Development of an Investigational Methionine-consuming Synthetic Biotic Medicine (SYNB1353) for the Treatment of Homocystinuria

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Introduction

• Classic homocystinuria (HCU) is a recessive inherited disorder caused by a defect in cystathionine β-synthase (CBS) which results in abnormal methionine metabolism and leads to an accumulation of homocysteine (Hcy) in the body.

• HCU is a multisystem disorder characterized by impairments of the eye, skeletal system, vascular system, and CNS. Early initiation of methionine-restricted diet significantly lowers the risk of developing complications in HCU mice and patients, but compliance to low protein diet is difficult (1-4).

• Synthetic biotic bacteria can be designed to consume toxic dietary metabolites to replicate the benefits of dietary restriction. Using Ginkgo’s proprietary metagenomic, codebase and protein engineering libraries, we have developed a potential therapeutic for HCU by engineering the probiotic E. coli Nissle (EcN) to consume methionine within the GI tract and prevent its absorption and conversion to Hcy.

Synthetic Biotic: a New Class of Medicine

Proven strategy with Phenylketonuria (PKU) lead strain SYNB1618 achieving prespecified 20% phenylalanine lowering in PKU patients

Results

Figure 1. Engineered E. coli Nissle SYNB1353 Consumes Methionine and Produces 3-methylthio propylamine (3-MTP) In Vitro. (A) Schematic of engineered E. coli Nissle SYNB1353 with its components. Optimal metP and metDC were identified using Ginkgo’s proprietary metagenomic, codebase and protein engineering libraries. (B) In vitro methionine consumption (solid line) and 3-MTP production (dotted line) by EcN (unengineered bacteria) or SYNB1353. Cells were incubated for the indicated time in M9 medium with 0.5% glucose and 10mM methionine at 37°C, supernatant was collected for methionine (HPLC) and 3-MTP (LC-MS/MS) measurements. *p<0.05 versus EcN. Met: methionine, metP: methionine ABC transporter permease, metDC: methionine decarboxylase, YjeH: methionine exporter.
Figure 2. Engineered E. coli Nissle SYNB1353 is a Non-colonizing Synthetic Biotic Medicine that is Active in Mice. (A) Kinetics of fecal excretion in healthy male mice. Antibiotic resistant EcN or SYNB1353 were orally administered at 1e10 CFU and fecal pellets collected at the indicated timepoints for CFU enumeration. (B) In vivo 3-MTP production by EcN or SYNB1353 in healthy male mice. Mice received a single oral dose of bacteria followed by 200 mg/kg D4-methionine 30 minutes later. Mice were immediately placed in metabolic cages (n=3/cage) and urine collected 5 hours later. *p<0.05 versus EcN.

Figure 3. Engineered E. coli Nissle SYNB1353 Produces 3-MTP and Consumes Methionine in Nonhuman Primates. (A) Nonhuman primate study design. Male cynomolgus monkeys (2-5 years old) were fasted overnight and received an oral methionine load (100 mg/kg) and vehicle or bacteria (1e12 live cells). Plasma was collected throughout, and urine was recovered 6 hours post dosing. (B) Urinary 3-MTP recovery at 6 hours post-dosing. (C) Plasma methionine and (D) total homocysteine levels before (0hrs) and after methionine/bacteria administration. *p<0.05 versus EcN, **p<0.05 versus vehicle.
Conclusions

• SYNB1353 is an engineered E.coli Nissle strain capable of consuming methionine and producing 3-methylthio propylamine (3-MTP) in vitro.

• SYNB1353, a non-colonizing bacterial strain, dose-dependently consumes methionine to produce 3-MTP in mice.

• Concomitant administration of SYNB1353 with an oral load of methionine blunts the appearance of methionine and total homocysteine in the blood of healthy nonhuman primates.

• SYNB1353 is expected to lower methionine and total homocysteine in HCU patients, with mathematical modeling predicting a doubling of natural protein intake and up to 58% lowering of total homocysteine in mild/moderate to severe HCU patients on 1e12 TID dosing.

References


