Tolerability and Kinetics of SYNB1020 in a Phase 1, First-in-human, Healthy Adult Volunteer Study

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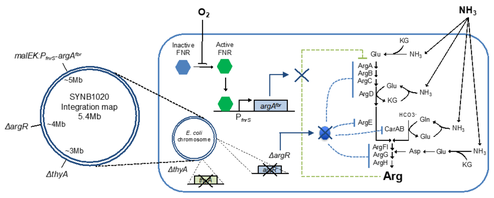
BACKGROUND

Patients with impaired liver function accumulate toxins in the blood stream, which presents as hepatic encephalopathy (HE) in approximately 55% of patients with chronic liver disease. The pathogenesis of HE is believed to be largely attributable to hyperammonemia. Probiotic bacteria have been postulated to have beneficial effects in patients with advanced liver disease. As a therapeutic strategy, ***E. coli*** Nissle (EcN), a well-characterized probiotic, was modified to convert ammonia (NH_3) to arginine (Arg) in the intestine by deleting a negative regulator of Arg biosynthesis and expressing a feedback-resistant Arg biosynthetic enzyme. To prevent colonization, the *thyA* gene was deleted to render the strain auxotrophic and dependent on exogenous thymidine to support replication. **(Figure 1).** The rationale for development of SYNB1020 was to create a commensal strain of EcN that would continuously consume excess ammonia where it is naturally produced, in the colon, before it can be absorbed into the blood. Based on preclinical safety and efficacy data, SYNB1020 advanced into human testing.

Figure 1: SYNB1020 modifications. 1) *argR* repressor gene deleted, 2) *argA* gene replaced with a feedback-resistant version, 3) Control of *argA* gene by anaerobic promoter (*fnr),* 4) *thyA* gene deleted to confer auxotrophy

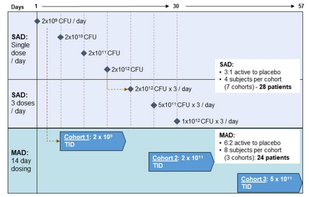


METHODS

Table 1. Study Endpoints

Primary Safety and tolerability of SYNB1020. The endpoints related to this objective include the nature and frequency of adverse events (AEs), laboratory assessments (blood chemistry, complete blood count [CBC], and urinalysis), and electrocardiograms (ECGs).
Secondary • Gastrointestinal (GI) tolerability as assessed by the Gastrointestinal Symptom Rating Scale (GSRS). • SYNB1020 kinetics (microbial kinetics, MK) in feces measured with quantitative polymerase chain reaction [qPCR] following dosing.
Exploratory A) Pharmacodynamic effects of SYNB1020, including measurements of • Plasma ammonia including ammonia profiling (10 samples over 24 hours) • Blood urea nitrogen (BUN) • Plasma amino acids • 24-hour urinary urea and nitrogen • Urinary orotic acid • Fecal nitrogen B) Stable isotope ^{15}N-ammonium chloride tracer study measuring • Urinary total ^{15}N, ^{15}N-urea (N1 and N2), ^{15}N-nitrate, ^{15}N-ammonia • Plasma ^{15}N-urea (N1 and N2), ^{15}N-nitrate, and ^{15}N-citrulline
Post-hoc analyses • *Ex vivo* activity in feces by ^{15}N-L-arg synthesis assay • Urinary total nitrate

Figure 2. Study design. Dosing was started in single-day (SAD) cohorts with increasing doses. Once a dose was determined safe and well tolerated, it was advanced into multiple day (MAD) cohorts.



METHODS

Preclinical Study Design: Balb/c mice with TAA-induced liver injury (3 x week 150 mg/kg) were treated with vehicle, $1x10^{10}$ CFU/dose twice daily (BID) EcN (SYN094), or $1x10^{10}$ CFU/dose BID of SYNB1020 for 4 weeks.

Clinical Study Design: A phase 1 single (SAD) and multiple (MAD) ascending dose study in healthy adult volunteers. Subjects who met the inclusion criteria and none of the exclusion criteria were admitted to a phase 1 unit and placed on a controlled diet designed to meet their calorie and macronutrient requirements. In the SAD portion of the study, following baseline assessments, subjects were randomized in a 3:1 ratio to receive either SYNB1020 or placebo. Subjects in the single dose portion of the study received either a single dose or three doses in a single day ranging from $2x10^9$ to $2x10^{12}$ CFU/dose. In the MAD portion of the study, subjects received SYNB1020 or placebo three times daily for 14 days with $2x10^9$ to $5x10^{11}$ CFU/dose three times per day (TID). (Figure 2) Study outcomes are listed in **Table 1**.

RESULTS

Preclinical data: Balb/c mice with TAA-induced liver injury (3 x week 150 mg/kg) were treated with vehicle or SYNB1020 for 4 weeks. A significant decrease in blood ammonia was seen compared to vehicle in Balb/c mice at 4 weeks. **(Figure 3)** Administration of $1x10^{10}$ CFU SYNB1020 BID for 4 weeks improved survival of TAA mice compared to the vehicle group **(Figure 4)**, and attenuated the toxin-induced increase in liver enzymes ALT and AST. **(Figure 5)**

Figure 3. Reduction in plasma was observed in Balb/c mice with TAA-induced liver injury ($p<0.05$).

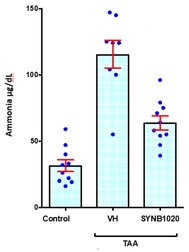


Figure 4. Effect of daily (BID) administration of $1x10^{10}$ CFU of SYNB1020 on survival observed at week XXX in BALB/c mice with TAA-induced liver disease

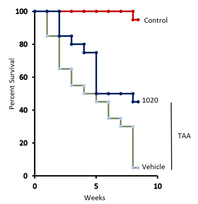
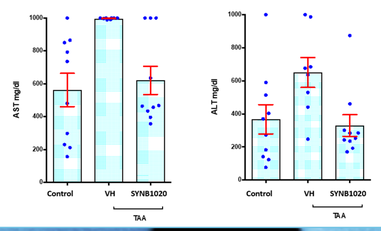


Figure 5. Effect of twice-daily (BID) administration of $1x10^{10}$ CFU of SYNB1020 on liver enzymes in BALB/c mice with TAA-induced liver disease



RESULTS

Demographics: 52 Healthy volunteers (40 male, 12 female) aged between 21 and 64 years were enrolled in Study SYNB1020-CP-001 and received at least 1 dose of SYNB1020 or matching placebo.

Safety: There were no deaths and no serious adverse events (AEs) in the study. Doses of SYNB1020 at or below $5x10^{11}$ CFU TID (total daily dose $1.5x10^{12}$ CFU) were well tolerated by healthy volunteers. Doses above this were associated with mild to moderate gastrointestinal AEs. In the SAD part, 3 subjects discontinued dosing due to mild-moderate nausea and vomiting (2 subjects in $2x10^{12}$ CFU TID cohort, 1 subject in $1x10^{12}$ CFU TID cohort), as did one subject in the highest MAD cohort ($5x10^{11}$ CFU TID). All AEs leading to discontinuation were reported after the first or second dose and resolved within the same day. Clinical chemistry, including liver enzymes, and hematology safety labs were unremarkable, and no change from baseline was observed in C-reactive protein **(Figure 6)**. There was no change from baseline in systolic or diastolic BP or heart rate **(Figure 7)**. No changes were seen in ECG parameters including QT interval.

Figure 7a. Time-matched Change from Baseline for Diastolic Blood Pressure

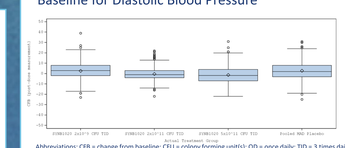
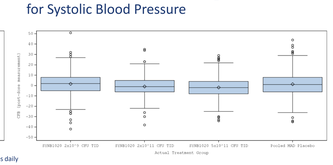


Figure 7b. Time-matched Change from Baseline for Systolic Blood Pressure



Abbreviations: CFB = change from baseline; CFU = colony forming unit(s); QD = once daily; TID = 3 times daily

Total GSRS scores prior to initiation of study dosing ranged from 1.0 to 1.5 across all cohorts. GSRS scores remained within the range of 1 to 2 at all post-dose assessments for most subjects. 4 subjects receiving SYNB1020 at a dose of ≥ 5×10^{11} CFU, 1 subject receiving SYNB1020 at $2x10^{11}$ CFU TID, and 1 placebo subject had an increase of ≥0.5 in the total GSRS score. The increase in GSRS score was associated with clinical GI AEs (nausea, vomiting, abdominal pain, bloating, flatulence) in all except one subject. **(Table 3)** GI symptoms were dose-limiting at doses >$5x10^{11}$ CFU.

Microbial kinetics: SYNB1020 was detected by strain-specific qPCR in all subjects in the treatment groups but none of the placebo subjects. The max qPCR copy number (CN) increased in a dose-dependent manner. Steady-state based on visual inspection was reached by 2 days. The mean residence time (MRT) was approximately 48 hours at all dose levels. There was no evidence of colonization, and all subjects cleared SYNB1020 within 2 weeks following the last SYNB1020 dose. **(Figure 8)**

Figure 6. Change from baseline in CRP

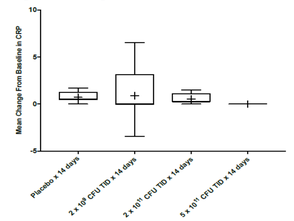
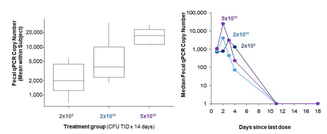


Table 3. Changes in total GSRS ≥0.5

Subject Number	Treatment Group	Baseline Total GSRS Score	Worst On-study Total GSRS Score (CFB)	Final On-study Total GSRS Score (CFB)	Concurrent Gastrointestinal TEAEs (CTCAE Grade)
0403	SAD: 2 × 10^{12} CFU QD for 1 day	1	3.9 (2.9)	3.9 (2.9)	Abdominal cramping (1) Bloating (1)
0404	SAD: 2 × 10^{12} CFU QD for 1 day	1	1.5 (0.5)	1.5 (0.5)	Nausea (1)
0501	SAD: 2 × 10^{12} CFU TID for 1 day	1	1.9 (0.9)	1.9 (0.9)	Nausea (2) Vomiting (1)
0706	MAD: 2 × 10^{11} CFU TID for 14 days	1	1.6 (0.6)	1.4 (0.4)	Abdominal pain (1) Nausea (1)
0808	MAD: 5 × 10^{11} CFU TID for 14 days	1.1	1.6 (0.5)	1.3 (0.3)	None
0806	MAD: Pooled placebo	1	1.6 (0.6)	1 (0)	Flatulence (1)

Figure 8. Steady-state qPCR copy number during 14 days of dosing increases with increasing SYNB1020 dose. All subjects cleared SYNB1020 within 12 days after the last dose.



RESULTS

Biomarkers: Baseline fasting venous ammonia ranged from 11 to 59 µmol/L (28.8 ± 8.8 µmol/L mean±SD; local laboratory ULN=32 µmol/L) in healthy volunteers. No diurnal variation in venous ammonia was observed. There was no change in ammonia profiles following administration of SYNB1020 in healthy volunteers. **(Figure 9)** No change from baseline was observed in BUN, plasma amino acids, 24 hr urinary urea and nitrogen, or urinary orotic acid. (data not shown) An increase in urinary nitrate was observed. **(Figure 10)** The ^{15}N-NH_4Cl tracer study showed a corresponding increase in both plasma and urinary ^{15}N-nitrate (data not shown).

Figure 9. Ammonia AUC at baseline (Day -2) and during SYNB1020 dosing (Days 7&14) in individual subjects was highly variable. Blue line represents mean ammonia AUC within a dose regimen.

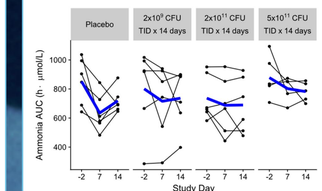
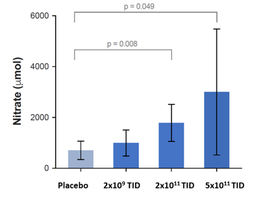
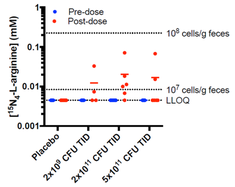


Figure 10. A dose-dependent increase in total urinary nitrate was observed.



Ex vivo: Feces from subjects in the MAD cohorts were homogenized and spiked with ^{15}N-NH_4Cl. Activity using the ^{15}N4-L-arg synthesis assay was detected in who were fecal samples at steady-state in subjects dosed with SYNB1020 but not in the placebo group. The amount corresponds to about 10^7-10^8 live cells/g of feces. **(Figure 11)**

Figure 11. Viability of SYNB1020 after excretion was demonstrated using the 15N4-L-arg synthesis assay.



CONCLUSIONS

- SYNB1020 is a modified probiotic that consumes ammonia in the intestine
- SYNB1020 reduced hyperammonemia and improved survival in the TAA mouse model
- SYNB1020 was well-tolerated in healthy volunteers at doses up to 5 X 10^{11} CFU TID for up to 14 days.
- No systemic toxicity was observed, GI symptoms (nausea, vomiting) were dose-limiting at doses >$5x10^{11}$ CFU.
- The bacteria were metabolically active in feces, and are cleared rapidly following discontinuation of dosing.
- There was a dose dependent increase in nitrate following dosing, implying that SYNB1020 can induce changes in systemic metabolism acting from within the lumen of the gut.
- Ongoing Ph1b/2a study will evaluate safety and tolerability in patients with cirrhosis as well as the ability of SYNB1020 to lower ammonia in patients with elevated ammonia.