Preventing Hospital Acquired Infections – review of clinical trials for Ribaxamase in the prevention of Clostridium difficile Infections

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Disruption of the Gut Microbiome Can Lead to Clostridium difficile Infection and the Emergence of Antimicrobial Resistance

Colonization resistance
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance
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Colonization resistance

Dysbiosis

IV β-lactam Antibiotics

Biliary excretion
Disruption of the Gut Microbiome Can Lead to Clostridium difficile Infection and the Emergence of Antimicrobial Resistance

Colonization resistance

Dysbiosis

IV β-lactam Antibiotics

Biliary excretion

AMR
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance

- **Colonization resistance**
- **IV β-lactam Antibiotics**
- **Dysbiosis**
- **C. difficile spores**
- **Biliary excretion**
- **AMR**
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance

Colonization resistance

Dysbiosis

IV β-lactam Antibiotics

Biliary excretion

C. difficile spores

AMR

CDI
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance

**CDI is Serious, Deadly, and Expensive**

- 29,000 US deaths/year within 30 days of diagnosis
- 1 in 5 (21,000) recurrences within 2 months
- CDI adds up to:
  - 12 days in the hospital
  - $27,160 per case in direct costs

**Colonization resistance**

**IV β-lactam Antibiotics**

**Dysbiosis**

**Biliary excretion**

**C. difficile spores**

**AMR**

**CDI**
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance

**Colonization resistance**

**Dysbiosis**

**C. difficile spores**

**IV β-lactam Antibiotics**

**Biliary excretion**

**CDI**

**AMR**

**C. difficile**
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance

**Colonization resistance**

**IV β-lactam Antibiotics**

**Probiotics and prebiotics**

**Dysbiosis**

**FMT & Bacterial Replacement Therapy**

**C. difficile spores**

**Biliary excretion**

**Antibiotics (Vaccines)**

**mAbs & Vaccines**

**AMR**

**CDI**

**29,000 US deaths/year within 30 days of diagnosis**

**1 in 5 (61,000) recurrences within 2 months**

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

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

**Synthetic Biologics**
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection and the Emergence of Antimicrobial Resistance
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

• 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 and prevent the emergence of AMR
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 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

Follow-up visit
- 4 week follow-up visit

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 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 is the community composition of two different samples. Each dot represents one patient sample.

Principle coordinate analysis of the $\beta$-diversity (unweighted Unifrac) of patient samples. 

- Bray-Curtis
- Unweighted Unifrac
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

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

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

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

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.

\[ p = 0.0064 \]

- 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
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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

![Bar chart showing colonization at 72 hours and 4 weeks with Placebo and Ribaxamase groups.](chart.png)

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
Loss of Microbial Diversity in VRE Colonized Patients

Microbial diversity is significantly reduced in VRE colonized patients

P<0.001

VRE +  VRE -
Loss of Microbial Diversity in VRE Colonized Patients

Microbial diversity is significantly reduced in VRE colonized patients

Appear to be dominated by enterococci

\[ P < 0.001 \]
Comparison of Patients with Enterococcal Mono-domination

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

P=0.004

![Bar chart showing comparison of Placebo and Ribaxamase groups with P-value](chart.png)
Comparison of Patients with Enterococcal Mono-domination

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

P=0.004

[Bar chart showing 6 VRE in Placebo vs 1 VRE in Ribaxamase]
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

T0

T1

Placebo

Ribaxamase

- 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

**LefSe Analysis**

**T0**

**Placebo**

**Ribaxamase**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Decreased</th>
<th>Increased</th>
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<tbody>
<tr>
<td>vanA</td>
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<tr>
<td>cfxA</td>
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<tr>
<td>vanD</td>
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Vancomycin resistance Genes
B-lactamase Genes
Analysis of the Change in Relative Abundance of AMR Genes

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

T0
Placebo

T1

Ribaxamase

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 cfxA1 and vanRD
Change in Mean Copy Number of *cfxA* from the T0 Screening Sample

![Diagram showing the change in copy number from T0 to T1 and T0 to T2 for Placebo and SYN-004.](image-url)
Change in Mean Copy Number of vanRD from the T0 Screening Sample

18 out of 19 patients with new VRE colonization had genetically detectable vanRD
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

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- **Bile**
- **Treat Infection**
- **Systemic Antibiotics**

- **Excess Antibiotic**

- **Oral antibiotics**

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

*Illustration of antibiotic movement through the digestive system.*
Ribaxamase Represents a Paradigm Shift
In the Use of Intravenous β-lactam Antibiotics

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Excess Antibiotic

Bile

Treat Infection

Systemic Antibiotics

Ribaxamase paradigm

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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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Questions?