Improvements of SYNB1353, an Engineered Bacteria for the Treatment of Homocystinuria, Lead to Increased *In vitro* and *In vivo* Degradation of Methionine

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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. Restriction of dietary methionine, a precursor to homocysteine, can reduce tHcy levels, but is extremely challenging for patients.
- Here we present preclinical and clinical results for SYNB1353, an engineered Synthetic Biotic designed to consume Met in the gut as a potential orally-administered, non-systemic biopharmaceutical treatment for HCU.

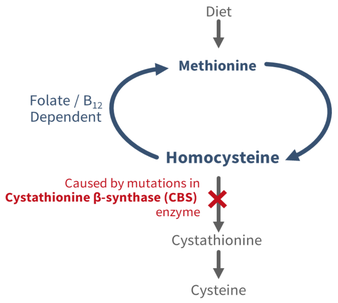

Figure 1. Diagram of dietary methionine cycle. This simplified diagram illustrates the cycling of dietary Met and conversion into homocysteine. Normally, homocysteine is converted into cystathionine by the cystathionine β-synthase enzyme. In HCU patients, this enzyme is absent or nonfunctional leading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

SYNB1353 was engineered to treat HCU by consuming Met in the GI tract, through its conversion to 3-MTP

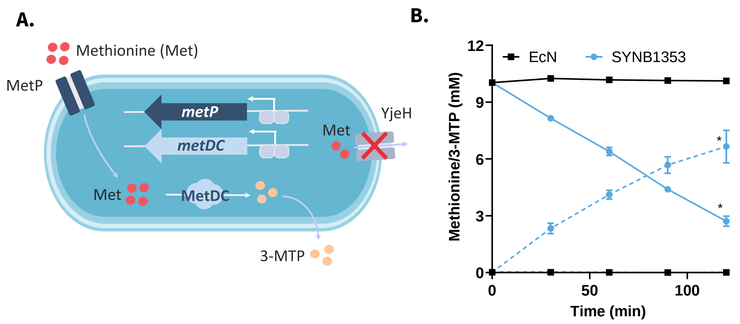

Figure 2. SYNB1353 metabolizes Met via MetDC. A) Schematic of SYNB1353. A MetDC *(Streptomyces* sp. 590; Q70D, N82H) and importer (MetP, *Flavobacterium segetis*) are integrated into EcN and controlled by inducible promoters; YjeH exporter is deleted. B) SYNB1353 consumes Met and produces 3-MTP at a significantly greater rate than EcN (*p<0.01).

Conclusions

- SYNB1353 completed Phase I in Healthy Volunteers and showed a significant reduction of plasma methionine
- Changes in fermentation process improved methionine degradation in SYNB1353
- Improved in vitro activity translated to improved in vivo activity in mice and NHPs
- These improvements will be incorporated into a subsequent Phase 2 for SYNB1353 in HCU, which is currently in development

Results

SYNB1353 metabolizes methionine in healthy volunteers

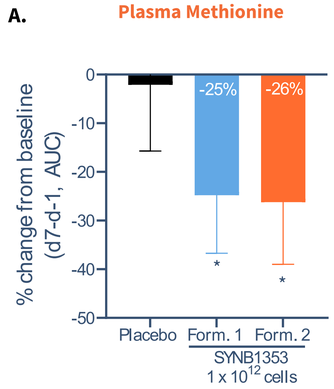

Figure 3. SYNB1353 lowers methionine absorption. SYNB1353 was evaluated in a double blind, placebo-controlled Phase 1 trial, with a multiple ascending dose design. Four cohorts using dose level $3x10^{11}$, $6x10^{11}$ and $1x10^{12}$ live cells were evaluated for safety, tolerability and ability to metabolize Met when challenged with 30 mg/kg Met before and after dosing with SYNB1353. Data show percent change from baseline on day 7 for plasma methionine $AUC_{0\text{-}24h}$ and for cohorts receiving $1x10^{12}$ live cell of SYNB1353 in two different formulations. FORM = formulation. LS mean change, 95% CI *p< 0.05

Changes in fermentation process increase SYNB1353 activity

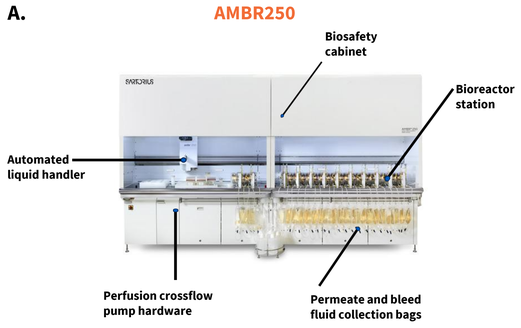

Figure 4. New process increases SYNB1353 in vitro activity > 2-fold. A. Utilizing AMBR250, we identified modifications in the fermentation process such as timing of induction and feed rate, which improve the ability of SYNB1353 to degrade methionine in vitro. **B.** SYNB1353 fermented in the previous process shows an in vitro activity of 1.49 ± 0.07 µmol Met/1e9 cells/hr compared to 3.29 ± 0.11 µmol Met/1e9 cells/hr for the new process. Error bars represent SD.

Proteomics indicate 4-fold change in a co-factor competitor protein

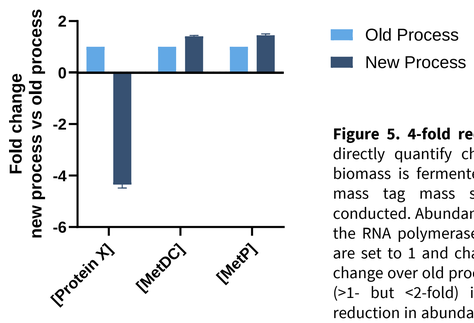

Figure 5. 4-fold reduction of Protein X in new process. To directly quantify changes in protein levels across SYNB1353 biomass is fermented in the old vs the new process, tandem mass tag mass spectrometry (TMT-MS) proteomics were conducted. Abundance of target protein levels are normalized to the RNA polymerase subunit, RpoB. Old process protein levels are set to 1 and changes in new process are expressed as fold change over old process. MetDC and MetP show a slight increase (>1- but <2-fold) in abundance. Protein X shows a 4-fold reduction in abundance in the new process over the old process. Protein X is a PLP-dependent enzyme and might compete with MetDC for PLP.

New process improves SYNB1353 activity in mice

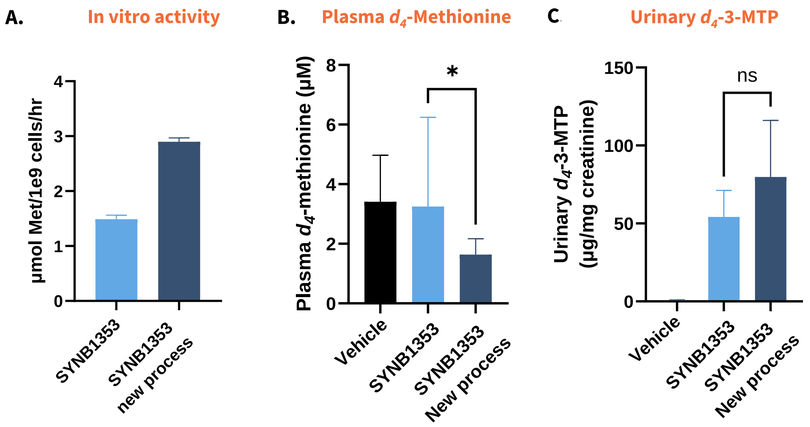

Figure 6. New process improves SYNB1353 activity in mice. Overnight fasted mice received vehicle or SYNB1353 (2.5 x 10^{10} cells) followed by 200 mg/kg d_4-methionine 30 minutes later. Plasma and urine samples were collected 5 hours post d_4-methionine **A.** In vitro activity of batches used for this study. **B.** Plasma d_4-methionine levels. *p < 0.01 **C.** Urinary d_4-3-MTP-glycine normalized to creatine levels. Error bars represent SD for A and SEM for B/C.

New process improves SYNB1353 activity in nonhuman primates

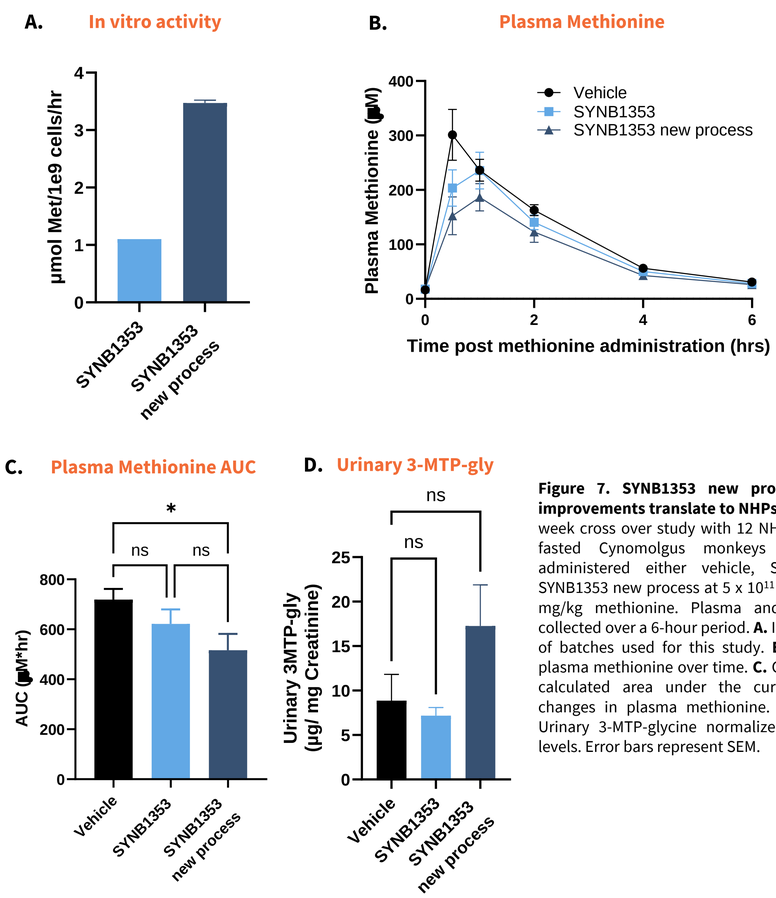

Figure 7. SYNB1353 new process activity improvements translate to NHPs. This was a 3-week cross over study with 12 NHPs. Overnight fasted Cynomolgus monkeys were orally administered either vehicle, SYNB1353, or SYNB1353 new process at 5 x 10^{11} cells with 100 mg/kg methionine. Plasma and urine were collected over a 6-hour period. **A.** In vitro activity of batches used for this study. **B.** Changes in plasma methionine over time. **C.** Corresponding calculated area under the curve (AUC) for changes in plasma methionine. *p < 0.01 **D.** Urinary 3-MTP-glycine normalized to creatine levels. Error bars represent SEM.

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