SYNB1353, a Synthetic Biotic engineered for the treatment of HCU, metabolizes methionine and lowers homocysteine in preclinical and clinical models

Jilllan Means, Michael James, Mary McDonald, Julie Blasbalg, Neal Sondheimer, Analise Reeves, Mylene Perreault, David Lubkowicz, David Hava
Synlogic Inc., Cambridge MA, USA

Introduction

• Homocystinuria (HCU) is a rare autosomal recessive disease caused by a loss of function of cystathionine β-synthase, leading to an accumulation of homocysteine (Hcy) in the plasma.

• Patients with high levels of Hcy are at risk for thromboembolism, lens dislocation, skeletal abnormalities, developmental delay, and intellectual disability. Current treatment options are limited due to efficacy and tolerability.

• Many patients must adhere to a heavily methionine (Met) restricted diet; however, lifelong compliance is challenging.

• Here, we present preclinical and clinical results for SYNB1353, an engineered Synthetic Biotic bacteria designed to consume methionine in the gut as a potential therapeutic for the treatment of HCU.

SYNB1353 is an engineered strain of E. coli Nissle (EcN) with optimized components that consumes Met and produces 3-MTP

Results

Protein abundance of MetDC<sub>1353</sub> is increased and exhibits minimal feedback inhibition by 3-MTP

SYNB1353 reduces plasma Met and Hcy in healthy non-human primates in a dose dependent manner

Conclusions

• The engineered MetDC enzyme in SYNB1353 exhibits an increase in protein abundance and minimal feedback inhibition by 3-MTP.

• SYNB1353 blunts plasma Met and Hcy in dose dependent manner in a preclinical NHP model.

• In a double blind, placebo-controlled Phase 1 study, SYNB1353 was generally safe and well tolerated and blunted Met absorption in healthy volunteers after a Met challenge.

SYNB1353 Phase 1 study design

A.

SYNB1353 metabolizes methionine and lowers homocysteine in healthy volunteers

Figure 1: Diagram of dietary methionine cycle. This simplified diagram illustrates the cycling of dietary Met and conversion into homocysteine. Normally, homocysteine is converted into cystathionine by the cystathionine β-synthase enzyme. In HCU patients, this enzyme is absent or nonfunctional leading to the accumulation of homocysteine in the plasma.

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Figure 2: Schematic of SYNB1353. A MetDC (Streptomyces sp. 590; Q70D, M2H2) and importor (MetP, Flavobacterium sp) are integrated into EcN. A) SYNB1353 consumes Met and produces 3-MTP at a significantly greater rate than EcN (*p=0.01). B) Homology model of wildtype Streptomyces MetDC versus the engineered MetDC<sub>1353</sub> with the two amino acid substitutions (Q70D, M2H2) at the interface of the dimer highlighted in red.

Figure 3: Characterizing SYNB1353 in vitro. A) Whole cell proteomics analysis of MetDC<sub>1353</sub> vs MetDC<sub>590</sub> accumulation in isogenic strains. B) Met consumption of SYNB1353 changes in increasing concentrations of 3-MTP to investigate feedback inhibition.

Figure 4: Preclinical activity of SYNB1353 in non-human primates. Dosing of healthy non-human primates (NHP) with either vehicle or two different doses of SYNB1353 by live cell count after a methionine challenge. Normalized AUC change from vehicle of a) plasma Met or b) plasma Hcy.

Figure 5: SYNB1353 was evaluated in a double blind, placebo-controlled Phase 1 trial, with a multiple ascending dose design. Four cohorts using dose level 5x10<sup>11</sup>, 1x10<sup>12</sup> and 5x10<sup>12</sup> live cells were evaluated for safety, tolerability and ability to metabolize Met in four cohorts of healthy volunteers challenged with 30 mg/kg Met before and after dosing with SYNB1353. A) Each cohort (n=6), included 6 subjects dosed with SYNB1353 and 2 dosed with placebo. Safety and tolerability was assessed prior to dose escalation. The 1x10<sup>12</sup> dose was repeated with an alternate formulation. B) In each cohort, SYNB1353 or placebo was administered over a 7 day period with meals, starting with a single dose on day 1, two doses on day 2 and 3 and three doses on days 4-7. A Met challenge (30 mg/kg) was administered on day 1 and day 7 and plasma Met and total Hcy was measured over 24 hrs.

Figure 6: SYNB1353 blocks methionine absorption. Percent change from baseline in day 7 A) plasma methionine and B) total plasma Hcy for cohorts receiving 1x10<sup>12</sup> live cell of SYNB1353. FORM = formulation, LS mean change, 95% CI *p=0.05