SYN-004, a Clinical-Stage, Orally Delivered β-Lactamase Therapy Protects the Gut Microbiome from IV Antibiotics

S. Connelly[1] J. A. Bristol[1], S. Hubert[1], N. A. Hasan[2], P. Subramanian[2], C. Furlan Freguia[1], J. Sliman[1], M. Kaleko[1]
[1]Synthetic Biologics, Inc., Rockville, MD, [2]CosmosID, Inc., Rockville, MD, USA

Abstract

Background: Disruption of the intestinal microbiome is a major, unintended consequence of antibiotic exposure that can lead to overgrowth of pathogenic organisms such as *Clostridium difficile*. SYN-004 is an orally-delivered beta-lactamase for use with intravenous (IV) penicillins and cephalosporins to degrade residual antibiotics within the gastrointestinal (GI) tract and thereby protect the microbiome. A Phase 2b study is in progress to assess the ability of SYN-004 to protect the microbiome and prevent antibiotic-associated diarrhea (AAD) and *C. difficile* infection (CDI) in patients receiving IV ceftriaxone (CRO). As SYN-004 has the potential to degrade orally-administered antibiotics in the GI tract prior to their absorption, a proof-of-concept study to evauate SYN-004 efficacy with oral amoxicillin was performed in normal pigs.

Materials/Methods: SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets that release enzyme in the duodenum (at pH >5.5). To verify that SYN-004 effectively protected the microbiomes of pigs with IV antibiotics, normal piglets (~20 kg, n=5 per cohort) were treated with IV CRO (50 mg/kg QD for 7 days) with a separate cohort receiving CRO+SYN-004 (75 mg QID). In parallel cohorts, animals received oral amoxicillin (20 mg/kg BID for 7 days) or amoxicillin+SYN-004. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

Results: For CRO, serum levels were similar in the antibiotic-alone and antibiotic+SYN-004 cohorts indicating that SYN-004 did not alter systemic antibiotic levels. Microbiome analyses demonstrated that SYN-004 prevented CRO-mediated dysbiosis. While the preliminary analyses using an HPLC-based amoxicillin detection assay demonstrated that SYN-004 did not affect amoxicillin serum levels, repeat analyses using an LC-MS-based assay revealed that amoxicillin was undetectable in the pig serum in the presence of SYN-004. Microbiome analyses demonstrated that SYN-004 prevented amoxicillin-mediated loss of species diversity and protected the microbiome, however, it is likely that SYN-004 degraded the amoxicillin prior to its systemic absorption. A Dirichlet-Multinomial model likelihood ratio test compared the microbiomes from pretreatment day -4 with post-treatment day 8. For the CRO-alone cohort, the p value was 7.5×10^{-25} indicating that the pre- and post-antibiotic fecal samples were significantly different. In contrast, for the CRO+SYN-004 cohort, the p value was 0.38, indicating that the two fecal sample populations were not significantly different.

Conclusions: SYN-004 protected the gut microflora in pigs from damage caused by IV CRO further supporting its clinical potential in humans. Notably, SYN-004 protected the microbiome from amoxicillin-induced dysbiosis, however SYN-004 interferred with amoxicillin systemic absorption. Novel, modified-release formulations of SYN-004, designed to release into the GI tract at a point distal to the absorption of amoxicillin but still proximal enough to degrade residual antibiotic, are being tested. Therefore, SYN-004 has the potential to become the first therapy designed to protect the microbiome from certain antibiotics and prevent AAD and CDI. The utility of SYN-004 to include oral as well as IV antibiotics is being explored.

Methods

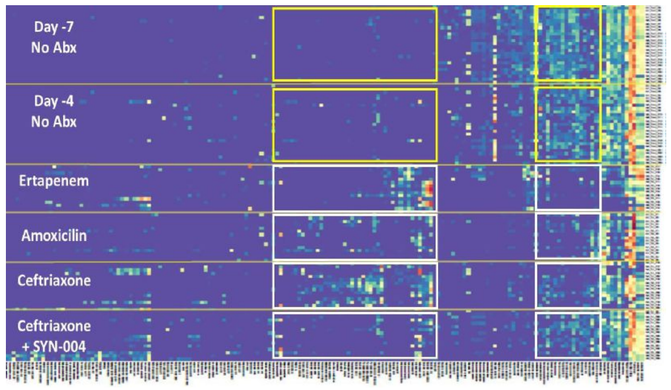
SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets that release enzyme in the duodenum (at pH >5.5). A piglet model of antibiotic-induced dysbiosis was established using three β-lactam antibiotics, ceftriaxone, a cephalosporin, ertapenem, a carbapenem, and amoxicillin, a penicillin. Normal piglets (~20 kg, n=5 per cohort) were treated for 7 days with ceftriaxone (50 mg/kg, IV, QD), ertapenem (30 mg/kg, IV, QD), or amoxicillin (20 mg/kg, PO, BID). SYN-004 (75 mg, PO, QID) was delivered for 9 days to separate cohorts that received ceftriaxone or amoxicillin starting the day before antibiotic treatment. Serum was collected on Day 2 of antibiotic treatment, and feces were collected on Days -7, -4, 4, and 8. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

Three additional formulations of the β-lactamase, designed to release the enzyme distal to the site of oral amoxicillin systemic absorption, were evaluted in pigs.

Results

Antibiotics Rapidly Disrupt the Gut Microbiome

Heat map analyses of the fecal microbial community based on their relative abundance. Each square represents a bacterial species present in individual animal microbiomes. The species are indicated horizontally, and the fecal collection day and animal are displayed vertically. The yellow and white boxes display changes in species diversity caused by antibiotic treatment.



Comparison of the bacterial species present in the microbiomes of pigs that received the β-lactam antibiotics (white boxes) to pretreatment (yellow boxes) reveals that antibiotic treatment caused the depletion of some species and the overgrowth of others. The ceftriaxone-mediated microbiome changes were reduced in the presence of SYN-004.

SYN-004 Protects the Microbiome from IV Ceftriaxone

The microbiome populations prior to antibiotic exposure (Day -4) and after antibiotic administration (Day 8) were compared using a Dirichlet-Multinomial model likelihood ratio test [1].

Likelihood Ratio Test (Day -4 vs Day 8)

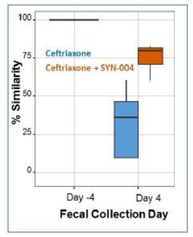
Treatment Group	Chi squared	P value
Ceftriaxone	469.93	7.5×10^{-25}
Ceftriaxone+SYN-004	79.22	0.38
Ertapenem	720.56	$<1.0 \times 10^{-25}$
Amoxicillin	102.64	2.8×10^{-4}

Each antibiotic caused microbiome dysbiosis as the microbiomes prior to antibiotic exposure were significantly different from the microbiomes after 8 days of antibiotic treatment. In contrast, SYN-004 prevented ceftriaxone-mediated dysbiosis, as the microbiomes before and after ceftriaxone exposure in the presence of SYN-004 were not significantly different.

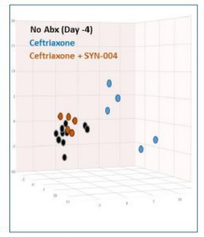
SYN-004 Protects the Microbiome from IV Ceftriaxone

Fecal DNA sequence data from pretreatment Day -4 samples were compared to Day 4 samples from cohorts that received ceftriaxone alone or ceftriaxone + SYN-004. Analyses included strain relative abundance using % similarity, principal components analysis, and changes in relative abundance.

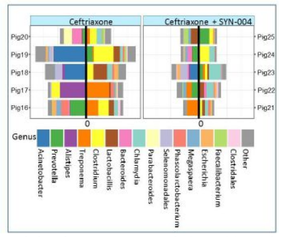
Strain Relative Abundance



Principal Components Analysis

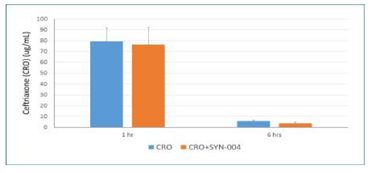


Changes in Relative Abundance



Ceftriaxone-mediated microbiome changes were reduced in the presence of 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) Fewer changes in species abundance were observed in the presence of SYN-004.

SYN-004 Does Not Affect Ceftriaxone Serum Levels



Serum was collected on Day 2 of antibiotic delivery. Ceftriaxone levels were assessed using a validated HPLC assay. Ceftriaxone levels were not significantly different in the presence or absence of SYN-004.

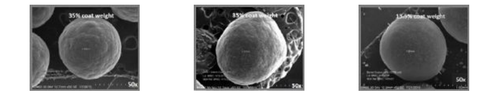
Extension of the SYN-004 Strategy to Use with Oral Amoxicillin

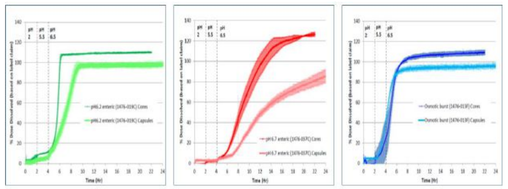
- Three new enteric-coated pellet formulations were designed to release β-lactamase in the lower GI tract at a point distal to amoxicillin systemic absorption
- These **M**odified-**R**elease **F**ormulations, targeting the ileocecal junction, are MRF6.2, MRF6.7 (pH-based) and MRF3 (timed release)
- Preliminary analyses in pigs demonstrated that SYN-004 and the 3 new MRFs all interferred with amoxicillin absorption into the blood
- Failure may have been caused by:
  - Damage to the pellets during transit through the stomach
  - Manufacturing flaws in the coatings of a small fraction of pellets
  - Retention of some pellets in the proximal intestinal tract
- New pellets are currently being generated with coatings designed to eliminate any early leakage of β-lactamase in the proximal small intestine. Pig studies are pending.

Modified-Release Formulations

The new formulations were designed to release distal to the site of oral amoxicillin systemic absorption.

MRF6.2 | **MRF6.7** | **MRF3**





In vitro dissolution demonstrates that each formulation was protected from acid and displayed the expected release profile, based on pH or time. Additional modified-release formulations are being evaluated.

Conclusions

- SYN-004 protected the gut microflora in pigs from dysbiosis caused by IV ceftriaxone treatment
- SYN-004 did not affect blood levels of ceftriaxone in pigs
- For use with oral amoxicillin, 3 new enteric-coated pellets, designed to delay β-lactamase release, were evaluated in pigs
- All 3 pellet formulations interfered with amoxicillin systemic absorption
- Pellets with more robust coatings are being generated

SYN-004 has the potential to become the first therapy designed to protect the microbiome from certain antibiotics and prevent AAD and CDI

References

1. La Rosa, et al. (2012). Hypothesis testing and power calculations for taxonomic-based human microbiome data. PLoS ONE, 7, e52087.