Beta-lactam antibiotics (abs) are excreted in bile and can damage the colonic microbiota leading to infection. SYN-006 is an orally-delivered beta-lactamase intended to degrade certain intravenous (IV) abs in the gut. A Phase 2 clinical study is in progress to assess SYN-006-mediated prevention of C. difficile infection (CDI) and abs-associated diarrhea (AAD). SYN-006 degrades penicillins and cephalosporins, but not carbapenems. We identified SYN-006, a carba-beta-lactamase derived from B. cereus, was produced in E. coli with yields of ~600 mg/L at 95% purity. Using a bacterial growth assay as a readout for abs degradation, SYN-006 displayed a broad degradation profile that included carbapenems, penicillins, and cephalosporins, and was resistant to beta-lactamase inhibitors. SYN-006 retained activity for at least 6 h in human chyme. The inactivation of the carbapenem, meropenem (30 mg/L), in the GI tract was evaluated in a pig model commensal microbiota (C57BL/6 R6, 17 kg). While SYN-006 showed variable levels, mostly likely due to its susceptibility to low pH at 4.5 for concentrations of ~0.15 U/mL, meropenem was unadsorbable. SYN-006 did not affect serum meropenem levels, verifying it functioned solely in the GI tract. SYN-006 is currently being formulated into enteric-coated pellets that releases at pH >5.5. To evaluate the effect of carba-beta-lactamase inhibition on the gut microbiota, animals (20 kg, n=5) were treated with enteripenem (30 mg/kg, IV, q.d.) for 7 days. Analysis of microbiota data from local DNA whole genome shotgun sequencing demonstrated that enteripenem caused dysbiosis, including loss of species diversity in the gut (Heatmap, p=0.04; Ratio Test, p=0.0014). Efficacy studies using the enteric-coated SY006 are being planned.

These data demonstrate that SYN-006 displays manufacturability and sufficient potency to continue to be developed into a potential oral prophylaxis. SYN-006 has the potential to protect the microbiome from all classes of beta-lactam to defend CDI and AAD.

### E. Coli Protein Expression and Purification

E. coli SYN-006 production strains were evaluated for expression via SDS/PAGE and Antibiotic Conc. (mL/L) was checked using C. E. coli and C. A. E. coli. SYN-006 was purified with a yield of 0.6 g. Purified SYN-006 retained full biological activity. Scale-up and production in a fermenter has the potential to substantially increase SYN-006 manufacturability.

### Antibiotic Degradation Profile

The purified beta-lactamase enzymes were assessed for antibiotic hydrolysis potency with a panel of antibiotics using E. coli, as the need for antibiotic inactivation. The total of 10 to 1000 μg of each antibiotic was mixed with 100 ng SYN-006 or 4% OVA. E. coli was added and grown quantitatively. The graph displays the highest antibiotic concentration at which bacterial growth was observed, indicating antibiotic inactivation.

### SYN-006 Stability in Human Chyme

Resistance to degradation by intestinal enzymes is a key attribute for orally-delivered enzymes. To determine if SYN-006 retained biological activity in human intestinal fluid, we evaluated SYN-006 stability in human chyme. SYN-006 was instilled in mixed human chyme or each of five human samples and enzyme activity was determined using the CENTA enzymatic assay.

### SYN-006 Degradates Meropenem in the Dog GI Tract

Jejuno-fistulated dogs (n=6) received meropenem (20 mg/kg, IV) alone or with a co-administration of SYN-006 (100 mg/kg, PO). Meropenem was delivered immediately after antibiotic injection. Meropenem and SYN-006 levels in the jejunal contents of each dog were evaluated.

### SYN-006 Protects the Microbiome from Antibiotic-Mediated Damage

Meropenem levels in the chyme of animals treated with meropenem alone were ~3.5-fold higher than in SYN-006-treated dogs, with levels of <50 μg/ml in all samples. SYN-006, at a concentration of 1 mg/kg, effectively protected the gut microbiome from meropenem-mediated dysbiosis. Levels of meropenem decreased by ~95% in SYN-006-treated dogs, compared to treatment with meropenem alone. This suggests that SYN-006 is a potent and effective therapeutic agent for preventing antibiotic-induced dysbiosis.

### Heatmap

The heatmap analyses compare the bacterial species present in the microbiomes of dogs prior to and after treatment with SYN-006. Treatment with SYN-006 significantly decreased the bacterial species diversity (Beta diversity) in the gut microbiome, indicating a reduction in antibiotic-mediated dysbiosis.

### Likelihood Ratio Test

The likelihood ratio test (LRT) was used to compare the microbiome populations prior to and after antibiotic treatment. The LRT values were calculated using the likelihood ratio test (LRT) with a significance level of 0.05. The LRT test showed a significant difference between the microbiome populations before and after antibiotic treatment.

### REFERENCES