The Oral β-Lactamase SYN-004 (ribaxamase), Designed to Protect the Gut Microbiome from Biliary Excreted IV Antibiotics, Efficiently Degrades Ceftriaxone in Two Phase 2a Clinical Trials

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John F. Kokai-Kun[*1], Tracey Roberts[1], Eric Sicard[2], Marianne Rufiange[2], Richard Fedorak[3], Christian Carter[3], Olivia Coughlin[1], Heidi Whalen[1], Klaus Gottlieb[1], Joseph Sliman[1]*

[1] Synthetic Biologics, Inc., Rockville, MD, [2] Algorithme Pharma, Laval, QC, [3] University of Alberta/Alberta Health Service , Edmonton, AB.

jkokai-kun@syntheticbiologics.com
301-417-4353

Synthetic BIOLOGICS

ABSTRACT

Background: SYN-004 (ribaxamase) is an orally-administered recombinant β-lactamase designed to be given during treatment with certain IV β-lactam antibiotics (ABX) for prevention of the adverse effects on the gut microbiome. SYN-004 is designed to be released in the small intestine when the pH > 5.5 to degrade β-lactam ABX excreted into the intestine via the bile. This is expected to protect the gut microbiome from disruption, prevent secondary infections like *C. difficile* and reduce emergence of ABX resistance in gut bacteria. SYN-004 must degrade β-lactam ABX in the GI, without affecting systemic ABX PK. Phase 1 clinical trials of SYN-004 have demonstrated good tolerability with no systemic absorption in healthy volunteers.

Methods: Two Phase 2a studies have now been conducted in subjects with functioning ileostomies to allow sampling of their intestinal chyme and support the mechanism of action of SYN-004. In the first study, subjects were administered 1 g of IV ceftriaxone (CRO) alone and in combination with one of two dose strengths of SYN-004. In the second study, CRO plus SYN-004 was administered in the presence or absence of the proton pump inhibitor (PPI), esomeprazole, to determine the effect of pH change on SYN-004 activity. Serial plasma and intestinal chyme samples from each study were analyzed for their concentrations of CRO and SYN-004.

Results:

1) SYN-004 effectively degrades CRO excreted into the small intestine
2) The plasma PK of CRO is unchanged by oral SYN-004 administration
3) SYN-004 is not detected in the plasma
4) The use of proton pump inhibitors does not reduce SYN-004 efficacy
5) SYN-004 is well tolerated when administered with IV CRO

Of particular importance is that co-administration with a PPI did not impact and may, in some cases, enhance SYN-004's ability to degrade CRO in the gut. In a hospital clinical setting, this is of importance since many patients are on concomitant PPI therapy.

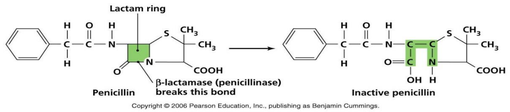
Conclusion: These two studies support SYN-004's ability to degrade certain IV β-lactam ABX in the human intestine and support progression into a currently enrolling Phase 2b clinical trial to examine its capacity to prevent *C. difficile*-associated disease and antibiotic-associated diarrhea in patients being treated with IV CRO for lower respiratory tract infection.

BACKGROUND

The use of intravenous β-lactam antibiotics, including cephalosporins, are a risk factor for the development of gastrointestinal infections like *Clostridium difficile*. These antibiotics are excreted, via the bile, into the intestine where they can disrupt the gut microbiome and potentially lead to the growth of opportunistic pathogens like *C. difficile*.

SYN-004 is a novel recombinant β-lactamase enzyme which is delivered orally with the intent of degrading the excess IV β-lactam antibiotics excreted into the intestine thus protecting the gut microbiome. The indications being pursued is prevention of *C. difficile* infection (CDI) and antibiotic associated diarrhea (AAD). The use of SYN-004 may also have the added benefit of reducing the development of antibiotic resistance in the gut microflora. Adding SYN-004 to any treatment with IV β-lactam antibiotics would represent a paradigm shift from the current paradigm where an antibiotic treats the primary infection but increases the risk for development of opportunistic infections like CDI, to a paradigm where highly-effective IV β-lactam antibiotics can be administered without this risk.

SYN-004 degrades β-lactam antibiotics (including most penecillins and cephalosporins) by cleaving the β-lactam ring.



Preclinical and nonclinical studies have demonstrated that SYN-004 can inactivate most β-lactam antibiotics *in vitro* and in animal models. Further, SYN-004 has demonstrated good stability in isolated human intestinal chyme.

Kaleko et al. 2016. Anaerobe (Published on line, 6 June 2016).

SYN-004 Non-clinical Experience

- SYN-004 effectively degrades IV administered ceftriaxone excreted into the intestine of dogs in a fistulated dog model.
- SYN-004 is well tolerated by dogs when orally administered t.i.d. for 28 days up to 57 mg/kg/day with minimal and sporadic systemic absorption of the enzyme detected.
- SYN-004 (t.i.d. dosing of 57mg/kg/day) is well tolerated by dogs when co-administered with IV ceftriaxone (1g, qd) for 14 days, with no significant change in the plasma PK of the ceftriaxone.

Kokai-Kun et al. 2016. International Journal of Toxicology. (Published on line, 23 Dec. 2015). 35: 309-316.

SYN-004 Phase 1 Clinical Experience

- SYN-004 was well tolerated in a Single Ascending Dose (SAD) study up to 750 mg in normal healthy volunteers.
- There was minimal and sporadic systemic absorption of SYN-004 detected (assay LLOQ = 0.8 ng/ml) and no anti-drug antibodies were detected in the SAD study.
- SYN-004 was well tolerated in a Multiple Ascending Dose (MAD) study up 300 mg, qh6 for 7 days in normal healthy volunteers.
- There was negligible systemic absorption of SYN-004 detected and no anti-drug antibodies were detected in the MAD study.

Roberts et al. 2016. Clinical Drug Investigation. 36: 725-734)

CURRENT STUDY DESIGNS

SYN-004 Phase 2a Clinical Mechanism of Action Studies

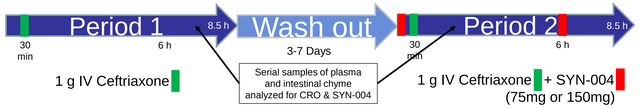
To advance the clinical development of SYN-004, two Phase 2a clinical studies were undertaken in otherwise healthy subjects with functioning ileostomies. The use of this subject group allowed for serial sampling of their intestinal chyme to answer several questions regarding SYN-004.

1. Is SYN-004 well tolerated in humans when co-administered with IV ceftriaxone?
2. Does SYN-004 effectively degrade IV-administered ceftriaxone excreted into the intestine?
3. Does SYN-004 affect the plasma concentrations of IV ceftriaxone?
4. Can SYN-004 be detected in the plasma of the ileostomy subjects?
5. Since the SYN-004 formulation is designed to release active enzyme at pH >5.5, does the use of the proton pump inhibitor (PPI), esomeprazole, affect the activity of SYN-004 *in vivo*?

SYN-004 Phase 2a Clinical Studies in Ileostomy Subjects

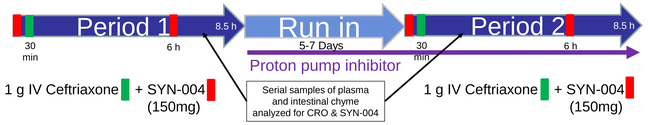
Study 1: IV Ceftriaxone (CRO) +/- SYN-004 (n=10)

A PHASE 2A, RANDOMIZED, MULTI-CENTER, OPEN-LABEL, SINGLE-DOSE, FIXED SEQUENCE STUDY TO EVALUATE THE EFFECT OF ORAL SYN-004 ON THE PHARMACOKINETICS OF INTRAVENOUS CEFTRIAXONE IN HEALTHY ADULT SUBJECTS WITH A FUNCTIONING ILEOSTOMY



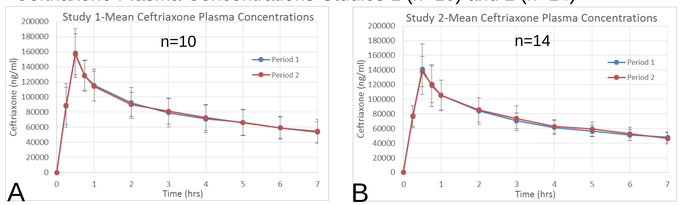
Study 2: IV Ceftriaxone & SYN-004 +/- Esomeprazole (n=14)

A PHASE 2A, MULTI-CENTER, OPEN-LABEL, 2 PERIOD, FIXED SEQUENCE STUDY EVALUATING THE EFFECT OF ESOMEPRAZOLE ON SYN-004 DEGRADATION OF CEFTRIAXONE IN HEALTHY ADULT SUBJECTS WITH A FUNCTIONING ILEOSTOMY



RESULTS

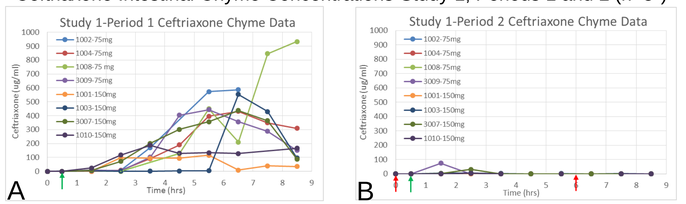
Ceftriaxone Plasma Concentrations-Studies 1 (n=10) and 2 (n=14)



Plasma concentrations of ceftriaxone (ng/ml) vs. time (hrs). Mean ceftriaxone concentration vs. time profiles in plasma were superimposable after a 1 g IV infusion with and without concomitant oral SYN-004 administration, Study 1 (A). Mean ceftriaxone concentration vs. time profiles in plasma were superimposable after a 1 g IV infusion in the presence of oral SYN 004 with and without concomitant oral esomeprazole at steady-state, Study 2 (B).

The ceftriaxone concentration in plasma was determined by a validated LC/MS-MS method. Assay LLOQ = 100 ng/ml

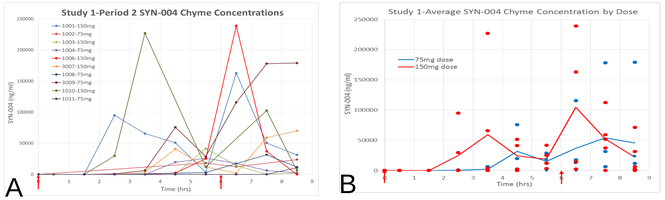
Ceftriaxone Intestinal Chyme Concentrations-Study 1, Periods 1 and 2 (n=8*)



Intestinal chyme concentrations of ceftriaxone (µg/ml) vs. time (hrs). Ceftriaxone (administered as a 1g IV infusion, green arrow) began to be detected in the chyme ~1 hr post infusion and peaked around 5 – 6 hrs for most subjects (A). Beginning ~3.5 hrs after SYN-004 oral administration (red arrows), mean ceftriaxone concentrations in intestinal chyme were substantially decreased by concomitant SYN-004 administration (B). Residual ceftriaxone in intestinal chyme was degraded when SYN-004 was present, and mean ceftriaxone concentrations were generally lower for the 150 mg dose regimen than for the 75 mg dose regimen. *Note, for subjects 1006 and 1011, the SYN-004 was detected later in their chyme (6.5 and 8.5 hrs), but still fully degraded ceftriaxone once it appeared, data not shown.

Intestinal chyme samples were collected from ileostomy bags of dosed subjects every hour (when present) and snap frozen for transport to the analytical laboratory. The chyme samples were thawed on ice and then extracted with 8M guanidine to neutralize any residual β-lactamase activity in the samples. The chyme samples were vigorously vortexed and then centrifuged to remove solids. The supernatants were extracted with acetonitrile and then the ceftriaxone concentrations in intestinal chyme were determined by a qualified LC/MS-MS method. Assay LLOQ = 1 µg/ml.

SYN-004 Intestinal Chyme Concentrations-Study 1, Period 2 (n=10)

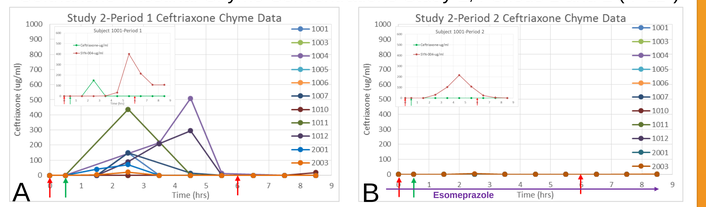


Intestinal chyme concentrations of SYN-004 (ng/ml) vs. time (hrs). Beginning ~2.5-4.5 hrs after SYN-004 oral administration (red arrows), SYN-004 began to be detected in intestinal chyme for some subjects and a second SYN-004 peak was detected at ~6.5-7.5 hrs following a second dose of SYN-004 (A). Concentrations of SYN-004 in intestinal chyme, though variable, were generally similar to or higher for the 150 mg dose regimen than for the 75 mg dose regimen (B).

The SYN-004 concentrations in intestinal chyme were determined in chyme supernatant by a qualified ECL method. Assay LLOQ = 10 ng/ml

The SYN-004 concentrations in plasma were determined by a validated ECL method. Assay LLOQ = 0.8 ng/ml (data not shown)

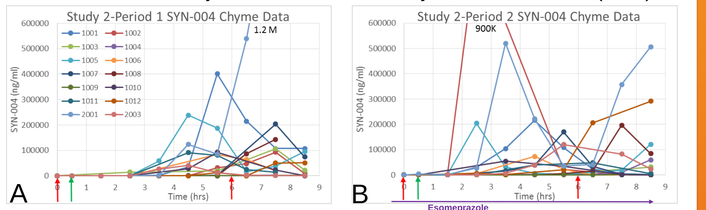
Ceftriaxone Intestinal Chyme Concentrations-Study 2, Periods 1 and 2 (n=11*)



Intestinal chyme concentrations of ceftriaxone (µg/ml) vs. time (hrs). Ceftriaxone began to be detected in the chyme ~1 hr post IV infusion (green arrow) but was degraded by 5 hrs post infusion in all subjects which coincided with the appearance of SYN-004 (A), administered at the red arrows. Ceftriaxone and SYN-004 concentrations overlaid for subject 1001 for comparison (inset). Beginning ~3 hrs after the first SYN-004 oral administration (red arrows), mean ceftriaxone concentrations in intestinal chyme were substantially lower in the presence of SYN-004 and steady-state oral esomeprazole (purple arrow) (B) than in the presence of SYN 004 alone (A). Ceftriaxone and SYN-004 concentrations overlaid for subject 1001 for comparison (inset). Residual ceftriaxone in intestinal chyme appeared to be degraded early when SYN-004 was co-administered with steady-state esomeprazole than when SYN-004 was administered alone over the course of the sampling period *Note, for subjects 1002, 1008 and 1009, the SYN-004 was detected later in their chyme, but fully degraded ceftriaxone once it appeared, data not shown.

The ceftriaxone concentrations in intestinal chyme were determined by a qualified LC/MS-MS method. Assay LLOQ = 1 µg/ml.

SYN-004 Intestinal Chyme Concentrations-Study 2, Periods 1 and 2 (n=14)



Intestinal chyme concentrations of SYN-004 (ng/ml) vs. time (hrs). Beginning ~2.5-4.5 hrs after SYN-004 oral administration (red arrows) in the absence of steady state esomeprazole, SYN-004 began to be detected in intestinal chyme with a second peak being detected at ~7 hrs following a second dose of SYN-004 (A). SYN-004 began to be detected in intestinal chyme ~1 .5 hours after oral administration of SYN-004 (red arrows) in the presence of steady state esomeprazole (purple arrow) with the second peak of SYN-004 also being detected ~ 1 hr after the second dose of SYN-004 (B). Lines colors for individual subjects are the same in graph B as in graph A.

The SYN-004 concentrations in intestinal chyme were determined in chyme supernatant by a qualified ECL method. Assay LLOQ = 10 ng/ml

CONCLUSIONS

- **SYN-004 is well tolerated when co-administered with IV ceftriaxone**
- **SYN-004 effectively degrades ceftriaxone in intestinal chyme to below the level of detection when SYN-004 is present**
- **SYN-004 does not significantly alter the plasma PK of ceftriaxone**
- **SYN-004 was not detected in the plasma of the subjects in Study 1**
- **SYN-004 can be administered with a PPI, and this appears to lead to earlier release of enzyme from the pH dependent formulation which correlates with earlier degradation of ceftriaxone after the 1st dose**

SYN-004 (ribaxamase) is now in a Phase 2b clinical trial for prevention of CDI and AAD in patients being treated with ceftriaxone for a LRTI