Digestive Disease Week
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Title: SYN-004, a Clinical Stage Oral Beta-Lactamase Therapy, Protects the Intestinal Microflora from Antibiotic-Mediated Damage in Humanized Pigs

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Antibiotics (abx) that are excreted into the intestine, such as ceftriaxone (CRO), can damage the microflora and lead to serious illnesses such as *Clostridium difficile* infection (CDI). SYN-004 is a clinical stage oral beta-lactamase therapy for use with IV abx to preserve the microbiome by degrading residual abx within the intestine. Phase I clinical studies demonstrated safety and tolerability at all dose levels. Phase 2, expected to commence in 2015, will assess intestinal CRO degradation in ileostomy subjects.

SYN-004 was engineered from the *B. licheniformis* PenP enzyme to broaden its abx hydrolysis spectrum. SYN-004 efficiently inactivates a broad range of cephalosporins, including CRO, cefazolin, cefuroxime, and cefoperazone, in addition to the penicillins. SYN-004 was manufactured in *E. coli* and formulated into enteric-coated pellets. *In vitro*, the pellets remained intact at low pH (0.1 N HCl) and complete dissolution occurred at pH >5.5. In human chyme, SYN-004 enzyme activity was maintained for at least 6 hrs, demonstrating enzyme stability in human intestinal contents. Efficacy studies performed with CRO-treated (IV, 30 mg/kg) jejunal-fistulated dogs revealed high intestinal CRO levels (mean C_{max} of 1500 ug/g chyme at 90 min) that were completely eliminated in the presence of SYN-004 ($\leq$5ug/g chyme). The ability of SYN-004 to protect the intestinal microbiome from CRO-induced damage was evaluated in a preliminary study in humanized pigs. The GI tract of 5 day old gnotobiotic pigs was populated with human adult fecal microflora. Two days later, animals received CRO (IP, 30 mg/kg) for 4 days. SYN-004 was delivered orally 4 times a day for 7 days beginning the day before CRO administration. Microbiome changes were monitored by high-throughput sequencing of the 16S rRNA gene V1V2 region using fecal DNA. The levels of a specific bacterial population expected to be sensitive to CRO, ampicillin-resistant aerobes including those of the phylum Proteobacteria, was assessed by plating equal amounts of feces on LB+amp plates. The figure displays the phylum-level taxonomic classifications and the quantification of the LB+amp bacterial growth. The Control (no abx) and CRO+SYN-004 cohorts showed good representation by Bacteroidetes, Proteobacteria and Firmicutes, while the CRO alone cohort displayed dysbiosis, with Bacteroidetes as the greatly predominant phylum. The LB+amp data corroborate these findings, as the CRO+SYN-004 cohort displayed similar, high bacteria levels as the Control, while the CRO alone cohort displayed at least 2 log lower levels, suggesting a reduction in the Proteobacteria population.

These data demonstrate that SYN-004 has the potential to protect the human microbiome and to become the first prophylactic therapy designed to prevent abx-mediated microbiome damage, including CDI, in patients receiving beta-lactam abx.

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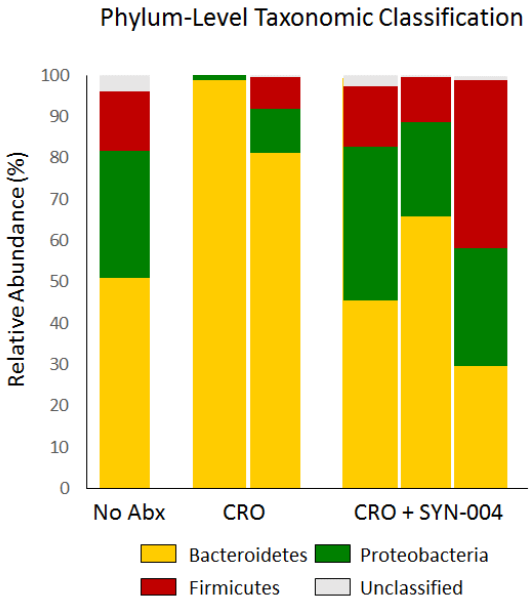
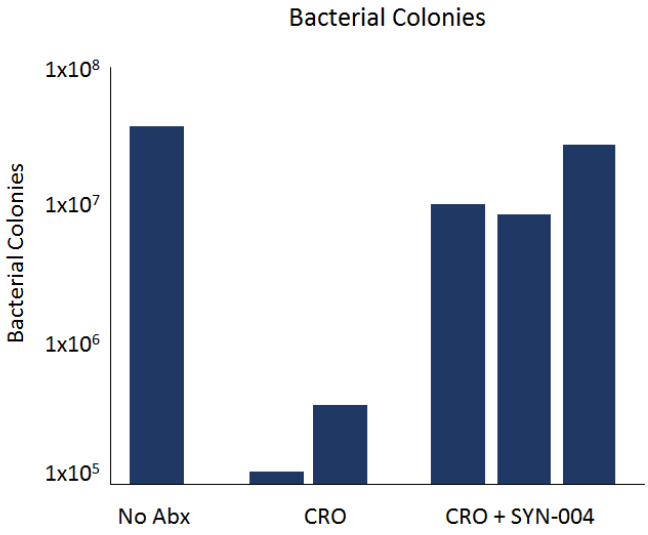

Phylum-Level Taxonomic Classification
Relative Abundance (%)
100
90
80
70
60
50
40
30
20
10
0
No Abx
CRO
CRO + SYN-004
Bacteroidetes
Proteobacteria
Firmicutes
Unclassified


Bacterial Colonies
Bacterial Colonies
1x10^8
1x10^7
1x10^6
1x10^5
No Abx
CRO
CRO + SYN-004