

Development of a Synthetic Biotic, SYN8802, for the Treatment of Enteric Hyperoxaluria.

Lauren Renaud, David Lubkowitz, Sarani Ghoshal, Nick Horvath, Pip Reeder, Chris Bergeron, Courtney Chen, Michael James, Pat Cantarella, Mark Charbonneau, Ron Shmueli, JR Gao, Vincent Isabella and Mylene Perreault.

Synlogic Operating Company, Inc., Cambridge, MA

Disclosures

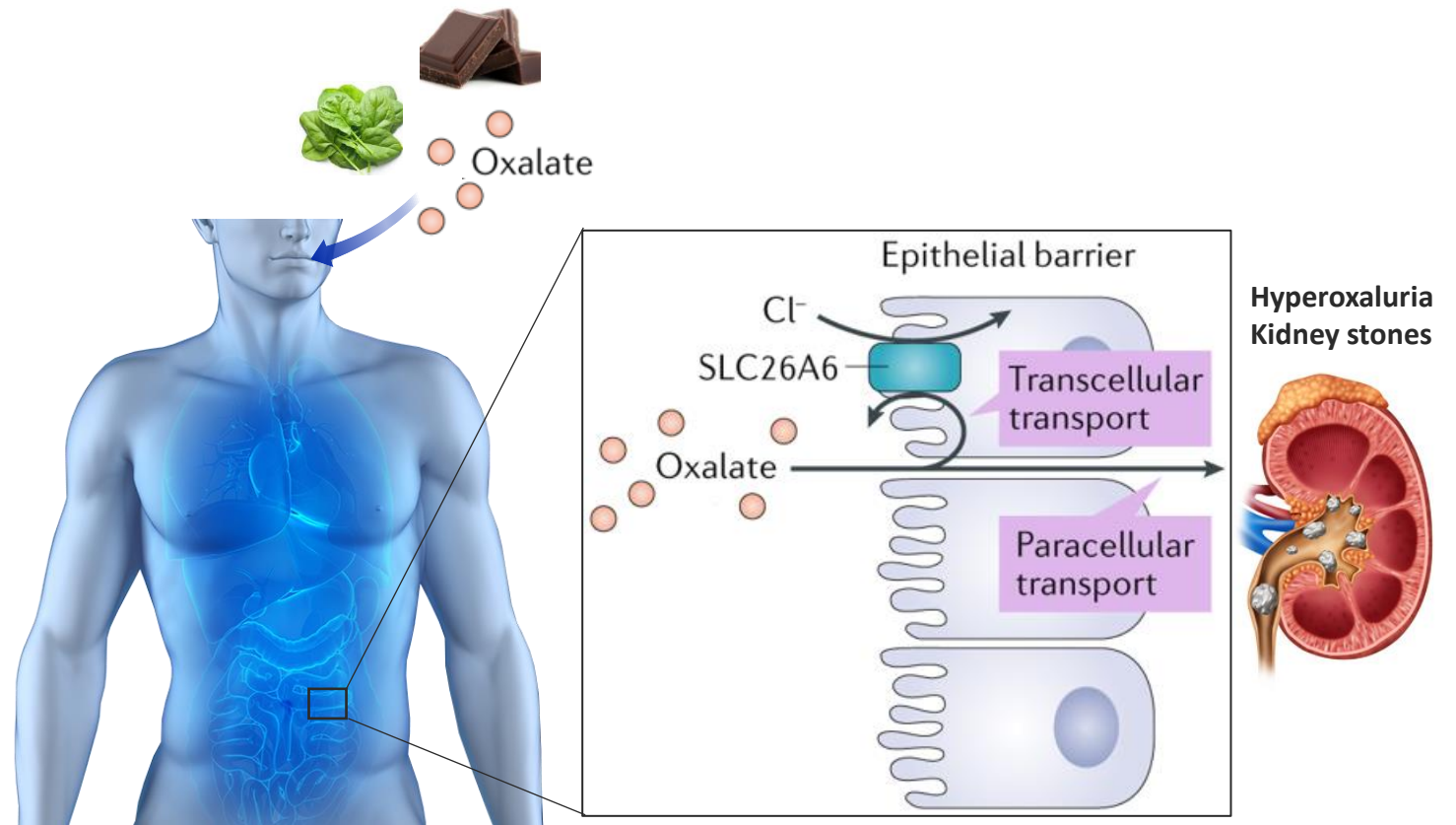
- All authors are employees of Synlogic Operating Company, Inc. and are stock and/or share owners of Synlogic, Inc.

Enteric Hyperoxaluria

Disease Overview

Enteric Hyperoxaluria (EH)

- Enteric hyperoxaluria (EH) is a metabolic disease that results from an excessive absorption of dietary oxalate.
- EH occurs mainly in patients with underlying gastrointestinal (GI) disorders, including post gastric bypass surgery, inflammatory bowel disease, Crohn's disease, pancreatitis, and short bowel syndrome.
- Increased GI oxalate absorption results in elevated urinary oxalate levels and contributes to kidney stone formation, nephrocalcinosis, crystallopathy and other adverse renal outcomes.



Objective: Engineer an Escherichia coli Nissle 1917 strain to metabolize oxalate for the treatment for Enteric Hyperoxaluria

Engineered EcN has the Potential to Consume Oxalate Throughout the GI Tract

GI Based Therapies Have Demonstrated Lowering of Systemic Oxalate

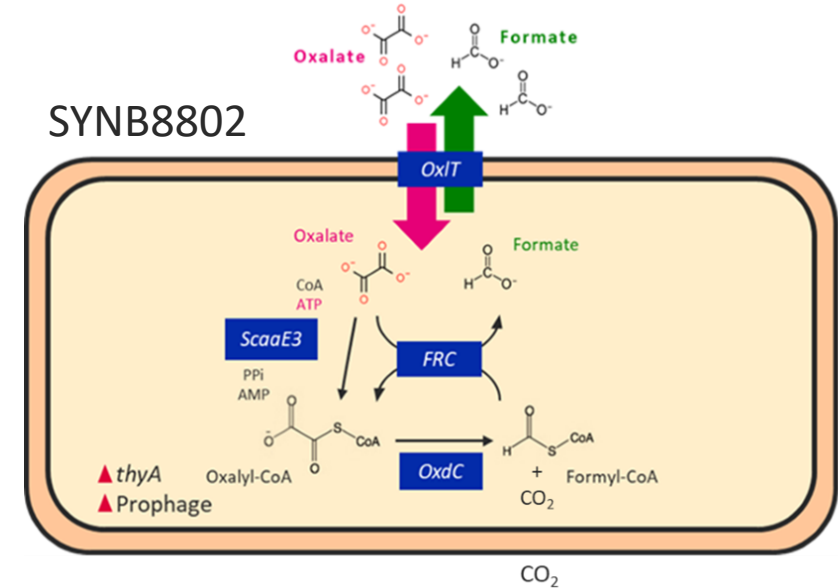
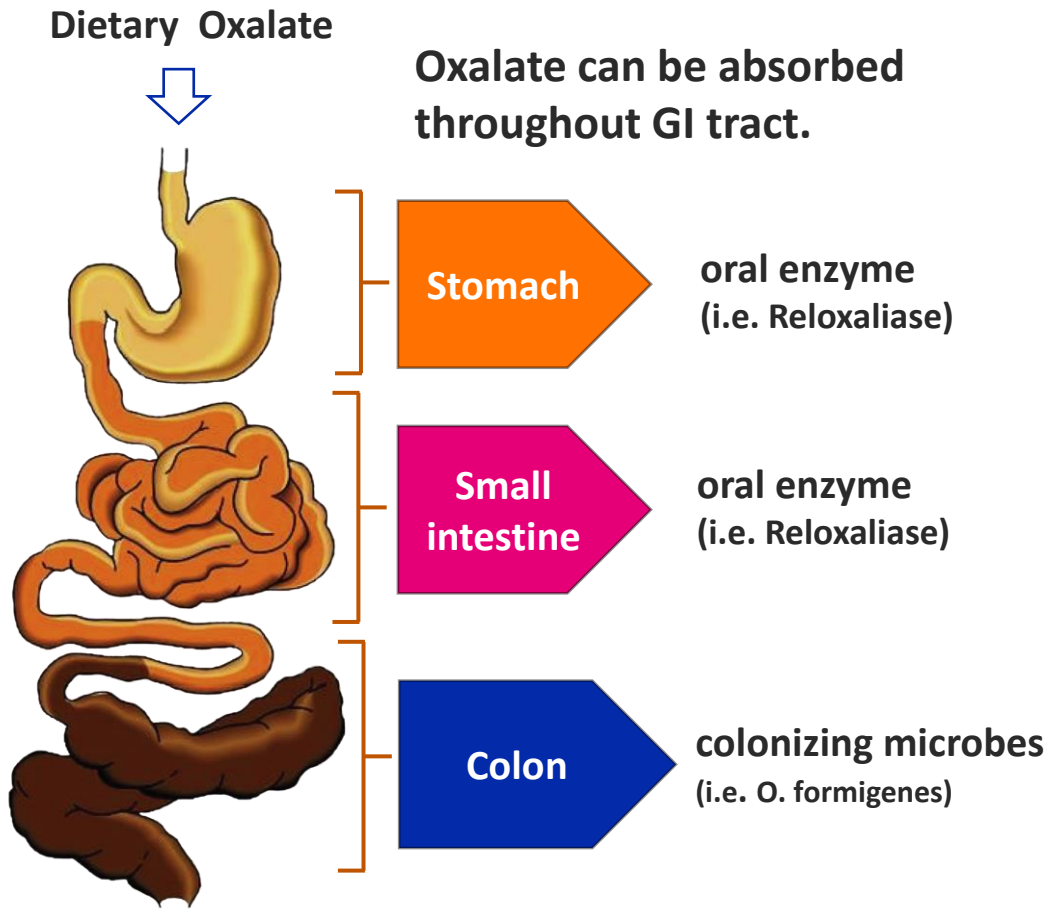


Figure 1: Schematic of SYN8802. EcN was chromosomally modified to integrate *OxIT*, *ScaaE3*, *OxdC*, and *Frc* under the control of PfnrS. The *thyA* and several structural genes within the endogenous prophage were deleted from the genome. Abbreviations: EcN: *E. coli* Nissle, OxIT = oxalate/formate antiporter; ScaaE3 = oxalyl-CoA synthetase; OxdC = oxalate decarboxylase; FRC = formyl-CoA transferase; ATP = adenosine triphosphate; AMP = adenosine monophosphate; PPI = pyrophosphate; Δ *thyA* = thymidylate synthase knock out.

Intestinal Degradation of Oxalate Throughout GI Tract Could Enhance Oxalate Lowering

Engineered Strain SYN8802 Consumes Oxalate and Produces Formate In Vitro

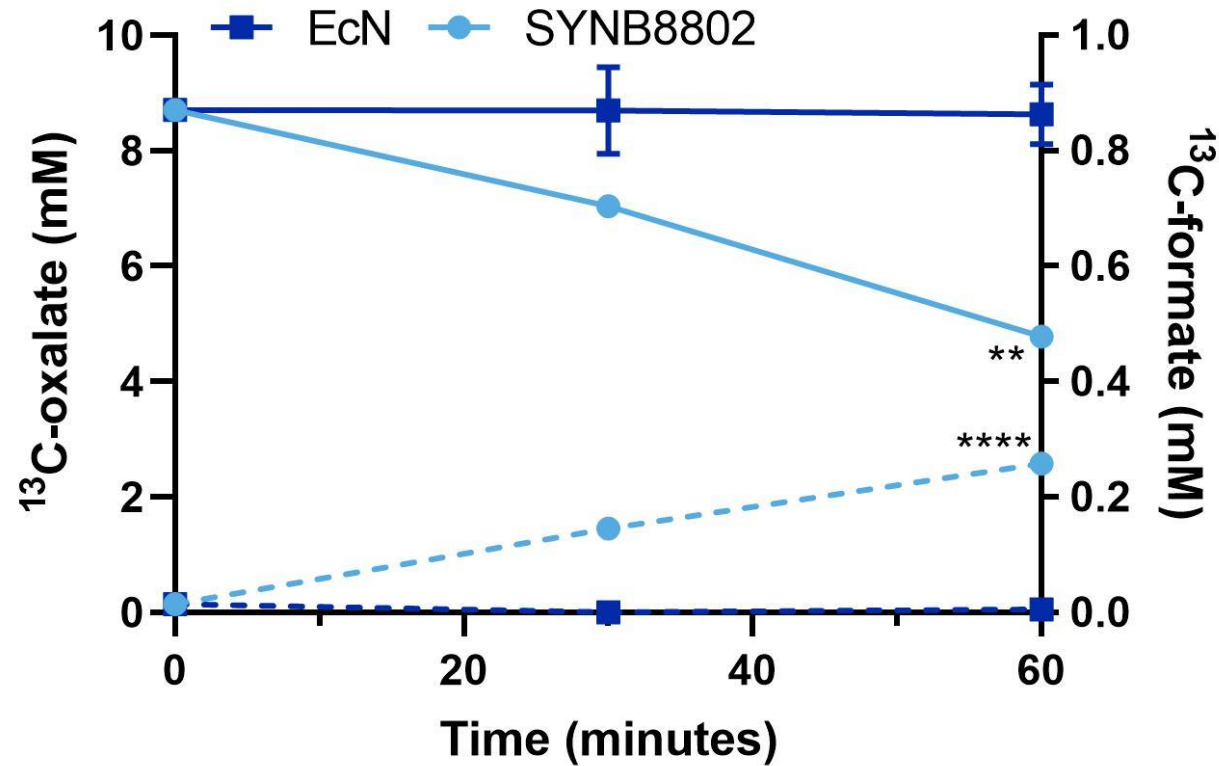


Figure 2: In Vitro ^{13}C -oxalate consumption and ^{13}C -formate production. Ecn and SYN8802 were grown and incubated statically and supernatant samples were removed at 30 and 60 minutes to determine the concentrations of ^{13}C -oxalate and ^{13}C -formate by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Statistical analysis was performed using two-way repeated measures analysis followed by Sidak's multiple comparison test. ** $p < 0.01$, **** $p < 0.0001$.

SYNB8802^R is Viable In Vivo and is Excreted in the Feces

SYNB8802^R is cleared within 24 hours in mice

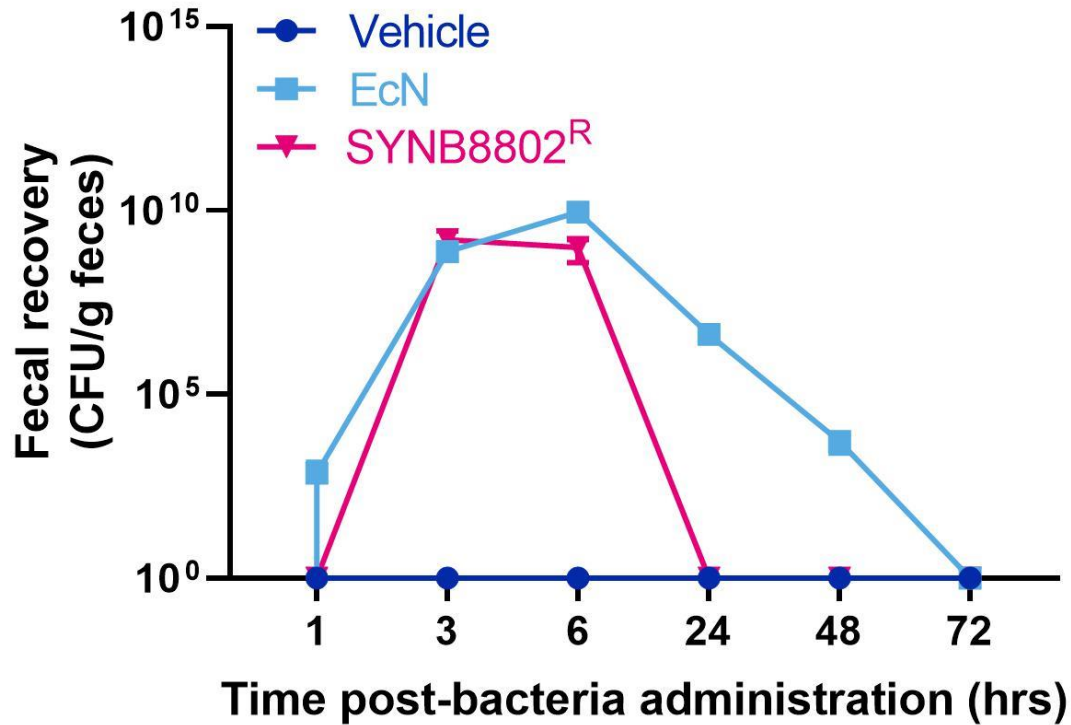


Figure 3: SYNB8802^R is viable In Vivo and cleared from feces by 24 hours in mice. C57BL/6J (n = 16) male mice received a single oral dose of the treatment (1.3 x 10¹⁰ CFU) and feces were collected and plated to determine colony-forming units (CFU). SYNB8802^R = antibiotic-resistant SYNB8802.

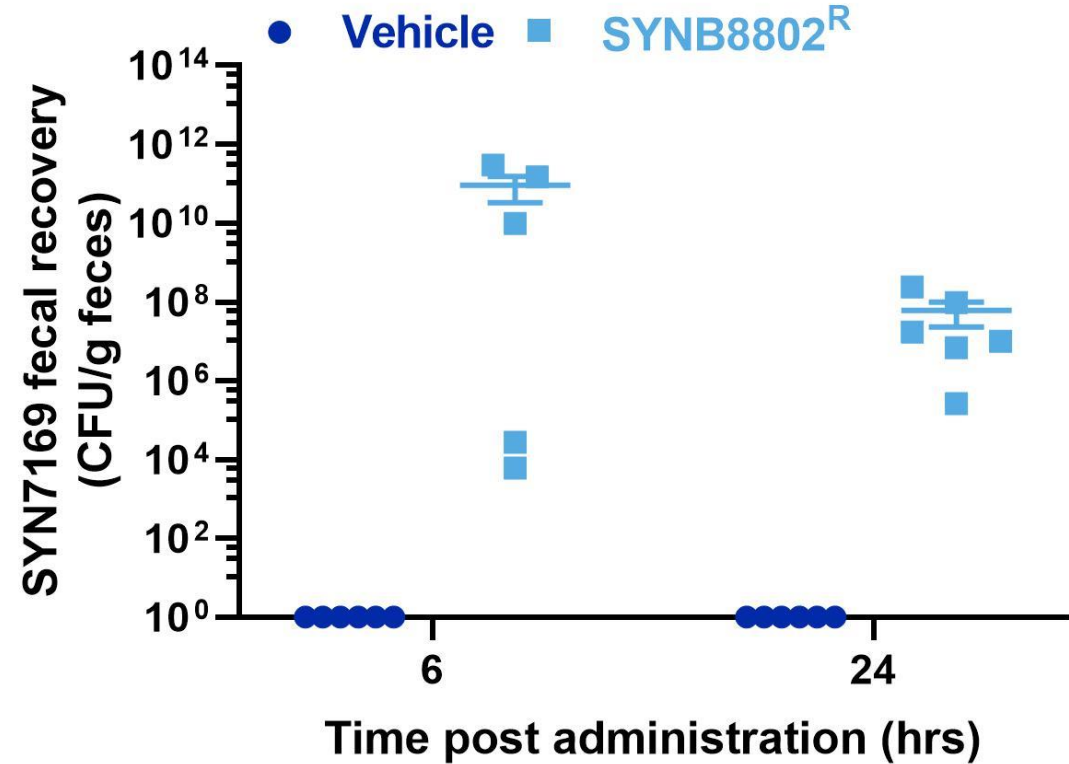


Figure 4: SYNB8802^R is viable In Vivo and excreted in feces of NHPs. Twelve cynomolgus monkeys were fasted overnight and administered a spinach suspension (~400 mg oxalate), sodium bicarbonate, ¹³C-oxalate, and formulation buffer or bacteria (1 x 10¹² CFU). Feces were collected at 6 and 24 hours postdosing and plated to determine viable colony-forming units (CFU). SYNB8802^R = antibiotic-resistant SYNB8802.

SYNB8802 Significantly Consumes ^{13}C -oxalate in the GI Tract of Mice

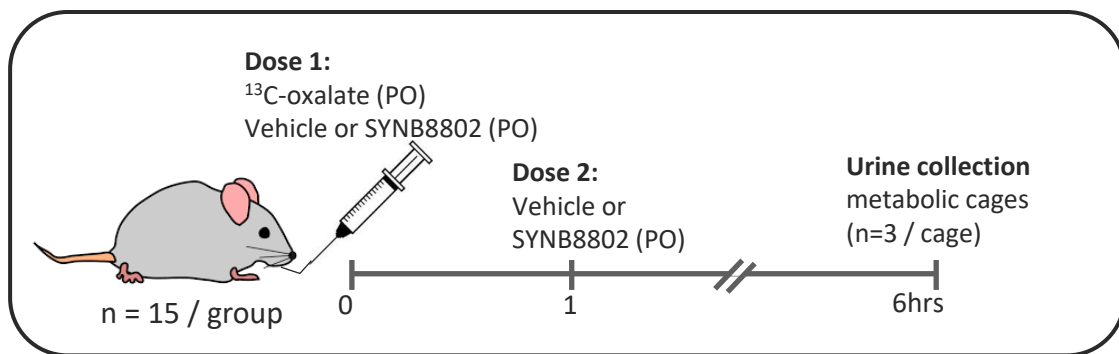


Figure 5: Study design. C57BL/6J male mice were orally administered a dose of ^{13}C -oxalate (100 μg) followed by vehicle (formulation buffer) or SYNB8802. Mice were immediately placed into metabolic cages (n = 3 / cage) and received another dose of vehicle or SYNB8802 1 hours post first dose. Urine was collected 6 hours following dose 1 and ^{13}C -oxalate and creatinine levels were quantitated by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

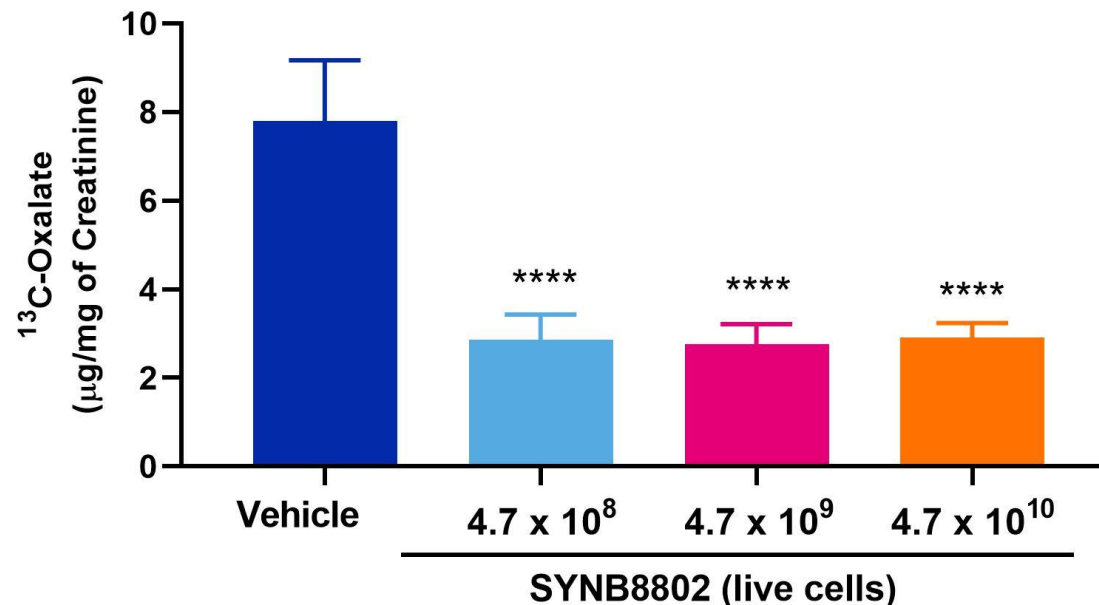


Figure 6: Consumption of ^{13}C -oxalate in the GI tract of healthy mice. Data presented as mean urinary ^{13}C -oxalate recovery normalized by creatinine \pm standard error of the mean. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test. **** p < 0.0001.

SYNB8802 Significantly Consumes Oxalate in the GI Tract of Nonhuman Primates

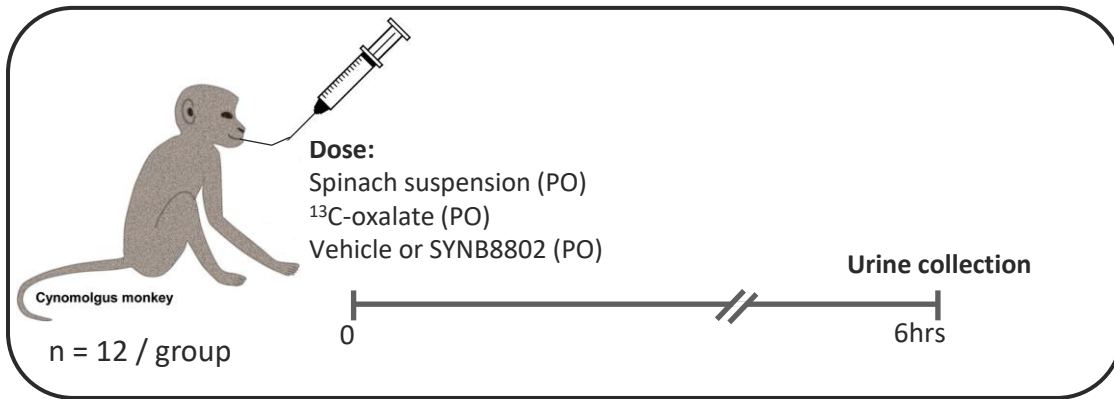


Figure 7: Study design. Male cynomolgus monkeys were fasted overnight and administered vehicle (water) or spinach suspension (~400 mg of oxalate), sodium bicarbonate, ¹³C-oxalate (50 mg), and vehicle (formulation buffer) or SYNB8802 (1 x 10¹² live cells). Urine was collected at 6 hours post-dosing, and the levels of oxalate, ¹³C-oxalate and creatinine were quantitated by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

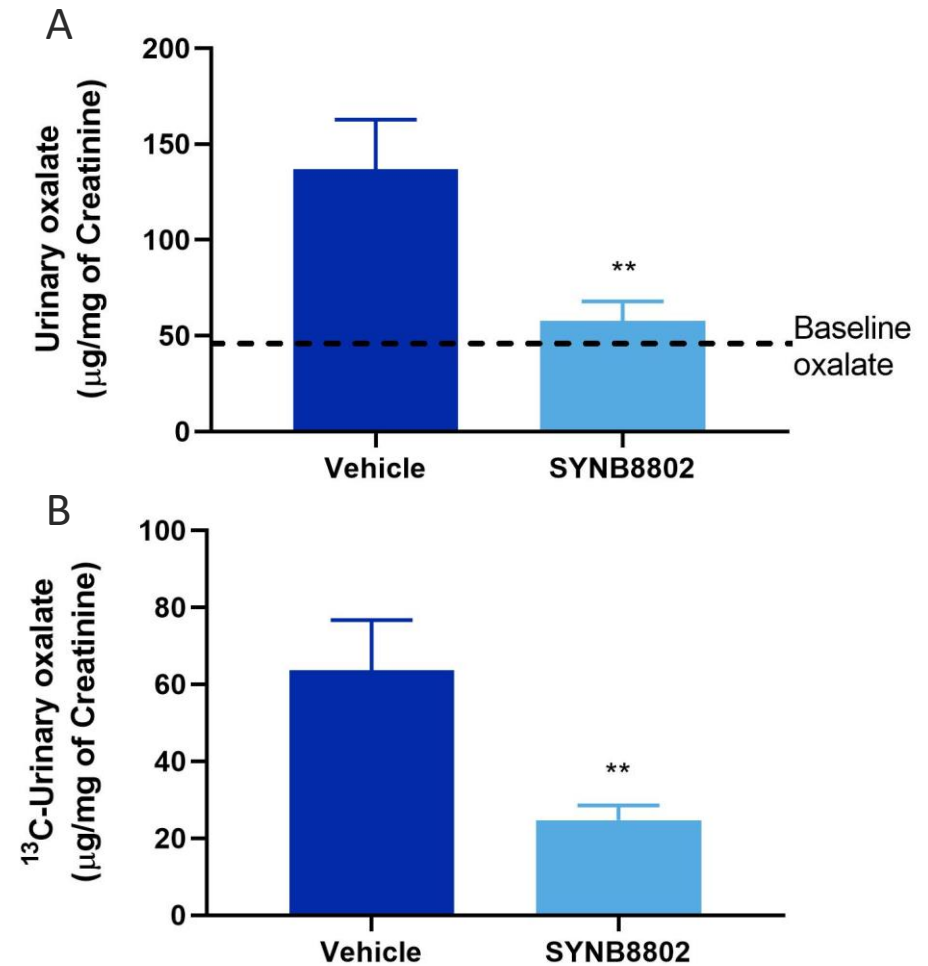


Figure 8: Consumption of oxalate (A) and ¹³C-oxalate (B) in the GI tract of cynomolgus monkeys with acute hyperoxaluria. Data presented as mean urinary oxalate or ¹³C-oxalate recovery normalized by creatinine ± standard error of the mean. Statistical analysis was performed using paired t-test. ***p* < 0.01.

Modeling Suggests SYN8802 Has Potential to Achieve >20% Urinary Oxalate Lowering at Target Dose Range

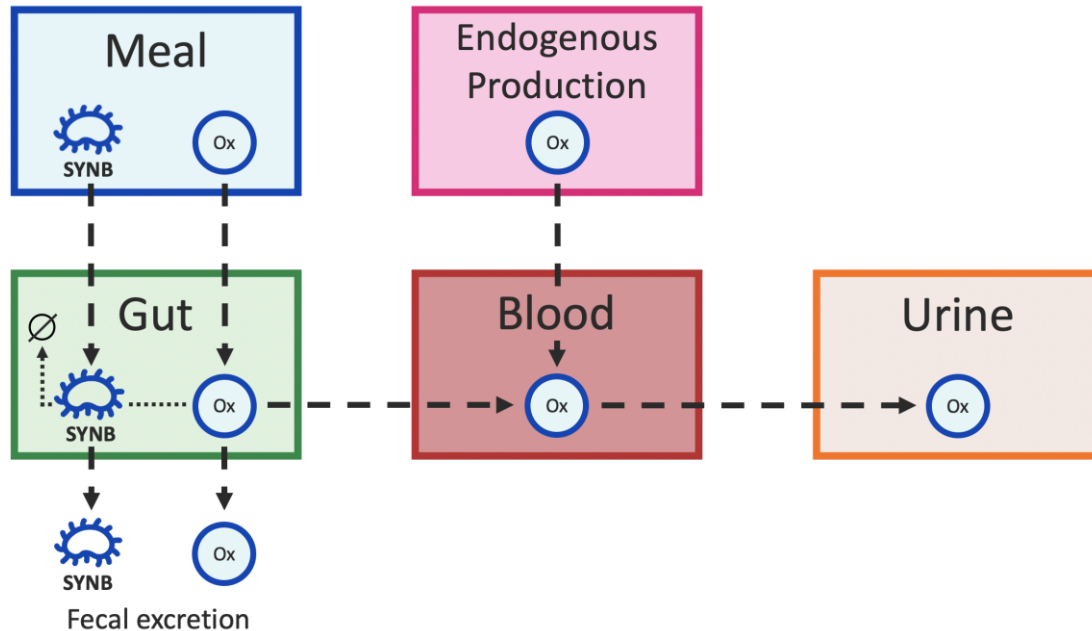


Figure 9: Enteric Hyperoxaluria *in silico* simulation (ISS) model schematic. ISS connects *in vitro* strain activity knowledge to host and disease biology. The **strain-side model** (green) simulates the consumption of oxalate by SYN8802 within the gastrointestinal physiology. The **host-side model** (overall schematic) simulates the impact of consumption by SYN8802 on the distribution of oxalate throughout the body.

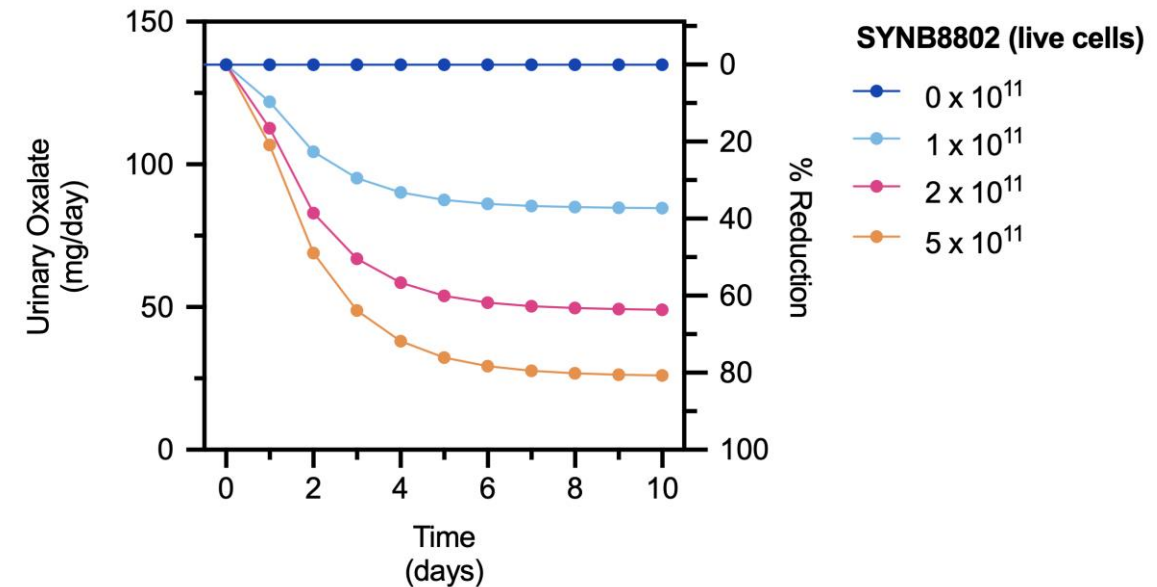


Figure 10: *In silico* simulation (ISS), urinary oxalate percent change from baseline after dosing with SYN8802. ISS predicts a dose-dependent lowering of urinary oxalate. Data presented as baseline assumption of increased dietary oxalate absorption in HOX patients (4x healthy absorption); bounded region represents the range of the assumption (3x-5x healthy absorption).

Conclusions

- Enteric hyperoxaluria is a disease caused by increased gastrointestinal oxalate absorption resulting in elevated urinary oxalate levels, which contributes to kidney stones formation and other adverse renal outcomes.
- *E. coli* Nissle strain SYN8802 is a Synthetic Biotic Medicine that metabolizes oxalate into formate in vitro and consumes gastrointestinal oxalate in healthy mice and monkeys with acute hyperoxaluria.
- Mathematical modeling predicts that SYN8802 has the potential to lower urinary oxalate by 20-50% in patients, suggesting that SYN8802 represents a promising new approach for the treatment of enteric hyperoxaluria.
- SYN8802 is moving into the clinic and will be assessed for safety and tolerability, microbial kinetics of strain, changes in plasma and urine biomarkers, and potential to reduce urinary oxalate.