SYN-004 (ribaxamase) prevents New Onset *Clostridium difficile* Infection by Protecting the Integrity Gut Microbiome in a Phase 2b Study

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

- IV Antibiotics
- Biliary excretion
- Dysbiosis
- Probiotics and prebiotics
- Antibiotics (Vaccines)
- mAbs & Vaccines
- FMT & Bacterial Replacement Therapy
- *C. difficile* spores
- ribaxamase

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

- 29,000 US deaths/year within 30 days of diagnosis
- 1 in 5 recurrences within 2 months

CDI adds up to:
- 12 days in the hospital
- $27,160 per case in direct costs

**C. difficile**

*FMT*
SYN-004 (ribaxamase)  rye bak’ sa mase

- 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 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*
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. for 7 days
  - Not systemically absorbed and no anti-drug antibodies were detected

• **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 intestine
  - Ribaxamase did not affect the plasma PK of the ceftriaxone
  - Ribaxamase can be administered in the presence of proton pump inhibitors
Ribaxamase: Efficacy 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

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

Modified intent to treat = 412 patients
Enriching for a Population at Risk for C. difficile Infection

- Patients 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)
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

4 week follow-up visit

US

Romania

Hungary

Poland

Canada

Bulgaria

Serbia

Fecal microbiome and fecal colonization samples taken for analysis

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)
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)
• AEs 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, Ottawa, Canada
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 \( \beta \text{-diversity} \) and enhanced microbiome recovery

Beta diversity compares the community composition of two different sample sets.

Principle coordinate analysis of the \( \beta \text{-diversity} \) (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.

\( p=0.0025 \)
\( p=0.0064 \)

- Bray-Curtis
- Unweighted UniFrac
Clostridium difficile Infection (CDI)

- 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

- Placebo vs Ribaxamase

- 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
New VRE Colonization at 72 hrs & 4 weeks

- P-values are 1-sided based on the pre-specified Z-test
Resistome Analysis of Longitudinal Fecal Samples

CDC Contract 200-2016-91935

- DNA extracted from 350 fecal samples sequenced by whole genome shotgun sequencing (Diversigen, Houston, TX)
- Interrogated against the CARD database
- 21,000,000 DNA matches
- 1300 AMR genes identified with ~60,000 matches per sample
- Including many genes of interest, 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
Analysis of the Change in Relative Abundance of AMR Genes

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

T0
\[\text{Placebo}\]

T1
\[\text{Ribaxamase}\]

LefSe Analysis

Sat. 12:30 Poster -1843
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
- Ribaxamase **reduced ceftriaxone-induced changes** in the gut resistome
  - β-lactamases and vancomycin resistance
Ribaxamase Represents a Paradigm Shift
In the Use of Intravenous β-lactam Antibiotics

Current paradigm

Stomach | Duodenum | Jejunum | Ileum | Cecum | Colon
---|---|---|---|---|---
Bile

Excess Antibiotic

Systemic Antibiotics

Treat Infection

Ribaxamase paradigm

Stomach | Duodenum | Jejunum | Ileum | Cecum | Colon
---|---|---|---|---|---

Ribaxamase

No Drug Release

Antibiotic Degraded

Oral antibiotics

Healthy, diverse microbiome
Suppresses secondary infections
Limits emergence of resistant species

× Disrupted microbiome
× Secondary infections such as C. difficile
× Selects for resistant species
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