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

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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.

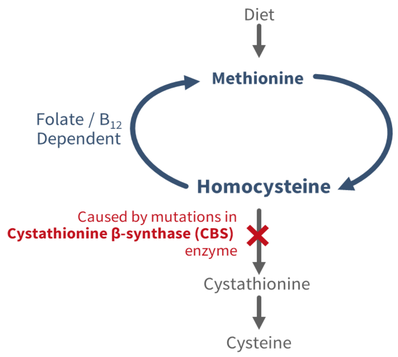

Figure 1. Diagram of dietary methionine cycle. This simplified diagram illustrates the cycling of dietary Met and conversion into homocysteine. Normally, homocysteine is converted into cystathionine by the cystathionine β-synthase enzyme. In HCU patients, this enzyme is absent or nonfunctional leading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

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

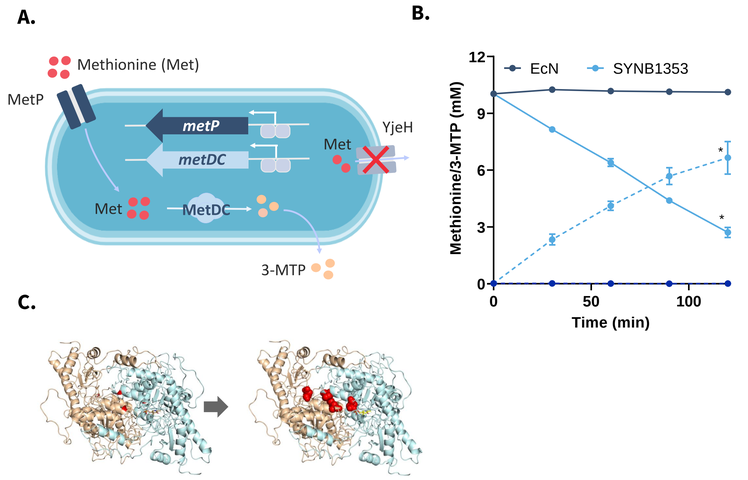

Figure 2. A) Schematic of SYNB1353. A MetDC *(Streptomyces* sp. 590; Q70D, N82H) and importer (MetP, *Flavobacterium segetis*) are integrated into EcN and controlled by inducible promoters; YjeH exporter is deleted. B) SYNB1353 consumes Met and produces 3-MTP at a significantly greater rate than EcN (*p<0.01). C) Homology model of wildtype *Streptomyces* MetDC versus the engineered $MetDC_{1353}$, with the two amino acid substitutions (Q70D, N82H) at the interface of the dimer highlighted in red.

Results

Protein abundance of $MetDC_{1353}$ is increased and exhibits minimal feedback inhibition by 3-MTP

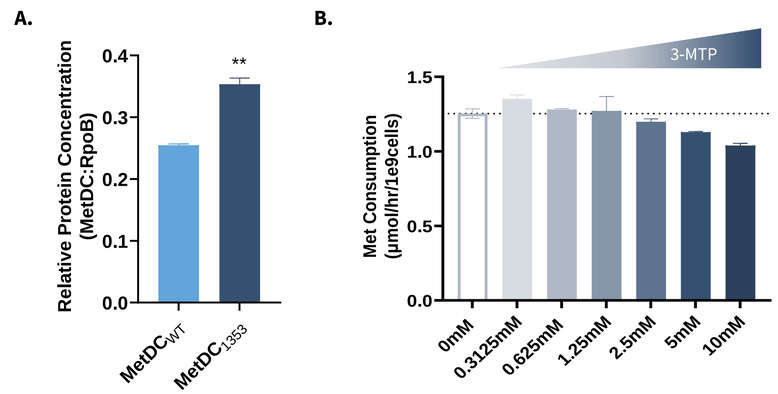

Figure 3. Characterizing SYNB1353 *in vitro*. A) Whole cell proteomics analysis of $MetDC_{1353}$ vs $MetDC_{WT}$ accumulation in isogenic strains. B) Met consumption of SYNB1353 lysate in increasing concentrations of 3-MTP to investigate feedback inhibition.

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

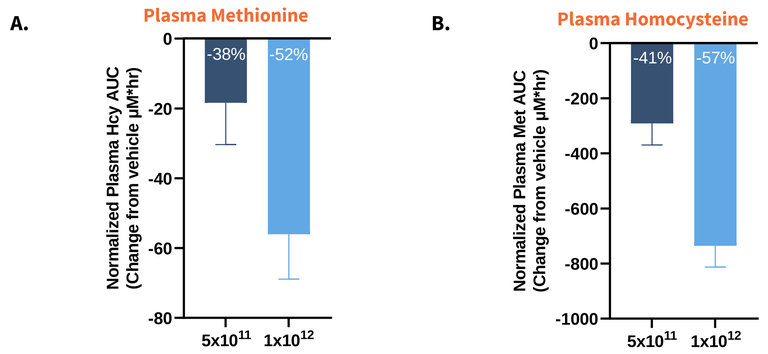

Figure 4. Preclinical activity of SYNB1353 in non-human primates. Dosing of healthy non-human primates (NHPs) with either vehicle or two different doses of SYNB1353 by live cell count after a methionine challenge. Normalized AUC change from vehicle of A) plasma Met or B) plasma Hcy.

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

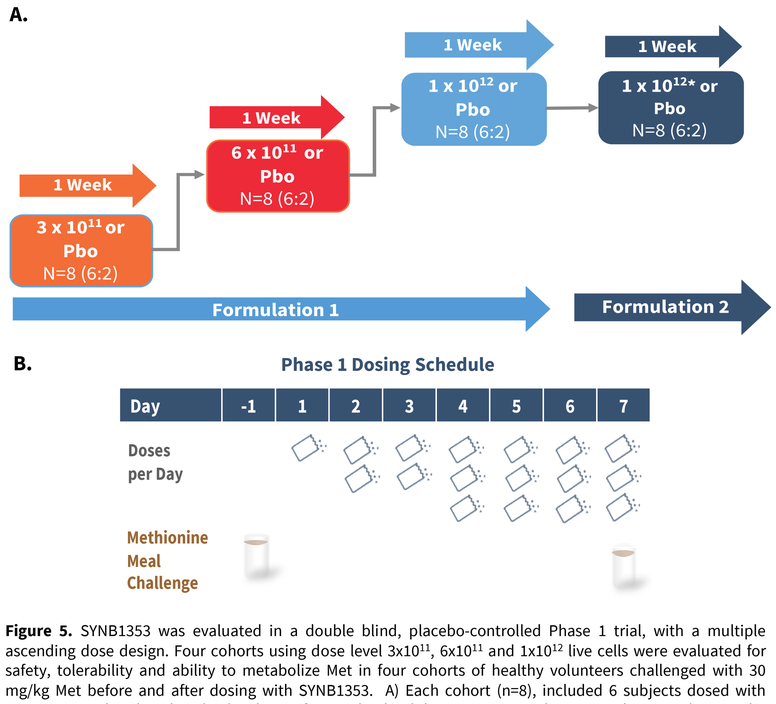

Figure 5. SYNB1353 was evaluated in a double blind, placebo-controlled Phase 1 trial, with a multiple ascending dose design. Four cohorts using dose level $3x10^{11}$, $6x10^{11}$ and $1x10^{12}$ live cells were evaluated for safety, tolerability and ability to metabolize Met in four cohorts of healthy volunteers challenged with 30 mg/kg Met before and after dosing with SYNB1353. A) Each cohort (n=8), included 6 subjects dosed with SYNB1353 and 2 dosed with placebo. Safety and tolerability was assessed prior to dose escalation. The $1x10^{12}$ 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.

SYNB1353 metabolizes methionine and lowers homocysteine in healthy volunteers

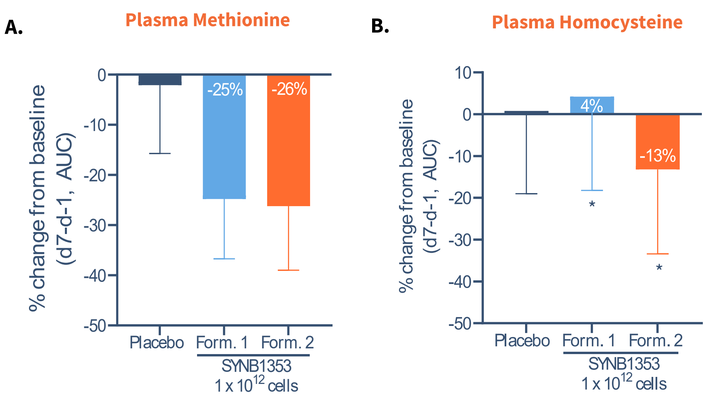

Figure 6. SYNB1353 blocks methionine absorption. Percent change from baseline in day 7 A) plasma methionine $AUC_{0\text{-}24h}$ and B) total plasma Hcy $AUC_{0\text{-}24h}$ for cohorts receiving $1x10^{12}$ live cell of SYNB1353. FORM = formulation. LS mean change, 95% CI *p< 0.05

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