Oral Beta-Lactamase Therapy to Prevent Antibiotic-Induced Disruption of the Gut Microbiome

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ABSTRACT

Background: Intravenous (IV) beta-lactam antibiotics, excreted via bile into the gastrointestinal (GI) tract, can damage the intestinal microflora and lead to opportunistic *Clostridium difficile* infections (CDI). SYN-004 is a clinical-stage, oral beta-lactamase enzyme therapy intended to preserve the gut microbiome by inactivating certain residual antibiotics in the intestine. SYN-004 completed two Phase 1 clinical studies. Phase 2 clinical studies are in progress.

Methods: SYN-004 was engineered from the *Bacillus licheniformis* penP enzyme (P1A) to improve its degradation of cephalosporins, including ceftriaxone (CRO), while maintaining its ability to degrade penicillins. SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets for oral delivery that release enzyme in the duodenum (at pH >5.5). SYN-004's safety and efficacy were evaluated in dogs and pigs, and human clinical trials are in progress.

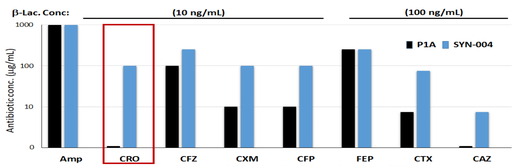
Results: In dogs, SYN-004 degraded IV CRO in the GI tract and did not alter CRO plasma levels. In pigs, SYN-004 protected the microbiome from IV CRO without affecting systemic antibiotic levels. Two Phase 1 clinical trials, single- and multiple-ascending dose studies in healthy adult volunteers, demonstrated SYN-004's safety and tolerability with negligible systemic bioavailability. Two Phase 2a studies are evaluating SYN-004's ability to degrade CRO in chyme collected from ileostomy subjects. A proof-of-concept Phase 2b trial is enrolling patients receiving IV CRO for lower respiratory tract infections to determine if SYN-004 reduces the incidence of CDI, prevents antibiotic-associated diarrhea (AAD), and protects the gut microbiome (ClinicalTrials.gov identifier: NCT02563106).

Conclusions: SYN-004 has the potential to become the first prophylaxis to eliminate many of the risks associated with the use of certain IV beta-lactam antibiotics, including protection from illnesses such as CDI, *Clostridium difficile*-associated diarrhea (CDAD), AAD, and secondary infections with drug-resistant pathogens. The ultimate goal of this antibiotic inactivation strategy is to preserve the symbiotic relationship between the patients and their GI microflora by enabling them to leave the hospital with the diversity of their gut microbiomes intact.

BACKGROUND

IV beta-lactam antibiotics, including cephalosporins, are excreted via the bile into the intestine where they can disrupt the intestinal microbiome and potentially lead to the overgrowth of pathogens like *Clostridium difficile*. SYN-004 is a recombinant beta-lactamase which is delivered orally with the intent of degrading the excess beta-lactam antibiotics in the gut to protect the intestinal microbiome and prevent *C. difficile* infection and antibiotic associated diarrhea.

Antibiotic Hydrolysis



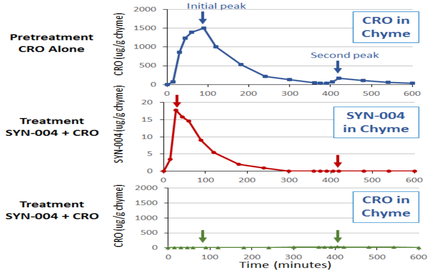
AMP:ampicillin; CRO:ceftriazone; CFZ:cefozolin; CXM:cefuroxime; CFP:cefoperazone; FEP:cefepime; CTX:cefotaxime; CAZ:cefoperazone

SYN-004 was engineered from the penP enzyme, named P1A, the first generation predecessor, by introducing a one amino acid change, D276N. SYN-004 displays a broader antibiotic degradation profile than P1A and efficiently degrades the cephalosporin, ceftriaxone. Use of ceftriaxone is a major risk factor for *C. difficile* infection.

RESULTS

SYN-004 Degraded Ceftriaxone in the GI Tract of Dogs

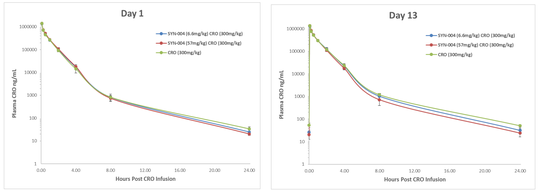
Jejunal-fistulated dogs (n=6) received SYN-004 (0.44 mg/kg; PO) and IV ceftriaxone (CRO, 30 mg/kg).



SYN-004 delivered orally 10 min prior to IV CRO eliminated the initial peak of CRO in the intestine of 4/6 dogs (graph displays data from the 4 dogs), and the second peak of CRO in 6/6 dogs, demonstrating that SYN-004 hydrolyzed the CRO in the intestines of all treated dogs.

SYN-004 Did Not Affect Systemic Ceftriaxone Levels

Two GLP toxicity studies conducted in dogs demonstrated that SYN-004 was well tolerated. In one of the studies, SYN-004 (6.6 mg/kg or 57 mg/kg; PO, TID) was delivered with IV ceftriaxone (300 mg/kg; QD) for 14 days. Serial plasma samples, collected on Days 1 and 13, were analyzed for ceftriaxone pharmacokinetics.

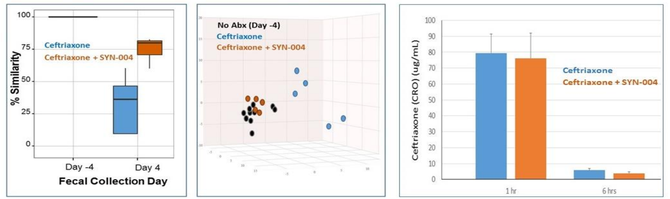


Daily oral dosing with SYN-004 did not have a significant effect on the PK of ceftriaxone. There were no test article-related effects on body weight, food consumption, clinical pathology parameters, organ weights, or test article-related macroscopic or microscopic findings. SYN-004 appears to be well tolerated when co-administered with 300 mg/kg of ceftriaxone. [J. Kokai-Kun et al. (2015). Int. J. Toxicol. pii: 1091581815623236].

SYN-004 Protected the Gut Microbiome in Pigs

Normal piglets (~20 kg, n=5 per cohort) were treated for 7 days with ceftriaxone (50 mg/kg, IV, QD). SYN-004 (75 mg, PO, QID) was delivered for 9 days to separate cohorts that received ceftriaxone starting the day before antibiotic treatment. Feces were collected on Days -4, 4, and 8. Fecal DNA was subjected to whole genome shotgun sequence analyses. Fecal DNA sequence data from Day -4 and Day 4 were compared from cohorts that recieved ceftriaxone alone or ceftriaxone + SYN-004. Ceftriaxone levels in serum collected on Day 2 was quantified using a validated HPLC assay.

Strain Relative Abundance | **Principal Components Analysis** | **Ceftriaxone Serum Levels**



Ceftriaxone-mediated microbiome changes were reduced in the presence of SYN-004 and ceftriaxone serum levels were unaffected by SYN-004. 1) Ceftriaxone caused a loss of species diversity by Day 4, that was reduced in the presence of SYN-004. 2) Pretreatment and ceftriaxone + SYN-004 Day 4 samples clustered together while the ceftriaxone alone cohort was distinct. 3) Ceftriaxone serum levels were similar in the presence or absence of SYN-004.

SYN-004 Phase 1 Clinical Trials Summary

Two Phase 1 studies were completed.

Study 1: Single-ascending sequential dose safety, tolerability, and PK study (40 subjects). This study was conducted as a double-blind, randomized, placebo-controlled (6 active and 2 placebo/cohort) study. SYN-004 (75, 150, 300, 600, or 750 mg capsule) was given as a single oral administration, to assess the safety, tolerability, and PK profile of SYN-004.

Results: There were no serious adverse events (SAEs), no discontinuations due to an AE, and no deaths. All treatment-emergent adverse events (TEAEs) were reported as Grade 1 intensity (does not interfere with normal activities) and resolved without intervention. **SYN-004 taken orally was not systemically bioavailable** and no anti-SYN-004 antibodies were detected in any subject.

Study 2: Multiple-ascending sequential dose safety, tolerability, and PK study (24 subjects). This study was conducted as a double-blind, randomized, placebo-controlled (6 active and 2 placebo/cohort) study. SYN-004 was delivered orally QID for 7 days at 75, 150, or 300 mg.

Results: Six subjects reported 7 TEAEs, all Grade 1 intensity that resolved without intervention. **SYN-004 was not systemically bioavailable even with dosing four times a day for 7 consecutive days** and no SYN-004 antibodies were detected in any subject.

SYN-004 Phase 2 Clinical Trials

Two Phase 2a and a Phase 2b studies are in progress.

Phase 2a: SYN-004 mechanism of action studies are being conducted in subjects with functioning ileostomies to allow sampling of intestinal chyme. In study 1, subjects receive ceftriaxone (CRO, 1 g) alone or in combination with SYN-004. In study 2, CRO plus SYN-004 is delivered in the presence or absence of a proton pump inhibitor to determine the effect of pH change on SYN-004 function. In both studies, serial plasma and chyme samples are analyzed for the concentrations of CRO and SYN-004. Study 1 is completed and study 2 is in progress.

Phase 2b: A SYN-004 proof-of-concept study is being conducted in ~372 patients being treated with CRO for a lower respiratory tract infection. Study is conducted as double-blind, placebo-controlled in patients randomized 1:1 to receive 150 mg of oral SYN-004 or placebo QID during CRO treatment and continuing for 72 hrs after CRO treatment. Patients are monitored for diarrhea over the next 6 weeks. The primary endpoints of the study are prevention of *Clostridium difficile* infection (CDI) and *Clostridium difficile*-associated diarrhea (CDAD) with secondary endpoints of prevention of antibiotic-associated diarrhea (AAD) and protection of the gut microbiome.

CONCLUSIONS

- SYN-004 efficiently degraded penicillins and a panel of cephalosporins, including ceftriaxone
- In dogs, oral delivery of SYN-004 pellets resulted in efficient degradation of intestinal ceftriaxone
- In dogs, SYN-004 did not affect systemic ceftriaxone levels
- SYN-004 was well tolerated in dogs at doses up to 57 mg/kg/day
- SYN-004 protected the intestinal microflora from dysbiosis caused by ceftriaxone in pigs
- In Phase 1 clinical studies, SYN-004 was well tolerated at a single dose of up to 750 mg and multiple doses of 300 mg QID for 7 days
- SYN-004 is not systemically bioavailable when administered orally
- Phase 2 clinical studies are in progress

SYN-004 has the potential to become the first prophylaxis designed to protect the microbiome from certain IV beta-lactam antibiotics and prevent CDI, CDAD, and AAD

DISCLOSURES

All authors except MS are employees of Synthetic Biologics, Inc. MS is a paid consultant for Synthetic Biologics, Inc.