SYN-004 (ribaxamase) Protects the Diversity of the Gut Microbiome in Patients Receiving Intravenous Ceftriaxone Treatment

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ABSTRACT

Background: The balance of the gut microbiome is linked to human health, and disruption of this balance is associated with many diseases from secondary infections like *Clostridium difficile* (CDI) to gastrointestinal and systemic disorders. Antibiotics are the most profound disrupters of the gut microbiome, eliminating more sensitive taxa and leading to a state of dysbiosis. IV β-lactam antibiotics, like cephalosporins, are a first line treatment for many infections, but a substantial portion of these antibiotics are excreted in the bile into the intestine disrupting the gut microbiome. SYN-004 (ribaxamase) is a β-lactamase designed to be orally administered with IV β-lactam antibiotics. Ribaxamase is formulated to remain in the GI tract to degrade the excess antibiotics before they reach the colon and thus protect the gut microbiome.

Methods: As part of a recent Phase 2b clinical study, longitudinal fecal samples were collected from patients receiving ≥5 days of IV ceftriaxone +/- ribaxamase and possibly an oral macrolide. Fecal samples were taken pre and post antibiotics, and extracted DNA was 16S rRNA sequenced to determine the composition of the gut microbiome. Comparisons of changes in the gut microbiome were made between patients who received ceftriaxone plus placebo or ribaxamase, and various sub-analyses were also performed.

Results: The primary endpoint of reduction of new onset CDI was achieved in this trial. Additionally, microbiome diversity comparisons demonstrated that patients who received ceftriaxone + placebo had a significant reduction (P<0.001) in measures of alpha and beta diversity as compared with both pre-antibiotic samples and patients who received ceftriaxone + ribaxamase. The gut microbiome of patients who received concurrent ribaxamase recovered to baseline while the gut microbiome of patients without ribaxamase remained disrupted at 4 weeks. On sub-analysis, patients who were positive for the clinical endpoints of CDI and antibiotic-associated diarrhea had more profound changes to their gut microbiome as compared with patients who didn't meet these endpoints.

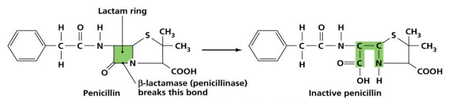
Conclusions: These results are consistent with the expected mechanism of action of ribaxamase and support its anticipated clinical activity in protecting the gut microbiome from β-lactam antibiotics.

BACKGROUND

The use of intravenous **β-lactam antibiotics**, including cephalosporins, are an **important risk factor** for the development of gastrointestinal infections like ***Clostridium difficile*** and the emergence of **antimicrobial resistance** (AMR). These antibiotics can be excreted, via the bile, into the intestine where they disrupt the balance of the gut microbiome and potentially lead to infections by opportunistic pathogens like *C. difficile* and the emergence of antimicrobial resistant organisms.

SYN-004 (ribaxamase) is a novel recombinant **β-lactamase** (an enzyme of ~29kDa) which is delivered orally with the intent of **degrading excess IV β-lactam antibiotics** excreted into the intestine thus **protecting the gut microbiome** from disruption. The primary indication being pursued is prevention of *C. difficile* infection (CDI). The use of SYN-004 may also have the added benefit of **reducing the development of antimicrobial resistance** in the gut microflora. Adding ribaxamase to any treatment with IV β-lactam antibiotics would represent a paradigm shift from the current model where an antibiotic treats the primary infection but often increases the risk for development of opportunistic infections like CDI and the emergence of AMR, to a paradigm where **highly-effective IV β-lactam antibiotics can be administered with substantially reduced risk**.

Ribaxamase degrades β-lactam antibiotics (including most penicillins and cephalosporins) by cleaving the β-lactam ring.



SYN-004 was granted Breakthrough Therapy designation for prevention of CDI by the FDA

Ribaxamase Preclinical Data

- Ribaxamase **inactivates most β-lactam antibiotics**
- Ribaxamase has **good stability** in isolated human intestinal chyme
- Formulated for a **pH dependent release at >5.5**-proximal small intestine
- Ribaxamase **protected the gut microbiome** in a pig model of antibiotic-mediated dysbiosis
- Ribaxamase **prevented the emergence of AMR** genes in pigs
- Ribaxamase is **well tolerated in dogs** up to 57 mg/kg/day for 28 days
- Ribaxamase **did not change the plasma PK** of IV ceftriaxone in dogs

Kaleko et al. 2016. Anaerobe 41:58-67.
Kokai-Kun et al. 2016. International Journal of Toxicology. 35: 309-316.
Connelly et al. 2017. Journal of Applied Microbiology. Open access

EFFICACY STUDY DESIGN

Ribaxamase Efficacy Study

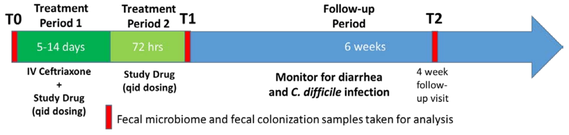
Patients admitted to the hospital for treatment of a lower respiratory tract infection

mITT = 412 patients

1:1

Ceftriaxone + ribaxamase | **Ceftriaxone + placebo**

+/- an oral macrolide at clinical site discretion



Diarrhea = 3 or more loose or watery stools in a 24 hour period, samples are collected
CDI = local lab results for presence of *C. difficile* toxins A and/or B by an approved test (confirmed at a central lab by toxin ELISA)

84 Multinational Clinical Sites

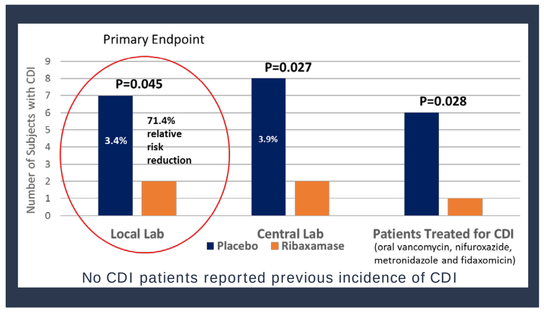
Primary Endpoint:
- **Prevention of *C. difficile* infection (CDI)**

Secondary Endpoint:
- **Prevention of *non-C. difficile* antibiotic-associated diarrhea (AAD)**

Exploratory Endpoints:
- **Evaluate ability to limit disruption of the gut microbiome and prevent emergence of AMR**

PRIMARY ENDPOINT

Ribaxamase significantly reduced the incidence of *C. difficile* infection



P-values are 1-sided based on the pre-specified Z-test. The study was powered at 80% with 1-sided alpha=0.05

Ribaxamase Early Phase Clinical Experience

- **Phase 1**-two studies in normal, healthy volunteers
  - **Well tolerated** up to 750 mg single dose and 300 mg q.i.d. for 7 days
  - **Not systemically absorbed** and no anti-drug antibodies were detected
  - Roberts et al. 2016. Clinical Drug Investigation 36: 725-734
- **Phase 2a**-two studies in subjects with functioning ileostomies, administered IV ceftriaxone ± oral ribaxamase
  - Ribaxamase **degraded ceftriaxone** to below the level of detection in the human intestine
  - Ribaxamase **did not affect the plasma PK** of the ceftriaxone
  - Ribaxamase can be administered in the presence of proton pump inhibitors
  - Kokai-Kun et al. 2017. Antimicrobial Agents and Chemotherapy. 41(3):e02197-16.

EXPLORATORY ENDPOINTS

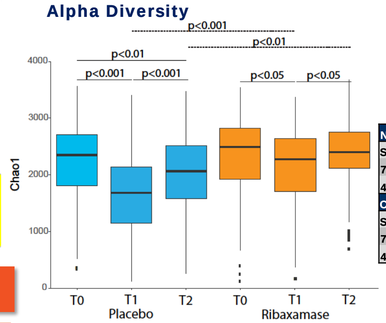
Protection of the Gut Microbiome

16S r RNA Sequencing (DNA Genotek)

Number of Samples	Count
Total Samples Collected	873
Samples Sequenced (screening sample required)	652
Sample Sequence failures	25

Number of Patients	Ribaxamase	Placebo
By Study Drug Assignment	112	117
Full 3 Sample Sets by Drug Assignment	91	96

Alpha Diversity

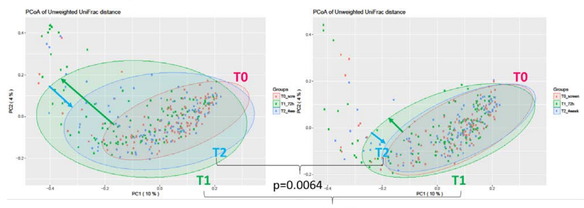


Alpha Diversity in CDI- vs. CDI+ Patients

	Avg Chao1	Avg OTUs	Avg Shannon
Non-CDI (n=223)			
Screening	2314	1665	8.034
72 hrs	1844	1336	7.609
4 weeks	2123	1549	7.879
CDI (n=9)			
Screening	1909	1384	7.92
72 hrs	1581	1200	7.36
4 weeks	1461	1141	7.5

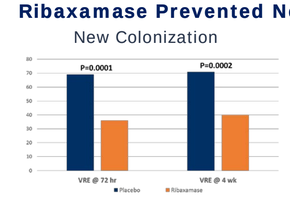
Alpha diversity is a measure of the community composition within an individual sample

Beta Diversity

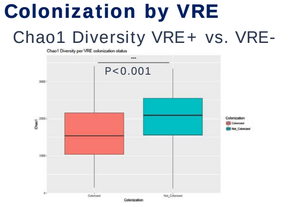


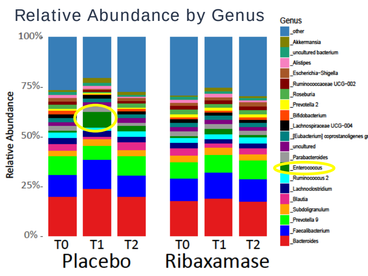
Beta diversity compares the community composition of two different sample sets

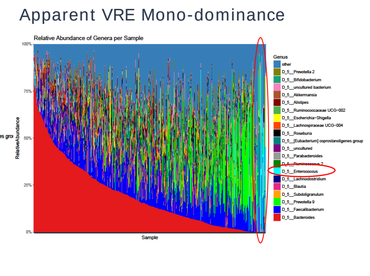
Ribaxamase Prevented New Colonization by VRE



New colonization was negative on screening (T0) and then positive on a subsequent sample (T1 or T2)







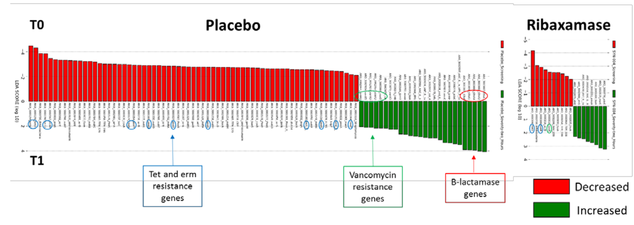
Protection of the Gut Resistome

Ribaxamase Prevented Emergence of Antimicrobial Resistance

- DNA extracted from 350 fecal samples sequenced by **whole genome shotgun sequencing** (Diversigen)
- Interrogated against the CARD database (https://card.mcmaster.ca)
- 21,000,000 DNA matches
- 1,300 AMR genes identified with ~60,000 matches per sample
- Identified many genes of interest including, **β-lactamases, vancomycin and macrolide resistance genes**
- Statistical analysis was performed to determine which genes significantly changed from the screening sample (T0) to the post antibiotic sample (T1) in the placebo vs. the ribaxamase patients-LefSe analysis
- This work was funded by contract 200-2016-91935, in response to The Centers for Disease Control and Prevention BAA 2016-N-17812

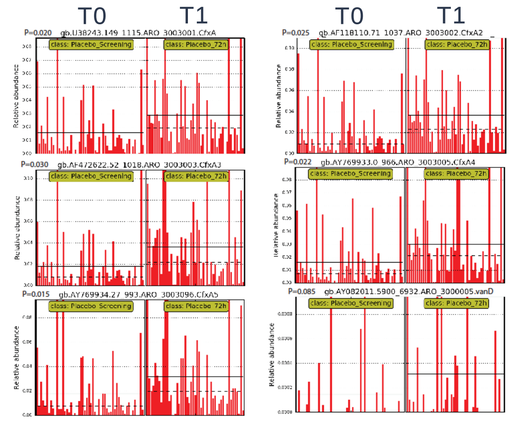
Analysis of the Change in Relative Abundance of AMR Genes

Collection point T0 to T1, Placebo vs. Ribaxamase-treated patients



Identified significant increases in the CfxA family of β-lactamases and the VanSD/VanRD vancomycin resistance genes in PBO group

Relative Abundance of Certain AMR Genes T0 vs. T1



CONCLUSIONS

- **Ribaxamase reduced the incidence of new onset CDI by 71% as compared with placebo (confirmed at the central lab), p=0.045**
- Ribaxamase **protected the diversity of the gut microbiome**
- Ribaxamase **reduced new colonization with VRE**
  - Patients with new VRE colonization had less diversity than non-colonized patients
  - Some VRE colonized patients appeared to display mono-dominance
- Ribaxamase **prevented ceftriaxone-mediated changes in the gut resistome**, consistent with reducing the selective pressure of the excreted ceftriaxone on the gut microbiome
  - Including β-lactamases and vancomycin resistance genes
  - CfxA family of β-lactamases and VanRD/VanSD