SYN-004 (Ribaxamase), an Oral β-lactamase, Prevented *Clostridium difficile* Infection and Protected Patients from Colonization by Antimicrobial Resistant Pathogens by Preserving Gut Microbiome Diversity in a Phase 2b Clinical Trial

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Forward-Looking Statements

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Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection

- **IV Antibiotics**
- **Probiotics and prebiotics**
- **Dysbiosis**
- **Biliary excretion**
- **Antibiotics (Vaccines)**
- **mAbs & Vaccines**
- **FMT & Bacterial Replacement Therapy**

*C. difficile* spores

**CDI IS SERIOUS, DEADLY, AND EXPENSIVE**

- 29,000 US deaths/year within 30 days of diagnosis
- 12 days in the hospital and $27,160 per case in direct costs

1 in 5 recurrences within 2 months

**ribaxamase**
SYN-004 (ribaxamase)

• An orally administered, β-lactamase (an enzyme of 29 kDa) that is designed to degrade penicillins and cephalosporins (engineered from P1A)

• Formulated for pH-dependent release at ≥ 5.5 (proximal small intestine)

• Expected to be orally administered during and after administration of certain intravenous (IV) β-lactam-containing antibiotics like ceftriaxone

• Intended to degrade the excess antibiotics that are excreted into the small intestine via the bile (ribaxamase is stable in human intestinal chyme)

• Designed to prevent disruption of the gut microbiome and thus protect from opportunistic GI pathogens like *C. difficile*
Pre-clinical Animal Models
Demonstrate the tolerability and in vivo activity of ribaxamase

• Fistulated dog model
  • Ribaxamase degraded IV β-lactam antibiotics excreted into the dog intestine

• Nonclinical toxicology in dogs
  • Ribaxamase was well tolerated up to 57 mg/kg/day
  • Ribaxamase was well tolerated when administered with IV ceftriaxone
  • Ribaxamase was not absorbed and did not change the plasma PK of the ceftriaxone

• Piglet Model of Antibiotic-Mediated Dysbiosis
  • Ribaxamase protected the gut microbiome from disruption by β-lactam antibiotics
  • Ribaxamase prevented the propagation of antibiotic resistance genes
    • Connelly et al. Applied and Environmental Microbiology, In press.
Clinical development
Early Phase Clinical Studies
Phase 1 and Phase 2a

• **Phase 1**-two studies in normal, healthy volunteers
  - Well tolerated up to 750 mg single dose and 300 mg q.i.d. – 7 days
  - Not absorbed and no anti-drug antibodies were detected

• **Phase 2a**-two studies in subjects with ileostomies, IV ceftriaxone ± oral ribaxamase
  - Ribaxamase degraded ceftriaxone to below the level of detection in the intestine
  - Ribaxamase did not affect the plasma PK of the ceftriaxone
  - Ribaxamase can be administered in the presence of proton pump inhibitors
Ribaxamase: Phase 2b Proof-of-Concept Study

84 Multinational Clinical Sites

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

Modified intent to treat = 412 patients

1:1

Ceftriaxone + Ribaxamase (plus a macrolide)

Ceftriaxone + Placebo (plus a macrolide)

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

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Enriching for a Population at Risk for *C. difficile* Infection

- Patient were admitted to a hospital for several days
- At least 5 days of ceftriaxone use expected
- Patients > 50 years old
- Patients with higher PORT scores
  (a measure of the severity of the primary infection)
**Phase 2b Proof of Concept Study**

**Study Design**

Randomized 1:1, 150 mg ribaxamase or placebo

**Treatment Period 1**
- 5-14 days
- IV Ceftriaxone + Study Drug (qid dosing)

**Treatment Period 2**
- 72 hrs
- Study Drug (qid dosing)

**Follow-up Period**
- 6 weeks
- Monitor for diarrhea and *C. difficile* infection

US
- Romania
- Hungary
- Bulgaria
- Poland

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)

Diarrhea = 3 or more loose or watery stools in a 24 hour period, samples are collected
Study Demographics and Safety Outcomes

• 206 patients per group in mITT
• Average age of patients ~70 years old
• ~2/3 males in each group
• ~1/3 of patients received macrolides
• ~1/3 patients received concurrent drugs for stomach acidity (PPIs)
• TEAEs and SAEs were similar between active and placebo and there was no trend associated with ribaxamase use
• Cure rate for the LRTI to the ceftriaxone treatment was ~99% in both groups at 72 hours post treatment and at 2 weeks post treatment
Analysis of Changes in the Gut Microbiome

16S rRNA sequencing of DNA extracted from fecal samples

652 samples sequenced, 229 patients, 187 full-3 sample sets

Sequencing and data analysis performed by DNA Genotek
SYN-004 (ribaxamase) Protected Microbial Diversity
Prevented ceftriaxone-mediated loss of $\alpha$-diversity and enhanced microbiome recovery

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

$\alpha$-diversity
- Observed OTUs
- Chao1 Diversity
- Shannon Diversity
**SYN-004 (ribaxamase) Protected Microbial Diversity**

Prevented ceftriaxone-mediated loss of **β-diversity** and enhanced microbiome recovery

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**Placebo**

**Ribaxamase**

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**β-diversity**
- Bray-Curtis
- Unweighted Unifrac

Beta diversity compares the community composition of two different sample sets

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Principle coordinate analysis of the β-diversity (unweighted Unifrac) of patient samples. By T2, the ribaxamase samples have recovered to their starting diversity, but the placebo samples still display a significant loss of diversity as compared with screening.
**Clostridium difficile** Infection

- No CDI patients reported previous CDI
- P-values are 1-sided based on the pre-specified Z-test
- The study was powered at 80% with 1-sided alpha=0.05
Antibiotic-associated Diarrhea

- P-values are 1-sided based on the pre-specified Z-test
New *C. difficile* Colonization at 72 hrs & 4 weeks

- New colonization is negative on screening and then positive on a subsequent sample
- $P$-values are 1-sided based on the pre-specified Z-test

![Graph showing colonization at 72 hours and 4 weeks with Placebo and Ribaxamase groups, and their respective $P$-values: $P=0.059$ for 72 hours and $P=0.088$ for 4 weeks.]
New VRE Colonization at 72 hrs & 4 weeks

- P-values are 1-sided based on the pre-specified Z-test
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 appeared to be well tolerated and not affect the cure rate for the primary infection
• Ribaxamase did not significantly reduce AAD as defined in the protocol, but there was a reduction in all cause diarrhea and in sub-analysis groups
• Ribaxamase reduced new colonization with C. difficile and VRE, p=0.0002
• Analysis of fecal samples for changes in the gut resistome are on-going (CDC contract)
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