# 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. Enteric Hyperoxaluria (EH) is caused by excessive absorption of dietary oxalate due to an underlying malabsorptive GI disease. Such as one of the most popular weight-loss surgeries in the United State, Roux-en-Y gastric bypass, as well as short bowel syndrome or IBD. 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* including one gene from *Saccharomyces cerevisiae* into a probiotic strain EcN. (**Figure 1)** SYNB8802 activity was assessed in media containing $^{13}C_2$-oxalate. While no oxalate consumption or formate production was observed for control EcN, SYNB8802 degraded $^{13}C_2$-oxalate in a linear fashion over the course of 60 min and concurrently produced $^{13}$C-labeled formate. **(Figure 2)**

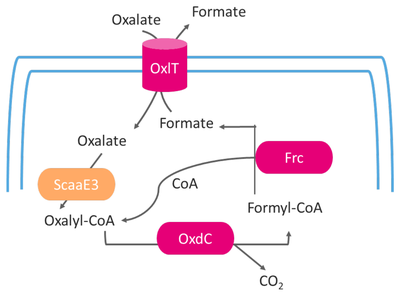



**Figure 1. Schematic of engineered pathway in SYNB8802**. Oxalate enters the cell via OxlT and is then converted to Oxalyl-CoA via ScaaE3. Oxalyl-CoA is decarboxylated to Formyl-CoA and $CO_2$ by OxdC. Frc transfers CoA from Formyl-CoA to an incoming oxalate, releasing formate that is exported via OxlT

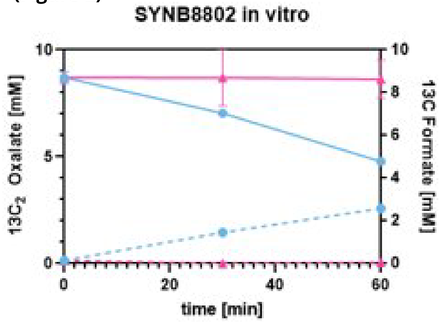



**Figure 2. in vitro activity of SYNB8802**. Left y-axis shows $^{13}C_2$-Oxalate. Right y-axis $^{13}$C Formate. SYNB8802 (light blue) degrades $^{13}C_2$-Oxalate (solid light blue line) in minimal media in the course of 60min. Subsequently $^{13}$C Formate is produced (dotted light blue line). Unengineered Nissle (pink) has no impact on $^{13}C_2$-Oxalate

**In vitro simulation (IVS)**

To estimate SYNB8802 activity under conditions representing the GI lumen, an in vitro simulation (IVS) system was developed, comprising a series of incubations in media representing human stomach, small intestine, and colon compartments by simulating luminal pH and oxygen, gastric and pancreatic enzymes, and GI transit times. The rate of oxalate degradation was estimated in each simulated compartment **(Figure 3)**.

Oxalate consumption was highest in simulated gastric fluid (SGF) (1.35±0.04 and 1.52±0.08 µmol oxalate/hr*$10^9$ cells at one and two hours post inoculation, respectively) and remained at similar levels after 1h incubation in simulated small intestinal fluid (SIF). Oxalate consumption decreased to 0.88±0.04 µmol oxalate/hr*$10^9$ cells after 2h incubation in SIF. SYNB8802 activity further decreased to 0.2±0.14 µmol oxalate/hr*$10^9$ cells in the completely anaerobic conditions of simulated colonic fluid (SCF), where it remained relatively stable over the 48h incubation period. These data suggest that SYNB8802 has the potential to metabolize oxalate throughout the human GI tract.

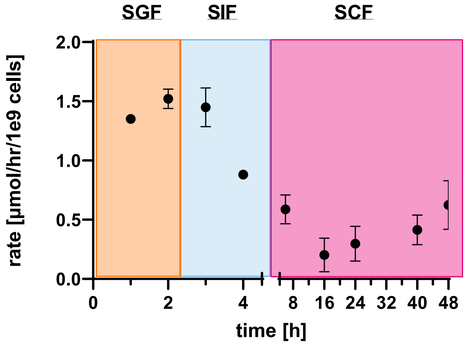
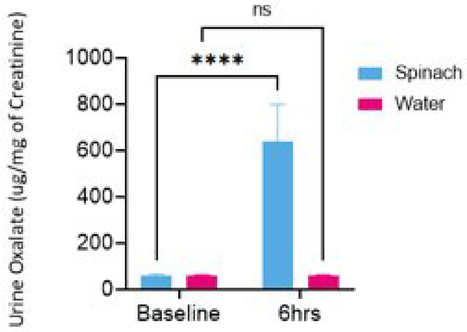



**Figure 3. In vitro simulation (IVS).** Left y-axis: Rate of oxalate degradation in µmol/h/$10^9$ cells. X-axis = time in hours. Left X-axis 0 – 4 hours, Right X-axis 6 – 48 h. Dots represent an average of a triplicate with error bars representing standard deviation. Data points in the orange box represent incubation in simulated gastric fluid (SGF). Data points in the light blue box represent incubation in simulated intestinal fluid (SIF). Data points in the pink box represent incubation in simulated colonic fluid (SCF).

***Acute Diet-induced Hyperoxaluria in Non-human Primates***




An acute model of Hyperoxaluria was developed in cynomolgus monkeys (non-human primates; NHP). Animals were orally administered vehicle (water) or a spinach suspension (1.5g/mL in water) with sodium bicarbonate, $^{13}C_2$-oxalate, and formulation buffer or bacteria. The spinach suspension resulted in a 10-fold increase in UOx recovery compared to controls 6 hours after administration ($p < 0.0001$, 2-way ANOVA; **Figure 4**).

**Figure 4. NHP model development.** Y-axis shows UOx normalized to creatine. NHPs (n=6 for each group) receiving either spinach (light blue) or water control (pink) show similar baseline UOx levels of around 45 mg/ug creatine. The spinach group (light blue) shows a significant increase in UOx levels over the control (pink) after 6 hours. Error bars represent SEM. **** $p < 0.0001$

Next, the ability of SYNB8802$^{CamR}$ to consume oxalate in vivo was evaluated. SYNB8802$^{CamR}$ lowered the recovery of unlabeled UOx compared to vehicle by 33%, 41%, 42%, and 73% at the 5 × $10^{10}$, 1 × $10^{11}$, 5 × $10^{11}$, and 1 x $10^{12}$ CFU dose levels, respectively (**Figure 5**). UOx lowering was statistically significant at the 1 x $10^{12}$ dose level compared to vehicle-treated animals ($p=0.013$, One-way ANOVA). 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 6**). Collectively, these studies indicate that orally administered SYNB8802 significantly lowers UOx levels in NHPs through the consumption of oxalate in the GI tract.

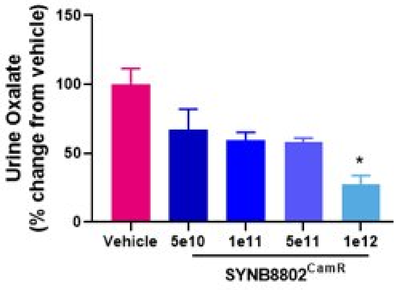



**Figure 5. Non-human primate study oxalate lowering.** The y - axis shows change in UOx from vehicle control. The x - axis shows vehicle (control, 100%) and the effect of increasing doses of SYNB8802$^{CamR}$ (n=6 for each group) on % change on UOx from vehicle with 1e12 being significant. (* $p < 0.05$). Error bars represent SEM

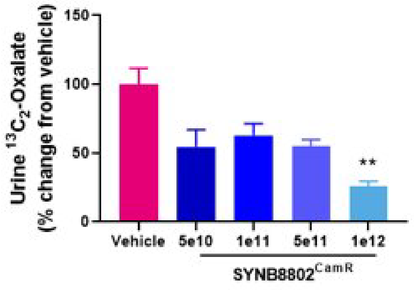



**Figure 6. Non-human primate study $^{13}C_2$ -oxalate lowering.** The y - axis shows change in urinary $^{13}C_2$ -oxalate from vehicle control. The x - axis shows vehicle (control, 100%) and the effect of increasing doses of SYNB8802$^{CamR}$ (n=6 for each group) on % change on UOx from vehicle with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

## Conclusions

- SYNB8802 is engineered to metabolize oxalate to formate and $CO_2$
- Development of IVS shows differential activity of SYNB8802 based on GI compartment
- 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 is open for enrollment.

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