SYNB1353, A Proposed Therapy for Homocystinuria, Lowers Plasma Methionine and Homocysteine in Healthy Volunteers Exposed to a Methionine Challenge

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Introduction

Classical homocystinuria (HCU) is an inherited disorder caused by pathogenic variants in the cystathionine beta-synthase (CBS) gene **(Figure 1)** resulting in excessive accumulation of homocysteine (Hcy) and multiorgan clinical manifestations. Early initiation of methionine-restricted diets significantly lowers the risk of developing complications in HCU. Elevated Hcy levels are associated with impairments of the eye, skeletal system, vascular system, and central nervous system.[1] 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[2] that is very low in natural protein are the current therapeutic options.

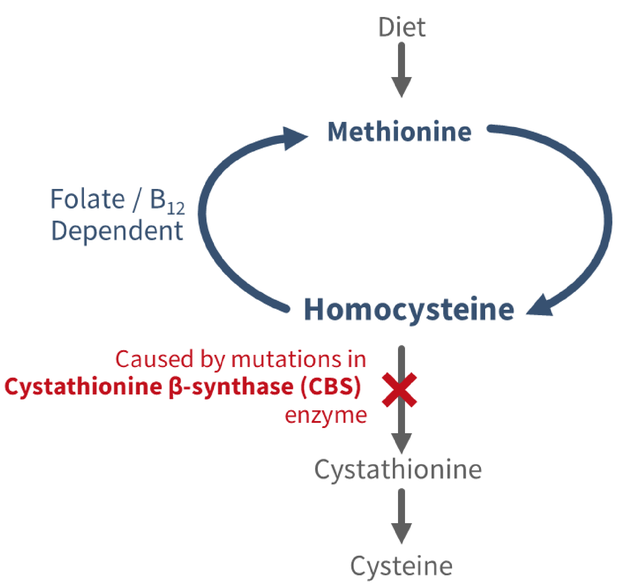


Figure 1. Mutations in the CBS gene cause the accumulation of Hcy and HCU. In HCU patients, mutations in the *CBS* gene result in accumulation of Hcy. Pharmacotherapeutic options for the treatment of HCU consist of vitamin B6 (pyridoxine), which can lower Hcy levels in B6-responsive patients, and betaine,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

SYNB1353: A methionine metabolizing synthetic biotic

SYNB1353 was engineered from the probiotic *E. coli* Nissle (EcN) to metabolize methionine (Met) via the methionine decarboxylase (MetDC) pathway, preventing its conversion into homocysteine. SYNB1353 converts Met to 3-methylthiopropylamine (3-MTP). To prevent the release of methionine once it enters the cell, the YjeH gene was deleted which is responsible for Met export in EcN.

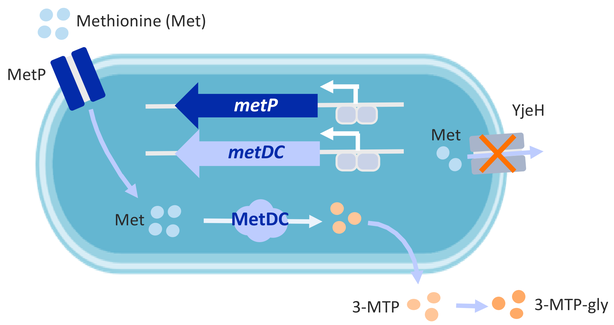


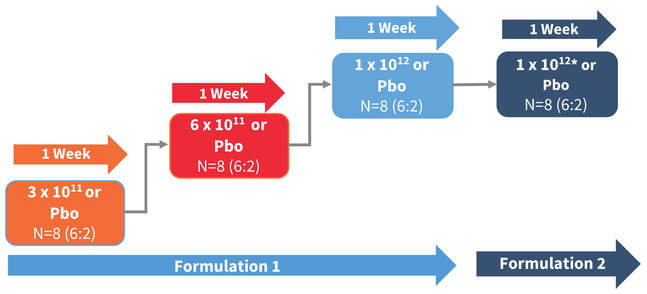
Figure 2. Schematic of SYNB1353. Met enters through the importer MetP into the cell and is converted via MetDC to 3-MTP. The strain contains a deletion of *yjeH*, a methionine exporter. 3-MTP is further metabolized *in* vivo to 3-MTP-glycine (3-MTP-gly) by the liver thus preventing further conversion to Hcy. Measurement of 3-MTP-gly provides evidence of strain activity *in vivo*

References:
1. Sacharow SJ, Picker JD, Levy HL. Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency. 2004 Jan 15 [updated 2017 May 18]. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2023. PMID: 20301697.
2. Morrison T, Bösch F, Landolt MA, Kožich V, Huemer M, Morris AAM. Homocystinuria patient and caregiver survey: experiences of diagnosis and patient satisfaction. Orphanet J Rare Dis. 2021 Mar 10;16(1):124. doi: 10.1186/s13023-021-01764-x. PMID: 33691747.
3. Perreault M *et al.* Development of an investigational methionine-consuming synthetic biotic medicine (SYNB1353) for the treatment of homocystinuria. Poster presented at: 14th International Congress of Inborn Errors of Metabolism meeting; Nov 21, 2021; Sydney, Australia.

Study Design

We evaluated SYNB1353 in a double-blinded, placebo-controlled Phase I trial utilizing a multiple ascending dose (MAD) design **(Figure 3A)**. Four cohorts using dose levels, $3x10^{11}$, $6x10^{11}$ and $1x10^{12}$ SYNB1353 live cells, were evaluated for safety, tolerability, and capacity to metabolize methionine in four cohorts of healthy volunteers challenged with 30 mg/kg methionine before and after exposure to SYNB1353. Study demographics are shown in **Table 1**. Subjects received a methionine challenge on day -1, followed by SYNB1353 using a dose ramp on days 1 to 7 **(Figure 3B).** The methionine challenge was repeated on day 7 before SYNB1353 dosing. Changes in plasma methionine and total Hcy were assessed over 24h after the methionine challenge.

A



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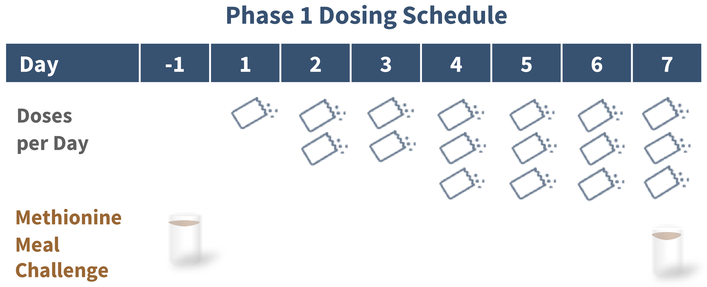


Figure 3. Phase 1 study design and dosing schedule. (A) Each cohort (n=8) included six subjects dosed with SYNB1353 and two subjects dosed with placebo. Safety and tolerability were assessed after each cohort prior to dose escalation. The $1x10^{12}$ live cell cohort was repeated with an alternate formulation. **(B)** In each cohort, SYNB1353 or placebo was administered over a seven-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 to 7. A methionine challenge (30 mg/kg) was administered on day -1 and day 7 and plasma methionine and plasma total Hcy were measured over 24h.

Table 1. Study demographics

Parameter	Placebo (N=8)	3×10¹¹ (N=6)	6×10¹¹ (N=5)	1×10¹² (N=6)	1×10¹²* (N=5)
Age (years)					
Mean (SD)	37.9 (8.4)	39.7 (12.8)	32.8 (4.7)	33.2 (11.3)	47.4 (14.0)
Min, Max	26, 47	25, 56	25, 36	23, 54	31, 63
Sex [F/M]	0/8	3/3	1/4	2/4	2/3
Race					
African American	4	4	2	0	5
White	4	2	3	6	0
Other	0	0	1	0	0
Ethnicity					
Hispanic	1	1	1	2	2
Not Hispanic	7	5	4	4	3
BMI (kg/m²)					
Mean (SD)	26.9 (3.7)	29.0 (4.2)	26.8 (4.6)	25.9 (3.4)	27.5 (3.4)
Min, Max	22.16, 32.70	24.56, 34.80	21.82, 33.65	21.07, 30.72	23.95, 31.83
Completed Dosing n (%)	8 (100)	5 (83.3)	5 (100)	6 (100)	5 (100)
Discontinued n (%)	0	1 (16.7)	0	0	0

Results

SYNB1353 was generally well-tolerated in healthy volunteers

Cohort	Dose 3E+11 (n=6)	Dose 6E+11 (n=5)*	Dose 1E+12 (n=6)	Dose 1E+12 (n=5)*	Total SYNB1353 (n=22)	rate per subject SYNB1353	Placebo (n=8)	rate per subject Placebo
Subjects with at least one TEAE	3	1	4	3	11	0.50	3	0.38
Maximum Grade 1	0	1	0	3	4	0.18	0	0.00
Maximum Grade 2	3	0	4	0	7	0.32	3	0.38
Total Number of TEAE	16	2	17	3	38	1.73	9	1.13
Subjects with at least one IMP-related TEAE	3	0	5	3	11	0.50	1	0.13
Maximum Grade 1	1	0	0	1	2	0.09	1	0.13
Maximum Grade 2	2	0	5	2	9	0.41	0	0.00
Any SAE	0	0	0	0	0	0.00	0	0.00
TEAE causing withdrawal	0	0	0	0	0	0.00	0	0.00

Table 2. Adverse events in healthy subjects receiving SYNB1353 or placebo. The majority of TEAE were GI related in nature. Rates and severity of TEAE were similar between SYNB1353 and placebo groups.

Results (continued)

Methionine Load Leads to Increase in Plasma Methionine and Plasma Homocysteine in Healthy Volunteers

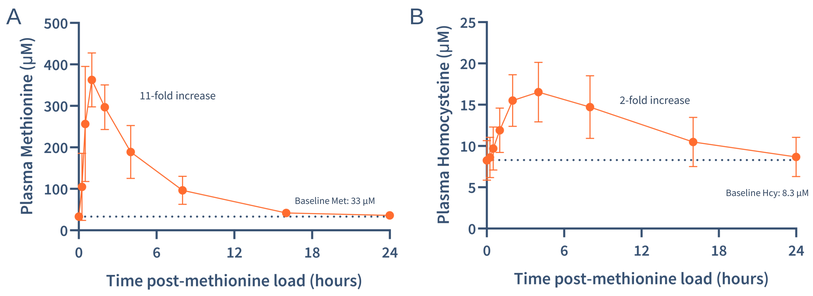


Figure 4. Increased plasma methionine and total Hcy following methionine challenge. (A) Plasma methionine and **(B)** total Hcy levels of all subjects (n=30) from all cohorts over 24 h after receiving a 30 mg/kg methionine load challenge. Data represent the mean ± SD. Methionine challenge resulted in an 11-fold increase in plasma methionine (C_{max}) and a 2-fold increase in plasma total Hcy (C_{max}) compared to baseline values.

Proof-of-Mechanism: SYNB1353 Blocks Methionine Absorption and Lowers Plasma Methionine

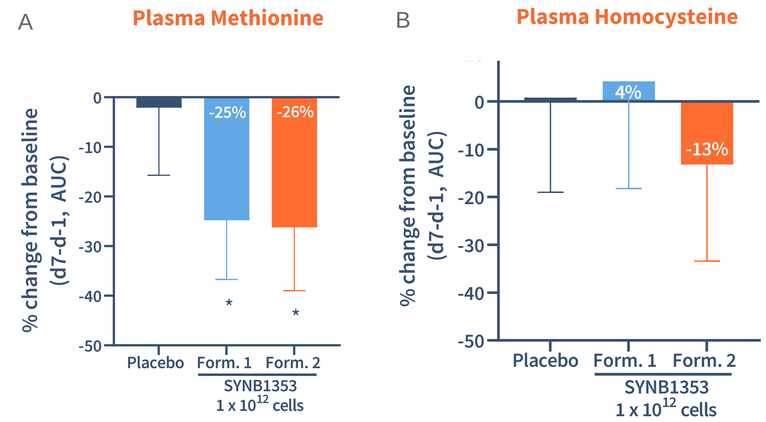
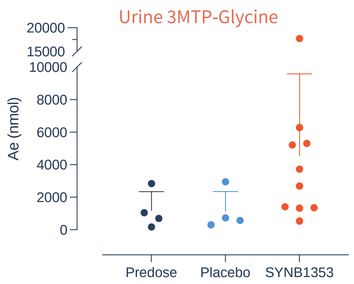


Figure 5. SYNB1353 blocks methionine absorption. Percent change from baseline in day 7 **(A)** plasma methionine $AUC_{0\text{-}24h}$ and **(B)** total plasma Hcy $AUC_{0\text{-}24h}$ for cohorts receiving $1x10^{12}$ live cell of SYNB1353. FORM = formulation. LS mean change, 95% CI *p< 0.05

SYNB1353 Metabolizes Methionine



Cohort	SYNB1353 Dose	% Positive Samples	Mean Ae (nmol)
Placebo	--	50% (4/8)	1139
SYNB1353	3x10¹¹	17% (1/6)	1330
	6x10¹¹	20% (1/5)	5303
	1x10¹²*	73% (8/11)	4865

Figure 6. 3MTP-glycine is increased in SYNB1353 dosed subjects. Total amount of excreted (Ae) 3-MTP-glycine in urine was determined for all subjects pre-dose on day -1 (n=30), all placebo dosed subjects (n=8) and all subjects dosed with SYNB1353 (n=22). Urine was collected for 24h after methionine challenge on either day -1 (predose) or on day 7. Data represent the mean and SD of all positive samples collected (n=4/30 predose, n=4/8 placebo and n=10/22 SYNB1353). The table shows the number and percentage of positive samples in each cohort and the corresponding mean. *pooled data from both cohorts receiving $1x10^{12}$ live cells.

Conclusions

- SYNB1353 was **well tolerated in healthy volunteers** with GI adverse event rates and severity similar between active and placebo groups
- SYNB1353 has **demonstrated methionine metabolism in the GI tract** of healthy volunteers, resulting in a **lowering of plasma methionine** and production of 3MTP-glycine, assessed following a meal challenge to elevate methionine levels
- Previously presented mechanistic modeling data suggests that SYNB1353 may increase protein intake and **lower plasma Hcy by up to 58% in HCU patients**[3]
- Based on this proof of mechanism in healthy volunteers, **SYNB1353 will be advanced to a Phase 2** proof of concept study in patients with HCU

Disclosures: This study was funded by Synlogic, Inc.

Presented at SIMD, Salt Lake City, UT, March 19, 2023