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Clinical Evaluation of SYN-004, an Oral Beta-Lactamase Therapy for the Prevention of Antibiotic-Induced Disruption of Intestinal Microflora

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Abstract

Antibiotics that are excreted into the intestine, such as ceftriaxone (CRO), can damage the microflora and lead to serious illnesses such as *Clostridium difficile* infection. SYN-004 is a clinical stage oral β-lactamase therapy for use with IV β-lactam antibiotics to preserve the microbiome by degrading residual antibiotics in the intestine. SYN-004 was engineered from the *Bacillus licheniformis* PenP enzyme to expand the hydrolysis of β-lactams to cephalosporins, including CRO, while maintaining its anti-penicillin activity.

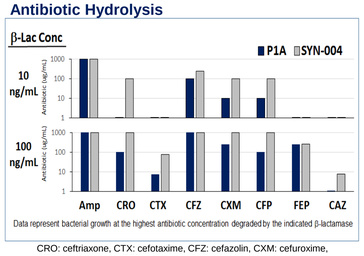
SYN-004, manufactured in *E. coli*, was formulated into enteric-coated pellets. In vitro, the pellets remained intact at low pH (0.1 N HCl) while complete dissolution occurred at pH >5.5. In human chyme, SYN-004 enzyme activity was maintained for at least 6 hrs, demonstrating enzyme stability in human intestinal contents. Efficacy studies were performed using jejunal-fistulated dogs (n=6). Following IV CRO (30 mg/kg), CRO was detected at high levels in the intestine (mean Cmax of 1500 ug/g at 90 min), and a second CRO peak (mean 167 ug/g) was observed six hours later, after feeding. When SYN-004 was delivered ten minutes prior to CRO, intestinal CRO levels remained low (<5 ug/g chyme) in 4/6 dogs. The second CRO peak was not detected in any SYN-004-treated animal demonstrating that SYN-004 was present, remained functional, and hydrolyzed the CRO in the intestines of all dogs. In a GLP toxicology study, dogs received SYN-004 capsules orally 3 times a day for 28 days at 0, 6.6, 18, and 57 mg/kg/day. Dosing was well tolerated with no indication of effects on any organ system and no histopathological findings. The NOAEL was 57 mg/kg/day. A second GLP dog study was conducted in which SYN-004 and CRO were administered together for 14 days.

Clinical evaluation of SYN-004 was initiated in late 2014 with single ascending and multiple ascending dose pharmacokinetic, safety and tolerability studies in humans. A proof-of-mechanism study in ileostomy subjects was initiated in 2Q 2015 to assess intestinal CRO degradation. The clinical program for SYN-004 will investigate the prevention of the undesirable effects associated with IV β-lactam antibiotics including antibiotic-associated diarrhea, *Clostridium difficile* infection, and intestinal colonization with resistant organisms and related infections. Clinical data are presented.

Background

IV β-lactam antibiotics, including cephalosporins, are excreted via the bile duct into the intestine where they can disrupt the intestinal microbiome and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. SYN-004 is a recombinant β-lactamase which is delivered orally with the intent of degrading the excess β-lactam antibiotics in the gut thus protecting the intestinal microbiome. The intended indication is prevention of *C. difficile* infection (CDI) and antibiotic associated diarrhea (AAD).

Antibiotic Hydrolysis



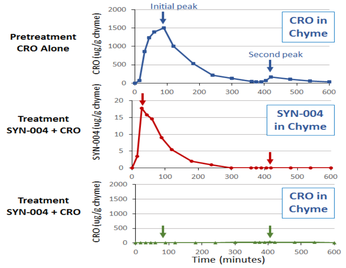
CRO: ceftriaxone, CTX: cefotaxime, CFZ: cefazolin, CXM: cefuroxime, CFP: cefoperazone, FEP: cefepime, CAZ: ceftazidime

SYN-004 was engineered from a predecessor, P1A, by introducing a single amino acid change, D276N. SYN-004 displays a broader antibiotic degradation profile than P1A and efficiently degrades cephalosporins, including ceftriaxone. Use of ceftriaxone is a major risk factor for CDI.

Results

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 (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 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)
- 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 and serum samples for possible anti-drug antibody determination were collected

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 safe and well tolerated with a NOAEL of 57mg/kg/day.

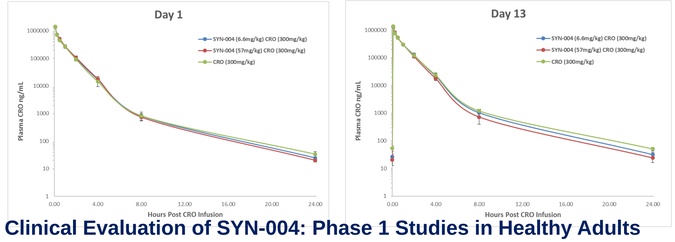
Oral (capsule) administration of SYN-004 three times daily at 2.2, 6.0, and 19mg/kg/dose (6.6, 18, and 57 mg/kg/day) to male and female Beagle dogs resulted in only sporadic exposure to SYN-004 in most animals, precluding assessment of toxicokinetic parameters. Where measurable, most plasma SYN-004 concentrations were within approximately 1- to 4-fold of the LLOQ (0.80 ng/mL) of the assay. No anti-SYN-004 antibodies were detected in the dogs.

Study 2: Fourteen 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, 300mg/kg CRO, CRO + 6.6mg/kg SYN-004, CRO + 57mg/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 300mg/kg of ceftriaxone.

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



Clinical Evaluation of SYN-004: Phase 1 Studies in Healthy Adults

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

- Double blind, randomized, placebo controlled (6 active and 2 placebo/cohort)-40 subjects
- Single oral SYN-004 administration by capsule at 75mg, 150mg, 300mg, 600mg and 750mg
- 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 0.8ng/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.

Phase 1: Multiple Ascending Sequential Dose Safety, Tolerance, and PK Study in Healthy Adults

- Double blind, randomized, placebo controlled (6 active and 2 placebo per cohort)-24 subjects
- Multiple oral SYN-004 doses by capsule (4 per day for 7 days) at 75mg, 150mg and 300mg
- 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 (0.8ng/ml). Multiple dosing did not result in any prolongation of exposure to SYN-004 levels. No anti-SYN-004 antibodies were detected in the subjects.

Conclusions

- In dogs, oral delivery of SYN-004 pellets resulted in efficient degradation of intestinal ceftriaxone
- SYN-004 was safe and well tolerated at doses up to 57mg/kg/day for 28 days in dogs
- Minimal systemic SYN-004 was detected in dogs
- Co-administration of SYN-004 with IV ceftriaxone for 14 days was safe and well tolerated in dogs and appeared not to change the plasma PK of ceftriaxone
- In Phase 1 clinical studies:
  - SYN-004 was safe and well tolerated at a single dose of up to 750mg and multiple doses of 300mg q.i.d. for 7 days
  - There were no identified safety or tolerability concerns attributable to SYN-004
  - SYN-004 is neither systemically bioavailable nor immunogenic at the clinical doses tested
- Two Phase 2a clinical studies of SYN-004 were initiated in 2Q 2015 and a Phase 2b clinical study is expected to begin in 2H 2015