**SYN-004 (ribaxamase) Protects the Diversity of the Gut Microbiome in Patients Receiving Intravenous Ceftriaxone Treatment**

**John F. Kokai-Kun*, Sheila Connelly, Charles Le, Ken Trout and Joseph Silman**

Synthetic Biosciences, Inc., Rockville, MD

---

**ABSTRACT**

**Background:** The balance of the gut microbiome is linked to human health, and disruption of this balance is associated with many diseases from obesity to chronic inflammatory conditions like gastrointestinal infections, antibiotic-mediated dysbiosis, and the emergence of antibiotic-resistant organisms (AMR). These antibiotics can be excreted in the bile into the small intestine, where they can disrupt the gut microbiome. SYN-004 (ribaxamase) is a novel recombinant β-lactamase (an enzyme of ~29kDa) which is delivered orally with the intent of degrading β-lactam antibiotics in the proximal ileum to protect the gut microbiome.

**Rifaxamase Efficacy Study**

**Ribaxamase Prevented New Colonization by VRE**

<table>
<thead>
<tr>
<th>New Colonization</th>
<th>Placebo</th>
<th>Ribaxamase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Abundance by Genus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent VRE Mono-dominance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New colonization prevented in 38% of samples sequenced by whole genome shotgun sequencing (Diversitree)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identified significant increases in the CHA family of β-lactamases and the VanS/VDanV vancomycin resistance genes in PBO group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXPLANATORY ENDPOINTS**

**Protection of the Gut Microbiome**

**Protection of the Gut Resistance**

**Ribaxamase**

**Conclusions:**

- Ribaxamase reduced the incidence of C. difficile infection by 71% as compared with placebo (confirmed at the central lab), p<0.043
- Ribaxamase protected the diversity of the gut microbiome
- Ribaxamase reduced new colonization with VRE
- Patients with new VRE colonization had less diversity than non-colonized patients
- Some VRE colonized patients appeared to display mono-dominance
- Ribaxamase prevented ceftriaxone-mediated changes in the gut microbiome

**Ribaxamase early phase clinical experience**

- Phase 1 - two studies in normal, healthy volunteers
  - Well tolerated up to 750 mg single dose and 300 mg q.d. for 7 days
  - Not systemically absorbed and no anti-drug antibodies were detected
- Phase 2a - two studies in subjects with functioning ileostomies, administered IV ceftriaxone or oral ribaxamase
  - Ribaxamase degraded ceftriaxone to below the level of detection in the human intestine
  - Ribaxamase did not affect the plasma PK of the ceftriaxone
  - Ribaxamase can be administered in the presence of pre-existing inhibitors

**Ribaxamase Prevented New Colonization by VRE**

**Chao1 Diversity VRE+ vs. VRE-**

<table>
<thead>
<tr>
<th>VRE+ vs. VRE-</th>
<th>Chao1</th>
</tr>
</thead>
<tbody>
<tr>
<td>New colonization prevented in 38% of samples sequenced by whole genome shotgun sequencing (Diversitree)</td>
<td></td>
</tr>
</tbody>
</table>

**Relative Abundance by Genus**

**Apparent VRE Mono-dominance**

**Relative Abundance of Certain AMR Genes T0 vs. T1**

- Identified significant increases in the CHA family of β-lactamases

---

**SYN-004 was granted Breakthrough Therapy designation for prevention of CDI by the FDA**

**Ribaxamase Preclinical Data**

- Ribaxamase inactivates most β-lactam antibiotics
- Ribaxamase is safe in isolated human intestinal chyme
- Formulated for a pH dependent release at >5.5, proximal small intestine
- Ribaxamase protected the gut microbiome in a pig model of antibiotic-mediated dysbiosis
- Ribaxamase prevented the emergence of AMR genes in pigs
- Ribaxamase was well tolerated in dogs up to 57 mg/kg/day for 28 days
- Ribaxamase did not change the plasma PK of IV ceftriaxone in dogs