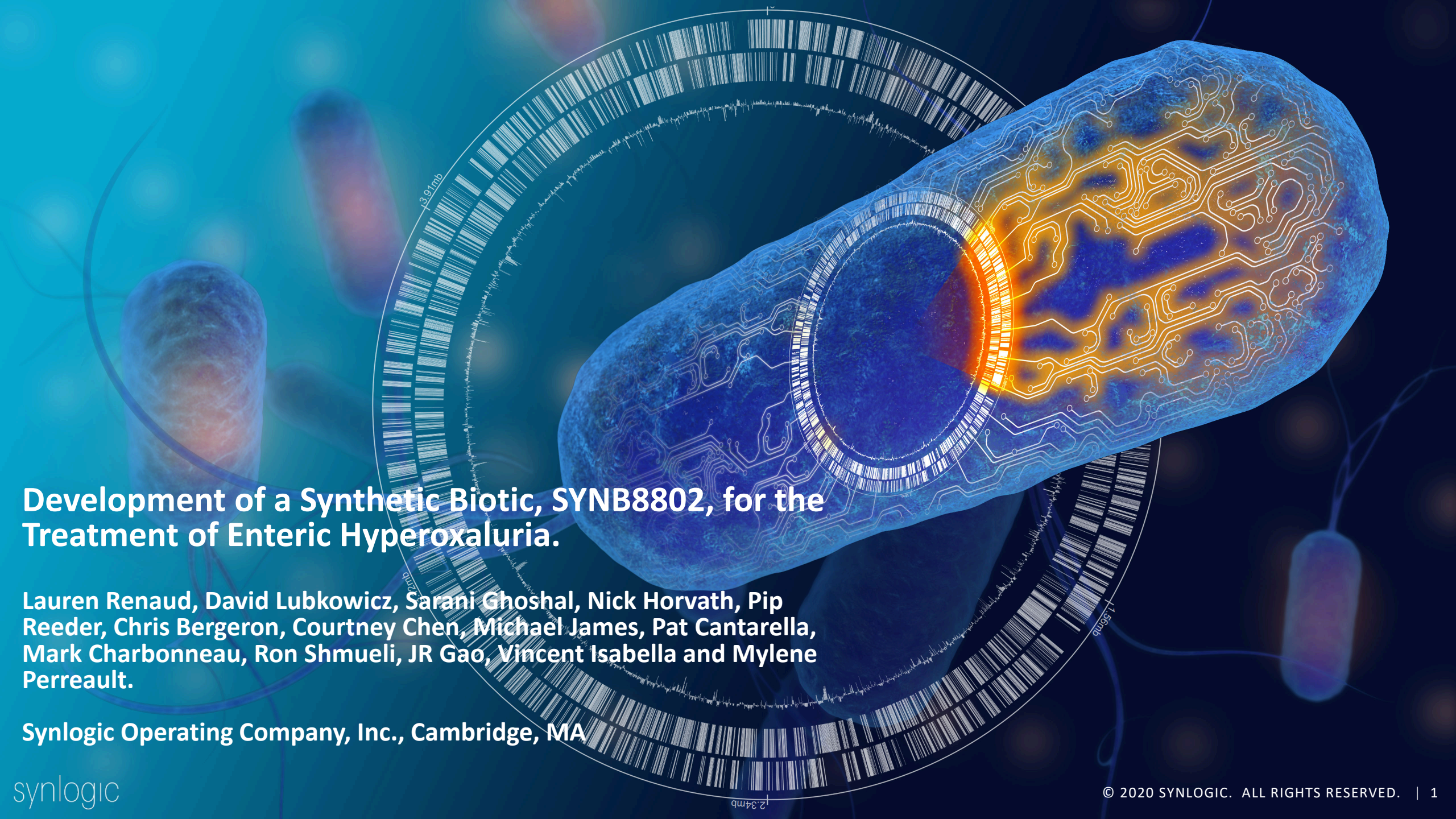

Development of a Synthetic Biotic, SYNB8802, for the Treatment of Enteric Hyperoxaluria.

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Disclosures

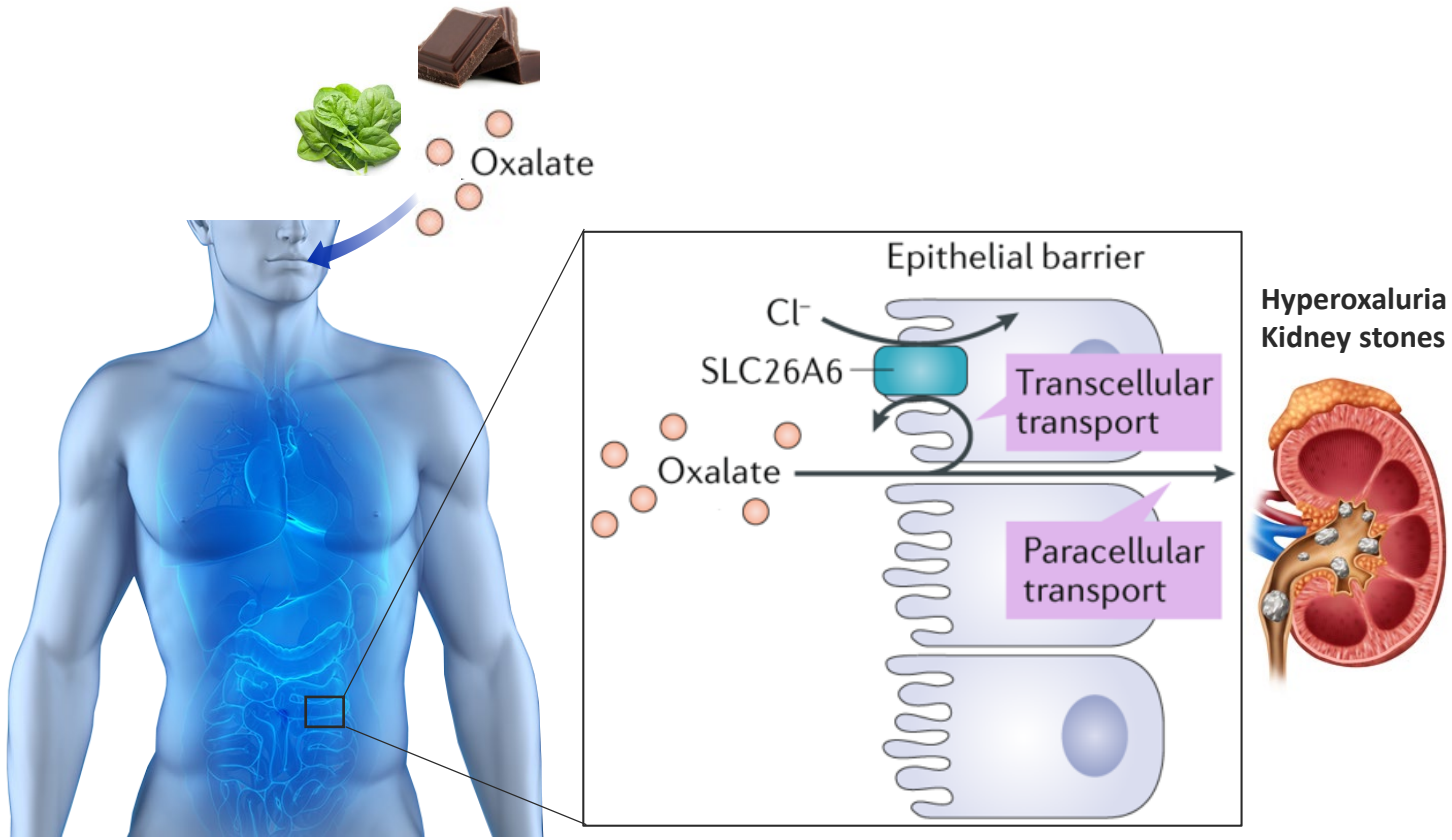
- All authors are employees of Synlogic Operating Company, Inc. and are stock and/or share owners of Synlogic, Inc.

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.

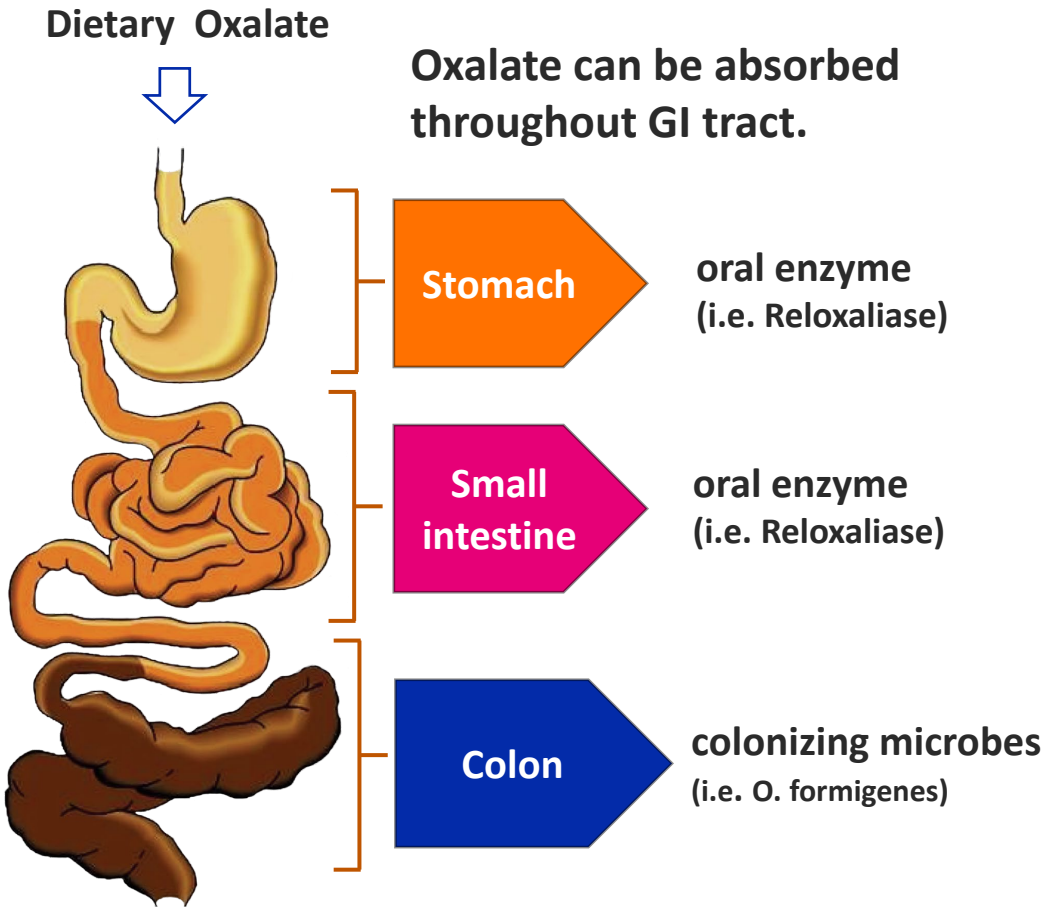


Objective: Engineer an Escherichia coli Nissle 1917 strain to metabolize oxalate for the treatment for Enteric Hyperoxaluria

Adapted from Immunity, microbiota and kidney disease. *Nat Rev. Nephrol* 2019.

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

GI Based Therapies Have Demonstrated Lowering of Systemic Oxalate



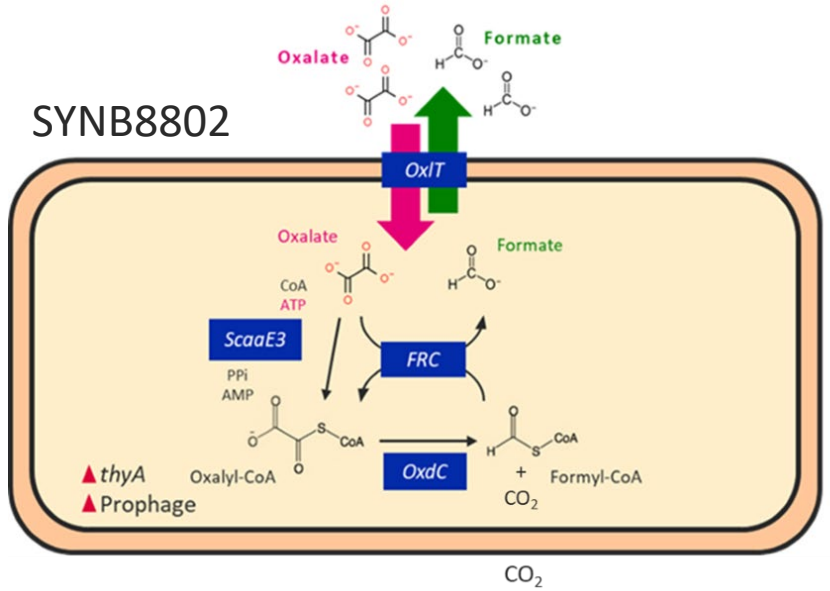


Figure 1: Schematic of SYNB8802. EcN was chromosomally modified to integrate *OxlT, ScaaE3, OxdC, and Frc* under the control of PfnrS. The *thyA* and several structural genes within the endogenous prophage were deleted from the genome. Abbreviations: EcN: *E. coli* Nissle, OxlT = oxalate/formate antiporter; ScaaE3 = oxalyl-CoA synthetase; OxdC = oxalate decarboxylase; FRC = formyl-CoA transferase; ATP = adenosine triphosphate; AMP = adenosine monophosphate; PPi = pyrophosphate; ΔthyA = thymidylate synthase knock out.

Intestinal Degradation of Oxalate Throughout GI Tract Could Enhance Oxalate Lowering

Engineered Strain SYNB8802 Consumes Oxalate and Produces Formate In Vitro

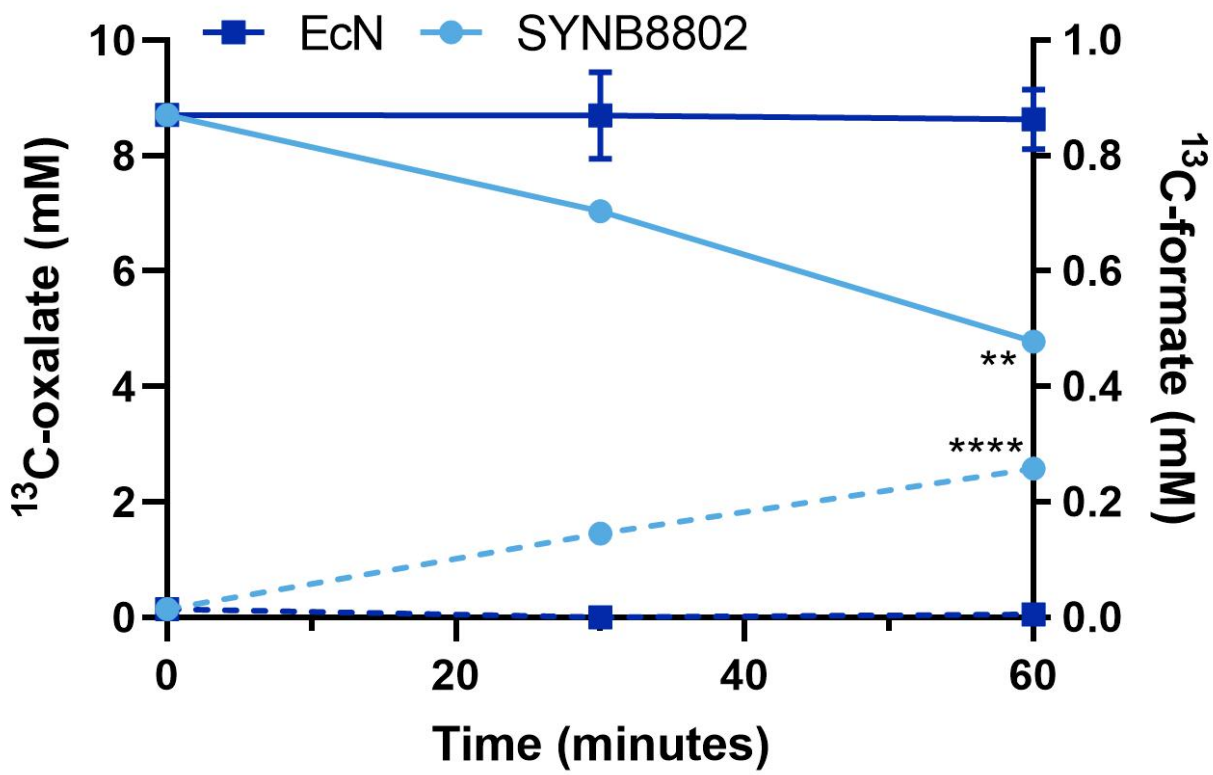


Figure 2: In Vitro ^{13}C-oxalate consumption and ^{13}C-formate production. EcN and SYNB8802 were grown and incubated statically and supernatant samples were removed at 30 and 60 minutes to determine the concentrations of ^{13}C-oxalate and ^{13}C-formate by liquid chromatography-tandem mass spectrometry (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

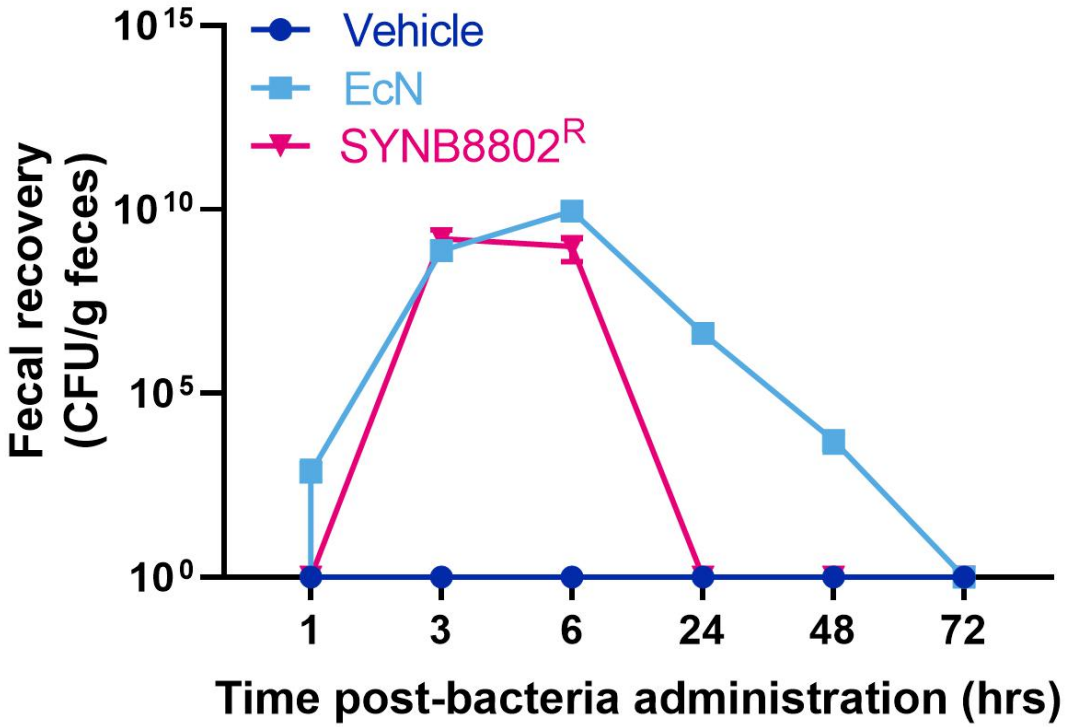


Figure 3: SYNB8802^{R} is viable In Vivo and cleared from feces by 24 hours in mice. C57BL/6J (n = 16) male mice received a single oral dose of the treatment (1.3 x 10^{10} CFU) and feces were collected and plated to determine colony-forming units (CFU). SYNB8802^{R} = antibiotic-resistant SYNB8802.

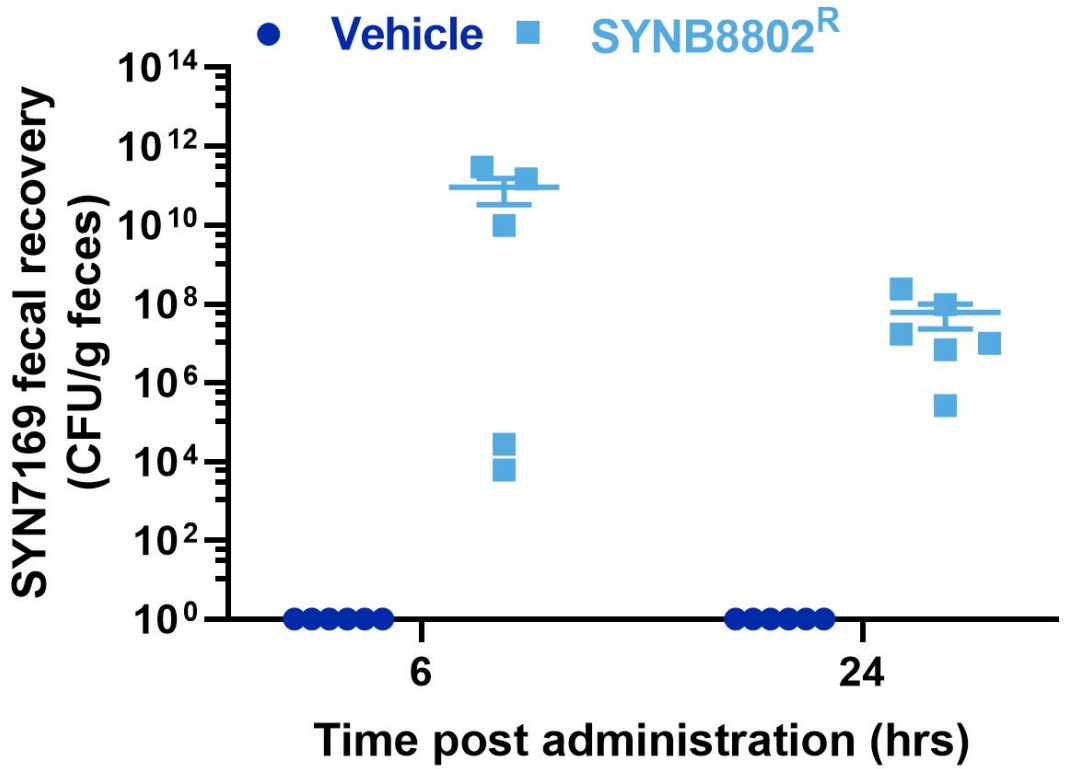


Figure 4: SYNB8802^{R} is viable In Vivo and excreted in feces of NHPs. Twelve cynomolgus monkeys were fasted overnight and administered a spinach suspension (~400 mg oxalate), sodium bicarbonate, ^{13}C-oxalate, and formulation buffer or bacteria (1 x 10^{12} CFU). Feces were collected at 6 and 24 hours 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

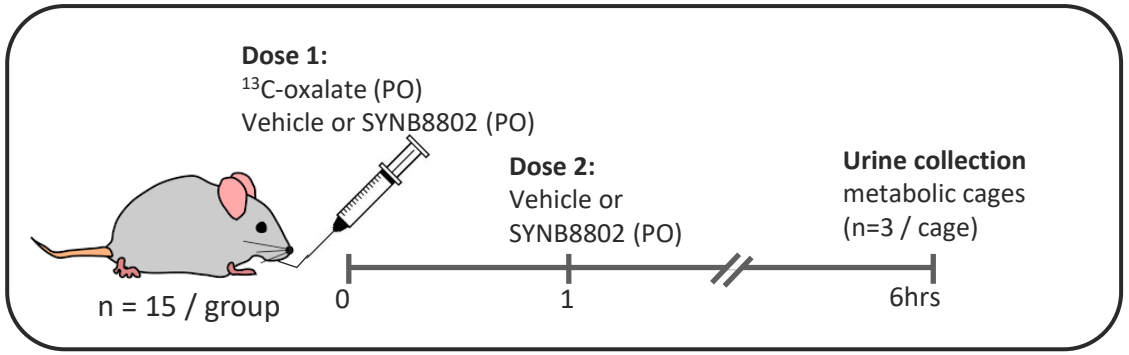


Figure 5: Study design. C57BL/6J male mice were orally administered a dose of ^{13}C-oxalate (100 µg) followed by vehicle (formulation buffer) or SYNB8802. Mice were immediately placed into metabolic cages (n = 3 / cage) and received another dose of vehicle or SYNB8802 1 hours post first dose. Urine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

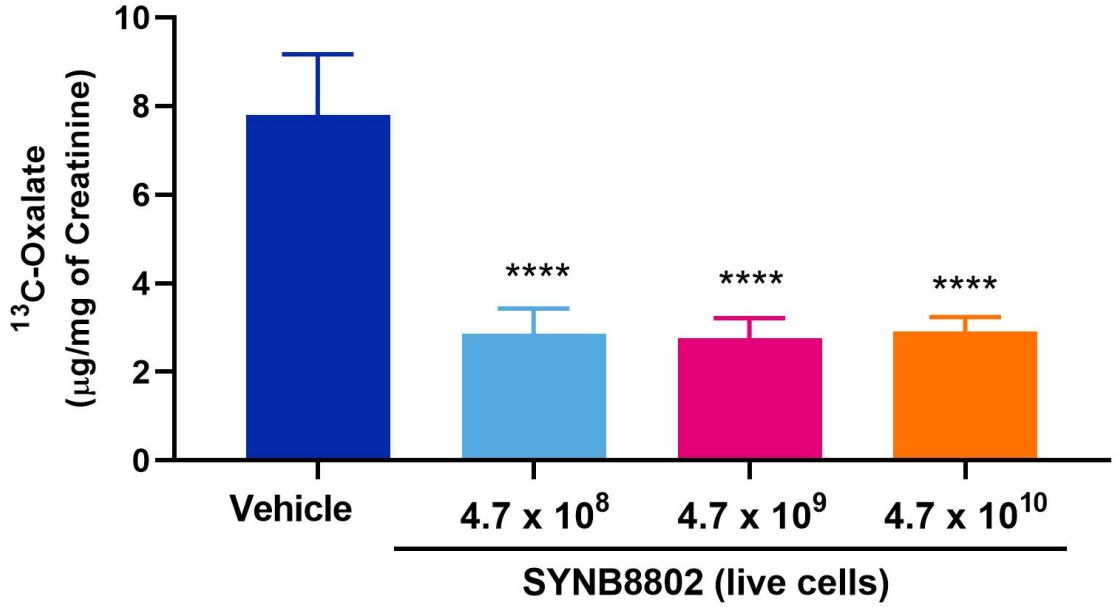


Figure 6: Consumption of ^{13}C-oxalate in the GI tract of healthy mice. Data presented as mean urinary ^{13}C- oxalate recovery normalized by creatinine ± standard error of the mean. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple comparison test.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

SYNB8802 Significantly Consumes Oxalate in the GI Tract of Nonhuman Primates

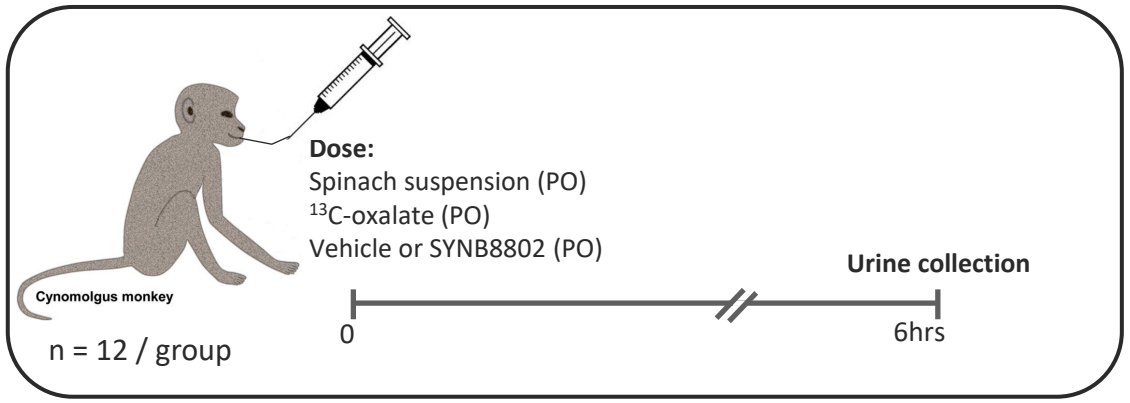


Figure 7: Study design. Male cynomolgus monkeys were fasted overnight and administered vehicle (water) or spinach suspension (~400 mg of oxalate), sodium bicarbonate, ^{13}C-oxalate (50 mg), and vehicle (formulation buffer) or SYNB8802 (1 x 10^{12} live cells). Urine was collected at 6 hours post-dosing, and the levels of oxalate, ^{13}C-oxalate and creatinine were quantitated by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

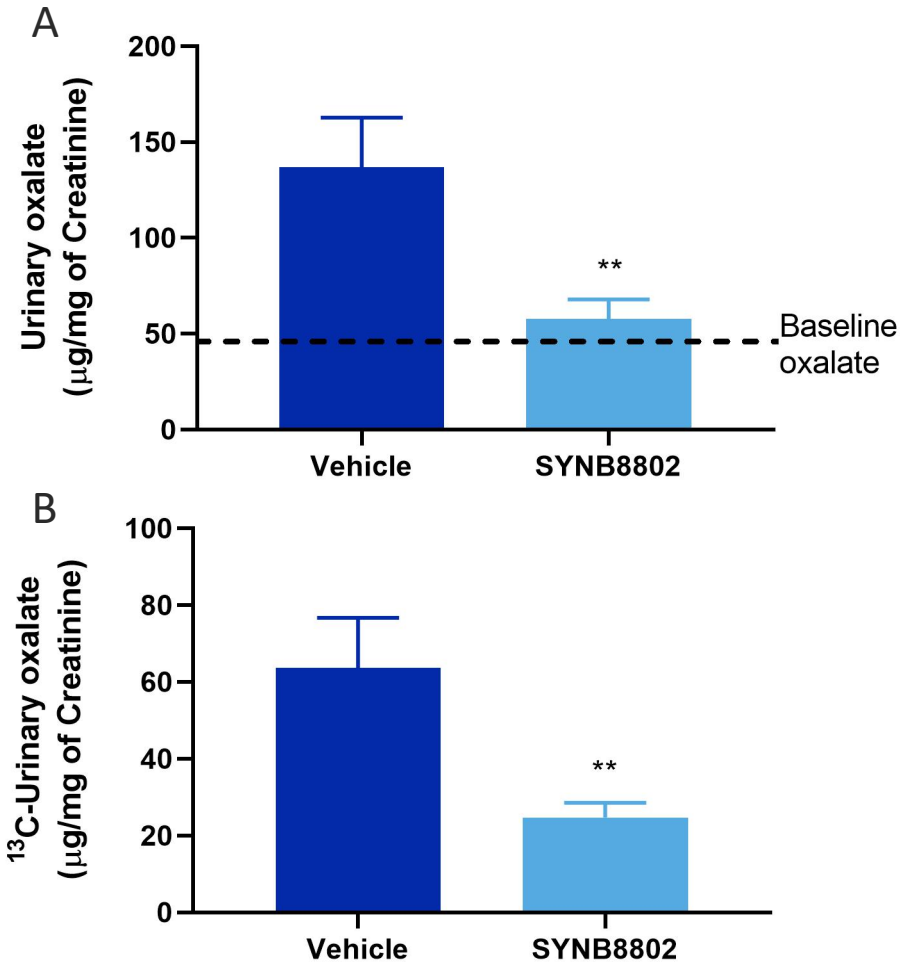


Figure 8: Consumption of oxalate (A) and ^{13}C-oxalate (B) in the GI tract of cynomolgus monkeys with acute hyperoxaluria. Data presented as mean urinary oxalate or ^{13}C-oxalate recovery normalized by creatinine ± standard error of the mean. Statistical analysis was performed using paired t-test.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

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Modeling Suggests SYNB8802 Has Potential to Achieve >20% Urinary Oxalate Lowering at Target Dose Range

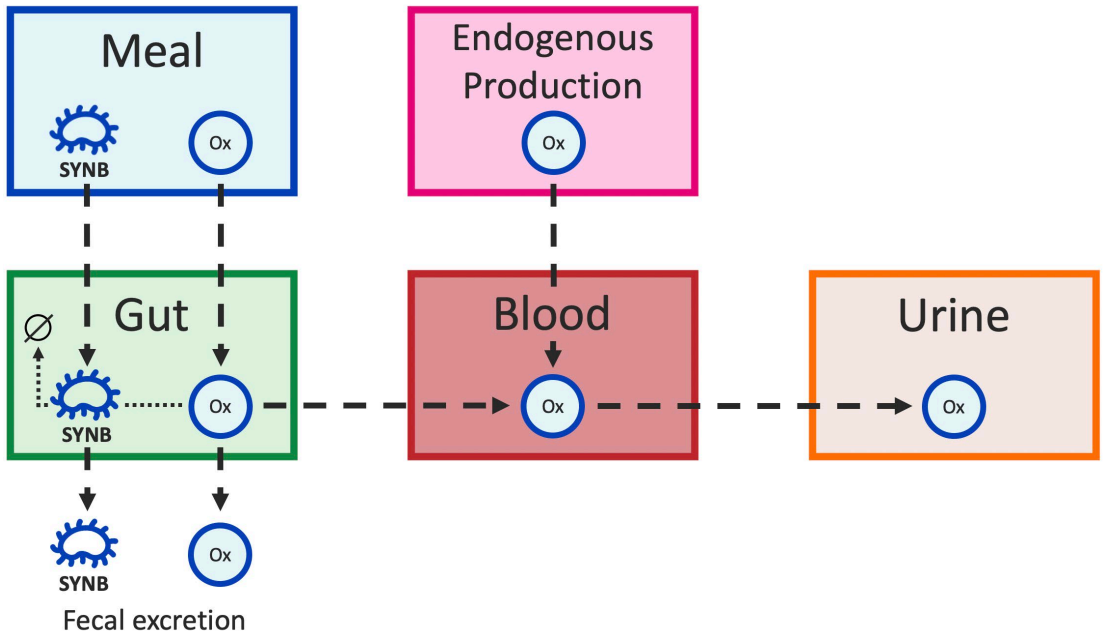

Figure 9: Enteric Hyperoxaluria *in silico* simulation (ISS) model schematic. ISS connects *in vitro* strain activity knowledge to host and disease biology. The **strain-side model** (green) simulates the consumption of oxalate by SYNB8802 within the gastrointestinal physiology. The **host-side model** (overall schematic) simulates the impact of consumption by SYNB8802 on the distribution of oxalate throughout the body.

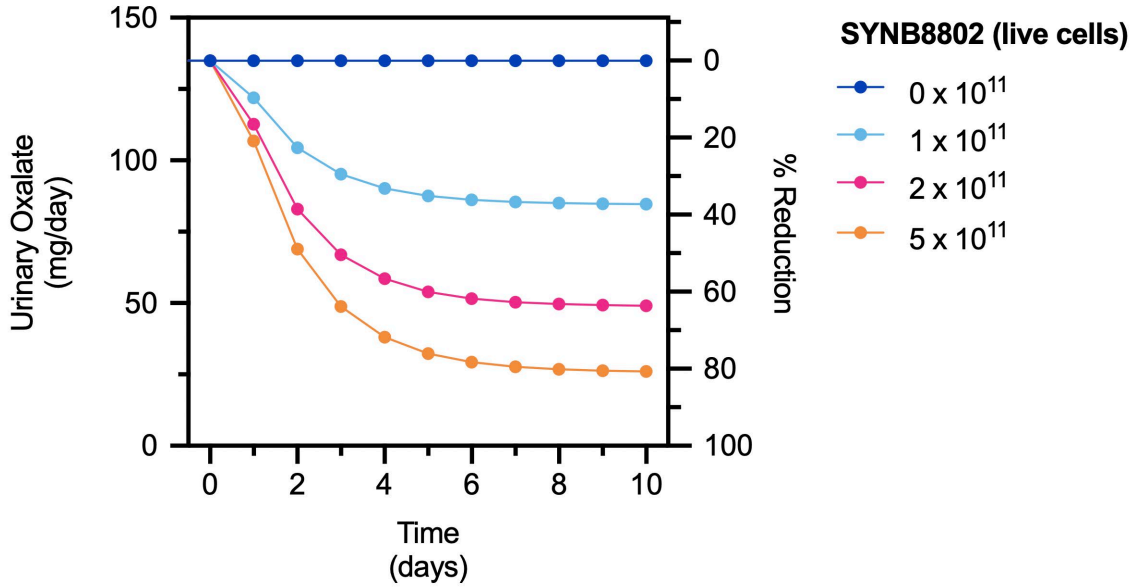

Figure 10: *In silico* simulation (ISS), urinary oxalate percent change from baseline after dosing with SYNB8802. ISS predicts a dose-dependent lowering of urinary oxalate. Data presented as baseline assumption of increased dietary oxalate absorption in HOX patients (4x healthy absorption); bounded region 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 SYNB8802 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 SYNB8802 has the potential to lower urinary oxalate by 20-50% in patients, suggesting that SYNB8802 represents a promising new approach for the treatment of enteric hyperoxaluria.

- SYNB8802 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.

