

# SYN-004 (Ribaxamase), an Orally Administered $\beta$ -Lactamase, Prevents *Clostridium difficile* Infection, Reduces New Colonization by Opportunistic Pathogens and Reduces Changes in the Gut Resistome

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## ABSTRACT

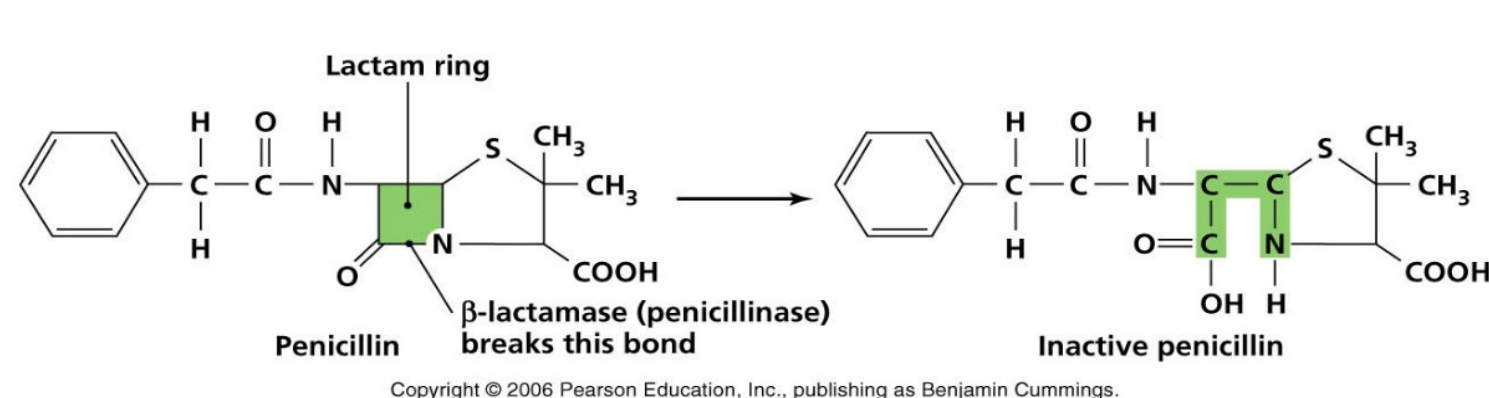
SYN-004 (ribaxamase) is an orally administered  $\beta$ -lactamase designed to be given with intravenous  $\beta$ -lactam antibiotics. Ribaxamase remains in the intestinal lumen to degrade  $\beta$ -lactam antibiotics which are excreted into the intestine. Degradation of excess antibiotics is expected to protect the gut microbiome from disruption and thus prevent secondary infections like *Clostridium difficile*, colonization by opportunistic pathogens and the emergence of antimicrobial resistance in the gut microbiome. Ribaxamase was shown to be well tolerated in Phase 1 clinical studies and efficiently degrade ceftriaxone excreted into the human intestine in Phase 2 studies. Ribaxamase did not alter the plasma pharmacokinetics of the ceftriaxone as it was not systemically bioavailable when delivered orally. A multinational, double blind, placebo controlled, efficacy study was conducted to determine whether ribaxamase could prevent *C. difficile* infection (CDI) with additional endpoints for antibiotic-associated diarrhea, colonization by opportunistic pathogens, changes in the balance of the gut microbiome and changes to the gut resistome. The mITT population was 412 patients who were admitted to the hospital for treatment of a lower respiratory tract infection. As treatment, the patients were expected to receive at least 5 days of IV ceftriaxone and were randomized 1:1 to receive either ribaxamase or placebo during ceftriaxone treatment and for a short time after. The patients could also receive an oral macrolide at the discretion of the clinical investigators. Fecal samples were collected at pre-specified points for determination of bacterial colonization with specific pathogens and to examine changes to the gut microbiome and resistome. The patients were monitored for diarrhea for 6 weeks during which CDI was defined as diarrhea (3 or more loose or watery stools in a 24 hour period) plus the presence of *C. difficile* toxin (as determined by the local clinical laboratory). The study was powered at 80% for the reduction in CDI with 1-sided alpha = 0.05. The study met its primary endpoint with a 71% relative risk reduction in CDI (1-sided p=0.045) in the ribaxamase group as compared with the placebo group and a statistically significant 44% relative risk reduction in new colonization by vancomycin resistant enterococci (1-sided p=0.0002) in patients who received ribaxamase. Ribaxamase also reduced changes in the gut resistome as compared with placebo. These data are consistent with ribaxamase maintaining the balance of the gut microbiome and preventing the deleterious effects of antibiotic treatment thus preventing CDI.

## BACKGROUND

The use of intravenous  $\beta$ -lactam antibiotics, including cephalosporins, are an important risk factor for the development of gastrointestinal infections like *Clostridium difficile*. These antibiotics can be excreted, via the bile, into the intestine where they disrupt the balance of the gut microbiome and potentially lead to the growth of opportunistic pathogens like *C. difficile* and the emergence of antimicrobial resistant organisms.

SYN-004 (ribaxamase) is a novel recombinant  $\beta$ -lactamase (an enzyme of ~29kDa) which is delivered orally with the intent of degrading excess IV  $\beta$ -lactam antibiotics excreted into the intestine thus protecting the gut microbiome from disruption. The primary indication being pursued is prevention of *C. difficile* infection (CDI). 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  $\beta$ -lactam antibiotics would represent a paradigm shift from the current model where an antibiotic treats the primary infection but often increases the risk for development of opportunistic infections like CDI, to a paradigm where highly-effective IV  $\beta$ -lactam antibiotics can be administered with substantially reduced risk.

SYN-004 degrades  $\beta$ -lactam antibiotics (including most penicillins and cephalosporins) by cleaving the  $\beta$ -lactam ring.



## SYN-004 Preclinical Experience

- SYN-004 inactivates most  $\beta$ -lactam antibiotics *in vitro* and in animal models
- SYN-004 has good stability in isolated human intestinal chyme
- SYN-004 protects the gut microbiome in a pig model of antibiotic-mediated dysbiosis
- SYN-004 prevents the emergence of antimicrobial resistance genes in pigs
- SYN-004 is well tolerated in dogs up to 57 mg/kg/day for 28 days
- SYN-004 does not change the PK of IV ceftriaxone in dogs

Kaleko et al. 2016. *Anaerobe* 41:58-67.  
Kokai-Kun et al. 2016. *International Journal of Toxicology*. 35: 309-316.  
Connelly et al. 2017. *Journal of Applied Microbiology*. Open access

## SYN-004 Early Phase Clinical Experience

### Phase 1 Studies

- 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 to 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.

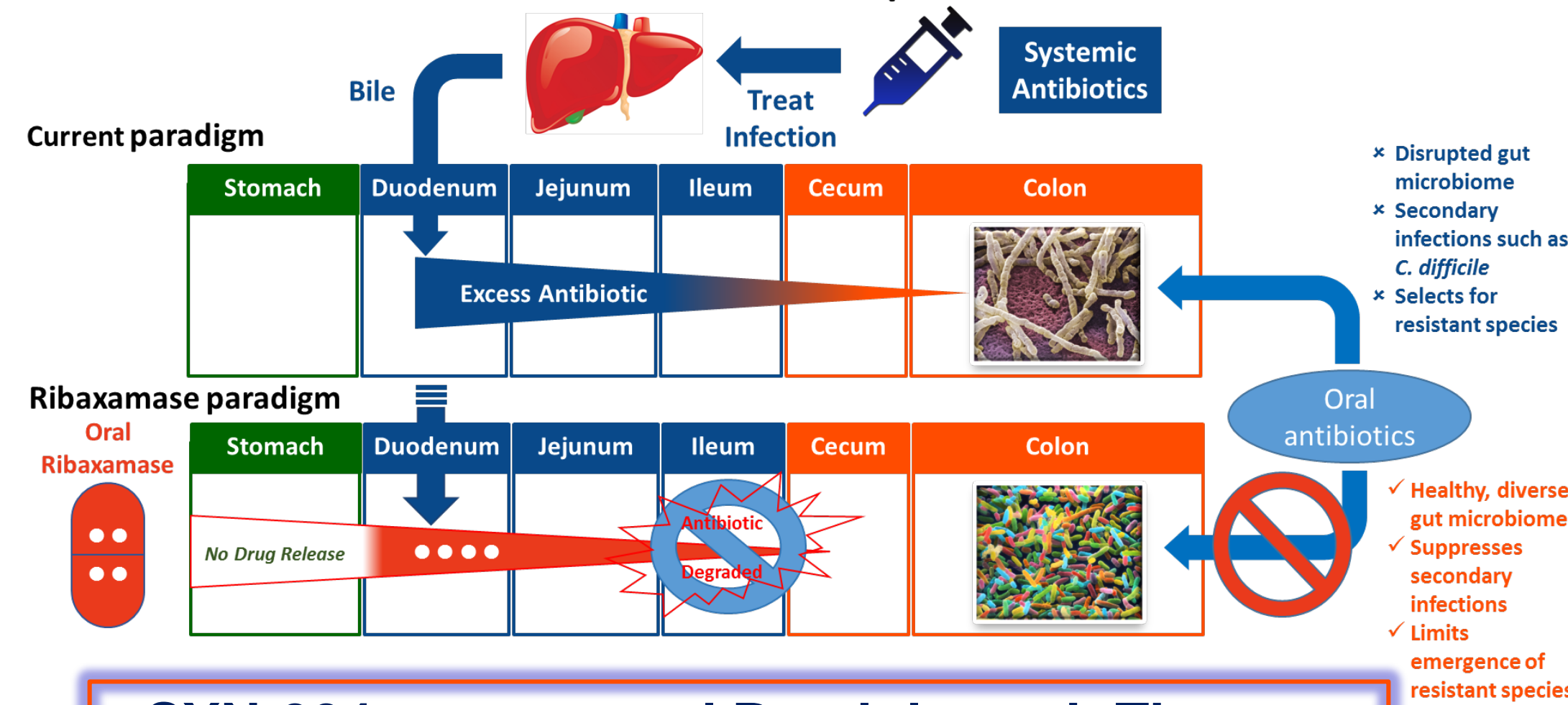
Roberts et al. 2016. *Clinical Drug Investigation*. 36: 725-734

### Phase 2a Studies-Mechanism of action studies in ileostomy patients

- SYN-004 was shown to **effectively degrade ceftriaxone** in intestinal chyme to below the level of detection when SYN-004 was present
- SYN-004 **did not alter the plasma PK** of ceftriaxone
- SYN-004 was not detectable in the plasma of the dosed subjects
- SYN-004 could be administered with a PPI, and this appeared to lead to earlier release of enzyme from the pH dependent formulation which correlated with earlier degradation of ceftriaxone after the 1<sup>st</sup> dose
- SYN-004 was well tolerated when co-administered with IV ceftriaxone

Kokai-Kun et al. 2017. *Antimicrobial Agents and Chemotherapy*. 41(3):e02197-16

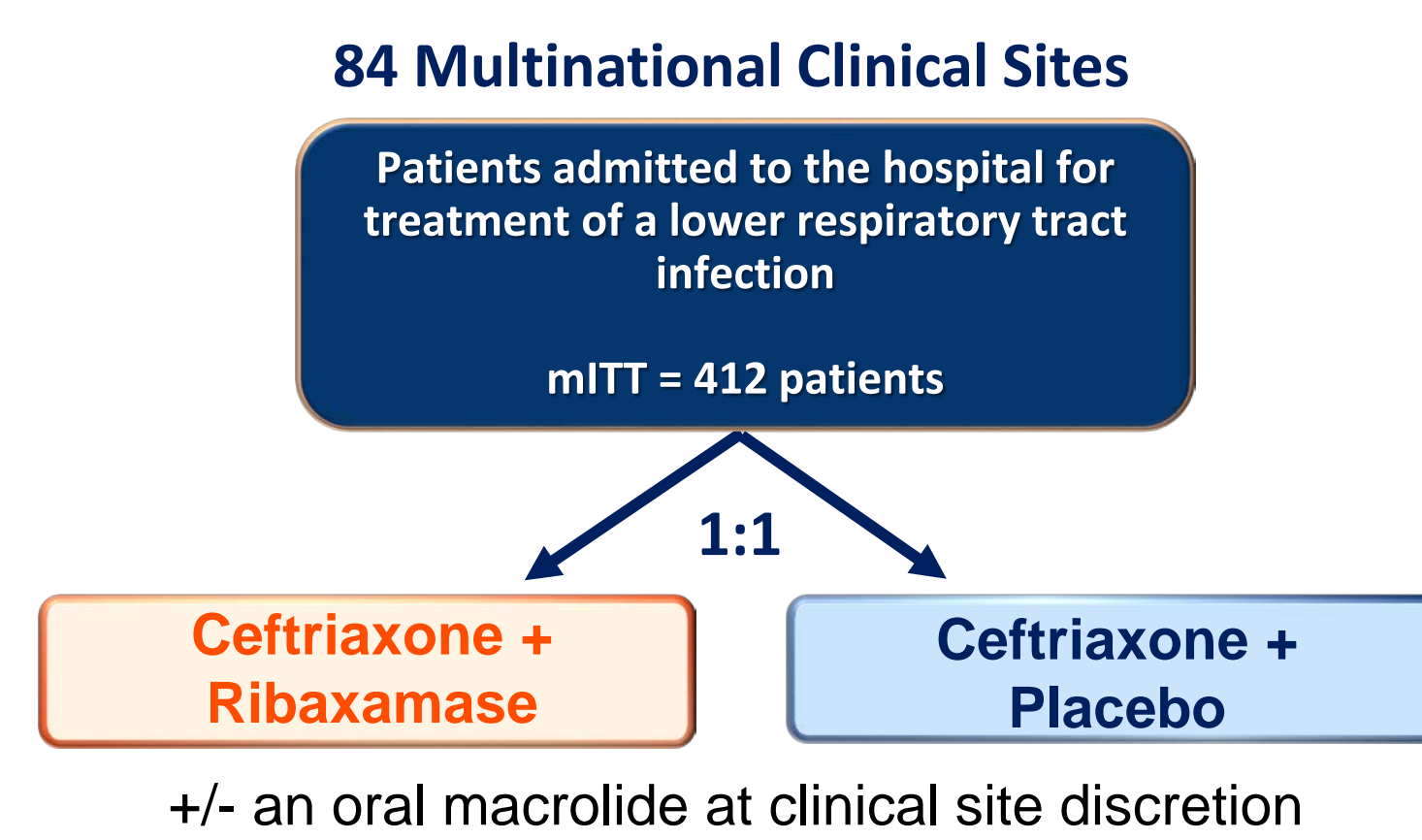
## Ribaxamase Represents a Paradigm Shift In the Use of Intravenous $\beta$ -lactam Antibiotics



**SYN-004 was granted Breakthrough Therapy designation for prevention of CDI by the FDA**

## STUDY DESIGN

### SYN-004 Phase 2b Proof of Concept Study



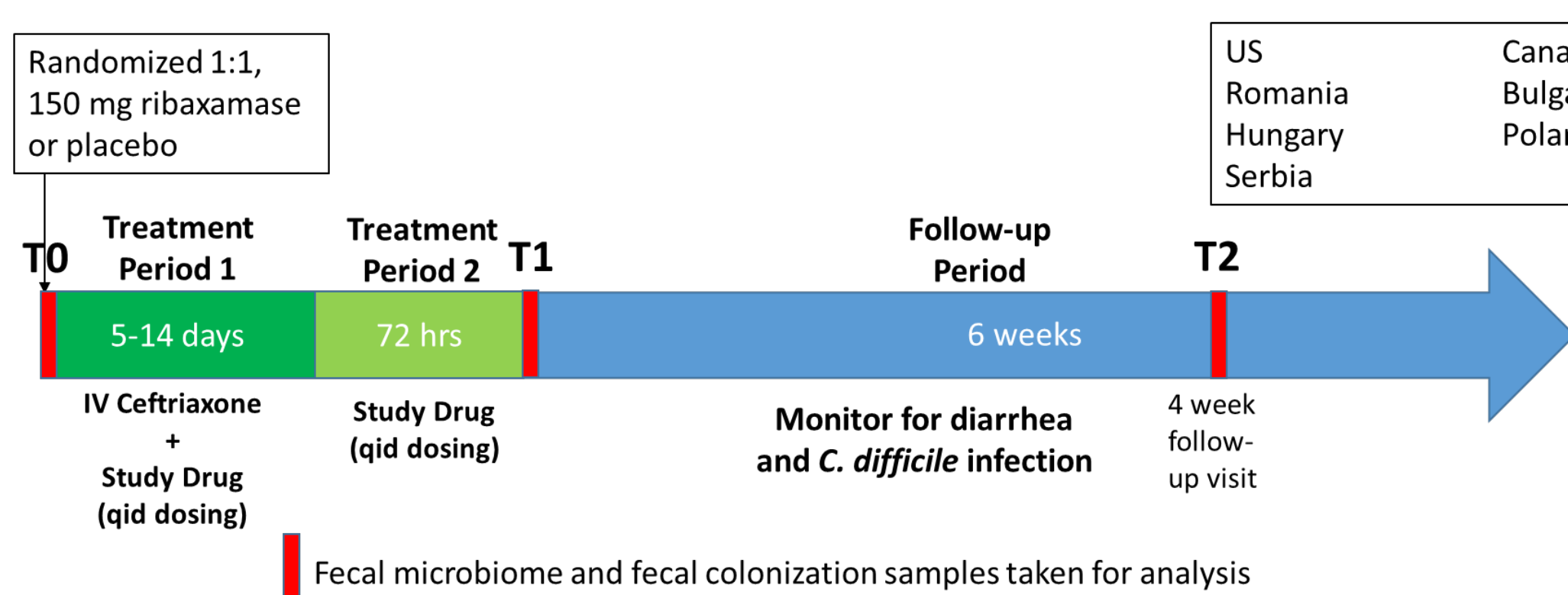
### Primary Endpoint:

- Prevention of *C. difficile* infection (CDI)

### Exploratory Endpoints:

- Evaluate ability to limit disruption of the gut microbiome

### Study Schematic



Diarrhea = 3 or more loose or watery stools in a 24 hour period, samples are collected  
CDI = local lab results for presence of *C. difficile* toxins A and/or B by an approved test (confirmed at a central lab by toxin ELISA)

### Patient Population Enriched for CDI Risk

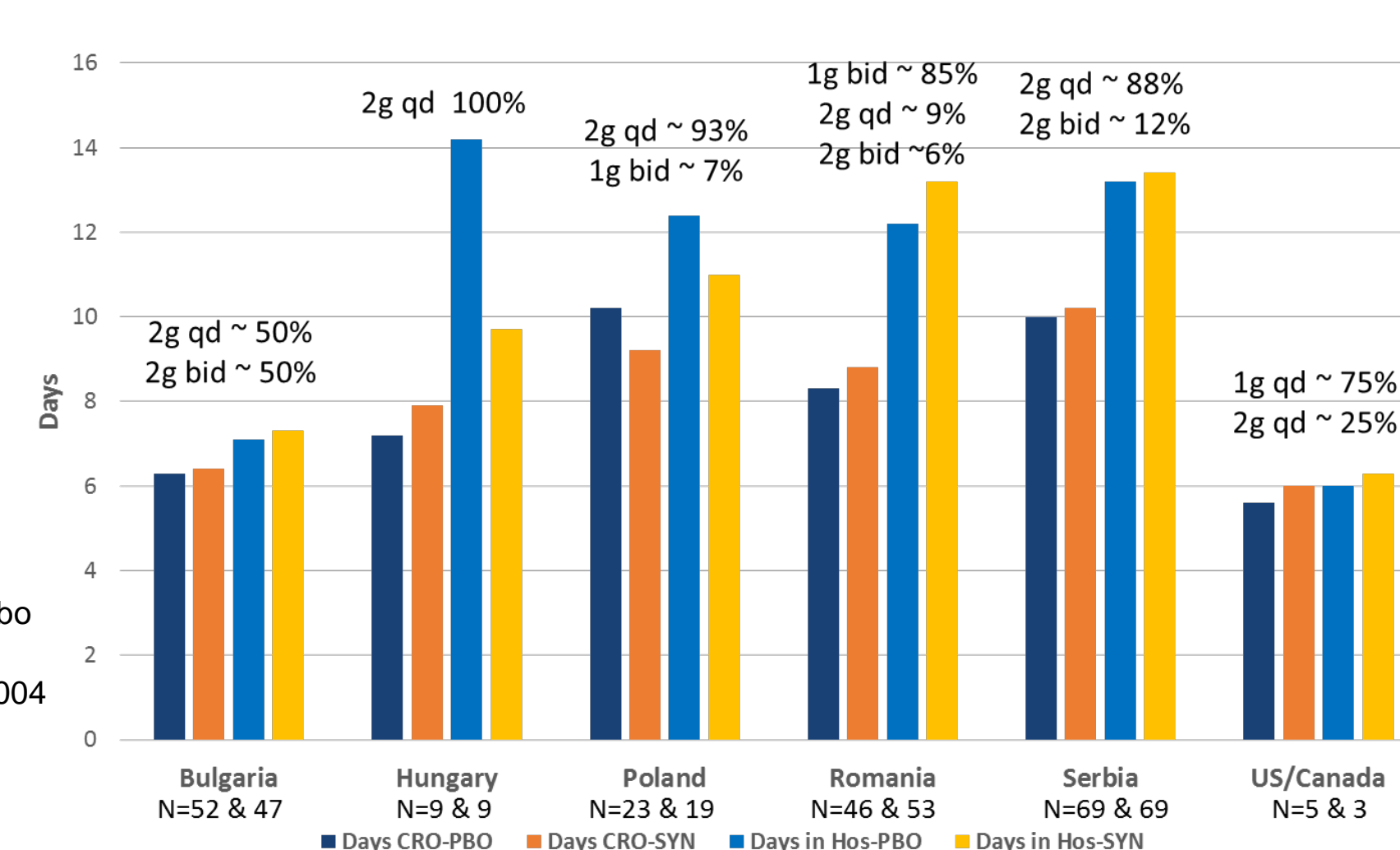
- Patient admitted to a hospital for several days
- At least 5 days of IV ceftriaxone use expected
- Patients > 50 years old
- Patients with higher PORT scores (a measure of the severity of the primary infection)

## STUDY RESULTS

### Demographics and Safety Outcomes

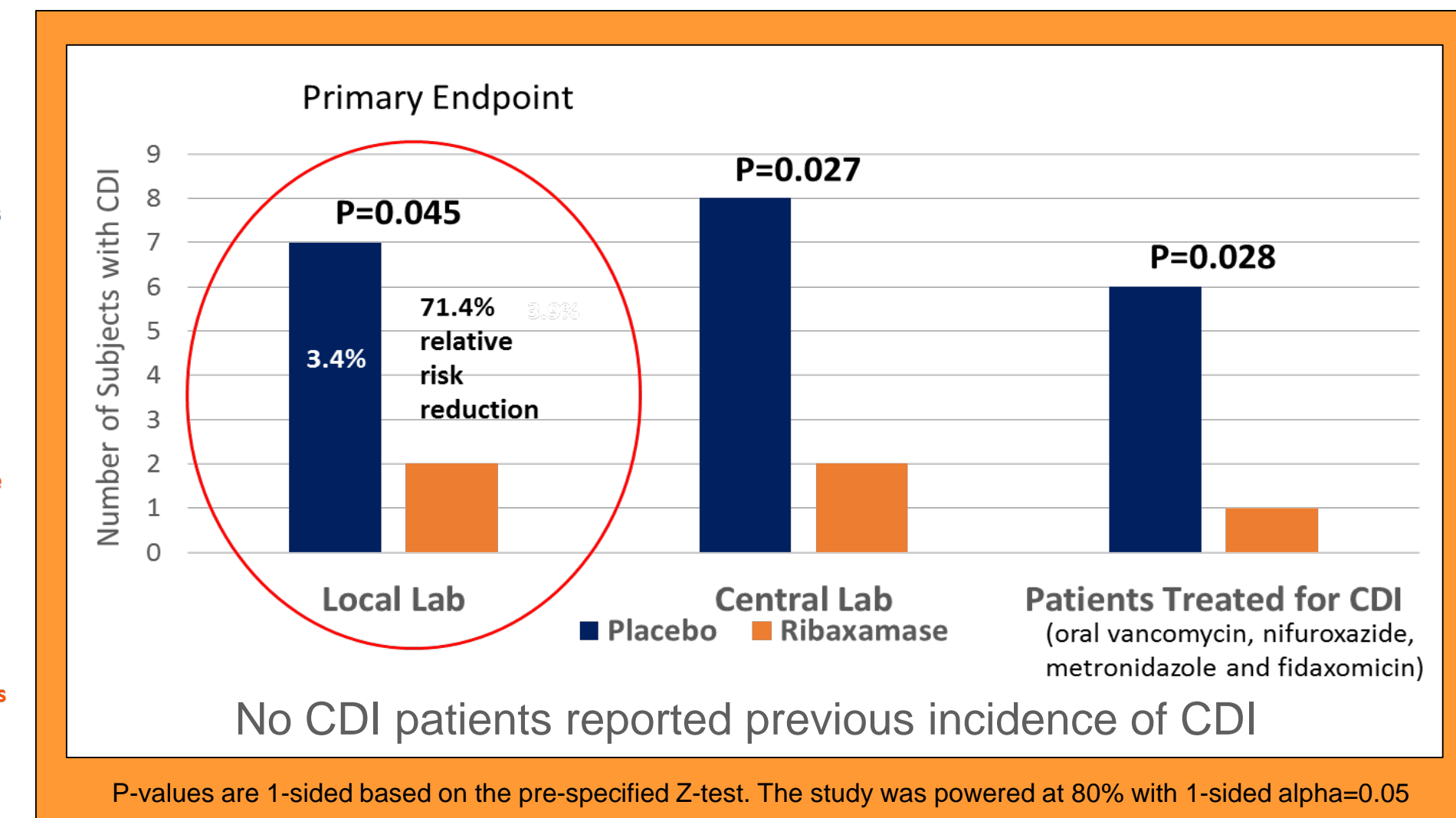
- Average age of patients **~70 years old**
- ~2/3 males in each group
- **~1/3 of patients received macrolides** in each group
- ~1/3 patients received concurrent drugs for stomach acidity (PPIs) in each group
- TEAEs and SAEs were similar between active and placebo and there was no trend associated with ribaxamase use
- **Cure rate for the LRTI to the ceftriaxone treatment was ~99% in both groups** at 2 weeks post treatment

### Comparison of Ceftriaxone Dosing and Hospital Stay by Country



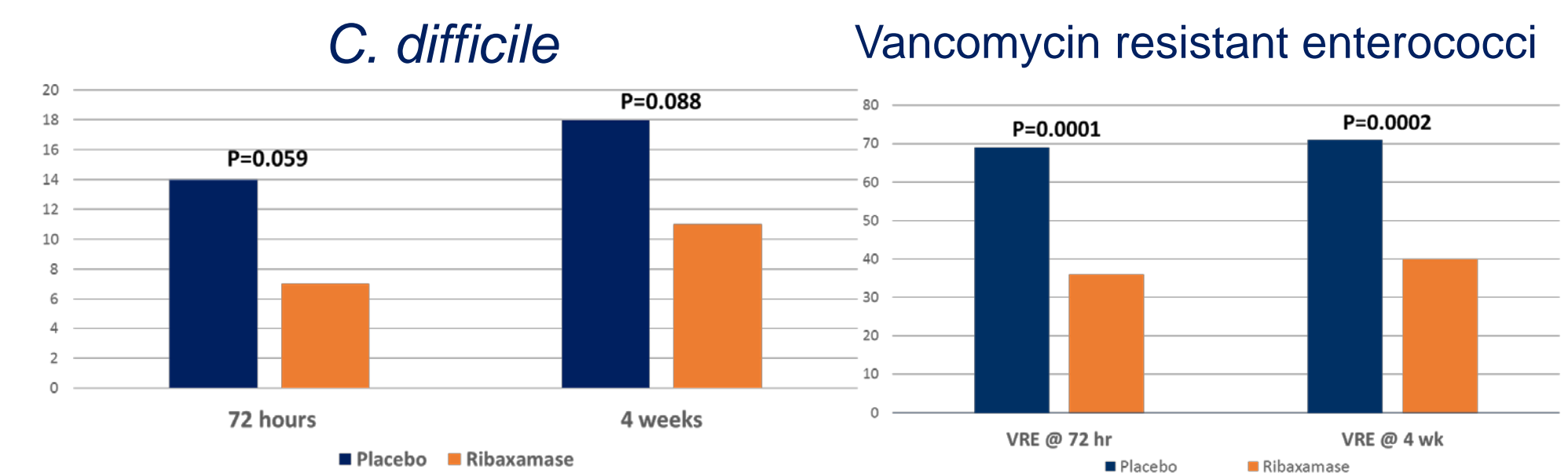
## PRIMARY ENDPOINT

**SYN-004 significantly reduced the incidence of *C. difficile* infection**



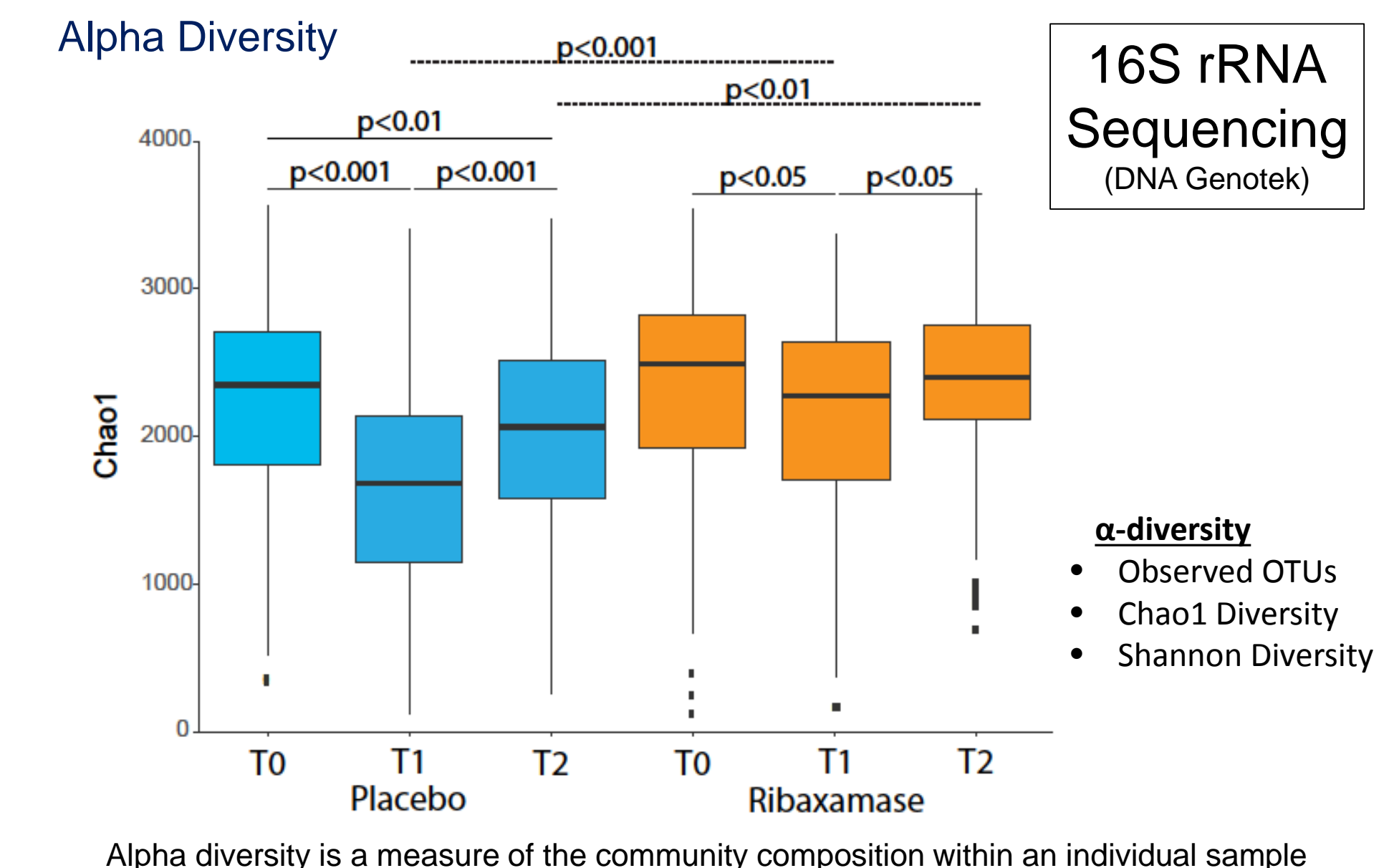
## EXPLORATORY ENDPOINTS

### SYN-004 Prevented New Colonization by Certain Pathogens

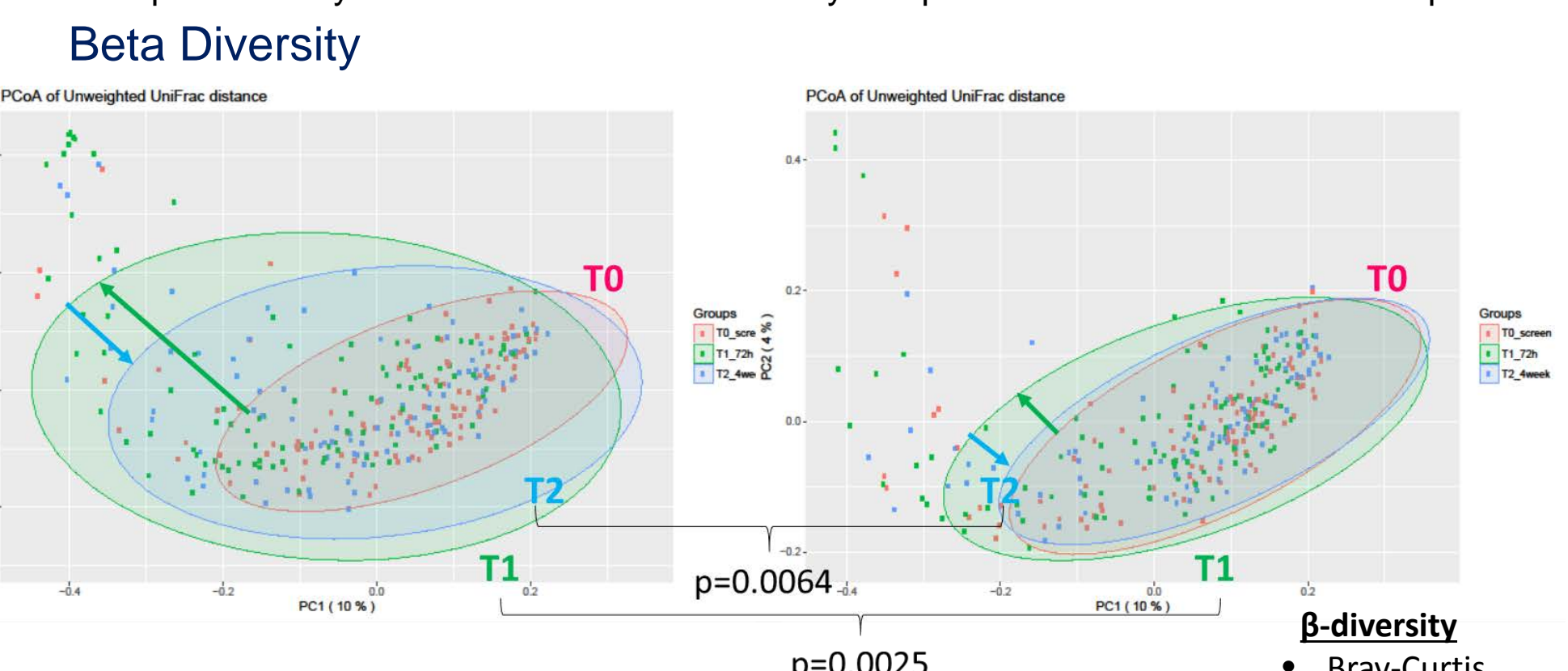


New colonization was negative on screening (T0) and then positive on a subsequent sample (T1 or T2)

### SYN-004 Protected the Diversity of the Gut Microbiome



Alpha diversity is a measure of the community composition within an individual sample

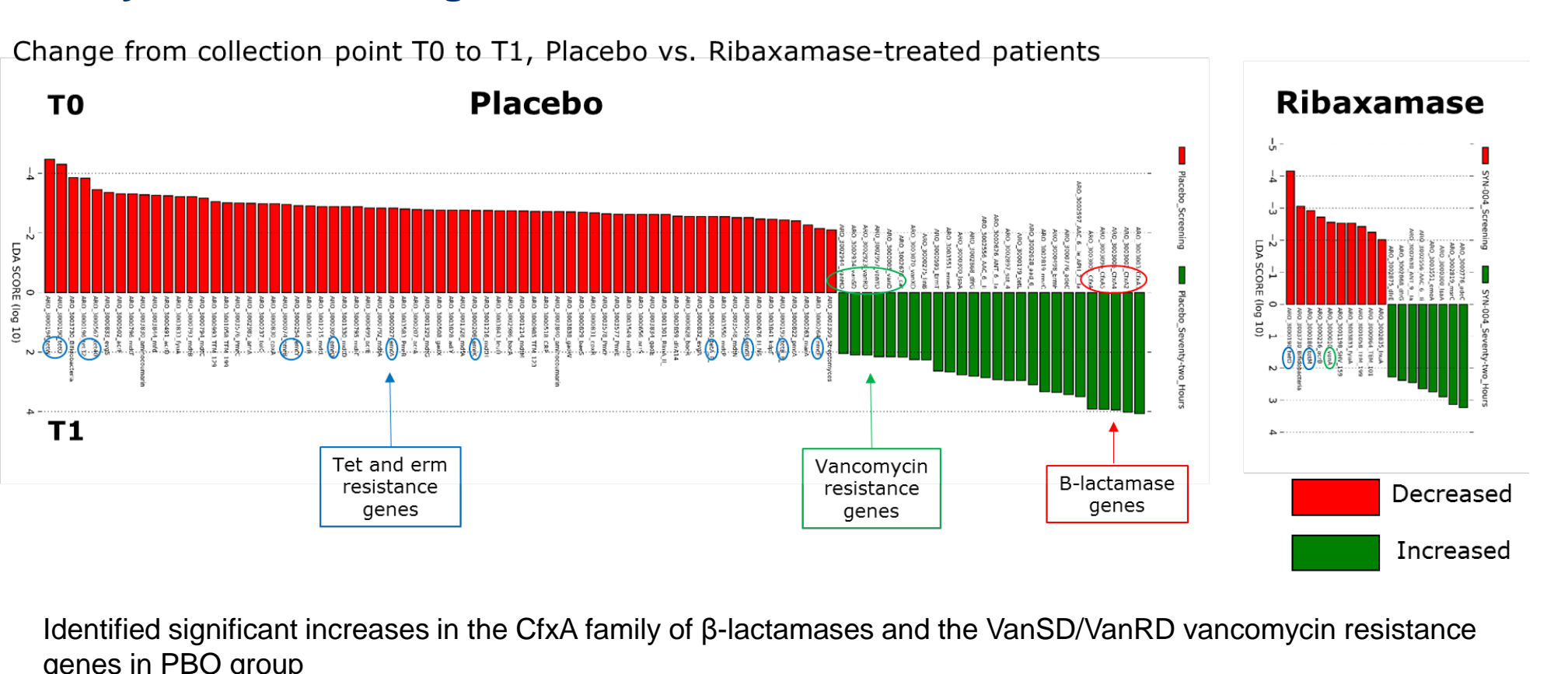


Beta diversity compares the community composition of two different sample sets

### Ribaxamase Prevented Emergence of Antimicrobial Resistance

- DNA extracted from 350 fecal samples sequenced by **whole genome shotgun sequencing** (Diversigen)
- Interrogated against the CARD database (<https://card.mcmaster.ca>)
- 1,300 AMR genes identified with ~60,000 matches per sample
- Identified many genes of interest including,  **$\beta$ -lactamases, vancomycin and macrolide resistance genes**
- Statistical analysis was performed to determine which genes significantly changed from the screening sample (T0) to the post antibiotic sample (T1) in the placebo vs. the ribaxamase patients-LefSe analysis
- This work was funded by contract 200-2016-91935 from the US CDC

### Analysis of the Change in Relative Abundance of AMR Genes



Identified significant increases in the CfxA family of  $\beta$ -lactamases and the VanSD/VanRD vancomycin resistance genes in PBO group

## CONCLUSIONS

- **SYN-004 reduced the incidence of new onset CDI by 71% as compared with placebo (confirmed at the central lab), p=0.045**
- SYN-004 **protected the diversity** of the gut microbiome
- SYN-004 appeared to be **well tolerated** and not affect the cure rate for the primary infection
- SYN-004 reduced all cause diarrhea and in sub-analysis groups reduced patient self-reported diarrhea and patients treated with anti-diarrheal drugs
- SYN-004 **reduced new colonization** with *C. difficile* and VRE
- SYN-004 **Reduced ceftriaxone-mediated changes in the gut resistome** (CDC contract)