Protection of the Gut Microbiome: Innovative Solutions to Dysbiosis and Antibiotic Resistance

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Forward-Looking Statements

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Clostridium difficile infection (CDI) Statistics:
Reducing CDI is a national priority with support from CDC & DHHS

CDI is the #1 hospital acquired infection in the U.S.

More than 453,000 patients are infected with C. difficile annually in the U.S.¹

Each year, 29,000 deaths are related to complications from CDI⁸

About 25% of patients experience CDI recurrence in the first 3 months³-⁵

On average, CDI patients spend an extra days in hospital²

$1.5B annual added cost to hospitals resulting from hospital-acquired CDI⁶-⁷

Linear Association Between Antibiotic use CDI risks

Brown et al. AAC 2013; 57:2326-2332
β-Lactam Antibiotics Are Widely Used

Overall IV β-Lactam Use - 2014

<table>
<thead>
<tr>
<th></th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td>Total IV ABx</td>
<td>23 million</td>
</tr>
<tr>
<td>IV β-lactams</td>
<td>17 million</td>
</tr>
<tr>
<td>% of Total</td>
<td>72%</td>
</tr>
</tbody>
</table>

Individual IV β-Lactam Antibiotics

- Ceftriaxone 30%
- Pip and Pip/Tazo 27%
- Cefazolin 23%
- Amp and Amp/Sulbactam 11%
- Other 9%
Disruption of the Gut Microbiome Can Lead to *Clostridium difficile* Infection

- **IV Antibiotics**
- **Biliary excretion**
- **Dysbiosis**
- **FMT & Bacterial Replacement Therapy**
- **Probiotics and prebiotics**
- **Antibiotics (Vaccines)**
- **mAbs & Vaccines**
- **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 and $27,160 per case in direct costs
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*
Ribaxamase Enteric-Coated Pellets

**pH Dissolution Profile**

- pH 1.0
- pH 5.8
- pH 6.8

Time (hours)

% Dissolution

**Stability in Human Chyme**

Relative RibA Activity (ΔAbs 405 nm)

Time (minutes)

**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
Ribaxamase Protected the Gut Microbiome in Piglets

doi:10.1111/jam.13432
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 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
Phase 2 Mechanism of Action Studies in Subjects with Ileostomies

Allows serial sampling of intestinal chyme

1 gram IV ceftriaxone
Oral ribaxamase (75-150 mg)

Ceftriaxone Plasma PK

Ceftriaxone Concentrations in Intestinal Chyme
Ribaxamase: 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
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)
**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
- Poland
- Bulgaria
- Canada

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)

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
- Cure rate for the LRTI to the ceftriaxone treatment 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
Heat Map Comparison of Taxa in Ribaxamase vs. Placebo at T0 & T1

Ribaxamase protected the gut microbiome from antibiotic-induced changes in taxa

Prevents Loss of Taxa

Prevents Overgrowth of Taxa

Loss of Taxa

Overgrowth of Taxa

¹ Data are a representative subset of all patients in each treatment group
² Each square represents the proportion of a particular taxa in that patient’s sample, each column is a patient and each row is a taxa
Synthetic Biologics 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

\[ \text{\( \alpha \)-diversity} \]

- Observed OTUs
- Chao1 Diversity
- Shannon Diversity
SYN-004 (ribaxamase) Protected Microbial Diversity
Prevented ceftriaxone-mediated loss of β-diversity and enhanced microbiome recovery

Placebo  T0  T1  T2  Ribaxamase  T0  T1  T2

p=0.0025  p=0.0064

Beta diversity compares the community composition of two different sample sets

β-diversity
- Bray-Curtis
- Unweighted Unifrac

Principle coordinate analysis of the β-diversity (unweighted Unifrac) of patient samples. β-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 β-diversity and enhanced microbiome recovery

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

Bias screening samples are similar with regards to β-diversity in both groups

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

Data are principle coordinate analysis of the β-diversity (unweighted Unifrac) of patient samples. β-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

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Ribaxamase</th>
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<tbody>
<tr>
<td>T0</td>
<td></td>
<td></td>
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<tr>
<td>T1</td>
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<tr>
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<td>T1</td>
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<tr>
<td>T2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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

Data are 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

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

### Data

- **Placebo**
  - T0
  - T1
  - T2

- **Ribaxamase**
  - T0
  - T1
  - T2

$\beta$-diversity is the community composition of two different samples. Each dot represents one patient sample.

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

Data are principle coordinate analysis of the $\beta$-diversity (unweighted Unifrac) of patient samples.
**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
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
Apparent Mono-domination by Enterococcal Species

Loss of microbial diversity in VRE colonized patients

P<0.001

VRE +  VRE -
Comparison of Patients with Enterococcal Mono-domination

> 30% of taxa present were enterococci at T1 or T2

- Placebo: 6 VRE
- Ribaxamase: 1 VRE
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

Placebo

T1

Ribaxamase

LefSe Analysis

Tet and erm resistance genes
Vancomycin resistance genes
B-lactamase genes

Decreased
Increased
## Change in relative abundance from T0 to T1 in genes of interest

<table>
<thead>
<tr>
<th>Gene of Interest</th>
<th>P value</th>
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### qPCR analysis

qPCR analysis of *cfxA* and *vanRD* determined that there was both new acquisition of AMR and expansion of existing AMR pools.
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
  - Including β-lactamases and vancomycin resistance genes
Ribaxamase Represents a Paradigm Shift
In the Use of Intravenous \(\beta\)-lactam Antibiotics

<table>
<thead>
<tr>
<th>Current paradigm</th>
<th>Ribaxamase paradigm</th>
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<tbody>
<tr>
<td><strong>Stomach</strong></td>
<td><strong>Stomach</strong></td>
</tr>
<tr>
<td><strong>Duodenum</strong></td>
<td><strong>Duodenum</strong></td>
</tr>
<tr>
<td><strong>Jejunum</strong></td>
<td><strong>Jejunum</strong></td>
</tr>
<tr>
<td><strong>Ileum</strong></td>
<td><strong>Ileum</strong></td>
</tr>
<tr>
<td><strong>Cecum</strong></td>
<td><strong>Cecum</strong></td>
</tr>
<tr>
<td><strong>Colon</strong></td>
<td><strong>Colon</strong></td>
</tr>
</tbody>
</table>

- **Excess Antibiotic**
- **Healthy, diverse microbiome**
- **Suppresses secondary infections**
- **Limits emergence of resistant species**

**Systemic Antibiotics**

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

**Oral antibiotics**

- **No Drug Release**
- **Antibiotic Degraded**

**Synthetic Biologics**
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Questions?