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

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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.

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 segnetis 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.

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

SYNB1353 is active in a nonhuman primate model of acute homocystinuria

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

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.