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

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METHODS

Primary Study Design: Balb/c mice with TAA-induced liver injury (3 x 1 week 150 mg/kg) were treated with vehicle, 1x10^7 CFU/dose twice daily (BID) E.coli (SYNB043), or 1x10^8 CFU/dose BID of SYNB1020 for 4 weeks.

Clinical Study Design: A phase 1 (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 SYNB010 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 2x10^6 to 2x10^7 CFU/dose. In the MAD portion of the study, subjects received SYNB1020 or placebo three times daily for 14 days with 2x10^6 to 5x10^7 CFU/dose three times per day (TID). (Figure 2) Study outcomes are listed in Table 1.

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.

Safety: There were no deaths and no serious adverse events (AEs) in the study. Doses of SYNB1020 at or below 5x10^9 CFU TID (total daily dose 1.5x10^10 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 2x10^7 CFU TID cohort, 1 subject in 1x10^8 CFU TID cohort). In the MAD portion (5x10^9 CFU TID cohort, as did one subject in the highest MAD cohort (5x10^10 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 8). 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.

RESULTS

Background: Patients with impaired liver function accumulate toxins in the blood stream, which presents as hepatic encephalopathy (HE) in approximately 5% 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 produce ammonia (NH3) to arginase (Arg) in the intestine by deleting a negative regulator of Arg biosynthesis and expressing a feedback-resistant Arg biosynthetic enzyme. To prevent colonization, the thyg 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 E.coli 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 gene repressor gene deleted, 2) argR gene replaced with a feedback-resistant version, 3) Control of argR gene by an arabinose promoter (Ar), 4) thyg gene deleted to confer auxotrophy.

Figure 2: Effect of daily (BID) administration of 1x10^8 CFU of SYNB1020 on survival observed at week 10 in Balb/c mice with TAA-induced liver disease

Figure 3: Effect of two-daily (BID) administration of 1x10^7 CFU dose of SYNB1020 in liver enzymes in Balb/c mice with TAA-induced liver disease

Figure 4: Reduction in plasma ammonia was seen compared to vehicle in Balb/c mice at 4 weeks. (Figure 3) Administration of 1x10^7 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 6: Change from baseline in Diastolic Blood Pressure

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

Figure 8: Time-matched Change from Baseline for Systolic Blood Pressure

Total GRS scores prior to initiation of study dosing ranged from 1.0 to 1.5 across all cohorts. GRS 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 x 10^9 CFU, 1 subject receiving SYNB1020 at 2x10^7 CFU TID, and 1 placebo subject had an increase of ≥0.5 in the total GRS score. The increase in GRS score was associated with clinical GI symptoms (nausea, vomiting, abdominal pain, bloating, flatulence) in all except one subject (150 GIL symptoms were dose-limiting at doses ≥5x10^9 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 tolerated SYNB1020 within 2 weeks following the last SYNB1020 dose. (Figure 6)

Figure 9: Ammonia AUC at baseline (Day -2) and during SYNB1020 dosing (Days TA4) in individual subjects (left) and averaged subject data (right). Due to the represents human ammonia AUC within a dose range

Ex view: Feces from subjects in the MAD cohorts were homogenized and spiked with 3N-NH4Cl. Activity using the 3N-NH4-L-arginine synthesis assay was defined in who fecal samples at steady-state in subjects dosed with SYNB1020 but not in the placebo group. The amount corresponds to about ≥10^-10 to 10^-9 mol/kg/day. (Figure 11)

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 10^9 CFU TID for up to 14 days.
• No systemic toxicity was observed. GI symptoms (nausea, vomiting) were dose-limiting at doses ≥5x10^9 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 reduce ammonia levels in systemic metabolism acting from within the luminal gut.
• Ongoing Ph1/2a 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.

RESULTS

Biometrics: Baseline fasting venous ammonia ranged from 11 to 59 μM (28.8 ± 8.8 μM; mean ±SD; local laboratory ULN=32 μM) 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 BLN, plasma amino acids, 24 hr urinary urea and nitrogen, or urinary oxalic acid. (data not shown) An increase in urinary nitrate was observed. (Figure 8) The 3N-NH4Cl tracer did not correspond with in both plasma and urinary 3N-nitrate (data not shown).

Figure 10: Steady-state qPCR copy number during 16 days of dosing with increasing SYNB1020 dose. All subjects cleared SYNB1020 within 12 days after the last dose.

Figure 11: A dose-dependent increase in total urinary nitrate was observed.

Figure 12: A dose-dependent increase in total urinary nitrate was observed.

Figure 13: Stability of SYNB1020 after administration was demonstrated using the 3N-NH4-L-arginine synthesis assay.