Development of Clinical Stage Oral β-Lactamase Therapies Designed for the Prevention of Antibiotic-Induced Disruption of the Intestinal Microbiome

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Abstract

OBJECTIVE: IV β-lactam antibiotics excreted into the gastrointestinal (GI) tract, such as ceftriaxone (CRO), can damage the microbiome and lead to opportunistic *Clostridium difficile* infections (CDI). SYN-004 is a potent oral β-lactamase enzyme designed to degrade certain residual IV β-lactam antibiotics in the GI tract while not affecting the systemic bioavailability of the antibiotic.
DESIGN AND METHODS: SYN-004 was engineered from the *Bacillus licheniformis* enzyme. Nonclinical *in vitro* and *in vivo* data demonstrated that SYN-004 degraded β-lactam antibiotics such as penicillins and certain cephalosporins in the GI tract. Clinical evaluation of SYN-004 with single- and multiple-ascending PK, safety and tolerability studies in humans were conducted. In Q1 2015, we started a Phase 2a study in ileostomy subjects, and are currently assessing intestinal CRO degradation by analyzing CRO concentrations in chyme.
RESULTS AND CONCLUSION:
SYN-004 was demonstrated to be safe in nonclinical studies in dogs and did not affect the systemic PK of IV CRO. SYN-004 was studied in two Phase 1 clinical trials (single-ascending dose and multiple-ascending dose) which demonstrated that oral SYN-004 was well tolerated, with negligible systemic bioavailability in the dose range tested.
Synthetic Biologics is continuing the clinical development of SYN-004 with the initiation of a Phase 2b clinical trial to evaluate the ability of SYN-004 to degrade residual IV antibiotic in the GI tract in hospitalized patients receiving IV CRO. The aim is prevention of undesirable effects associated with IV β-lactam antibiotics including CDI, antibiotic-associated diarrhea, and intestinal colonization with resistant organisms. The study design will be presented and discussed.

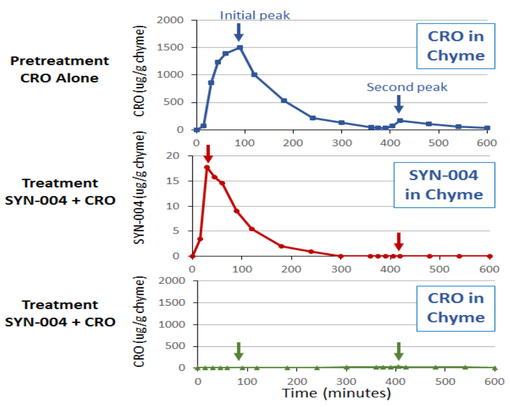
Background

The use of intravenous β-lactam antibiotics, including cephalosporins, are a risk factor for the development of GI infections like *Clostridium difficile*. These antibiotics are excreted via the bile into the intestine where they can disrupt the intestinal microbiome and potentially lead to the growth of opportunistic pathogens like *C. difficile*. SYN-004 is a novel recombinant β-lactamase which is delivered orally with the intent of degrading the excess β-lactam antibiotics excreted into the intestine thus protecting the gut microbiome. The intended indication for SYN-004 is prevention of *C. difficile* infection (CDI) and antibiotic associated diarrhea (AAD). The use of SYN-004 may also have the added benefit of reducing the development of antibiotic resistance in the gut microflora. **Adding SYN-004 to any treatment with IV β-lactam antibiotics would represent a paradigm shift from the current paradigm where an antibiotic treats the primary infection but increases the risk for development of opportunistic infections like CDI, to a paradigm where highly-effective IV β-lactam antibiotics can be administered without this increased risk.**

Non-clinical Studies

SYN-004 Degraded Ceftriaxone in the GI Tract of Dogs

SYN-004 was tested in the intestinal tract of jejunal-fistulated dogs (n=6) following oral delivery of SYN-004 enteric-coated pellets, designed to release active drug at pH >5.5 (0.44 mg/kg), and IV ceftriaxone (30 mg/kg).



This dog study revealed that ceftriaxone (CRO) was excreted at high levels into the intestine following IV delivery (mean C_{max} of 1500 μg/g of jejunal chyme), and a second CRO peak (mean 167 μg/g) was observed six hours later, after an additional feeding. When SYN-004 was delivered orally 10 min prior to IV CRO, SYN-004 was present, remained functional, and hydrolyzed the CRO. The CRO concentration stayed low (< 5 μg/g chyme) for ten hours.

SYN-004 was Safe in Two GLP Toxicology Studies in Beagle Dogs

Study 1: Twenty Eight Day Oral SYN-004 Dosing Study

- Administered for 28 consecutive days, 3 times daily, by oral capsule
- Placebo, 6.6, 18, and 57 mg/kg/day (2.2, 6.0, and 19 mg/kg/dose)
- Six or ten dogs/group, 5 males and 5 females
- Evaluate the toxicity potential and toxicokinetic profile of SYN-004
- Plasma samples for toxicokinetic evaluation were collected on study days 0 and 27

All animals survived to the scheduled necropsies; there were no test article-related clinical findings observed at any dosage level. Test article administration did not result in test article-related alterations in ophthalmic or ECG parameters, gross findings, organ weights, clinical pathology parameters, or histologic findings. SYN-004 was well tolerated with a NOAEL of 57mg/kg/day.

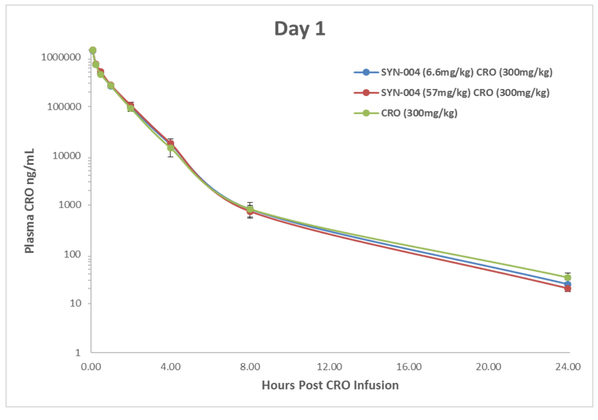
Oral (capsule) administration of SYN-004 three times daily at 2.2, 6.0, and 19mg/kg/dose (6.6, 18, and 57 mg/kg/day) to male and female dogs resulted in only sporadic systemic exposure to SYN-004 in most animals, precluding assessment of toxicokinetic parameters. Where measurable, most plasma SYN-004 concentrations were within approximately 1- to 4-fold of the LLOQ (0.80 ng/mL) of the assay.

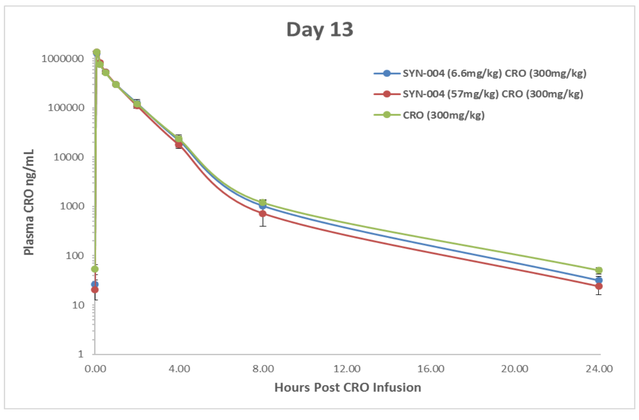
Study 2: Fouteen Day SYN-004 Dosing Study with IV Ceftriaxone Administration

- SYN-004 administered for 14 consecutive days , 3 times daily, by oral capsule
- Ceftriaxone (CRO) administered once per day by slow bolus IV, 15min post SYN-004
- Saline control, 300 mg/kg CRO, CRO + 6.6 mg/kg SYN-004, CRO + 57 mg/kg SYN-004
- Six dogs/group, 3 males and 3 females
- Evaluate the toxicity potential SYN-004 administered with CRO
- Plasma samples for toxicokinetic evaluation of CRO were collected on study days 1 and 13

All animals survived to the scheduled necropsy. There were no test article-related effects on body weight, food consumption, clinical pathology parameters, or organ weights. There were no test article-related macroscopic or microscopic findings. SYN-004 (at 57mg/kg/day) appears to be safe and well tolerated when co-administered with 300 mg/kg of ceftriaxone.

Analysis of the pharmacokinetics of ceftriaxone, as presented in the figures below, indicated that daily oral dosing with SYN-004 did not have a significant effect on the plasma PK of ceftriaxone.





Clinical Studies

Completed Phase 1 Studies in Healthy Adults

Phase 1: Single Ascending Sequential Dose Safety, Tolerability, and PK Study

- Double blind, randomized, placebo controlled (6 active and 2 placebo/cohort) - 40 subjects
- Single oral SYN-004 administration by capsule at 75 mg, 150 mg, 300 mg, 600 mg and 750 mg
- Assess the safety, tolerability and PK profile of SYN-004 in healthy adults.
- Blood samples for PK evaluation and determination of anti-SYN-004 Abs were collected

Of 40 subjects, 11 (27.5%) had 12 TEAEs. There were no SAEs, no discontinuations due to an AE, and no deaths. Eight of 30 (26.7%) SYN-004 subjects reported 9 TEAEs (flatulence 5, headache 3, and somnolence 1) and 3 of 10 (30%) PBO subjects reported 3 TEAEs (headache 2 and neck pain 1). All TEAEs were reported as Grade 1 in intensity (does not interfere with normal activities) and resolved without intervention.

SYN-004 taken orally was not systemically bioavailable. In the highest dose group (750 mg), all 6 subjects had at least one SYN-004 plasma concentration above the LLOQ of 0.8 ng/ml (C_{max} 1.4ng/ml, T_{max} 1-4 hours post dose), but none of the lower dose groups had consistent PK parameters. No anti-SYN-004 antibodies were detected in any subject.

Phase 1: Multiple Ascending Sequential Dose Safety, Tolerability, and PK Study

- Double blind, randomized, placebo controlled (6 active and 2 placebo per cohort) - 24 subjects
- Multiple oral SYN-004 doses by capsule (4 times per day for 7 days) at 75 mg, 150 mg and 300 mg
- Assess the safety, tolerability and PK profile of SYN-004
- Blood samples for PK evaluation and anti-SYN-004 Abs were collected

Of the 24 subjects randomized (18 received SYN-004; 6 received placebo), 6 subjects (25%) had a total of 7 TEAEs; all occurred in subjects receiving SYN-004. The 7 TEAEs included 2 flatulence, 2 headache, 1 pollakiura, 1 pyuria, and 1 hematuria. All TEAEs were reported as Grade 1 in intensity (does not interfere with normal activities) and resolved without intervention. The TEAEs of pyruia and of hematuria were in different patients, were both observed on microscopy only, and all TEAEs resolved without intervention.

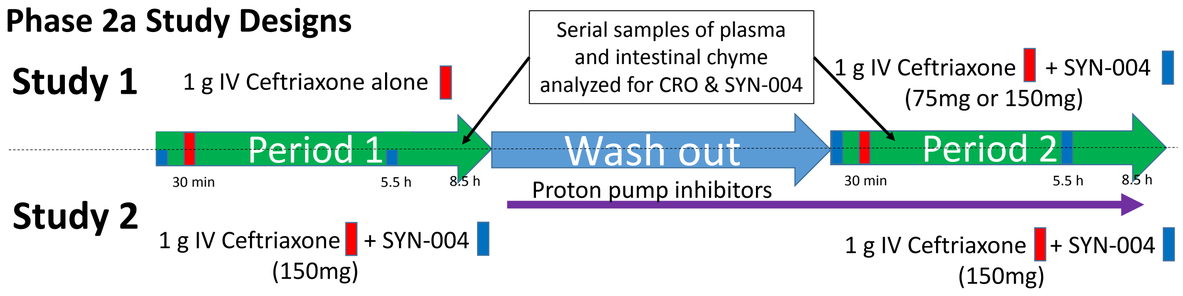
SYN-004 was not systemically bioavailable even with dosing four times a day for 7 consecutive days. Any measurable peak plasma concentrations tended to occur in the 2-4 hour post-dose and the peak concentrations were at or near the assay LLOQ (0.8 ng/ml). Multiple dosing did not result in any prolongation of exposure to SYN-004 levels. No anti-SYN-004 antibodies were detected in any subjects.

Ongoing Phase 2 Clinical Studies

Phase 2a Studies in Subjects with Functioning Ileostomies

Two phase 2a studies are being conducted in subjects with ileostomies to allow sampling of their intestinal chyme which provides a unique *in vivo* approach to support the mechanism of action of SYN-004. In the first study, subjects are being administered 1 g of IV ceftriaxone (CRO) alone or in combination with two dose strengths of oral SYN-004. The oral SYN-004 formulation is designed to release active drug in the intestine at pH > 5.5. Serial plasma and intestinal chyme samples are then analyzed for their concentrations of ceftriaxone and SYN-004. In the second study, ceftriaxone plus SYN-004 is administered in the presence and absence of a proton pump inhibitor to determine the effect of pH change on SYN-004 function.

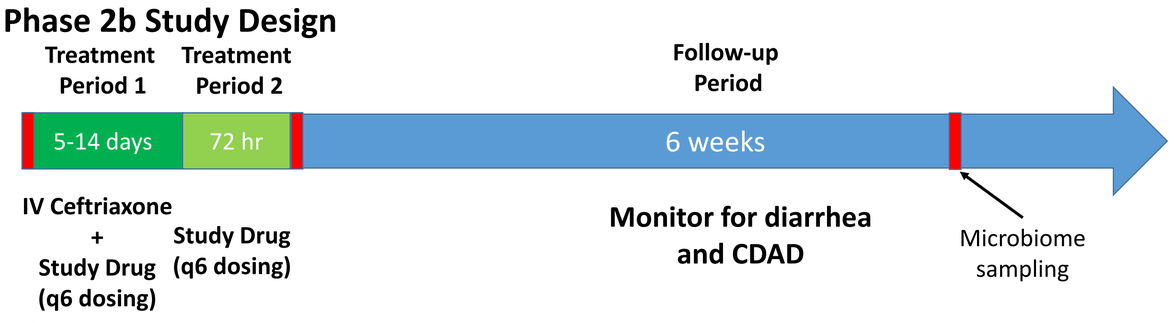
Phase 2a Study Designs



Phase 2b Study in Patients being Treated with Ceftriaxone for LRTI

A phase 2b clinical study has been initiated in ~372 patients who are being treated with IV ceftriaxone for at least 5 days for a lower respiratory tract infection (LRTI). In this double blind, placebo controlled study patients are randomized 1:1 to receive either 150 mg of oral SYN-004 or placebo four times per day during ceftriaxone treatment and for 72 hours after and then monitored for diarrhea over the next 6 weeks. The primary endpoint of the study is prevention of *Clostridium difficile*-associated disease with secondary endpoints of prevention of antibiotic-associated diarrhea and protection of the gut microbiome.

Phase 2b Study Design



Conclusions

- **In non-clinical studies in dogs:**
  - **Oral delivery of SYN-004 efficiently degrades ceftriaxone in the intestine**
  - **SYN-004 is safe and well tolerated at doses up to 57 mg/kg/day for 28, with minimal systemic absobtion**
  - **Co-administration of SYN-004 with IV ceftriaxone for 14 days is well tolerated and appears not to change the plasma PK of the ceftriaxone**
- **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 q.i.d. for 7 days with only minor treatment emergent adverse events**
  - **SYN-004 was neither systemically bioavailable nor immunogenic at the doses tested**
- **Two Phase 2a clinical studies of SYN-004 are ongoing to examine the *in vivo* activity of SYN-004 in subjects with functioning ileostomies**
- **A Phase 2b clinical study with prevention of *C. difficile*-associated disease and antibiotic-associated diarrhea as end points has been initiated**
- **SYN-004 is expected to protect the gut microbiome from the adverse effects of β-lactam antibiotics, reduce the incidence of opportunistic GI infections like CDI and reduce the pressure for the development of resistance to these antibiotics**