Clinical-Stage, Oral β-Lactamase Enzyme to Prevent *Clostridium difficile* Infection Triggered by Antibiotic-Mediated Gut Microbiome Disruption

Sheila Connelly[1], J. Andrew Bristol[1], Steven Hubert[1], Christian Furlan-Freguia[1], Poorani Subramanian[2], Nur Hasan[2], Joseph Sliman[1], Michael Kaleko[1]

[1]Synthetic Biologics, Inc., Rockville, MD, [2]CosmosID, Inc., Rockville, MD

sconnelly@syntheticbiologics.com

ABSTRACT

Background: Antibiotic-mediated disruption of the gut microbiome can lead to serious infections such as *Clostridium difficile* (CDI). SYN-004 (ribaxamase), previously called SYN-004, in Phase 2b clinical testing, is a β-lactamase enzyme for oral use with certain IV β-lactam antibiotics intended to preserve the gut microbiome by inactivating antibiotics in the GI tract. The β-lactamase strategy for microbiome protection from both IV and orally-delivered antibiotics was explored using pig models of antibiotic-mediated gut dysbiosis.

Methods: The β-lactamase was produced in *E. coli* and the clinical formulation, ribaxamase, was manufactured as enteric-coated pellets for duodenal release. Pig models (20 kg, n=5 per cohort) of ceftriaxone- (CRO) and amoxicillin- (AMX) +/- ribaxamase mediated gut dysbiosis were established. Fecal DNA whole genome shotgun sequence analyses assessed microbiome preservation and systemic antibiotic absorption was quantified by HPLC or LC/MS/MS. New formulations of the β-lactamase, engineered to be released in the GI tract at a point distal to oral antibiotic absorption but proximal enough to protect the microbiome, were tested *in vitro.*

Results: In pigs, ribaxamase was shown to protect the gut microbiomes from IV CRO and oral AMX. CRO serum levels were unaffected by ribaxamase. In contrast, no systemic AMX was detected in the presence of ribaxamase, suggesting that the β-lactamase degraded AMX prior to its absorption. Therefore, pH-dependent release formulations, designed to release enzyme more distally in the GI tract, were produced and tested *in vitro*. Dissolution analyses demonstrated no leakage at pHs below the target and the expected release profiles. The most promising formulations are being scaled up for evaluation with oral AMX in pigs.

Conclusion: Ribaxamase protected the gut microflora in pigs from damage caused by IV CRO, further supporting the ribaxamase clinical program focused on microbiome protection in humans. New formulations to target enzyme release distal to AMX absorption displayed the expected *in vitro* release profiles and are being tested in pigs. Ribaxamase has the potential to become the first therapy designed to protect the microbiome from certain β-lactam antibiotics and to prevent antibiotic-associated diarrhea and CDI.

BACKGROUND

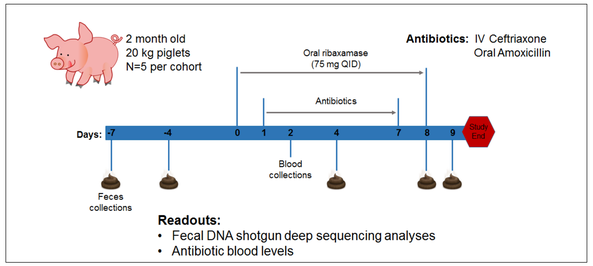
IV β-lactam antibiotics, including cephalosporins, are excreted via the bile into the intestine where they can disrupt the intestinal microflora and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. SYN-004 (ribaxamase), previously called SYN-004 [1,2] is a clinical stage, oral β-lactamase enzyme therapy for use with IV β-lactam antibiotics designed to preserve the gut microflora by degrading residual antibiotics in the intestine [1]. The intended indications are prevention of *C. difficile* infection (CDI) and antibiotic-associated diarrhea (AAD). Phase I clinical studies demonstrated that ribaxamase was well tolerated [2]. Ribaxamase was neither systemically bioavailable nor immunogenic in humans [2]. Phase 2a clinical studies conducted in subjects with functioning ileostomies demonstrated that ribaxamase effectively degraded ceftriaxone in the GI tract without affecting systemic antibiotic levels, and that the use of proton pump inhibitors did not affect ribaxamase efficacy. A Phase 2b clinical study has recently completed enrollment and is examining the ability of ribaxamase to prevent CDI, AAD, and the emergence of antibiotic-resistant organisms in patients hospitalized with a lower respiratory tract infection and receiving IV ceftriaxone.

SYN-004 clinical data were presented in Poster 1357, Friday October 28, 2016

RESULTS

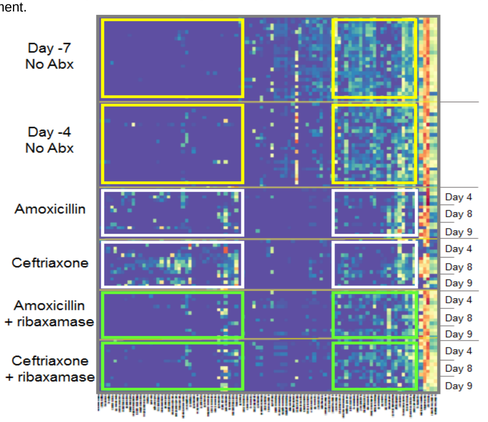
Porcine Model of Antibiotic-Mediated Gut Dysbiosis

A pig model of antibiotic-mediated dysbiosis was established. Normal piglets (20 kg, n=20) were treated with ceftriaxone (50 mg/kg, IV, SID), or oral amoxicillin (20 mg/kg, PO, BID) for 7 consecutive days. Cohorts (n=5) were also treated with SYN-004 (ribaxamase) (75 mg, PO, QID) for 9 days. Blood was collected on Day 2. Feces were collected at two pre-antibiotic time points (Days -7 and -4), during treatment (Day 4), and after antibiotics were stopped (Days 8 and 9). Fecal DNA was subjected to whole genome shotgun sequence analyses.



Antibiotics Rapidly Disrupt the Gut Microbiome

Heat map analyses of the fecal microbial community based on species 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, white, and green boxes highlight changes in species abundance 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. Amoxicillin and ceftriaxone-mediated microbiome changes were reduced in the presence of ribaxamase (green boxes).

Ribaxamase Protects the Microbiome

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 [3].

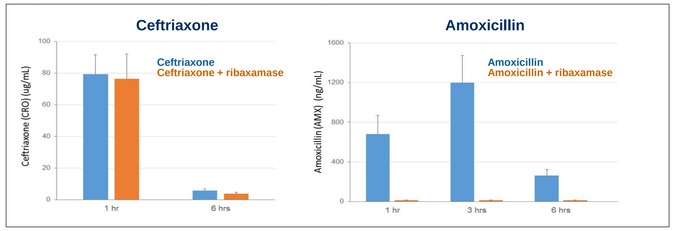
Likelihood Ratio Test (Day -4 vs Day 8)

Treatment Group	Chi squared	P value
Ceftriaxone	469.93	<0.0001
Ceftriaxone + ribaxamase	79.22	0.38
Amoxicillin	720.56	<0.0001
Amoxicillin + ribaxamase	102.64	0.13

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

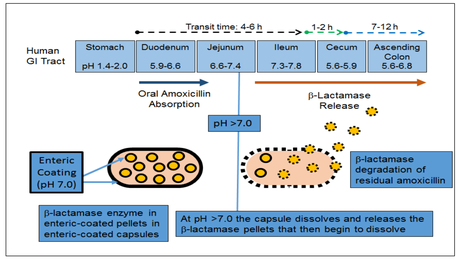
Ribaxamase Does Not Affect Ceftriaxone Serum Levels

Serum was collected on Day 2 of antibiotic delivery. Ceftriaxone levels were assessed using a validated HPLC assay and amoxicillin levels using a LC/MS/MS assay. Data: mean ± SD.



Ceftriaxone levels were not significantly different in the presence or absence of ribaxamase at 1 or 6 hrs (Two-tailed Student's T-test, p=0.76 or p=0.08, respectively). In contrast, amoxicillin was not detected in the serum of any animal that received ribaxamase, suggesting that the amoxicillin was degraded in the GI tract prior to being absorbed.

Distal Release Ribaxamase Formulations



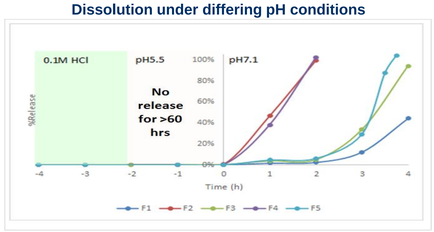
Early release of the β-lactamase enzyme from the clinical ribaxamase formulation resulted in the degradation of the orally-delivered amoxicillin in the GI tract prior to absorption.

New formulations to prevent premature β-lactamase release were produced. The five new formulations are composed of enteric-coated enzyme pellets contained within enteric-coated capsules.

In Vitro Dissolution of New Ribaxamase Formulations

Five distal-release formulations (F1-F5) composed of enteric-coated pellets within enteric-coated capsules were tested *in vitro* for β-lactamase release. The capsules were held in 0.1M HCl for 2 hrs to simulate conditions in the stomach, and for 2->60 hrs at pH 5.5, conditions of the upper small intestine, and at pH 7.1, conditions of the lower small intestine and colon. Enzyme release was monitored by absorption at 300 nm.

Dissolution under differing pH conditions



No enzyme release was detected in 0.1M HCl and at pH 5.5. Notably, capsules remained intact for over 60 hrs at pH 5.5. At pH 7.1 two patterns of release were detected, a fast release that initiated upon exposure to pH 7.1 (F2, F4) and a slower release delayed for ~2 hrs after exposure to pH 7.1 (F1, F3, F5).

Evaluation of these formulations in the porcine gut dysbiosis model with oral amoxicillin demonstrated amoxicillin absorption into the blood with several formulations, however, serum amoxicillin levels were 3-10 fold lower than that detected with the amoxicillin alone control animals. Follow-up dose response studies are evaluating the use of lower doses of the β-lactamase to identify a dose that allows complete absorption of amoxicillin into the blood while maintaining gut microbiome protection.

CONCLUSIONS

- Ribaxamase is intended as an orally-delivered β-lactamase to protect the gut microbiome from IV β-lactam antibiotic-mediated dysbiosis
- Ribaxamase is progressing through Phase 2 clinical trials
- Ribaxamase was shown to protect the gut microbiome from dysbiosis caused by IV ceftriaxone in pigs
- Ribaxamase was released prior to oral amoxicillin absorption in the pig GI tract
- New formulations of ribaxamase are being tested to allow use with oral β-lactam antibiotics

Ribaxamase is a first-in-class oral enzyme designed to degrade certain IV β-lactam antibiotics within the GI tract and maintain the natural balance of the gut microbiome for the prevention of CDI, AAD, and the emergence of antibiotic-resistant organisms

REFERENCES

1. Kaleko, M. *et al.* (2016) Development of SYN-004, an Oral Beta-Lactamase Treatment to Protect the Gut Microbiome from Antibiotic-Mediated Damage and Prevent *Clostridium difficile* infection. *Anaerobe Epub,* http://dx.doi.org/10.1016/j.anaerobe.2016.05.015
2. Roberts, T. *et al. (2016)* Tolerability and Pharmacokinetics of SYN-004, an Orally Administered β-Lactamase for the Prevention of *Clostridium difficile*-Associated Disease and Antibiotic-Associated Diarrhea, in Two Phase 1 Studies. *Clin. Drug Investig.* **36**, 725-734. doi: 10.1007/s40261-016-0420-0
3. La Rosa, P.S. *et al.* (2012) Hypothesis Testing and Power Calculations for Taxonomic-Based Human Microbiome Data. *PLoS ONE* **7,** e52078