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SYNB1353 for Homocystinuria (HCU)

Findings from Proof of Mechanism Phase 1 Study in Healthy Volunteers,

November 30, 2022

SYNB1353: Potential for a Breakthrough in HCU

Novel Mechanism Targets GI-Based Methionine for a Differentiated Treatment Approach

Rare metabolic disease with risks of acute and chronic complications - and severe need for new treatment options

- Elevated homocysteine (tHcy) in HCU can cause acute thromboemboli and chronic multisystem complications
- Methionine restricted diet is the mainstay of treatment but **majority of patients above targets for tHcy**
- Direct **synergies with PKU** for clinical development, commercial operations: shared KOLs, metabolic clinics, connected patient groups

SYNB1353 offers potential 1st-of-its-kind treatment option that works with HCU patients' lives

- A genetically engineered probiotic**, SYNB1353 is designed to consume methionine, a precursor to homocysteine**
- Patients and KOLs share enthusiasm for target product profile: **safe, orally-administered, convenient – and viable across age groups**
- **Proof of mechanism established:** Phase 1 results in HVs confirmed that SYNB1353 can consume methionine in the GI tract and lower absorption
- Safety, tolerability profile consistent with other Synlogic programs: adverse events were all **mild to moderate**, **no SAEs**

Forward Development Planning Underway

- **Planning for ph. 2 study in HCU patients underway**
- CMC activities to support scale-up for phase 2 and registration studies underway

tHcy = total homocysteine

HCU: An Inborn Error of Metabolism Resulting in Multisystem Burden

CBS Enzyme Deficiency Results in Accumulated Homocysteine[1]

Diet

Methionine

Folate / B_{12} Dependent

Homocysteine

Caused by mutations in **Cystathionine β-synthase (CBS)** enzyme

Cystathionine

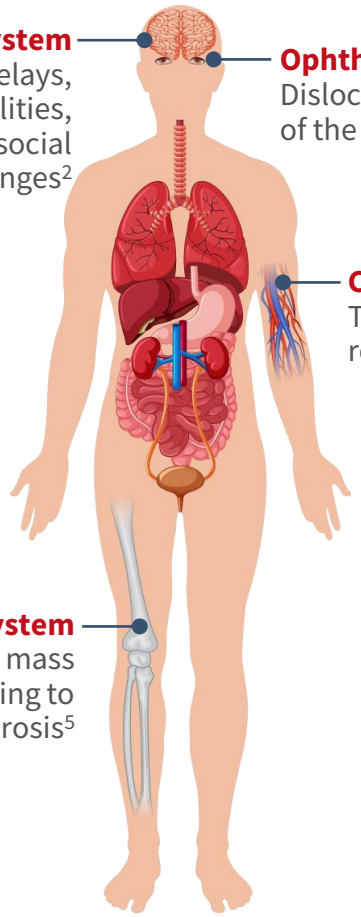
Cysteine

Estimated prevalence for CBS or "Classical" HCU, US & Europe[6,7]: ~5,000

Acute Risks, Progressive Complications

Central Nervous System
Developmental delays, intellectual disabilities, neuropsych/psychosocial challenges[2]

Ophthalmological System
Dislocation or displacement of the natural lens[2]



Circulatory System
Thromboembolism, resulting in stroke[2,3,4]

Skeletal System
Reduction of bone mass and quality leading to scoliosis and osteoporosis[5]

"I think about my HCU patients like potential time bombs due to their risk of acute events"
- HCU KOL

1. Development of an Investigational Methionine-consuming Synthetic Biotic Medicine (SYNB1353) for the Treatment of Homocystinuria, International Congress of Inborn Errors of Metabolism, November 23, 2021; 2. Mudd SH. Disorders of transulfuration. In: Scriver CR (ed). *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. McGraw-Hill: New York, 2001, pp 2007–2205; 3. Saposnik G, et al. Heart Outcomes Prevention Evaluation 2 Investigators. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke.* 2009;40(4):1365-1372; 4. Ding R, et al. The association of cystathionine β synthase (CBS) T833C polymorphism and the risk of stroke: a meta-analysis. *J Neurol Sci.* 2012;312(1-2):26-30; 5. reviewed in: Saito M, Marumo K. The Effects of Homocysteine on the Skeleton. *Curr Osteoporos Rep.* 2018;16(5):554-560. 6. Weber Hoss GR, Sperb-Ludwig F, Schwartz IVD, Blom HJ. Classical homocystinuria: A common inborn error of metabolism? An epidemiological study based on genetic databases. Mol Genet Genomic Med. 2020 Jun;8(6):e1214. doi: 10.1002/mgg3.1214. Epub 2020 Mar 30. PMID: 32232970; PMCID: PMC7284035. 7. Synlogic Data on File: Key Opinion Leader Conversations 2021-2022.

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Total Homocysteine (tHcy): Clinical Biomarker to Manage HCU

Difficulties with Current Standard of Care Leave Levels Uncontrolled for Most Living with HCU

HCU Treatment Goal: Reduce, Control Total Homocysteine (tHcy)

Predictor of outcomes, treatment target

Treatment of HCU focuses on tHcy control;[1] Loss of tHcy control in later life is associated with serious complications[2]

"Lower is better"

Guidelines recognize -20% for clinical response[1]

Normal (healthy) range: 5-15 µmol/L)

In HCU, levels can be >200 µmol/L

Guidelines recommend <100 µmol/L if possible[1]

Regulatory precedent as basis for approval

Per indication language for Cystadane® (betaine)[3]

Current Treatment Options Leave Majority with Uncontrolled tHcy

1) Low-methionine diet (low in natural protein), **along with supplemental formula** (Met-free L-AA mixture)

- Complexity, difficulty yields poor adherence[1]

2) Betaine (Cystadane®)

- Fishy taste/odor hurts compliance[1]

Majority of patients remain with tHcy levels far above goals[4], at risk for both acute and chronic complications

1. Morris AAM, et al. Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency
2. Walter 1998 3.. U.S. Prescribing Information for Cystadane® (betaine) 4. De Biase et al. 2020 & Synlogic patient & KOL Insights

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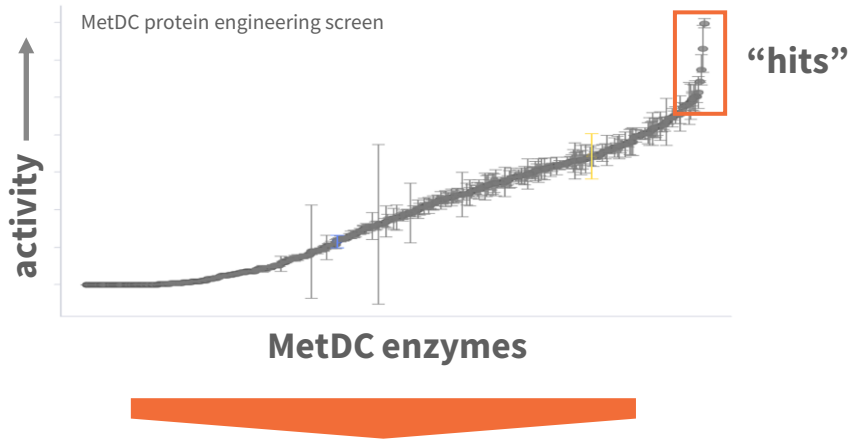
SYNB1353 for HCU: Targeting Methionine for a New Approach

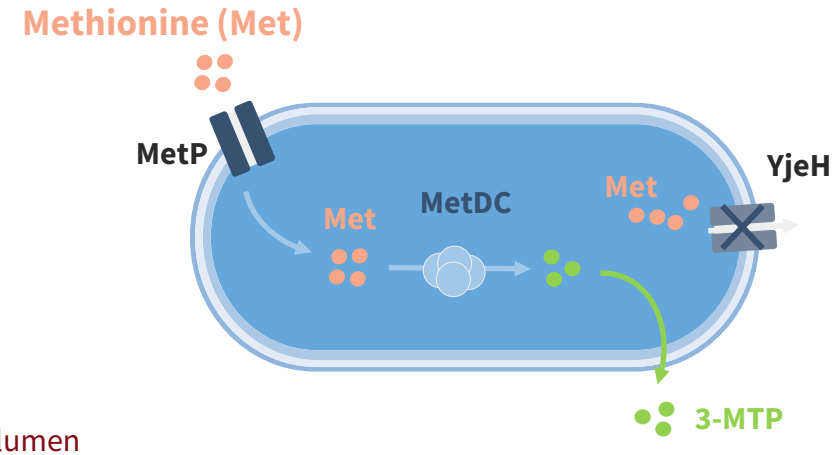
Developed from Ginkgo Collaboration

synlogic + GINKGO BIOWORKS



- Optimized, engineered components identified:
  - **MetDC** methionine decarboxylase from protein engineering screen
  - **MetP** transporter from metagenomic screen

A Methionine-Consuming Synthetic Biotic

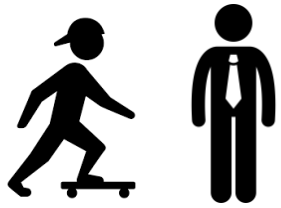
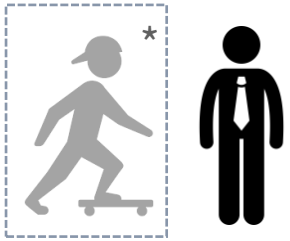
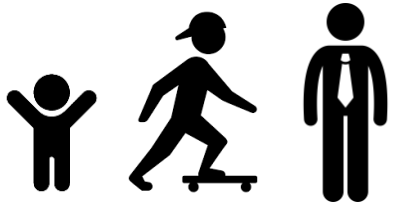


- Engineered probiotic E. coli Nissle, designed to **metabolize methionine** (Met) via the methionine decarboxylase (**MetDC**) pathway, preventing its conversion into homocysteine
- Converts Met to 3-methylthiopropylamine (**3-MTP**); **YjeH** gene deleted to prevent the release of methionine once it enters the cell

1. Means, et al. Activity of SYNB1353, in a Mouse Model of Homocystinuria, 2022 Synthetic Biology: Engineering, Evolution & Design (SEED)

A Differentiated Biotherapeutic for HCU

SYNB1353 Potentially Integrates Efficacy, Safety, Convenience to Enable Use Across Ages

	TRAVERE™ THERAPEUTICS	aeglea™	synlogic
	Pegtibatinase (TVT-058) **Phase 1/2**	Pegtarviliase (AGLE-177) **Phase 1/2**	SYNB1353 ✓ **Phase 1, Proof of Mechanism**
Dosing & Administration[1, 2, 3]	 1.5 mg/kg biweekly Injection	 1.35 mg/kg weekly Injection	 *Sachet of lyophilized powder mixed with ~3 oz liquid taken with meals*
Safety Considerations	ERT mechanism associated with potential for allergic/immunological challenges		Transient/reversible, mild GI-related side effect
Potentially Applicable Patient Population[1, 2, 3]			

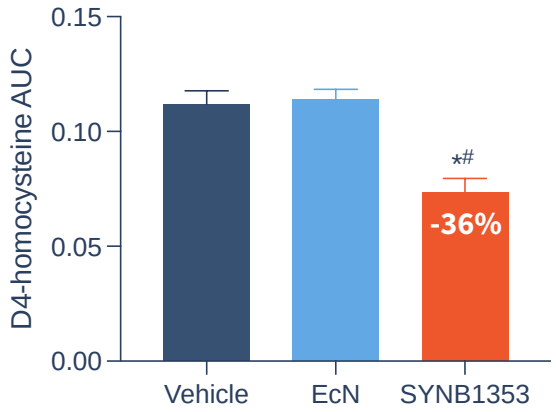
*>12 in UK/Australia only, >18 in US https://www.aeglea.com/clinical-trials/

1. https://hcuconnection.com/trials/1. 2. https://www.aeglea.com/clinical-trials/ 3. Synlogic data on file.

SYNB1353: Program Progress to Date

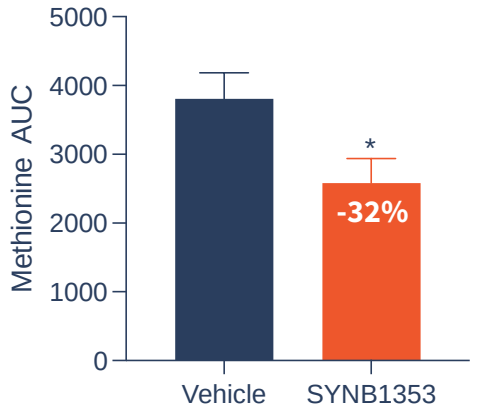
Validated Preclinically in Multiple Models

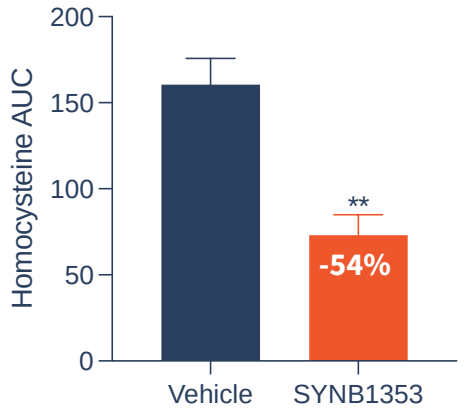
Hcy Lowering in CBS-Knockdown Mice



*p<0.05 versus vehicle, #p<0.05 versus EcN

Met and Hcy Lowering in Met-Challenged NHPs+





*p<0.05 versus vehicle, **p<0.01 versus EcN

Rapid Progress: Candidate to POM in ~12 Months

November 2021
Candidate announced
Preclinical data presented at ICIEM 2021

July 2022
First dose in Phase 1 HV Study

August 2022
Granted Fast Track Designation by FDA

November 2022
Proof of Mechanism achieved in Phase 1

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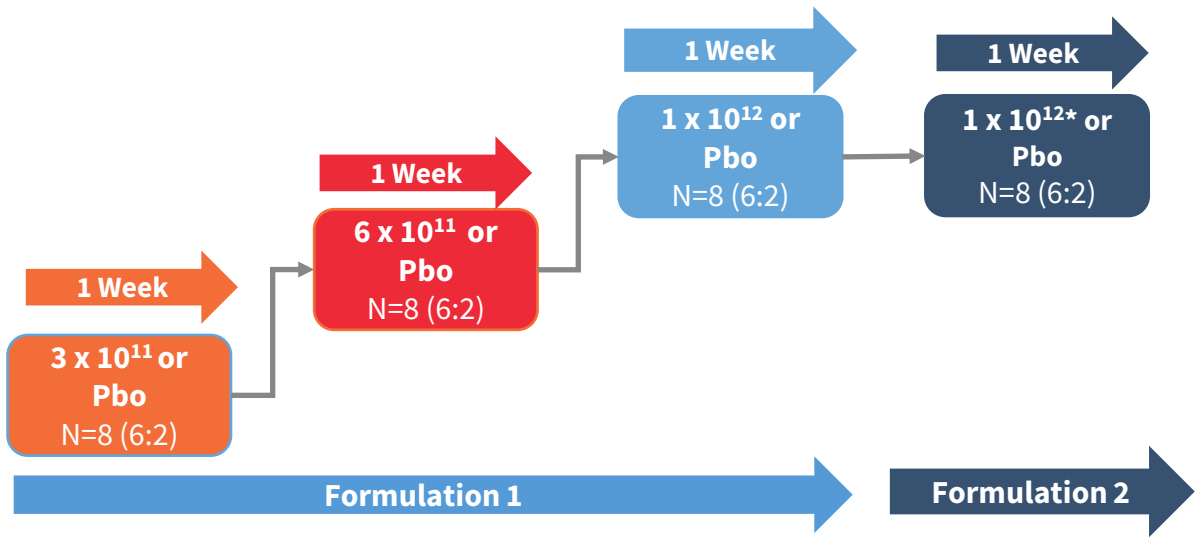
+Similar design and model as used in Phase 1 healthy volunteer (HV) study

Study Design for SYNB1353 Phase 1 in Healthy Volunteers

Methionine Meal Challenge Used to Simulate Severely Elevated Methionine, Homocysteine in HCU

Phase 1 MAD Design in Healthy Volunteers

Double-blind, placebo-controlled, randomized, dose-escalation, inpatient study



*$1x10^{12}$ dose cohort repeated with alternate formulation

Dosing Schedule

Day	-1	1	2	3	4	5	6	7
Doses per Day								
Methionine Meal Challenge								

- **Objectives:** To assess the safety, tolerability, and PD of SYNB1353 in HVs, in a dietary model of homocystinuria (HCU), and to evaluate two different formulations at the maximum dose.
- **Endpoints for Each Cohort & Dose Level Studied:**
  - Safety and tolerability
  - Clearance of SYNB1353 by day 28 (measured in feces)
  - Plasma methionine, measured over 24 hours as area under the curve (AUC) following a methionine meal challenge

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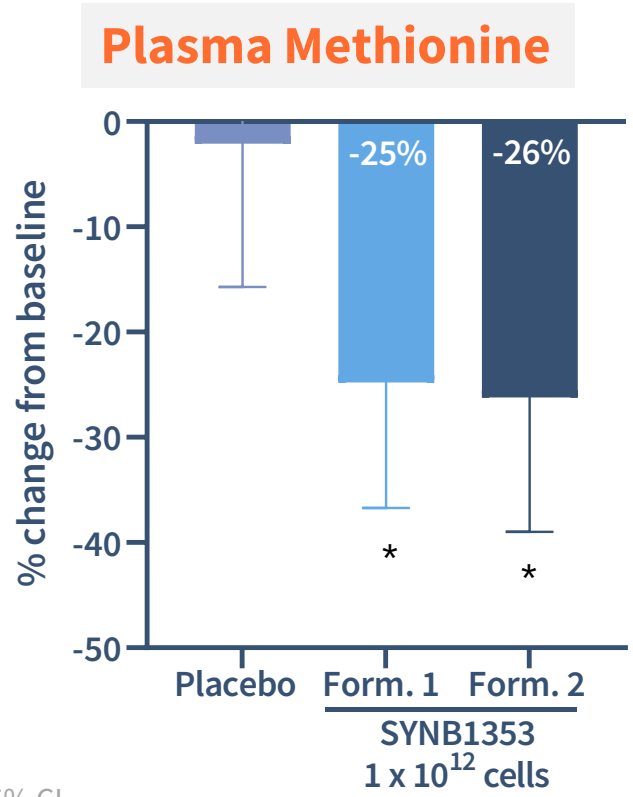
SYNB1353: Proof-of-Mechanism Achieved via Met Meal Challenge

Change vs. Baseline, Measured Following a Methionine Meal Challenge as Area Under the Curve (AUC) over 24 hours



Data provided proof of mechanism by demonstrating the effects of SYNB1353's GI-based metabolism of methionine on plasma methionine, in healthy volunteers

LS mean change, 95% CI

*$p < 0.05$ Form = formulation; placebo n=8, 1x10^{12} form 1 n=6, 1x10^{12} form 2 n=5

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Safety & Tolerability – Summary of Top-Line Findings

SYNB1353 was generally well-tolerated in healthy volunteers.

There were **no serious adverse events** (SAEs)

Adverse events (AEs) were all **mild to moderate**, transient, and predominantly GI in nature.

- One subject discontinued dosing due to an adverse event.

Frequency and severity of **GI-related AEs were similar in the active and control group**

- 7 of 22 SYNB1353 compared to 3 of 8 placebo subjects had at least 1 GI-related AE

All subjects completing the 28-day analysis cleared SYNB1353 in feces

GI = gastrointestinal

SYNB1353 & HCU: Program Summary and Key Highlights

- The goal of treatment in HCU is to **lower plasma levels of total homocysteine (tHCy)**, reducing the risk of acute and chronic, multisystem complications. tHcy has been accepted as a regulatory endpoint for efficacy in HCU patients

- SYNB1353 is an engineered probiotic **designed to consume methionine**, **a precursor to homocysteine**, in the GI tract and lower total plasma homocysteine for patients with HCU

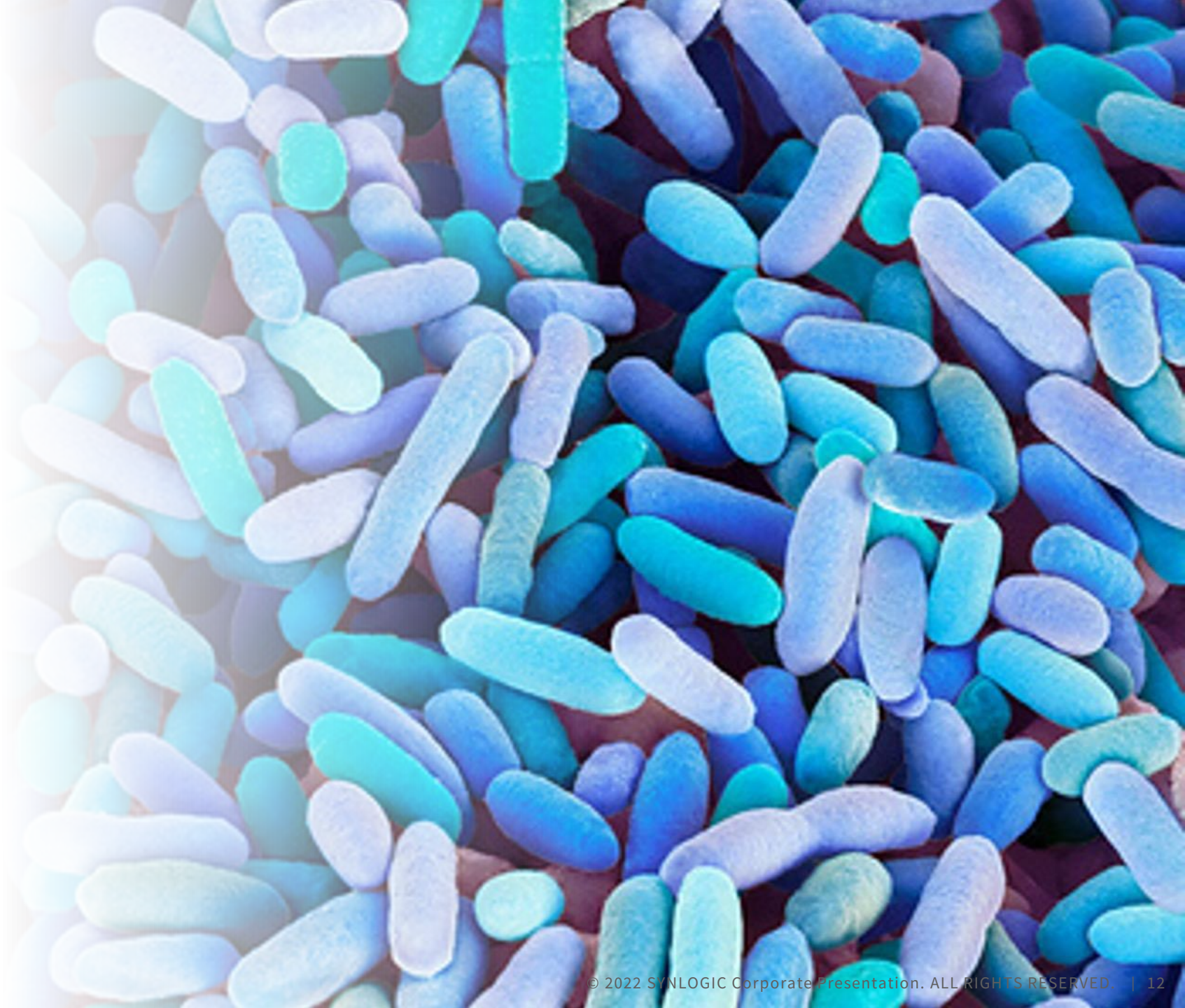
- SYNB1353 has achieved FDA **Fast Track designation** (August 2022), and **Orphan Drug designation** (November 2022)

- SYNB1353 has **demonstrated methionine consumption in the GI tract** of healthy volunteers, resulting in a **lowering of plasma methionine**, assessed following a meal challenge to elevate methionine levels

- SYNB1353 was **well tolerated in healthy volunteers** with GI adverse event rates and severity similar between active and placebo groups

- 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

Thank You



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