An Engineered E. coli Nissle for the treatment of Phenylketonuria (PKU)

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Synthetic Biotic Medicines: A Novel Class of Living Medicines

**Synthetic**
- Engineered bacteria
- With designed genetic circuits
- To degrade metabolites that induce disease or synthesize substances to treat disease

**Biotic:** *E. coli* Nissle as chassis:
- Widely-used oral probiotic
- Leverage the safety of probiotic
- Found within natural human microbiome
- Amenable to genetic manipulation

**Synthetic Biology + Bacteria = Synthetic Biotic Medicine**

Therapeutic delivered locally to treat systemic diseases
Synlogic Synthetic Biotic Platform:
Bringing Rational Drug Development to the Microbiome

Build Potency

Rational design:
- Synthetic biology tools applied
- Engineer potency
- Exceed endogenous bacterial activity

Apply Pharmacological Principles

Pharmacologically tractable:
- Non-colonizing
- Measurable dose-response

Develop Reliable Manufacturing

GMP manufacturing:
- Single strain
- Reproducible yield
- Formulation & delivery
SYNB1618 for Phenylketonuria (PKU):
Facilitating Normalization of Plasma Phe Levels

- **Rare Inherited amino acid metabolism disorder**
  - Build up of amino acid phenylalanine (Phe) in the blood and organs caused by mutation/loss of function of Phenylalanine hydroxylase (PAH), which normally converts Phe to Tyr

- **Diagnosed:** 16,500 in US, similar in Europe
  - If left untreated, symptoms include cognitive impairment, convulsions, behavior problems, skin rash, musty body odor

- **Treatment:**
  - Low protein diet (no meat, dairy, nuts, eggs)
    - Difficult to maintain lifelong compliance
  - Kuvan: PAH cofactor (Only for patients with some residual PAH activity)
    - Cofactor of PAH enzyme (20-40% of patients are responsive)
SYNB1618 Mechanism of Action:
Designed to Convert Toxic Phenylalanine to non-toxic metabolites

Key strain design elements:
• PAL (Phenylalanine ammonia lyase) – Breaks down Phe to non toxic byproduct, trans-cinnamate (TCA)
• pheP – High affinity Phe transporter – increase rate of Phe uptake into engineered cells, alleviating transport bottleneck
• FNR (fumarate and nitrate reductase regulator) promoter – Activates transcription of payload in vivo
• AΔdapA auxotrophy as biocontainment element
SYNB1618 Mechanism of Action: Designed to Convert Toxic Phenylalanine to Trans-cinnamic Acid

Amino acids from protein [Absorption and Recirculation]

Healthy

Phenylalanine Hydroxylase (PAH): converts Phe into Tyrosine

Impaired PAH

Accumulation of Phe to toxic levels

SYNB1618

Normalized levels of Phe

Phenylalanine

High-Affinity Uptake

Syntetic Genetic Circuit

Phenylalanine

t-Cinnamic Acid

Metabolic Conversions

Probiotic bacteria: E. Coli Nissle

When Phe is not efficiently metabolized (PKU) SYNB1618 provides an alternative mechanism
Mechanism of Action:
Functional analysis of PAL and pheP in vitro in E. coli Nissle

- Expression of PAL leads to production of trans-cinnamate (TCA) as a product of Phe degradation
- Uptake of Phe is rate-limiting; Expression of transporter, pheP, led to a 7-fold increase in the rate of TCA production/Phe degradation
Pah^{enu2/enu2}: A mouse model of PKU
Profiling the mouse model and the small intestine as a “Phe sink”

A. Standard diet (SD) vs. Phe-deficient diet (PDD) in Pah^{enu2/enu2} mice

B. Stable elevation of Phe post-SQ injection in Pah^{enu2/enu2} mice

C. High Phe in small intestine of Pah^{enu2/enu2} mice

D. High Phe in small intestine of C57BL/6 mice
Dietary vs Non-Dietary sources of Phe
Enterorecirculation as a source of free Phe

Enterorecirculation and Phe:
- Dietary protein is not the only source of Phe in small intestine
- Amino acids recycled into the GI tract for reabsorption
- High steady-state levels of free Phe in the small intestine

Endogenous proteins
- 65-200g/day
  - Digestive enzymes
  - Mucins
  - Serum albumin
  - Immunoglobulins
  - Epithelial proteins

Dietary proteins
- 50-100g/day
  - Free amino acids
  - Oligopeptides

Chang TM, Nature Reviews in Drug Discovery, 2005
Dave AL et al., Peptides, 2016
Enterorecirculation of Phe in mice
Isotopically-labeled Phe in blood appears in the GI tract

Enterorecirculation and Phe:
- Phe delivered to the blood was found in the small intestine of both PKU and WT mice as early as 20 min
Hippurate (HA): A biomarker of SYNB1618 activity in vivo
The fate of orally dosed TCA

- Essentially all orally dosed TCA recovered as urinary hippurate (HA)
- HA could serve as a biomarker of SYNB1618 activity in vivo
Dose-dependent activity of SYNB1618 in $Pah^{enu2/enu2}$ mice:
HA is a biomarker of SYNB1618 activity in vivo

Urine collection over 4h for determination of HA recovery

Each dot represents urine collected from a metabolic cage of 3 mice/cage
In vivo efficacy of SYNB1618 in \( \text{Pah}^{enu2/enu2} \) mice

SYNB1618 reduces enterorecirculating Phe with concomitant production of urinary HA

**Results:**
- Enterorecirculating (non-dietary) Phe is accessible within the GI tract; its degradation can lead to significant serum Phe reduction
In vivo activity of SYNB1618 in healthy non-human primates
Evidence for a “Phe sink” and enterorecirkulation in a primate model

Results:
- Significant HA recovered from fasted animals, even those that did not receive a peptide bolus
- IV $^{13}$C$_6$-Phe was recovered in the urine as $^{13}$C$_6$-HA, demonstrating enterorecirkulation and SYNB1618 activity
**In vivo efficacy of SYNB1618 in healthy NHPs**

SYNB1618 results in significant blunting in serum Phe following challenge

**Results:**
- SYNB1618 administration led to significant decrease ($p = 0.015$) in $d_5$-Phe AUC with corresponding increase in $d_5$-HA recovered in the urine
Dose-responsive activity of SYNB1618 in healthy NHPs
SYNB1618 exhibits dose-dependent pharmacokinetics

Results:
- Dose-responsive recovery of urinary HA
- Dose-responsive serum AUC for TCA and HA
- Significant blunting of serum Phe elevation at the 3 highest doses of SYNB1618 (p < 0.05)
Conclusions

• A chromosomally integrated, modified *E. coli* Nissle strain, SYNB1618, was created and could degrade Phenylalanine to the non-toxic product *trans*-cinnamate
  • Activity of the strain could be enhanced by co-expression of high affinity transporter, *pheP*

• Phenylalanine is abundant in the small intestine
  • Both dietary and non-dietary Phe make up a “reservoir” of Phe in the GI tract
  • Phe from the blood can re-enter the GI tract through enterorecirculation

• The product of SYNB1618, *trans*-cinnamate, is converted to hippurate and excreted in urine, which can be used as a quantitative biomarker of in vivo strain activity

• SYNB1618, administered orally, can result in significant decreases in serum Phe in both mice and NHP
  • SYNB1618 also exhibits dose-responsive pharmacokinetics

• SYNB1618 has entered Phase 1 trials in healthy volunteers
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Back Up
Pharmacokinetics of SYNB1618 in mice

SYNB1618 exhibits rapid transit

- SYNB1618 gavaged to C57BL/6 mice and GI compartments plated over time
- Complete clearance from all animals within 48h
- Transit of SYNB1618 through the small intestine was rapid

- Progression to primates anticipated to be a more ideal translation model