Development of Orally-Delivered Therapeutics to Protect the Gut Microbiome from Antibiotic-Mediated Damage

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ABSTRACT

Beta-lactam antibiotics (abx) are excreted in bile and can damage the colonic microflora leading to serious adventitious infections. SYN-004 is an orally-delivered beta-lactamase intended to degrade certain intravenous (IV) abx in the gut to protect the microbiome. A Phase 2b clinical study is in progress to assess SYN-004-mediated prevention of *C. difficile* infection (CDI) and abx-associated diarrhea (AAD). SYN-004 degrades penicillins and cephalosporins, but not carbapenems. We identified SYN-006, a carbapenemase, to expand this prophylactic approach to all beta-lactam abx classes.

SYN-006, a metallo-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 abx 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 hrs in human chyme. The inactivation of the carbapenem, meropenem (30 mg/kg, IV), in the GI tract was evaluated in fistulated dogs (n=6) using SYN-006 formulated in PBS, pH 7.5 (1 mg/kg). While SYN-006 jejunal levels were variable, most likely due to its sensitivity to low pH, at SYN-006 concentrations of $\geq$ 0.5 U/ml, meropenem was undetectable. 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 release at pHs >5.5. To evaluate the effect of carbapenems on the gut microbiome, a piglet model was developed. Animals (20 kg; n=5) were treated with ertapenem (30 mg/kg, QD, IV) for 7 days. Analysis of microbiome data from fecal DNA whole genome shotgun sequencing demonstrated that ertapenem caused dysbiosis, including loss of species diversity and changes in species abundance (Likelihood Ratio Test, p=7.0 x 10^{-16}). Pig efficacy studies using the enteric-coated SYN-006 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 abx to defend against CDI and AAD.

BACKGROUND

IV beta-lactam antibiotics, including cephalosporins, are excreted via the bile into the intestine where they can disrupt the intestinal microflora and potentially lead to the outgrowth of pathogens like *Clostridium difficile*. SYN-004 is a clinical stage, oral beta-lactamase enzyme therapy for use with IV beta-lactam antibiotics to preserve the gut microflora by degrading residual antibiotics in the intestine [1]. The intended indications are prevention of *C. difficile* infection (CDI) and antibiotic-associated diarrhea (AAD). Phase I clinical studies demonstrated SYN-004 was well tolerated. SYN-004 was neither systemically bioavailable nor immunogenic in humans. Phase 2a clinical studies conducted in subjects with functioning ileostomies demonstrated that SYN-004 effectively degraded ceftriaxone in the GI tract without affecting systemic antibiotic levels, and that the use of proton pump inhibitors did not affect SYN-004 efficacy. A Phase 2b clinical study is currently enrolling to examine the ability of SYN-004 to prevent CDI and AAD in patients being treated with IV ceftriaxone for a lower respiratory tract infection.

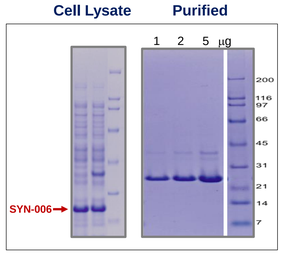
SYN-004 preclinical and clinical data are being presented in Poster 456.

While SYN-004 degrades penicillins and cephalosporins, it has not been shown to inactivate carbapenems. To expand this prophylactic approach to all classes of beta-lactam antibiotics, we are developing SYN-006, a broad spectrum carbapenemase.

RESULTS

E. Coli Protein Expression and Purification

E. coli SYN-006 production strains were evaluated for expression via SDS/PAGE and antibiotic hydrolysis activity using the CENTA chromogenic assay.



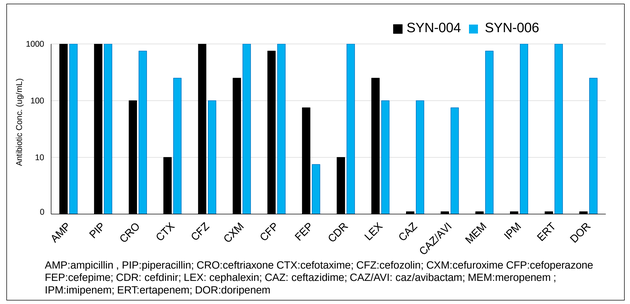
- ✓ Generated high-expressing *E. coli* cell line
- ✓ Verified biological activity of the SYN-006 protein
- ✓ Developed a facile purification process
- ✓ Scale-up and formulation in progress

SYN-006 is a class B metallo-enzyme that requires zinc for antibiotic hydrolysis activity. The addition of zinc to the bacterial growth media was found to shift expression from inclusion bodies to the soluble fractions.

SYN-006 was purified with a yield of 0.6 g/L. Purified SYN-006 retained full biological activity. Scale-up and production in a fermenter has the potential to substantially increase SYN-006 yield. These data demonstrate that SYN-006 is manufacturable.

Antibiotic Degradation Profile

The purified beta-lactamase enzymes were assessed for antibiotic hydrolysis potency with a microtiter plate activity assay using *E. coli* growth as the read-out for antibiotic inactivation. A total of 10 to 1000 μg/ml of each antibiotic was mixed with 100 ng/ml of SYN-004 or SYN-006. *E. coli* was added and growth quantified. The graph displays the highest antibiotic concentration at which bacterial growth was observed, indicating antibiotic inactivation.

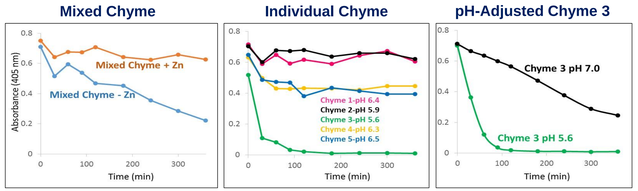


AMP:ampicillin , PIP:piperacillin; CRO:ceftriaxone CTX:cefotaxime; CFZ:cefozolin; CXM:cefuroxime CFP:cefoperazone FEP:cefepime; CDR: cefdinir; LEX: cephalexin; CAZ: ceftazidime; CAZ/AVI: caz/avibactam; MEM:meropenem ; IPM:imipenem; ERT:ertapenem; DOR:doripenem

SYN-006 displayed a broader antibiotic degradation profile that included the carbapenems (MEM, IPM, ERT, DOR), compared to SYN-004. SYN-006 showed good activity against the penicillins (AMP, PIP) and the cephalosporins (CRO, CTX, CFZ, CXM, CFP, CDR, LEX, CAZ) and was not inhibited by the beta-lactamase inhibitor avibactam (AVI).

SYN-006 Stability in Human Chyme

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

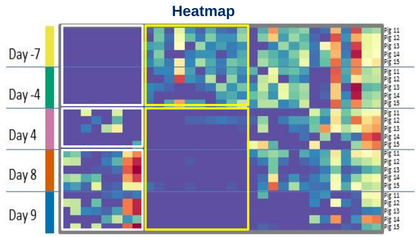


SYN-006 displayed stable biological activity in human mixed chyme and 4 of 5 individual chyme samples with 100 μM $ZnSO_4$. Notably, SYN-006 was rapidly inactivated in Chyme 3. As Chyme 3 had the lowest pH, SYN-006 was evaluated in pH-adjusted Chyme 3. Increasing the pH to 7.0 improved SYN-006 stability. SYN-006 is sensitive to low pH and retains activity in human chyme.

Ertapenem Rapidly Disrupts the Pig Gut Microbiome

To evaluate SYN-006 efficacy in protecting the microbiome from the effect of beta-lactam antibiotics, including carbapenems, a pig model of ertapenem-mediated dysbiosis was established. Normal piglets (20 kg, n=5) were treated with IV ertapenem (30 mg/kg, IV, SID) for 7 consecutive days. Feces were collected at two pre-antibiotic time points (Days -7 and -4), during treatment (Day 4), and after antibiotics were stopped (Days 8 and 9). Fecal DNA was subjected to whole genome shotgun sequence analyses.

Heat map analyses of the fecal microbial community was based on species relative abundance. Each square represents a bacterial species present in individual animal microbiomes. The species are indicated horizontally, and the fecal collection day and animal are displayed vertically. The yellow and white boxes display changes in species diversity caused by ertapenem treatment;. The Dirichlet-Multinomial model likelihood ratio test [2] was used to compare the microbiome populations prior to and after antibiotic treatment.



Likelihood Ratio Test

Treatment Group	Chi squared	P value
Day -4	-31.72	1.00
Day 4	387.73	$1.7x10^{-9}$
Day 8	720.56	$<1x10^{-25}$
Day 9	655.16	$7.0x10^{-16}$

The heatmap analyses compare the bacterial species present in the microbiomes of pigs prior to and after ertapenem treatment and reveal that the antibiotic caused the depletion of some species (yellow boxes) and the overgrowth of others (white boxes). The likelihood ratio test compares the microbiomes prior to ertapenem treatment (Day -4) to the microbiomes after antibiotic exposure. The microbiomes at all time points after antibiotic treatment were significantly different from the pre-exposure microbiomes. A pig model of ertapenem-mediated dysbiosis was successfully established.

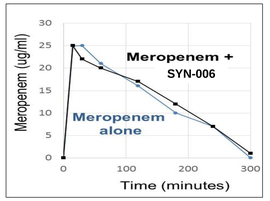
SYN-006 Degrades Meropenem in the Dog GI Tract

Jejunally-fistulated dogs (n=6) received meropenem (30 mg/kg, IV) alone or with a liquid formulation of SYN-006 (1 mg/kg, PO). The SYN-006 was delivered immediately after antibiotic injection. Meropenem and SYN-006 levels in the jejunal contents were measured, and meropenem serum levels were evaluated.

Chyme Meropenem and SYN-006

Treatment (n=3)	Dog	SYN-006 (U/g)	Meropenem (ug/g)
Meropenem Alone	1	NA	3.0
	2	NA	3.2
	3	NA	3.0
Meropenem + SYN-006	4	80	0
	5	0.5	0
	6	0.2	2.0

Serum Meropenem



Meropenem levels in the chyme of animals treated with meropenem alone were ~3.0 ug/g. In the presence of SYN-006, meropenem levels were undectable in two animals, or 2.0 ug/g in one animal. SYN-006 chyme levels were extremely variable, ranging from 80 U/g to 0.2 U/g. This variability is likely due to SYN-006's sensitivity to acid, as the enzyme was delivered orally in a non-enteric-coated liquid formulation. SYN-006 had no effect on systemic meropenem levels. Therefore, when present in the chyme at levels of 0.5 U/g or higher, SYN-006 completely degraded the meropenem in the dog GI tract without affecting meropenem serum levels.

CONCLUSIONS

- SYN-006 demonstrated manufacturability in *E.* coli
- SYN-006 has a broad antibiotic degradation profile including penicillins, cephalosporins, and carbapenems
- A pig model of ertapenem-mediated dysbiosis was established
- In dogs, oral SYN-006 resulted in degradation of intestinal meropenem and did not affect meropenem serum levels
- An enteric-coated formulation of SYN-006 is currently in production and is expected to be tested in the pig dysbiosis model

SYN-006 has the potential to become a prophylaxis to protect the microbiome from certain IV beta-lactam antibiotics including carbapenems and to prevent CDI and AAD

REFERENCES

1. Kaleko, M. *et al.* (2016) Development of SYN-004, an Oral Beta-Lactamase Treatment to Protect the Gut Microbiome from Antibiotic-Mediated Damage and Prevent *Clostridium difficile* infection. *Anaerobe Epub*, http://dx.doi.org/10.1016/j.anaerobe.2016.05.015
2. La Rosa, P. S. *et al.* (2012) Hypothesis Testing and Power Calculations for Taxonomic-Based Human Microbiome Data. *PLoS ONE* **7,** e52078