SYN-004 (ribaxamase)

An Orally-Delivered Beta-Lactamase Protects the Gut Microbiome from Antibiotic-Mediated Damage and Mitigates Propagation of Antibiotic-Resistance Genes in a Porcine Dysbiosis Model

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Digestive Disease Week 2017
Chicago, IL
May 7, 2017
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The Gut Microbiome Regulates Human Physiology

"All disease begins in the gut!" - Hippocrates

400 B.C.

Gut Microbiome Involved in:
- Digestion
- Immune system
- Protection from pathogens
- Metabolic, cardiovascular, neurological diseases

Reservoir of antibiotic resistance

Disrupted by:
- Opportunistic infections
- C. difficile
- VRE
- MDR

Synthetic Biologics is developing therapies to protect the gut microbiome from antibiotic damage.
SYN-004 (ribaxamase): Paradigm Shift Through Protection and Prevention

Antibiotic Inactivation to Preserve the Gut Microbiome

Current Approach

Antibiotics
- β-lactam
- Fluoroquinolone
- Clindamycin
- Other

Ribaxamase Paradigm

Antibiotics
Beta-lactam antibiotic + ribaxamase

C. difficile

Recurrence of C. difficile infection

More Antibiotics
- Metronidazole
- Vancomycin
- Fidaxomicin

Protection of the microbiome
Prevention of C. difficile infection

Ribaxamase: protection of the gut microbiome during antibiotic use

Intact gut microbiome
Beta-Lactamases: From Enemies to Therapies

**Strategy:** SYN-004 (ribaxamase) is a beta-lactamase enzyme designed to be taken orally to degrade selected beta-lactam antibiotics in the GI tract to protect the microbiome.

**Product:** Capsule with enteric-coated enzyme

**Intended Protection:** Protection of the gut microbiota, prevention of *C. difficile* infection, and reduction of antibiotic-resistance propagation.

**Outcome:** Orally-delivered ribaxamase is intended to degrade residual antibiotics in the GI tract to protect the gut microbiome without affecting antibiotic efficacy.
Completed Phase 1 (2 trials), Phase 2a (2 trials), and a Phase 2b trial

**Phase 1:** Demonstrated good tolerability with no systemic absorption of ribaxamase*

**Phase 2a:** Demonstrated ribaxamase degraded ceftriaxone in the GI tract without affecting systemic levels**

**Phase 2b:** Demonstrated a significant reduction in *C. difficile* disease and a significant reduction in new colonization by vancomycin-resistant enterococci (VRE) in patients receiving IV ceftriaxone for a lower respiratory tract infection

- Tuesday May 9 at 10:30 am, oral presentation of Abstract 874j in Clinical Science: Late-Breaking Abstract Plenary, Room S103. John Kokai-Kun: An Oral Beta-Lactamase Prevented *Clostridium Difficile Infection* and Protected Patients from Colonization by Antimicrobial Resistant Pathogens by Preserving Gut Microbiome Diversity in a Phase 2B Clinical Trial
- Through CDC funding, microbiome assessments are in progress to evaluate ribaxamase’s ability to reduce the emergence of antibiotic resistance

**Phase 3:** Expected 1H2018

Piglet Model of Antibiotic-Mediated Dysbiosis

2 month old 20 kg piglets N=5 per cohort

Days:
-7  -4  0   1   2   4   7   8   9

Feces collections
Blood collections

Oral ribaxamase (75 mg QID)

Antibiotics: IV Ceftriaxone IV Ertapenem Oral Amoxicillin

Readouts:
- Fecal DNA whole genome shotgun sequencing analyses
- Antibiotic blood levels
Ribaxamase Protected the Microbiome in Piglets

- Piglet Fecal DNA whole genome shotgun sequencing and taxonomic profiling
- Heatmap of bacterial strains displayed as the relative abundance

Ribaxamase reduced antibiotic-mediated changes to the microbiome

A broad spectrum of antibiotic-resistance genes were propagated in response to ceftriaxone, not just those conferring resistance to beta-lactams.

Ribaxamase reduced emergence of antibiotic-resistance genes.
Ribaxamase Prevented Propagation of a Broad Range of AR Genes

Change in the frequency of AR genes

- **Ceftriaxone Alone**
- **Ceftriaxone + Ribaxamase**

Ceftriaxone caused an increase in AR gene frequency while ribaxamase reduced AR gene frequency.
Ribaxamase Prevented Emergence of Non-Beta-Lactamase AR Genes

Aminoglycoside _strA_

Ribaxamase significantly reduced emergence of genes conferring resistance to antibiotics other than beta-lactams.

Tetracycline _tet39_

P<0.05
Beta-Lactam Antibiotics Caused Dysbiosis in Piglets

Heatmap of bacterial strains displayed as the relative abundance

Antibiotics caused depletion of some species and overgrowth of others
A broad spectrum of antibiotic-resistance genes were propagated in response to antibiotic exposure, not just those conferring resistance to beta-lactams.
Emergence of Antibiotic Resistance Genes after Amoxicillin Exposure

ESBL Genes

- OXA-347
- CblA-1

Other AR Gene

- Aminoglycoside_strA

ESBL and AR genes were rapidly propagated after amoxicillin exposure.
Oral Amoxicillin Exposure Causes Propagation of a Broad Range of AR Genes

A broad spectrum of antibiotic-resistance genes were propagated in response to amoxicillin exposure.
Emergence of AR Genes after Ertapenem Exposure

ESBL Genes

- OXA-227
- IMP-27

Relative Gene Frequency (%)

Day -7  Day -4  Day 4  Day 9

Other AR Genes

- mphE
- adeC

Relative Gene Frequency (%)

Day -7  Day -4  Day 4  Day 9

ESBL and AR genes were rapidly propagated after ertapenem exposure

Mollenkopf DF. et al., Ohio State University (2017) AAC 61: e01298-16; Johnson, TJ (2017) AAC 61: e02348-16

"Nightmare" bacteria resistant to last-resort antibiotics discovered on farm. http://civileats.com/2016/12/15/26075
Vancomycin-resistance genes were rapidly propagated after ertapenem exposure.
A broad spectrum of antibiotic-resistance genes were propagated in response to ertapenem exposure.
Future Directions

• Ribaxamase
  • Continuing Phase 2b data analysis including exploratory end points, as well as fecal microbiome and resistome data (CDC contract)
  • Oral presentation of Phase 2b data, Tuesday May 9 at 10:30 am Clinical Science: Late-Breaking Abstract Plenary, Room S103
  • Planning for Phase 3 pivotal trials

• Ribaxamase and oral beta-lactam antibiotics
  • Formulations that release distal to the site of oral antibiotic absorption are in progress
  • Testing in pig model of oral amoxicillin dysbiosis
  • Has the potential to expand indications to include oral beta-lactams

• Carbapenemase (SYN-006)
  • Recombinant protein produced in E. coli
  • Formulation for oral delivery in progress
  • Testing in pig model of ertapenem dysbiosis

• Additional strategies to protect the microbiome from antibiotics
Ribaxamase is intended as an orally-delivered beta-lactamase to protect the gut microbiome from IV penicillins and cephalosporins to prevent C. difficile infection (CDI).

Phase 2b proof-of-concept study demonstrated a statistically significant reduction in CDI and new VRE colonization in patients that received ribaxamase with ceftriaxone compared to placebo.

Ribaxamase protected the gut microbiome from ceftriaxone-mediated dysbiosis in pigs.

Ribaxamase reduced the emergence and propagation of antibiotic-resistance genes in pigs.

Goal of this antibiotic-inactivation strategy is to enable patients to leave the hospital with their gut microbiomes intact:
- Protect from CDI and secondary infections with MDR organisms
- Reduce antibiotic resistance
- Diminish risks associated with beta-lactam antibiotics

Ribaxamase has the potential to become the first prophylactic therapy designed to prevent antibiotic-mediated microbiome damage including C. difficile infection.
Acknowledgements

Synthetic Biologics, Inc.

Research
Michael Kaleko
Christian Furlan-Freguia

Development
Ray Stapleton, Jr.
J. Andrew Bristol
Steven Hubert

Clinical and Nonclinical
Joe Sliman
Olivia Coughlin
Lara Guzman
John Kokai-Kun
Heather McFall
Tracey Roberts
Scott Shapot
Amy Sloan
Heidi Whalen

CosmosID, Inc.

Rita R. Colwell
Nur Hasan
Poorani Subramanian

Protection
Preservation
Prevention

Graphic by Hyperbiotics