Protecting the Gut Microbiome from Antibiotics

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
# Microbiome Protection Pipeline

<table>
<thead>
<tr>
<th>Product Candidate</th>
<th>Description</th>
<th>Preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
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<tbody>
<tr>
<td>SYN-004 (ribaxamase)</td>
<td>Oral β-lactamase enzyme co-administered with IV β-lactam designed to degrade excess antibiotic</td>
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<tr>
<td>SYN-007</td>
<td>Delayed release oral β-lactamase enzyme co-administered with ORAL β-lactam</td>
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<td>SYN-006</td>
<td>Oral carbapenemase enzyme co-administered with IV carbapenem antibiotic</td>
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<tr>
<td>SYN-020 (IAP)</td>
<td>Oral intestinal alkaline phosphatase enzyme co-administered with IV or oral antibiotic to reverse dysbiosis (not antibiotic degrading)</td>
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</table>
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

Degrading excess $\beta$-lactam antibiotic excreted into the GI tract

**SYN-004 (ribaxamase)**

*Designed to Prevent CDI, AMR*

- **SYN-004 (ribaxamase)**
  - Concomitant with IV $\beta$-lactam

- Antibiotic Excreted in Bile
- Treat Primary Infection (e.g. pneumonia)
- Systemic (IV) $\beta$-lactam antibiotic

- Disrupted microbiome
- Proliferation of pathogens such as *C. difficile*
- Emergence of antimicrobial resistant species (AMR)

**Diagrams:**
- Treating primary infection
- Antibiotics excreted in bile
- Concomitant with IV $\beta$-lactam
Degrading excess β-lactam antibiotic excreted into the GI tract

Enteric protection intended to prevent gastric release and limit acid degradation

- Disrupted microbiome
- Proliferation of pathogens such as *C. difficile*
- Emergence of antimicrobial resistant species (AMR)
SYN-004 (ribaxamase) Designed to Prevent CDI, AMR

Degrading excess β-lactam antibiotic excreted into the GI tract

- Ribaxamase enzyme released into the upper small intestine degrades β-lactam antibiotic
- Systemic (IV) β-lactam antibiotic
- Antibiotic Excreted in Bile
- Treat Primary Infection (e.g. pneumonia)

Stomach - Duodenum - Jejunum - Ileum - Cecum - Colon
Degrading excess β-lactam antibiotic excreted into the GI tract

TARGET OUTCOMES:
- Restore healthy, diverse microbiome
- Suppress proliferation of pathogens e.g. C. difficile
- Limit emergence of AMR

Ribaxamase enzyme released into the upper small intestine degrades β-lactam antibiotic

SYN-004 (ribaxamase) Designed to Prevent CDI, AMR
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

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

Randomized to 150 mg ribaxamase or placebo qid

Feces collected

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
Phase 2b Study Demographics

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 Against *C. difficile* Infection

No CDI patient reported previous CDI

P-values are 1-sided based on the pre-specified Z-test
Study was powered at 80% with 1-sided alpha=0.05
A Trend Towards Diminished New *C. difficile* Colonization

Number of patients negative for *C. diff.* on screening and positive in a following sample

![Bar chart showing comparison between Placebo and Ribaxamase groups at 72 hours and 4 weeks]

- **72 hours:** Placebo: 14, Ribaxamase: 6, \( P = 0.059 \)
- **4 weeks:** Placebo: 20, Ribaxamase: 12, \( P = 0.088 \)

*P*-values are 1-sided based on the pre-specified Z-test
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 Protected the Gut Microbiome From CRO

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
Ribaxamase Protected the Gut Microbiome From CRO

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Ribaxamase Protected the Gut Microbiome From CRO

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
Phase 2b fecal samples were analyzed by 16S sequencing

Alpha Diversity measure of the composition within an individual sample
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
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
Ribaxamase Attenuated Changes to the Abx Resistance Genes

Placebo

Ribaxamase

LefSe Analysis

- Increased
- Decreased
Ribaxamase Attenuated Changes to the Abx Resistance Genes

LefSe Analysis
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.
Ribaxamase Mitigated Expansion of Abx Resistance Genes

Ceftriaxone increased the abundance of resistance genes for multiple Abx. Ribaxamase reduced the emergence of antibiotic resistance.
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
SYN-007 Enables Absorption of Oral Amoxicillin in Dogs

Amoxicillin Absorption

Ribaxamase Release
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 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)
SYN-007 Protects the Dog Gut Microbiome from Oral Amoxicillin

Principal Component Analysis
By attribute: Relative Abundance

- Amox alone pre
- Amox+SYN-007 pre
- Amox alone post
- Amox+SYN-007 post

PC3
SYN-007 Protected the Microbiome and Suppressed AMR
Pipeline Products

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 tested in pigs
SYN-006 does not Interfere with Ertapenem Serum 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
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

• Ertapenem alone pre
• Ertapenem + SYN-006 pre
• Ertapenem alone post
• Ertapenem + SYN-006 post
SYN-006 Reduces Propagation of Antibiotic Resistance Genes
SYN-006 Reduces Propagation of Antibiotic Resistance Genes

SYN-006 attenuated ertapenem-induced increased frequency of aminoglycoside, macrolide, tet, and efflux pump AR genes
Oral intestinal alkaline phosphatase enzyme (IAP) → SYN-020

• Endogenous enzyme co-administered with IV or oral antibiotic to mitigate dysbiosis and facilitate microbiome recovering (not antibiotic degrading)
IAP to Protect and Restore the Gut Microbiome

Naturally-occurring enzyme produced by enterocytes in the proximal small intestine

Well-studied for decades

Dephosphorylates a broad spectrum of substrates

IAP maintains gut health via multiple mechanisms
  • Detoxifies inflammatory mediators
  • Tightens the gut barrier
  • Promotes the growth of commensal flora

Efficacious in several animals models of GI inflammation and in humans with colitis

Manufacturing and formulations have limited IAP clinical uses
  • We solved these problems!
IAP Protects Mice Against CDI

- SYN-020 (IAP)
- Streptomycin
- COL
- C. difficile

**Study Day**

- Time point
- Follow up

**Graphs**

- Percent survival over time (hours)
- Total symptom score over time (days post infection)

- * vehicle
- SYN-020

[Synthetic Biologics logo]
IAP may Promote a Preferential Gut Microenvironment

Stacked bar graph, filtered, frequency, genus level
Summary and Conclusions

In a Phase 2b trial, Ribaxamase protected the gut microbiome from CRO and significantly diminished:
- the incidence of CDI
- overgrowth with VRE
- the emergence of resistance to multiple classes of antibiotics

Ribaxamase did not interfere with systemic antibiotic levels or antibiotic efficacy.

Currently working with the FDA to define the remaining elements of the Phase 3 trial protocol.

Pipeline products:
- use with oral antibiotics
- microbiome protection from all classes of beta-lactams
- antibiotic “agnostic” --- IAP has the potential to be efficacious in indications beyond CDI

Similarities between the human clinical and preclinical data facilitate the develop of new products.
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