SYN-004 (ribaxamase) Prevents Clostridium difficile Infection and Antimicrobial Resistance

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Anaerobe
Las Vegas, NV
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IV β-lactam Antibiotics

Biliary excretion
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Dysbiosis

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- **C. difficile spores**
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- CDI

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

- 29,000 US deaths/year within 30 days of diagnosis
- CDI adds up to: 12 days in the hospital and $27,160 per case in direct costs
- 1 in 5 (20%) recurrences within 2 months
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FMT & Bacterial Replacement Therapy

C. difficile spores

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Antibiotics (Vaccines)

mAbs & Vaccines

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C. difficile spores

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

IV β-lactam Antibiotics

*Biliary excretion*

*C. difficile* spores

*ribaxamase*
**SYN-004 (ribaxamase)**

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

- 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*
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
    • Roberts et al. 2016. Clinical Drug Investigation 36: 725-734

• **Phase 2** - 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: 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)

Exploratory Endpoints:

• Evaluate ability to limit disruption of the gut microbiome
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

- **4 week follow-up visit**

**Countries Involved**:
- US
- Romania
- Hungary
- Poland
- Bulgaria
- Serbia

**Diarrhea**
- Diarrhea = 3 or more loose or watery stools in a 24 hour period, samples are collected

**CDI**
- 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 in each group also received **macrolides**
- ~1/3 patients received concurrent drugs for stomach acidity (**PPIs**)

**Adverse Events**

- Percentage of subjects reporting at least one treatment emergent adverse event (TEAE) was similar between ribaxamase and placebo groups (40.8% vs. 44.2%)
- SAEs, including fatal AEs, were **not considered drug-related** by investigators at the clinical sites, or by an independent third-party expert, each of whom determined that SAEs were attributable to **disparities in underlying health and comorbidities** between the groups
- PI’s assessment of **resolution of the LRTI was equivalent** in both groups 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 \(\beta\)-diversity and enhanced microbiome recovery

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

**\(\beta\)-diversity**
- Bray-Curtis
- Unweighted Unifrac

Principle coordinate analysis of the \(\beta\)-diversity (unweighted Unifrac) of patient samples. \(\beta\)-diversity is the community composition of two different samples. Each dot represents one patient sample.
SYN-004 (ribaxamase) Protected Microbial Diversity
Prevented ceftriaxone-mediated loss of $\beta$-diversity and enhanced microbiome recovery

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

**$\beta$-diversity**
- Bray-Curtis
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Screening samples are similar with regards to $\beta$-diversity in both groups
SYN-004 (ribaxamase) Protected Microbial Diversity
Prevented ceftriaxone-mediated loss of $\beta$-diversity and enhanced microbiome recovery

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

Placebo samples display a significant loss of $\beta$-diversity as compared with ribaxamase

**p=0.0025**

- Bray-Curtis
- Unweighted Unifrac
SYN-004 (ribaxamase) Protected Microbial Diversity
Prevented ceftriaxone-mediated loss of $\beta$-diversity and enhanced microbiome recovery

**Beta diversity** compiles the community composition of two different sample sets

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Ribaxamase</th>
<th>p=0.0064</th>
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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.

$\beta$-diversity
- 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
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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

\[ P = 0.059 \]

\[ P = 0.088 \]
New VRE Colonization at 72 hrs & 4 weeks

- P-values are 1-sided based on the pre-specified Z-test
Comparison of Patients with Enterococcal Mono-domination

> 30% of taxa present were enterococci at T1 or T2 based on 16S sequencing

\[ P = 0.004 \]
Comparison of Patients with Enterococcal Mono-domination

> 30% of taxa present were enterococci at T1 or T2 based on 16S sequencing

P = 0.004

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<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Ribaxamab</th>
<th>VRE</th>
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<tbody>
<tr>
<td></td>
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<td>1</td>
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<td>Placebo group</td>
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<td>Ribaxamab group</td>
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Resistome Analysis of Longitudinal Fecal Samples

- 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
- Total hits per AMR gene ranged from 1 to 2.3M (tetQ, tetW)
- 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
Analysis of the Change in Relative Abundance of AMR Genes

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

**T0**

**Placebo**

**Ribaxamase**

B-lactamase Genes

cfxA

Decreased

Increased
Analysis of the Change in Relative Abundance of AMR Genes

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

T0

Placebo

Ribaxamase

T1

Vancomycin resistance Genes
vanD

B-lactamase Genes
cfxA

vanA

Decreased

Increased
Analysis of the Change in Relative Abundance of AMR Genes

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

LefSe Analysis
Analysis of the Change in Relative Abundance of AMR Genes

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

Confirmed by qPCR analysis of \(cfxA\) and \(vanR\)D
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 **reduced new colonization** with *C. difficile* and VRE, (p=0.0002), reduced enterococcal mono-domination
• Ribaxamase **reduced ceftriaxone-induced changes** in the gut resistome which could result in a general reduction of AMR
Ribaxamase Represents a Paradigm Shift
In the Use of Intravenous β-lactam Antibiotics

Current paradigm

- Bile
- Treat Infection
- Systemic Antibiotics
- Excess Antibiotic
- Oral antibiotics

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

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

- Stomach
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
Ribaxamase Represents a Paradigm Shift
In the Use of Intravenous β-lactam Antibiotics

Current paradigm

Ribaxamase paradigm

- **Excess Antibiotic**
- **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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Questions?