A Clinical-Stage Oral β-Lactamase Therapy Prevents Antibiotic-Mediated Damage of the Gut Microbiome

Sheila Connelly, PhD
Vice President of Research
ICAAC/ICC September 20, 2015
Importance of Intestinal Health Has Long Been Recognized

“ALL DISEASE BEGINS IN THE GUT!”
-Hippocrates 400 B.C.

Gut Microbiome Involved in:
• Digestion
• Nutrient absorption
• Vitamin synthesis
• Bile salt metabolism
• Stimulation of immune system

Disrupted by:
• Disease
• Antibiotics

Synthetic Biologics is developing therapies to protect the gut microbiome from the damage caused by antibiotics
β-Lactamases: From Enemies to Therapies

Strategy: β-lactamase enzyme is intended to degrade residual antibiotics in the GI tract to protect the microbiome

Product: Capsule with enteric-coated enzyme

Outcome: Prevention of *Clostridium difficile* infection and antibiotic-associated diarrhea

Orally-delivered β-lactamases intended to degrade residual antibiotics in the GI tract to protect the gut microbiome without affecting antibiotic efficacy
SYN-004 Degrades Cephalosporins

- SYN-004 was engineered from P1A
- P1A is a clinical isolate from *Bacillus licheniformis*

*E. coli* growth microtiter plate assay

SYN-004 efficiently degrades cephalosporins, including ceftriaxone, cefuroxime, cefoperazone, ceftazidime, and cefotaxime
SYN-004 Degraded Ceftriaxone (CRO) in Dog GI Tract

In the presence of SYN-004 no CRO was detected in chyme
Neonatal Pigs with Human Gut Microflora

Readouts:

- Direct measure of CRO-sensitive bacterial population
- Fecal DNA 16S rRNA V6 region sequence analyses
- Shotgun deep sequencing analyses

Days: 0  5  6  7  10  11  20

- Birth
- Populate GI tract with human adult fecal microflora
- Oral SYN-004 (75 mg QID)
- CRO (IP, 50 mg/kg)
- Feces collections
SYN-004 Protected the Gut Microflora in Pigs

- Monitored CRO-sensitive bacterial population, Amp\(^R\) aerobes

**Bacterial Growth on LB+Amp Plates**

<table>
<thead>
<tr>
<th></th>
<th>No Abx</th>
<th>CRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYN+004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Bacterial Colonies**

- SYN-004 protected the CRO-sensitive fecal bacterial population
SYN-004 Prevented CRO-Induced Dysbiosis

- 16S rRNA V6 region sequence analyses

**Phylum-Level Taxonomic Classification**

SYN-004 protected the Proteobacteria and Firmicutes and prevented overgrowth of Bacteroidetes in the presence of CRO.
SYN-004 Protected the Pig Microbiome

- Whole genome shotgun sequencing and taxonomic profiling
- Heatmap of bacterial strains based on compositional similarity and relative abundance
- Dendrogram clustering samples with similar compositions

The SYN-004-treated animals clustered with the controls demonstrating that SYN-004 protected the GI microflora from the effects of CRO.
SYN-004 Prevented Overgrowth of *M. smithii*

- Whole genome shotgun sequencing and analyses
- Nearest shrunken centroid classification of fecal bacterial species

The SYN-004-treated animals were more similar to the no antibiotic controls demonstrating that SYN-004 protected the GI microflora from the effects of CRO.
SYN-004 is in Phase 2 Clinical Trials

Preclinical Results
- Safe in two GLP toxicity studies in dogs
- Well tolerated with a NOAEL of 57 mg/kg/day, highest dose tested
- Not detected systemically
- Did not affect ceftriaxone blood levels

Clinical Results
- Phase 1 clinical studies demonstrated SYN-004 safety and tolerability with a single dose of up to 750 mg and multiple doses of 300 mg 4X a day for 7 days
- SYN-004 was not systemically bioavailable
- SYN-004 was not immunogenic
- Phase 2a studies are in progress
- Phase 2b study to initiate 3Q 2015
Conclusions

- SYN-004 degraded ceftriaxone in the dog GI tract
- SYN-004 protected the intestinal microbiome from ceftriaxone in pigs
- SYN-004 prevented the overgrowth of *M. smithii* in antibiotic-treated pigs
- *M. smithii* was reported to be associated with constipation, IBS, and obesity
- SYN-004 is progressing through Phase 2 clinical trials

SYN-004 has the potential to become the first prophylactic therapy designed to prevent antibiotic-mediated microbiome damage including *C. difficile* infection and antibiotic-induced diarrhea
Acknowledgements

Synthetic Biologics, Inc.
Research
John Monahan
Michael Kaleko

Development
J. Andrew Bristol
Steven Hubert

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

Tufts Cummings School of Vet. Med.
Giovanni Widmer
Saul Tzipori

CosmosID, Inc.
Poorani Subramanian
Nur Hasan

Ipsat Therapies, Ltd
Pertti Koski

SynPhaGen, LLC
Todd Parsley