Title: Development of Therapeutic Agents That Protect the Colonic Microflora from Beta-Lactam Antibiotics for the Prevention of *Clostridium difficile* Infection

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Beta-lactam antibiotics (abx) are excreted in the bile, which can damage the colonic microflora and lead to serious illnesses such as *Clostridium difficile* infection. SYN-004 is a beta-lactamase (BLA) currently in human trials that is given orally to degrade abx in the small intestine to protect the microbiome. SYN-004 degrades penicillins and cephalosporins, but not carbapenems. To expand this prophylactic approach to beta-lactam abx, we are evaluating the potential to develop product candidates from three broad spectrum carbapenemases, P2A, NDM-1, and KPC-1. Here we determined how effectively each could be manufactured and then assessed their relative BLA potencies *in vitro* for 16 antibiotics.

Over 100 different *E. coli* production strains for P2A, NDM, and KPC were evaluated for expression (SDS/PAGE) and activity (CENTA chromogenic assay). For the metallo-enzymes, P2A and NDM, the addition of zinc was found to shift expression from inclusion bodies to the soluble fractions. The highest expressing strains for each BLA were chosen for 5L bioreactor fermentation and chromatographic purification (95%). Final yields for each were ~600 mg/L. A microtiter assay using *E. coli* growth as the read-out for abx degradation was used to assess the potencies of each BLA with 16 abx. A total of 10 to 1000 µg/ml of each abx was mixed with 10 ng/ml of each BLA. *E. coli* was added and growth quantified. The graph displays the highest concentration of each abx with bacterial growth. Compared to SYN-004, P2A, NDM, and KPC all displayed broader degradation profiles that included carbapenems. NDM was the most potent BLA and efficiently degraded all tested cephalosporins and carbapenems. NDM was resistant to the inhibitors sulbactam and tazobactam. KPC was the only BLA with activity against the monobactam, aztreonam, albeit at a 10-fold higher BLA concentration.

These data indicate that all three BLAs can be manufactured and have sufficient potency to be developed into oral therapeutics. Each has the potential to protect the microbiome from most, if not all beta-lactam antibiotics and provide prophylaxis against *Clostridium difficile* infection. We are currently assessing the stability of each in human chyme and plan to advance the best candidate to animal modeling and clinical application.
Beta-Lactamase Concentration, 10 ng/ml

AMP: ampicillin; SAM: ampicillin/sulbactam; PIP: piperacillin; TZP: piperacillin/tazobactam; CRO: ceftriaxone; CTX: cefotaxime; CFZ: cefozolin; CXM: cefuroxime; CFP: cefoperazone; FEP: cefepime; CAZ: cefazolin; MEM: meropenem; IPM: imipenem; ERT: ertapenem; DOR: doripenem; ATM: aztreonam

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