Activity of SYNB1353, an Investigational Methionine-Consuming Synthetic Biotic Medicine, in an Acute Nonhuman Primate Model of Homocystinuria

Mylene Perreault[1], Erik Gerson[1], Jillian Means[1], Michael James[1], Ted Moore[2], Analise Reeves[1], David Lubkowicz[1], and David Hava[1]
[1]Synlogic Inc, Cambridge, MA, USA [2]Ginkgo Bioworks Boston, MA, USA.

Introduction

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 (**Figure 1**). Elevated Hcy levels are associated with impairments of the eye, skeletal system, vascular system, and central nervous system. In patients with residual CBS activity (~50% of HCU population), vitamin B6 (pyridoxine) is effective at reducing Hcy levels. For pyridoxine unresponsive patients, betaine (involved in remethylation of Hcy to methionine) and a low-methionine diet that is very low in natural protein are the current therapeutic options. Early initiation of a low-methionine diet significantly lowers the risk of developing complications in HCU patients, but compliance to low protein diet is difficult.

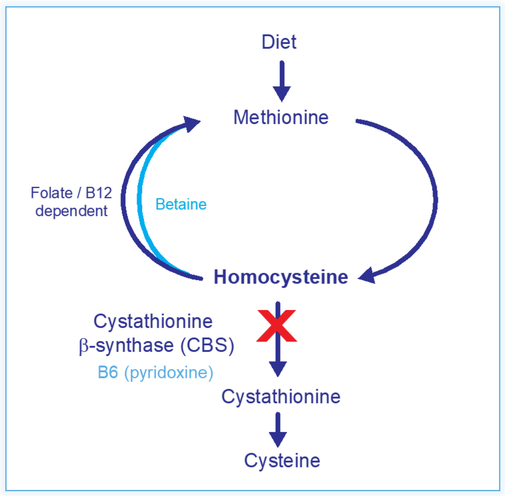

Figure 1. In HCU patients, mutations in the *CBS* gene result in accumulation of Hcy. Pharmacotherapeutic options for the treatment of HCU consist of vitamin B6 (pyridoxine), which can lower Hcy levels in B6-responsive patients, and betaine, which is involved in Hcy remethylation to methionine.

Study Design

The probiotic *E. coli* Nissle (EcN) was engineered to metabolize methionine within the gastrointestinal (GI) tract via the methionine decarboxylase (MetDC) pathway (**Figure 2**). Using proprietary codebase and metagenomic libraries, combined with protein engineering strategies, MetDC from *Streptomyces sp. 590* and methionine importer MetP from *Flavobacterium segetis* were identified by metagenomic screen and MetDC was further optimized via protein engineering. Genes encoding these proteins were chromosomally integrated under the control of a chemically inducible promoter, P_{tac}, which is induced by isopropyl β-D-1-thiogalactopyranoside (IPTG). To prevent the release of methionine in the GI tract once it enters the cell, the *yjeH* gene that encodes a methionine/branched chain amino acid exporter was deleted. The resulting strain, SYNB1353, converts methionine to carbon dioxide (CO_2) and 3-methylthiopropylamine (3-MTP), which is used as a biomarker of strain activity.

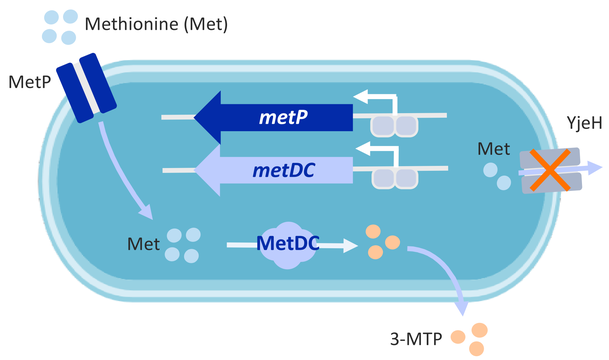

Figure 2. Schematic of engineered *E. coli* Nissle SYNB1353 with its components. Optimal metP and metDC were identified using proprietary metagenomic, codebase and protein engineering libraries.

Results

Engineered EcN SYNB1353 consumes methionine and produces 3-MTP *in vitro*

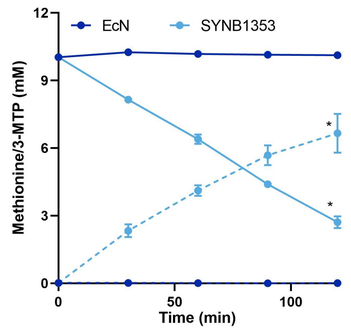

Figure 3. In vitro methionine consumption (solid line) and 3-MTP production (dotted line) by EcN (control bacteria) or SYNB1353. Cells were incubated for the indicated time with 10 mM methionine at 37°C, and supernatants were collected. *p<0.05 versus EcN.

An oral methionine load increases plasma methionine (A) and plasma total Hcy (B) in healthy cynomolgus monkeys

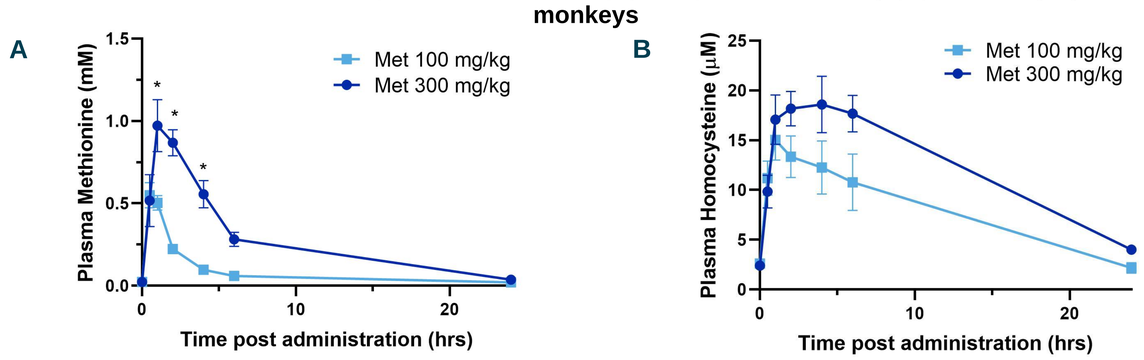

Figure 4. Monkeys were fasted overnight and received a single oral dose of methionine (100 or 300 mg/kg). Blood was collected for methionine and Hcy measurements. Data presented as mean ± SEM (n = 6/group). Statistical analysis was performed using two-way repeated ANOVA with Sidak's multiple comparison test. *p < 0.05 versus 100 mg/kg methionine.

SYNB1353 is active in a nonhuman primate model of acute homocystinuria

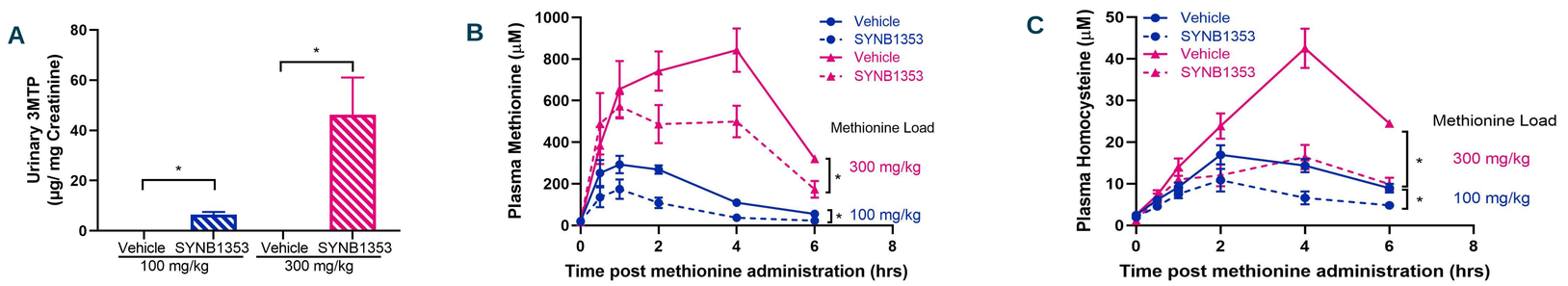

Figure 5. Monkeys were fasted overnight and received a single oral dose of methionine (100 or 300 mg/kg) with vehicle or SYNB1353 (1 x 10^{12} live cells). Urine was collected for 3-MTP, and blood was collected for methionine and Hcy measurements. Data presented as mean ± SEM (n=12/group for 100 mg/kg methionine, n=6/group for 300 mg/kg methionine). Statistical analysis was performed using unpaired t-test with Welch's correction (A) and two-way ANOVA with Sidak's multiple comparison test (B-C). *p < 0.05.

SYNB1353 dose-dependently increases urinary recovery of 3-MTP (A) and decreases plasma methionine (B) and plasma homocysteine (C) in a nonhuman primate model of acute homocystinuria

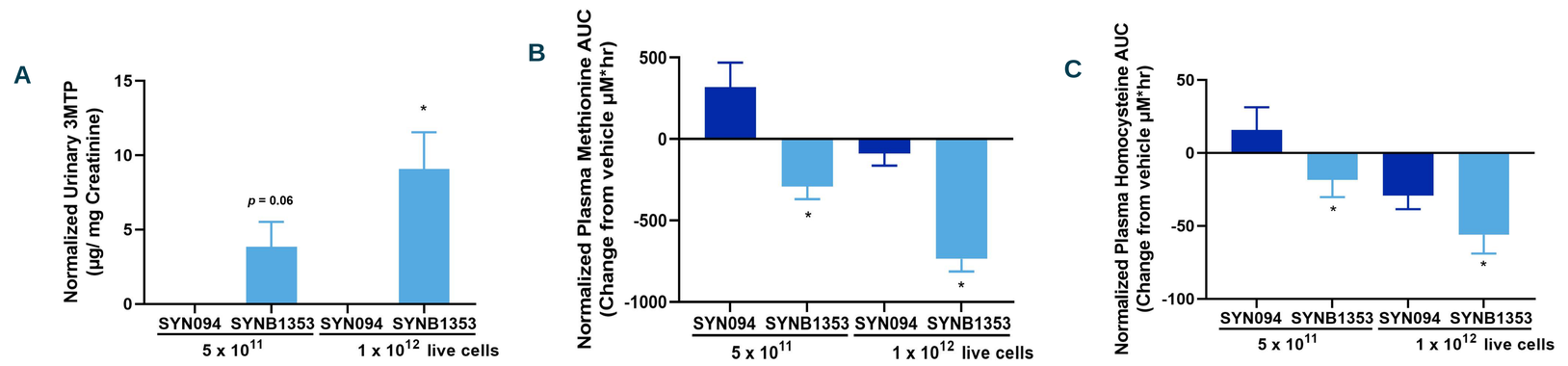

Figure 6. Monkeys were fasted overnight and received a single oral dose of methionine (100 mg/kg) with vehicle, EcN (control strain) or SYNB1353 (5 x 10^{11} or 1 x 10^{12} live cells). Urine was collected for 3-MTP, and blood was collected for methionine and Hcy measurements. Data was normalized to the study-respective vehicle and presented as mean SEM (n = 12/group). Statistical analysis was performed using paired t-test. *p < 0.05.

Conclusions

SYNB1353 is an engineered *E.coli* Nissle strain capable of metabolizing methionine and producing 3-MTP in vitro.

Concomitant administration of SYNB1353 with an oral load of methionine blunts the appearance of plasma methionine and plasma total homocysteine in nonhuman primates. Thus, SYNB1353 represents a promising approach for the treatment of HCU.