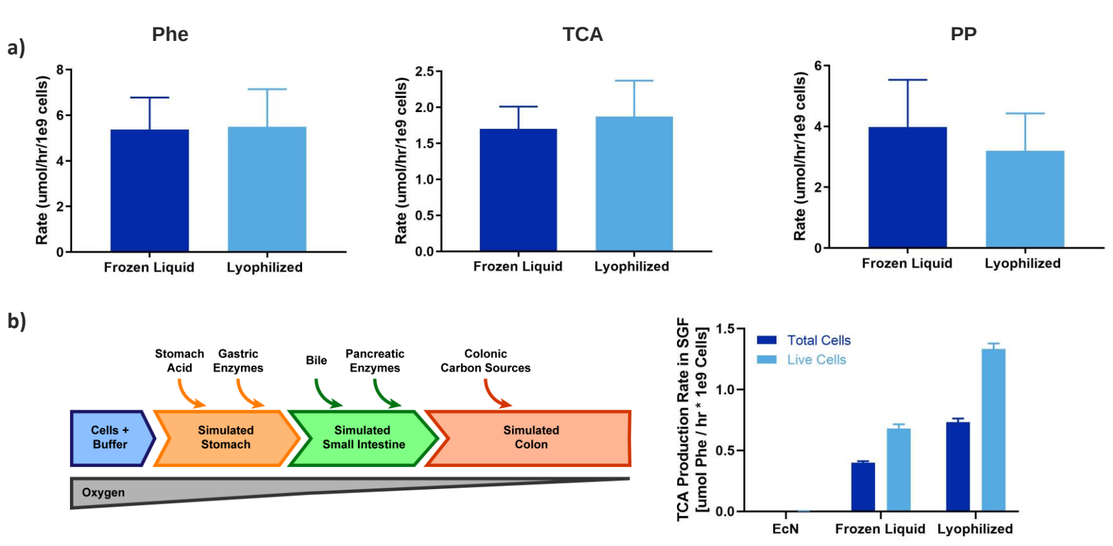


Figure 4. Activity of Lyophilized SYNB1618 cells *in vitro* in media containing Phe (a), or in a *in vitro* gut simulation system (IVS) mimicking oxygen and pH conditions of the human GI tract (b).

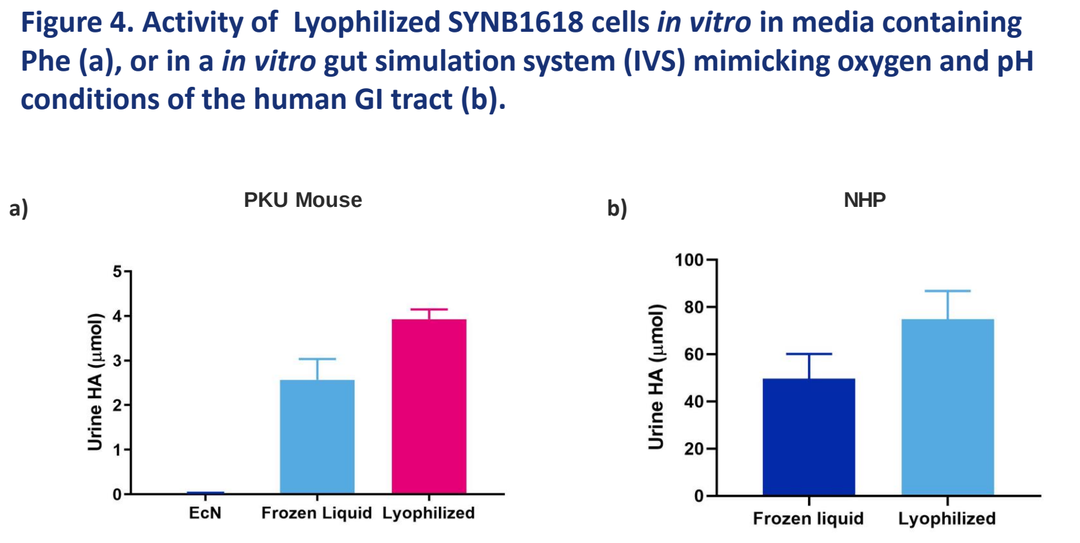


Figure 5. *In vivo* activity of solid formulation in a PKU Mouse model and in NHPs a) Mice were administered 4.1e10 live cells over 3 doses which is the equivalent of 1.3e10, 2.1e10, and 1.4e9 CFU for EcN, frozen liquid and lyophilized groups respectively b) NHP were administered 1.3e11 live cells in a single dose which is equivalent to 6.8e10 and 4.2e9 CFU for frozen liquid and lyophilized groups.

RESULTS

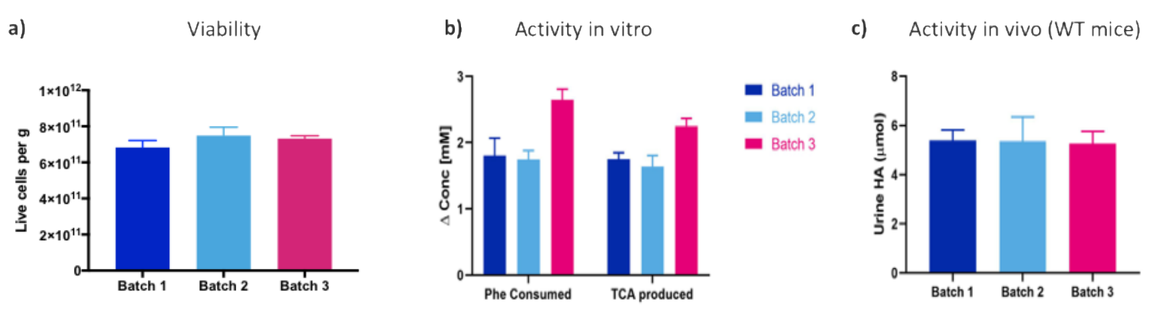


Figure 6. Batch to batch consistency of solid process. Three independent batches were evaluated for viability (a), activity *in vitro* (b), or activity *in vivo* (c) in a WT mouse

	Process 1	Process 2		
Characteristics (Unit)	Frozen Liquid	Solid Batch 1	Solid Batch 2	Solid Batch 3
CFU (mL)	8.07×10^{10}	5.65×10^{10}	1.49×10^{10}	2.15×10^{10}
Total Cell (mL)	2.59×10^{11}	1.29×10^{11}	1.38×10^{11}	1.34×10^{11}
Live Cell (mL)	2.2×10^{11}	1.08×10^{11}	1.19×10^{11}	1.16×10^{11}
Viability (%)	89%	84%	86%	87%
Activity (TCA) µmol/hr/1e9 cells	4.34	2.14	2.36	1.81
Activity (PPA) µmol/hr/1e9 cells	4.07	2.82	2.61	3.05
Free DNA (µg/mL)	207	195	111	85
Free Protein (µg/mL)	11,808	5,804	3,328	3,677
Free Endotoxin (EU/mL)	4.6×10^{7}	1.78×10^{7}	3.91×10^{7}	3.66×10^{7}
Viscosity (cP)	445	34	36	31

Figure 7. Analytical characterization of batches made with new solid process compared to previous frozen liquid batch

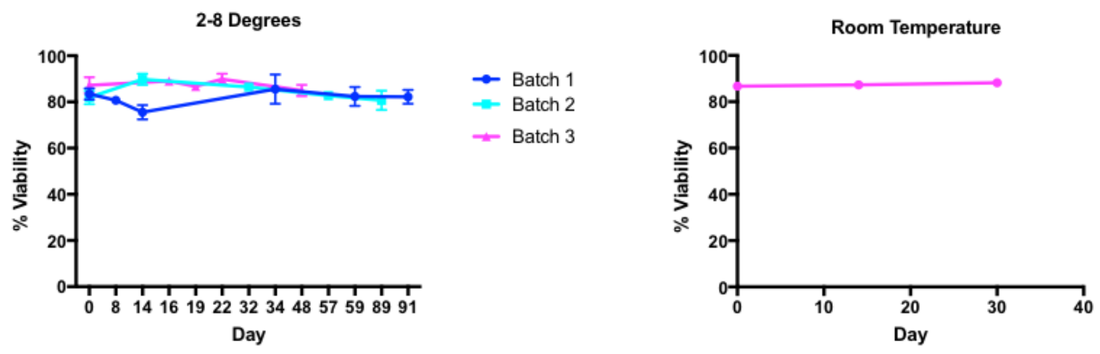


Figure 8. Stability of solid SYNB1618 formulation either at 2-8 degrees or room temperature.

CONCLUSIONS

- We have developed a process for a solid formulation of SYNB1618 with minimal impact on cell viability or activity.
- Lyophilized SYNB1618 is similarly active to frozen liquid in consumption of Phe or production of TCA/HA *in vitro* and *in vivo.*
- Process robustness has shown batch to batch reproducibility in cell viability and activity at the 30L scale.
- Lyophilized SYNB1618 is stable for ≥90 days at 2-8 C and ≥30 days at room temperature.
- The new solid process is expected to have improved quality attributes including less free protein and reduced viscosity.
- A solid oral SYNB1618 formulation with improved stability and convenience has potential as a new therapy for managing blood Phe levels in PKU patients.