Development of a Synthetic Biotic, SYNB8802, for the treatment of Enteric Hyperoxaluria

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Background

Oxalate arises from a variety of dietary and endogenous sources and is an end-product of human metabolism. In the healthy individual, the majority of urine oxalate is derived from endogenous hepatic production. An increase in either GI oxalate absorption or hepatic oxalate production increases plasma oxalate and thus urinary oxalate. Enteric Hyperoxaluria (EH) is caused by excessive absorption of dietary oxalate due to an underlying malabsorptive GI disease. Malabsorptive bariatric surgeries such as Roux-en-Y can lead to increased luminal free fatty acids resulting in increased oxalate solubility and absorption. (Lieske 2015, Canales 2014, Valezi 2013, Canales 2014) Chronic hyperoxaluria is associated with recurrent kidney stones, nephrocalcinosis, and progressive renal damage. In severe cases untreated EH can progress to systemic oxalosis, a condition in which oxalate accumulates in joints, bones, eyes, heart, and other organs.

There are currently no approved treatments for EH. Disease management aims to decrease the risk of recurrent kidney stones and progressive disease by limiting the intake of dietary oxalate and fat, increasing dietary calcium intake, and maintaining adequate fluid intake. However, the efficacy of dietary treatments alone is limited and there is an unmet medical need for novel therapies. We describe here the development of SYNB8802, a Synthetic Biotic medicine engineered to consume oxalate within the GI tract and convert it to nontoxic metabolites as a novel treatment approach to EH.

Methods & Results

Strain Design

SYNB8802 is a genetically engineered, non-colonizing strain of *Escherichia coli* Nissle 1917 (EcN). It was developed by engineering a pathway for oxalate degradation derived from the human commensal microorganism *Oxalobacter formigenes* into a probiotic strain EcN. SYNB8802 was engineered to be an auxotrophic strain through deletion of the *thyA* gene that encodes for thymidine synthetase, which is essential for replication. **(Figure 1)**

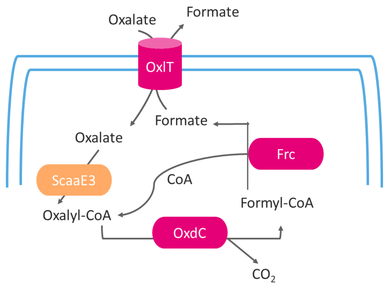

Figure 1. Schematic of engineered pathway in SYNB8802. Oxalate enters the cell via OxlT and is then converted to Oxalyl-CoA via ScaaE3. Oxalyl-CoA is decarboxylated to Formyl-CoA and CO_2 by OxdC. Frc transfers CoA from Formyl-CoA to an incoming oxalate, releasing formate that is exported via OxlT

Acute Diet-induced Hyperoxaluria in Non-human Primates

An acute model of Hyperoxaluria was developed in cynomolgus monkeys (non-human primates; NHP). Animals (n=6 per group) were fasted the night prior to the study for approximately 16-18 hours. On the morning of the experiment urine was collected and each monkey was removed from its cage and orally administered a spinach suspension (1.5g/mL in water) with sodium bicarbonate (1.8 mmol), $^{13}C_2$-oxalate (50 mg), and formulation buffer (13.8%w/v Trehalose, 68mM Tris, 55mM HCl, 1× phosphate-buffered saline (PBS)) followed by either vehicle (water) or SYNB8802^{CamR}.

Animals were then returned to their cages and a clean urine collection pan was placed at the bottom of each cage. Urine was collected at 6 hours postdosing, and the total urine volume was recorded. Cumulative urinary oxalate and creatinine levels were measured by LC/MS-MS. SYNB8802^{CamR} lowered the recovery of unlabeled UOx compared to vehicle by 73% at the 1 x 10^{12} CFU dose level. (p=0.013, One-way ANOVA; **Figure 2**).

A similar relationship was observed for $^{13}C_2$-oxalate excretion, where the 1 x 10^{12} CFU dose group also exhibited significant lowering compared to vehicle (75%; p<0.01; **Figure 3**). Collectively, these studies indicate that orally administered SYNB8802 significantly lowers UOx levels in NHPs through the consumption of oxalate in the GI tract.

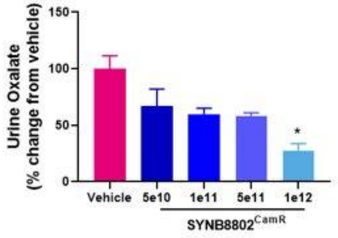

Figure 2. Non-human primate study oxalate lowering. The y - axis shows change in UOx from vehicle control. The x - axis shows vehicle (control, 100%) and the effect of increasing doses of SYNB8802^{CamR} (n=6 for each group) on % change on UOx from vehicle with 1E12 being significant. (* p < 0.05). Error bars represent SEM

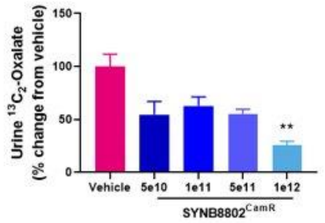

Figure 3. Non-human primate study $^{13}C_2$-oxalate lowering. The y - axis shows change in urinary $^{13}C_2$-oxalate from vehicle control. The x - axis shows vehicle (control, 100%) and the effect of increasing doses of SYNB8802^{CamR} (n=6 for each group) on % change on UOx from vehicle with 1E12 being significant. (** p < 0.01). Error bars represent SEM

First-in-Human Study

Mean dietary intake of oxalate in the Western diet has been estimated at 152 mg/day (Holmes and Kennedy, 2000). The estimated 90th percentile intake is approximately 300 mg. A small fraction (typically 10%) of dietary oxalate is absorbed by the gastrointestinal (GI) tract, and systemic oxalate is secreted by the kidneys as urinary oxalate (UOx). (Holmes 2001) In healthy volunteers UOx level is normally below 40 mg/24hr. In EH, UOX levels are significantly higher primarily due to increased absorption of dietary oxalate from the gut. Hyperoxaluria can also be induced by high dietary oxalate intake; an approach that has been used in healthy volunteer studies assessing the potential of novel treatments to lower UOx levels.

SYNB8802 is being investigated in an ongoing Phase 1a/b study comprising two Parts.

Part A is a diet-induced Hyperoxaluria model in healthy volunteers. Subjects will consume a controlled high oxalate (400 to 600 mg) low calcium (400 mg) (HOLC) diet for 4 days prior to starting dosing of investigational product TID in a placebo-controlled, randomized design. Cohorts of N=9 (6 active: 3 placebo) are enrolled in a multiple ascending dose (MAD) study. (**Figure 4**)

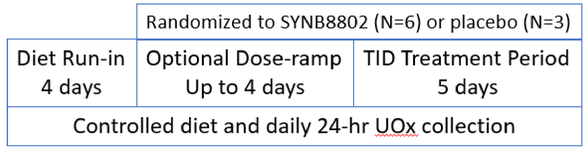

Figure 4. Part A MAD study in healthy volunteers. Hyperoxaluria is induced during a 4-day diet run-in period. High oxalate low calcium diet is continued throughout the study period. 24-hr UOx is collected daily.

Part B is a placebo-controlled crossover study of up to 20 patients with a history of Roux-en-Y (at least 12 months ago) and elevated UOx >70 mg/day. Patients will follow their normal diet. Treatment period is 6 days preceded by a dose-ramp period up to 4 days. (**Figure 5**) The primary outcome is change from baseline in 24-hour UOx amount excreted with SYNB8802 treatment versus placebo.

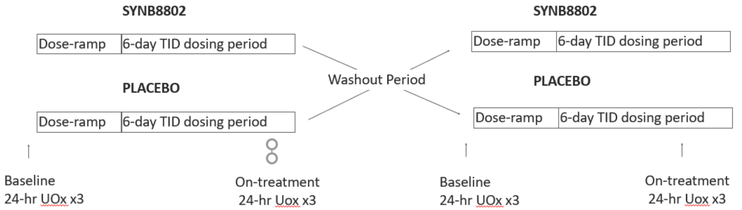

Figure 5. Part B crossover study in Roux-en-Y patients. The mean of 3 UOx values collected at baseline and at the end of the dosing period is used to assess the PD effect of SYNB8802.

Conclusions

- **SYNB8802 is engineered to metabolize oxalate to formate and CO_2**
- **Urinary oxalate could be elevated in NHPs using a dietary model.**
- **SYNB8802 leads to a decrease in UOx level in dietary induced Hyperoxaluria in NHPs in a dose-dependent manner.**
- **Preclinical data support further clinical development.**
- **Safety and tolerability of SYNB8802 is being explored in a Phase 1a/b study [NCT04629170].**
- **Part B seeking proof-of-concept in Roux-en-Y patients open for enrollment.**

Select References

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