Clinical Development of Ribaxamase, an Oral-Beta Lactamase Intended to Protect the Gut Microbiome and Prevent *C. difficile* Infection

Christian Furlan Freguia
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The Gut Microbiome Regulates Human Physiology

**Gut Microbiota Involved in**

- Digestion
- Nutrient absorption
- Vitamin synthesis
- Protection from MDR organisms
- Immune, Metabolic, CV, Neuro Physiology

Discuss a strategy to protect the gut microflora from antibiotic damage
Ribaxamase Program Overview

- Orally administered β-lactamase for use with IV penicillins and cephalosporins
- Enteric coated pellets that release the β-lactamase in the duodenum
- Designed to degrade the β-lactam antibiotic in the upper GI tract
- To remove the antibiotic from the chyme before it reaches the colon
- Ribaxamase is not absorbed, so it does not alter systemic antibiotic levels

**Clinical Objectives**

1. Diminish the risk of *Clostridium difficile* infection (CDI)
2. Prevent colonization by MDR pathogens (and secondary infections)
3. Slow the emergence and/or spread of MDR pathogens
4. Prevent changes to the microflora
Engineered for Improved Potency Against Cephalosporins

Class A serine $\beta$-lactamase

Isolated from *Bacillus licheniformis*

D276N aa substitution

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<thead>
<tr>
<th>IV $\beta$-Lactam Use</th>
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<td><strong>Days on Therapy</strong></td>
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<tr>
<td>Total IV Abx</td>
<td>23 million</td>
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Individual β-Lactam Antibiotics

Days on Therapy

- Ceftriaxone: 30%
- Cefazolin: 23%
- Pip and Pip/Tazo: 27%
- Other: 9%
- Amp and Amp/Subbactam: 11%

Arlington Medical Resources (AMR), a Decision Resources Group Company 2014 audits of acute care hospital antibiotic utilization
Ribaxamase Formulation

Ribaxamase Enteric-Coated Pellets

pH Dissolution Profile

Stability in Human Chyme

Six fistulated dogs were treated with IV ceftriaxone (CRO) +/- oral ribaxamase.

Chyme was collected and assayed for CRO and ribaxamase.

Kaleko et al. (2016) Anaerobe 41:58
Ribaxamase Protected the Microbiome in Pigs

Ribaxamase Protected the Microbiome in Pigs

Ribaxamase Mitigated Expansion of Abx Resistance Genes

Ceftriaxone increased the abundance of resistance genes for multiple Abx. Ribaxamase reduced the emergence of antibiotic resistance.

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Clinical Development
Early Clinical Trials --- Safety and Mechanism

Two Phase 1 studies in 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

Two Phase 2a studies in volunteers with ileostomies

- Subjects received IV ceftriaxone +/- oral ribaxamase
- Ribaxamase did not affect the plasma PK of ceftriaxone
- Ribaxamase removed ceftriaxone from the chyme
- Ribaxamase was efficacious with proton pump inhibitors

Early Clinical Trials --- Safety and Mechanism

Ceftriaxone Alone

Ceftriaxone with Ribaxamase

CRO with Ribaxamase + PPI

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

Exploratory Endpoint
Protection of the gut microbiome

US, Canada, Romania, Bulgaria, Hungary, Poland, Serbia
Inclusion Criteria to Enriched for Risk of C. diff. Infection

Patients were admitted to a hospital for several days
At least 5 days of ceftriaxone use expected
Patients > 50 years old
Patients with high PORT scores
Design of the Phase 2b Study

Diarrhea → 3 or more loose stools in a 24 hour period, samples were collected

CDI → Local lab reported toxins A and/or B by an approved test
Sent to a central lab for confirmation by toxin ELISA
206 patients per group

Average age ~70

~2/3 of each group were males

~1/3 of each group received macrolides

~1/3 of each group received drugs for stomach acidity (PPIs)

The cure rates for the LRTI for both groups were comparable
Ribaxamase Protected Microbial Diversity in the Phase 2b Study

Phase 2b fecal samples were analyzed by 16S sequencing.

Alpha Diversity measure of the composition within an individual sample.

[Graph showing Chao1 diversity comparison between Placebo and Ribaxamase groups at different time points (T0, T1, T2).]
Ribaxamase Protected Microbial Diversity in the Phase 2b Study

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Alpha Diversity measure of the composition within an individual sample.
Ribaxamase Protected Microbial Diversity in the Phase 2b Study

Principle coordinate analysis of the β-diversity of the patient samples
Each dot represents one patient sample

T0  T1  T2
p=0.0025  p=0.0064

Placebo  Ribaxamase
Ribaxamase Protected Against *C. difficile* Infection

No CDI patient reported previous CDI
A Trend Towards Diminished New *C. difficile* Colonization

Number of patients negative for *C. difficile* on screening and positive in a subsequent sample

![Graph showing comparison between Placebo and Ribaxamase](image)
Ribaxamase Protected from Colonization by VRE

New colonization by Vancomycin-Resistant Enterococcus at 72 hours and 4 weeks

P-values are 1-sided based on the pre-specified Z-test
Ribaxamase Attenuated Changes to the Abx Resistance Genes

LefSe Analysis

- Green: Increased
- Red: Decreased
Ribaxamase Attenuated Changes to the Abx Resistance Genes

LefSe Analysis

- **Placebo**
  - Tet and erm resistance genes
  - Vancomycin resistance genes

- **Ribaxamase**
  - B-lactamase genes

- **Increased**
- **Decreased**
Pipeline Products

Use with oral antibiotics → SYN-007

- Beneficial for patients transferred from IV
- Potentially beneficial for all outpatients on oral β-lactams
- Developed a delay-released formulation of ribaxamase in the GI tract
- Preliminary data in dogs look encouraging

Use with carbapenems → SYN-006

- Increasing in usage and is very damaging to the microbiome
- The potential to diminish the emergence of carbapenem resistance
- Developing a metallo-β-lactamase with broad activity
- Currently testing in pigs
SYN-007 Enables Absorption of Oral Amoxicillin in Dogs

Oral amoxicillin +/- SYN-007 TID for 16 doses

Serum amoxicillin PK after the first and last dose

Feces for whole genome sequencing before and after the antibiotic regimen
SYN-007 Protects the Dog Gut Microbiome from Oral Amoxicillin

Principal Component Analysis (Bray-Curtis Dissimilarity)
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SYN-006 does not Interfere with Ertapenem Plasma Levels in Pigs

IV Ertapenem +/- SYN-006 TID for 4 days
Serum ertapenem PK collected at day 3
Feces for whole genome sequencing before and after the antibiotic regimen

Ertapenem Serum Levels
SYN-006 Protects the Gut Microbiome in Pigs

Principal component analysis

- Ertapenem alone pre
- Ertapenem + SYN-006 pre
- Ertapenem alone post
- Ertapenem + SYN-006 post
SYN-006 Protects the Gut Microbiome in Pigs

Principal component analysis

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Summary

Ribaxamase was shown in a Phase 2b clinical trial to protect the gut microbiome from CRO and diminish
  ➢ the incidence of CDI --- and potentially diminish its spread within a hospital
  ➢ overgrowth with VRE
  ➢ the emergence of resistance to multiple classes of antibiotics

Ribaxamase did not interfere with systemic antibiotic levels or antibiotic efficacy

The simple mechanism of action, validated in dog chyme and the pig microbiome, and similarly validated in human Phase 2 trials, supports the potential for success in future clinical trials

Goal is to enable a patient to leave the hospital with the same microbiome that he came in with
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