SYN-004, a Clinical-Stage, Orally Delivered β-Lactamase Therapy Protects the Gut Microbiome from IV Antibiotics

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Abstract

**Background:** Disruption of the gastrointestinal microbiota is a major, underappreciated consequence of antibiotic exposure that can lead to overgrowth of pathogenic organisms such as Clostridium difficile (CD), keystone bacteria that are central to normal gut ecology and epithelial health. Prior studies have demonstrated that antibiotics cause rapid and marked changes in the gut microbiome, which are associated with the emergence of antimicrobial resistance (AMR) and increased severity of antibiotic-associated diarrhea (AAD). Prevention of these effects is of high importance, especially as the resistance crisis deepens.

**Aims:** To evaluate the potential of SYN-004, a Novel β-Lactamase Inhibitor (BLI) and phosphate release formulation of amoxicillin, to prevent the antibiotic-induced disruption of the intestinal microbiome in piglets.

**Conclusions:** SYN-004 protected the gut microbiome in pigs from damage caused by IV CRO treatment further supporting its clinical potential in humans. Notably, SYN-004 protected the microbiome from amoxicillin-induced pathways, however SYN-004 interfered with the enteric pathogen E. coli, a proposed mediator of antibiotic related diarrhea. Novel, modified-release formulations of SYN-004 designed to release into the GI tract in a timed delay to the absorption of amoxicillin or colistin enough to degrade enough to maintain systemic antibiotic activity, are being tested. Therefore, SYN-004 has the potential to become the first therapy designed to protect the microbiota from enteric antibiotics and prevent AAD and CDI. The utility of SYN-004 to include not only as an IV antibiotic is being explored.

**Methods**

SYN-004 was manufactured in E. coli and formulated into enteric-coated pellets that release enzyme in the duodenum (at pH ~6.5). A pilot model of antibiotic-induced diarrhea was established using three strains (lactamase bacteria: ceftriaxone, a cephalosporin, etearamipen, a carbapenem, and amoxicillin, a penicillin). Normal piglets (~20 kg, n = 5 per cohort) were treated with 30 µg/kg bw ceftriaxone (in CRO), amoxicillin (30 µg/kg bw, PO; QID), SYN-004 (75 mg QID), or amoxicillin (200 mg/kg bw, PO, QID). SYN-004 was delivered for 5 days to separate cohorts that received ceftriaxone or amoxicillin starting the day before antibiotic treatment. Serum was collected on Day 2 of antibiotic treatment, and feces were collected on Days 1 and 2. Two additional antibiotic treatment groups were included: amoxicillin (200 mg/kg bw, PO, QID) and ceftriaxone (500 mg/kg bw, IV daily) in order to compare the effects of oral versus IV antibiotic treatments. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

**Results:** For CRO, serum levels were similar in the antibiotic-alone and antibiotic-SYN-004 cohorts indicating that SYN-004 did not alter systemic antibiotic levels. Microbiome analyses demonstrated that SYN-004 prevented CRO-mediated dysbiosis. While the preliminary analyses using an HPLC-based amoxicillin detection assay demonstrated that SYN-004 did not affect amoxicillin serum levels, repeat analysis using an LC-MS-based assay revealed that amoxicillin was undetectable in the pig in the presence of SYN-004. Microbiome analyses demonstrated that SYN-004 prevented amoxicillin-mediated loss of diversity and increased functional richness. SYN-004 increased stability in the gut microbiome, decreasing the number of days with phase changes from CRO to SYN-004 (75 mg QID). In parallel cohorts, animals received oral amoxicillin (200 mg/kg bw for 7 days) or amoxicillin-SYN-004. Serum antibiotic levels were measured and whole genome shotgun sequence analyses of pig fecal DNA were performed.

**Conclusion:** SYN-004 protected the gut microbiome in pigs from damage caused by IV CRO further supporting its clinical potential in humans. Notably, SYN-004 protected the microbiome from amoxicillin-induced pathways, however SYN-004 interfered with the enteric pathogen E. coli, a proposed mediator of antibiotic related diarrhea. Novel, modified-release formulations of SYN-004 designed to release into the GI tract in a timed delay to the absorption of amoxicillin or colistin enough to degrade enough to maintain systemic antibiotic activity, are being tested. Therefore, SYN-004 has the potential to become the first therapy designed to protect the microbiota from enteric antibiotics and prevent AAD and CDI. The utility of SYN-004 to include not only as an IV antibiotic is being explored.

**References**