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

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**ABSTRACT**

**Background:** Antibiotic-mediated disruption of the gut microbiome can lead to serious clinical outcomes, including *Clostridium difficile* (CD) infection. Synthetic Biologics Inc. has previously called SYN-004, Phase 2a clinical testing, is a β-lactamase enzyme for oral use with co-administration of β-lactam antibiotics to prevent the gut microbiome by tricking antibiotics in the GI tract. The β-lactamase strategy for microbiome protection has been previously tested with ribaxamase. A proof of concept of this strategy 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 oral administration. Pig models (20 kg; n=9 per cohort) of antibiotic dysbiosis included with (test) or without (control) ribaxamase-mediated gut dysbiosis were validated. Fecal DNA whole genome shotgun sequence analyses assessed microbiome preservation and systemic antibiotic absorption was quantified by HPLC or LCMS/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 to protect against β-lactam antibiotics, were also investigated in vitro.

**Results:** In pigs, ribaxamase was shown to protect the gut microbiomes from C. difficile and oral AMX. CRX 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 microbiome in pigs from damage caused by IV CRX, further supporting the ribaxamase strategy for preventing antibiotic-mediated gut 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.

**REFERENCES**

**In Vitro Dissolution of New Ribaxamase Formulations**

Four β-lactam antibiotics (IV, PDL, amoxicillin) were administered either enterically or orally in vivo for β-lactamase release. The capsules were held in 0.1 M HCl for 2 hrs with drug contained in the powder, and then pH was adjusted to 5.5, simulating the upper small intestine, and at pH 7.1, conditions of the lower small intestine and colon. Enzyme release was monitored by absorption at 260 nm.

**DISCUSSION**

- **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 ceftiraxone in pigs.**
- **Ribaxamase is released prior to oral amoxicillin absorption in the pig gut 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 β-lactam antibiotics within the GI tract and maintain the normal balance of the gut microbiome for the prevention of CDI, AAD, and the emergence of antibiotic-resistant organisms.**

**SYN-004 clinical data were presented in Poster 1307, Friday October 28, 2016.**

**Porcine Model of Antibiotic-Mediated Gut Dysbiosis**

- A model of antibiotic-mediated gut dysbiosis was used in AMX-treated pigs (20 kg, n=2) were treated with ceftiofur (50 mg/kg IV, SID), or oral amoxicillin (25 mg/kg PO, BID) for 7 days via intravenous injection. After antibiotic treatment, daily fecal DNA was collected for 5 days.

**Ribaxamase Protects the Microbiome**

The microbiome populations prior to antibiotic exposure (Day-0) and after antibiotic administration (Day-8) were compared using a Drichlet/Multinomial model likelihood ratio test (DMLRT).

**Ribaxamase Does Not Affect Ceftriaxone Serum Levels**

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

**Distal Release Ribaxamase Formulations**

Early release of the β-lactamase enzymes from the clinical distal release formulation in the degradation of the antibiotic-labile microbiome in the GI tract prior to absorption.

**New formulations to prevent systemic antibiotic absorption were produced.** The five new formulations tested were designed to release enteric-coated enzyme pellets intraduodenally or enterocutaneously.