Use of ribaxamase (SYN-004), a β-lactamase, to prevent *Clostridium difficile* infection in β-lactam-treated patients: a double-blind, phase 2b, randomised placebo-controlled trial

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Summary

Background Infections with *Clostridium difficile* are a health threat, yet no products are currently licensed for prevention of primary *C difficile* infections. Intravenous β-lactam antibiotics are considered to confer a high risk of *C difficile* infection because of their biliary excretion into the gastrointestinal tract and disruption of the gut microbiome. ribaxamase (SYN-004) is an orally administered β-lactamase that was designed to be given with intravenous β-lactam antibiotics to degrade excess antibiotics in the upper gastrointestinal tract before they disrupt the gut microbiome and lead to *C difficile* infection. We therefore aimed to determine whether administration of ribaxamase could prevent *C difficile* infection in patients being treated with intravenous ceftriaxone for a lower respiratory tract infection, thereby supporting continued clinical development.

Methods In this parallel-group, double-blind, multicentre, phase 2b, randomised placebo-controlled trial, we recruited patients who had been admitted to a hospital with a lower respiratory tract infection with a pneumonia index score of 90–130 and who were expected to be treated with ceftriaxone for at least 5 days. Patients were recruited from 54 clinical sites in the USA, Canada, Bulgaria, Hungary, Poland, Romania, and Serbia. We randomly assigned patients older than 50 years to groups (1:1) in blocks of four by use of an interactive web portal; these groups were assigned to receive either 150 mg ribaxamase or placebo four times per day during, and for 72 h after, treatment with ceftriaxone. All patients, clinical investigators, study staff, and sponsor personnel were masked to the study drug assignments. The primary endpoint was the incidence of *C difficile* infection, as diagnosed by the local laboratory, in patients who received at least one treatment dose, and this outcome was assessed during treatment and for 4 weeks after treatment. This study is registered with ClinicalTrials.gov, number NCT02563106.

Findings Between Nov 16, 2015, and Nov 10, 2016, we screened 433 patients for inclusion in the study. Of these patients, 20 (5%) patients were excluded from the study (16 [4%] patients did not meet inclusion criteria; four [1%] patients because of dosing restrictions). We enrolled and randomly assigned 413 patients to groups, of whom 207 patients were assigned to receive ceftriaxone plus ribaxamase and 206 patients were assigned to receive ceftriaxone plus placebo. However, one (<1%) patient in the ribaxamase group withdrew consent and was not treated with ribaxamase. During the study and within the 4 weeks after antibiotic treatment, two (1·0%) patients in the ribaxamase group and seven (3·4%) patients in the placebo group were diagnosed with an infection with *C difficile* (risk reduction 2·4%, 95% CI –0·6 to 5·9; one-sided p=0·045). Adverse events were similar between groups but more deaths were reported in the ribaxamase group (11 deaths *vs* five deaths in the placebo group). This disparity was due to the higher incidence of deaths attributed to cardiac-associated causes in the ribaxamase group (six deaths *vs* one death in the placebo group).

Interpretation In patients treated with intravenous ceftriaxone for lower respiratory tract infections, oral ribaxamase reduced the incidence of *C difficile* infections compared with placebo. The imbalance in deaths between the groups appeared to be related to the underlying health of the patients. Ribaxamase has the potential to prevent *C difficile* infection in patients treated with intravenous β-lactam antibiotics, and our findings support continued clinical development of ribaxamase to prevent *C difficile* infection.

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See Comment page 451

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Introduction

In 2014, *Clostridium difficile* caused more than 450 000 infections and 44 500 deaths in the USA.[1,2] *C difficile* infections are responsible for about US$6 billion in healthcare costs annually;[3] however, there are no licensed products to prevent initial infection. A healthy gut microbiome confers resistance against pathogenic colonisation, including by *C difficile*, but damage to the gut microbiome by antibiotics eliminates this protection and enables *C difficile* infection.[4–6] Most intravenously administered β-lactam antibiotics (such as penicillins and cephalosporins) are at least partly excreted through the bile

Research in context

Evidence before this study

Ribaxamase (SYN-004) is a second-generation, orally administered β-lactamase that was designed to protect the gut microbiome from intravenous β-lactam antibiotics that have been excreted into the gastrointestinal tract. The first-generation orally administered β-lactamase and ribaxamase predecessor, P1A, was primarily a penicillinase and was not active against cephalosporins. P1A did, however, demonstrate protection of the gut microbiome in patients treated with piperacillin and tazobactam, and it prevented the outgrowth of antimicrobial-resistant bacterial variants by reducing piperacillin to below a detectable concentration in faeces. P1A did not effectively degrade ceftriaxone, however, and prevention of *Clostridium difficile* infection was not evaluated for P1A use. P1A was modified by a single amino acid substitution to create ribaxamase. Ribaxamase has increased enzymatic activity against a broader range of β-lactam antibiotics relative to P1A and thus increases the number of β-lactam antibiotics that can be broken down in the gut by this enzyme. We searched PubMed for papers published before Aug 21, 2018, with the search term "orally-administered β-lactamase and CDI [*C difficile* infection]", and with no language restrictions. Our search did not reveal any other publications beyond those related to ribaxamase and P1A. We found that, in phase 1 clinical studies, ribaxamase was well tolerated and was not systemically bioavailable and, in phase 2a studies, ribaxamase reduced ceftriaxone concentrations to below a detectable level in the intestine but did not alter their concentration in plasma.

Added value of this study

We studied ribaxamase in a randomised clinical trial of 412 patients who were treated with ceftriaxone for a lower respiratory tract infection and either ribaxamase (206 patients) or placebo (206 patients). We found an incidence of *C difficile* infection of 3·4% in the placebo group versus 1·0% in the ribaxamase group (risk reduction 2·4%, 95% CI –0·6 to 5·9; one-sided p=0·045). To our knowledge, our findings represent the first evidence that degrading antibiotics in the upper intestine could protect against *C difficile* infection.

Implications of all available evidence

Results from this study support continued clinical development of oral ribaxamase for administration with intravenous β-lactam antibiotics, such as ceftriaxone, to protect the gut microbiome and reduce the risk of *C difficile* infection.

into the intestine,[7,8] thereby causing damage to the gut microbiome, and these antibiotics are therefore strongly associated with *C difficile* infection.[9–11] It is the cumulative effect of repeat doses of these antibiotics that significantly increases the risk of *C difficile* infection.[11,12] Ceftriaxone is highly excreted in bile, leading to intestinal concentrations of this drug up to 1 mg/mL,[13] and this excretion can continue for 48 h after ceftriaxone treatment.[14] Since ceftriaxone is excreted in bile and because of the activity of ceftriaxone against anaerobic organisms in the gut, this β-lactam antibiotic confers a high risk of *C difficile* infection. There is therefore an unmet medical need to protect the gut microbiome against intravenous β-lactam antibiotics, such as ceftriaxone, and thus reduce the risk of *C difficile* infection.

Ribaxamase (SYN-004) is an orally administered β-lactamase designed to degrade excess intravenous β-lactam antibiotics that have been excreted into the upper gastrointestinal tract, thus protecting the gut microbiomes of patients from disruption and preventing infection by *C difficile*. Ribaxamase is a second-generation orally administered β-lactamase and was modified from its predecessor enzyme, P1A,[15,16] to expand its degradation profile to include cephalosporins.[17]

In phase 1 studies,[18] ribaxamase was well tolerated up to a single dose of 750 mg or four doses of 300 mg ribaxamase daily for 7 days, and only mild adverse events (grade 1; ie, did not interfere with daily activity) were reported, including flatulence and headache in 11% of the participants. Ribaxamase was not systemically absorbed and no anti-drug antibodies were detected. In phase 2a studies,[13] ribaxamase degraded ceftriaxone that had been excreted into the intestine to below detectable concentrations but did not affect the plasma pharmacokinetics of ceftriaxone. In this placebo-controlled phase 2b study, we aimed to determine whether administration of ribaxamase could prevent *C difficile* infection in patients being treated with intravenous ceftriaxone for a lower respiratory tract infection, thereby supporting continued clinical development.

Methods

Study design and participants

In this parallel-group, double-blind, multicentre, phase 2b randomised placebo-controlled trial, we screened potential participants who had been admitted to hospital for treatment of a lower respiratory tract infection. Patients older than 50 years were recruited from 84 clinical sites in the USA, Canada, Bulgaria, Hungary, Poland, Romania, and Serbia. Participants were expected to be admitted to hospital and to receive at least 5 days of intravenous ceftriaxone treatment. Patients could also receive macrolides at the discretion of the clinical investigator as needed to treat their lower respiratory tract infection. We required that the infection was moderate to severe, with a pneumonia index score[19] of 90–130. We excluded patients with any diarrhoeal illness within the 72 h before randomisation, a *C difficile* infection within the previous 4 weeks, more than one *C difficile* infection in the previous 4–12 weeks, or currently receiving treatment for a *C difficile* infection. Patients were also excluded if they received any antibiotics, other than those

to treat their current lower respiratory tract infection, during the month before enrolment in the study or if they had inflammatory bowel disease or evidence of an active gastrointestinal infection. The study protocol and informed consent forms were approved by the appropriate institutional review boards or ethics committees, and all study patients provided written consent to participate in the study.

Randomisation and masking

Patients were randomly assigned (1:1) to receive either 150 mg ribaxamase or placebo (both as two capsules) during, and for 72 h after, ceftriaxone treatment. Randomisation was done with an interactive voice and web response system in blocks of four, and a statistician not otherwise involved in the study produced the randomisation schedule. Active and placebo capsules looked identical and were provided to the clinical sites in numbered bottles that were based on the masked randomisation scheme. All patients, clinical investigators, study staff, and sponsor personnel were masked to the study drug assignments.

Procedures

Ribaxamase was expressed in *Escherichia coli*, purified, and formulated as oral 75-mg capsules containing enteric-coated pellets that were designed to release active enzyme at a pH of more than 5·5—ie, within the intestine.[20] Placebo pellets contained formulation buffer and the other excipients but no active drug.

This study was done in three time periods (appendix). Treatment period 1, in which participants received ceftriaxone plus study drug every 6 h (range 5–7 h), was expected to last from 5 to 14 days, and the duration and dose of ceftriaxone was based on the local standard of care. Treatment period 2 involved a continuation of the study drug (every 6 h) for an additional 72 h after the end of ceftriaxone treatment. Patients were monitored for diarrhoea and *C difficile* infection and assessed for safety for an additional 6 weeks (the follow-up period), during which patients returned to the clinic for a visit at 2 and 4 weeks, and a follow-up phone call was made at 1, 3, and 6 weeks.

Daily vital signs were monitored during treatment period 1, at the end of treatment period 2, and at the 2-week and 4-week follow-up visits for safety analyses. Physical examinations and 12-lead electrocardiograms were also done and samples for laboratory analyses (chemistry, haematology, and urinalysis) were collected at screening (before the first dose of study drug), at the end of treatment period 2, and at the 4-week follow-up visit. Adverse events and serious adverse events, and concomitant use of medication, were recorded throughout the study. If diarrhoea (three or more loose or watery stools in 24 h) occurred, a sample was collected and immediately refrigerated. Within 36 h, each faecal sample from a patient with diarrhoea was split into two: one sample was tested at the local clinical laboratory by use of an approved test for either *C difficile* toxins (immunoassay) or their genes (nucleic acid amplification assay), as per the local protocol. The second sample was frozen and sent to a central laboratory (ACM Global Laboratories) for confirmatory testing by a *C difficile* toxin ELISA (Premier Toxins A&B; Meridian Bioscience, Cincinnati, OH, USA). Faecal samples from patients with diarrhoea that were shipped to the central laboratory were frozen and thawed only once before testing.

At three timepoints during the study (screening, end of treatment period 2, and at the 4-week follow-up; appendix), faecal samples were collected (BD ESwab Collection and Transport System) for analysis of colonisation by pathogens. These samples were sent to the central laboratory (at ACM) for identification (via culture testing) of *C difficile*, vancomycin-resistant enterococci (VRE), and extended-spectrum β-lactamase producing Gram negative bacilli with appropriate CHROMagar selective media. *C difficile* isolates from central laboratory-confirmed infections with *C difficile* or faecal samples showing *C difficile* colonisation were collected for future analysis. We defined new colonisation by an opportunistic pathogen as a negative result in the screening sample, followed by a positive result for that pathogen in a subsequent sample. Faecal samples for gut microbiome analysis were collected at screening, the end of treatment period 2, and at the 4-week follow-up in OMNIgene GUT collection tubes (DNA Genotek; Ottawa, ON, Canada) and sent frozen to a central laboratory (DNA Genotek) for later analysis.

See Online for appendix

Outcomes

The primary endpoint was the incidence of *C difficile* infection, as confirmed in a local laboratory, between the time of the first dose of study drug and the 4-week follow-up visit in a modified intention-to-treat population. This population included all patients who received at least one dose of study drug. A faecal sample from a patient with diarrhoea that was negative for *C difficile* toxins or genes by the local laboratory was defined as antibiotic-associated diarrhoea. Central laboratory results identifying *C difficile* infections were a secondary outcome and were used for confirmation and not reported to the clinical sites.

The results of prespecified safety assessment outcomes were reported for both groups. The safety analysis population also included all patients who received at least one dose of study drug (the modified intention-to-treat population). The clinical investigators assessed the treatment of the primary lower respiratory tract infection on the third day of ceftriaxone treatment, 72 h after the final ceftriaxone treatment, and at the 2-week follow-up visit; this outcome was determined on the basis of whether the patient's infection was adequately treated with the prescribed antibiotic regimen or whether a change in regimen was required. We also did a post-hoc

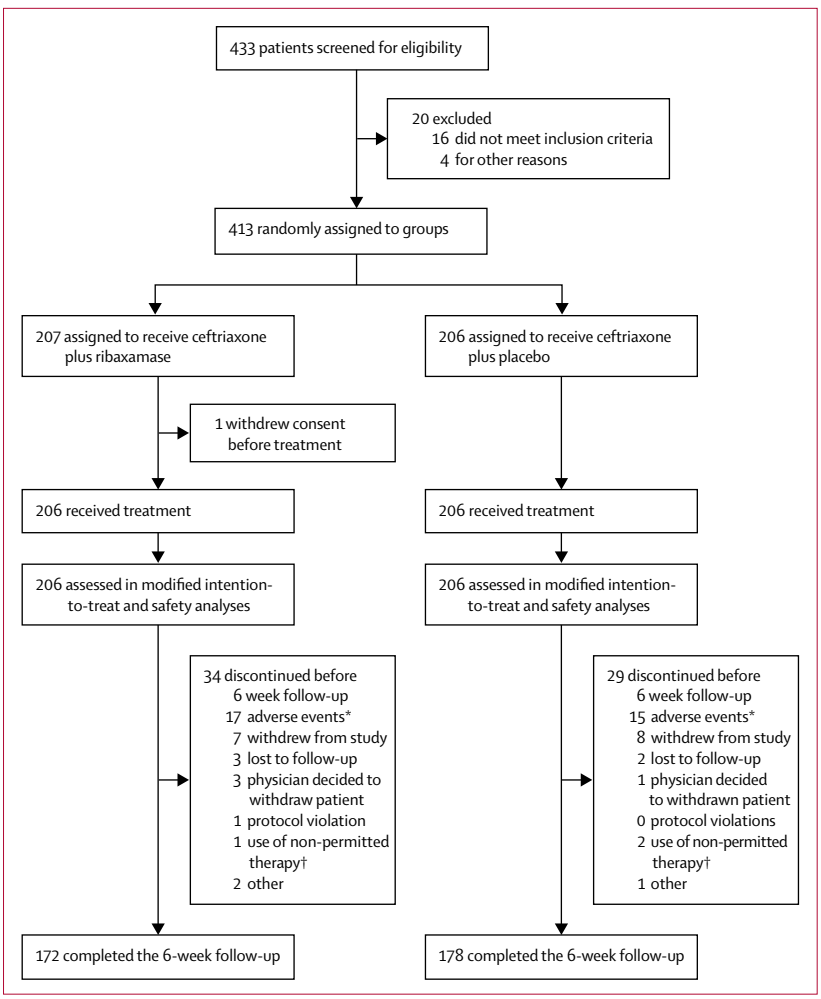

Figure 1: **Trial profile**
*Includes patients who were found to have a *C difficile* infection and were withdrawn from the study.
†Patients were switched to orally administered β-lactam antibiotics during study drug administration, which is contraindicated for ribaxamase.

analysis to review the causes of all deaths during the study. This analysis involved reviewing all available medical histories and other safety assessments for all patients who died during the study. The period covered by the analysis included the patients' reported medical histories upon admission to the study until the time of their death.

Statistical analysis

The planned sample size for our study was based on a speculative assumption that 10% of patients in the placebo group and 3% of patients in the ribaxamase group would develop a *C difficile* infection. A sample size of 155 patients in each treatment group was expected to provide 80% power to detect a treatment difference. This calculation was based on a one-sided test at an overall α of 0·05. A prespecified interim analysis was done on the primary endpoint by an unmasked data monitoring board when 80% of the patients had attended the 4-week follow-up visit. When this analysis had returned a finding of "continue the trial per protocol", we decided to close enrolment because the enrolment target had been attained, at which point 412 patients had been enrolled.

All efficacy analyses were done in the modified intention-to-treat dataset. As prespecified in the protocol and statistical analysis plan, all hypothesis testing was one-sided at a significance level of 0·05. A Z test was used to compare the difference between ribaxamase versus placebo in patients with study endpoints at the prespecified times. The p value and one-sided 95% Newcombe-Wilson CI for the treatment difference were also calculated. The original statistical analysis plan, which was based on the assumed 10% incidence of *C difficile* infection in the placebo group, considered all patients who were lost to follow-up (ie, who died or discontinued) as treatment failures; however, since the study was designed to indicate prevention of a relatively rare outcome (*C difficile* infection), we amended the protocol and statistical analysis plan to incorporate a sensitivity analysis that only included local laboratory-confirmed *C difficile* infection as the primary endpoint, without considering all those lost to follow-up as treatment failures. This modification to the protocol and statistical analysis plan was made before unmasking of the study groups.

Baseline was defined as the last available assessment before the first dose of study drug, and for the analyses of all endpoints, patients with missing data were imputed as not having an outcome event. Data at each timepoint was pooled for all sites, and the change from baseline to each timepoint after baseline were summarised by treatment group. The data monitoring committee comprised two medical doctors and a consultant statistician provided by the contract research organisation (INC). This study is registered with ClinicalTrials.gov, number NCT02563106.

Role of the funding source

The funder of the study and its employees were responsible for study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 16, 2015, and Nov 10, 2016, we screened 433 patients for inclusion in the study (figure 1). Of these patients, 20 (4·6%) patients were excluded from the study (16 [3·7%] patients did not meet inclusion criteria; four [0·9%] patients because of dosing restrictions). We enrolled 413 patients from 54 of the 84 included clinical sites (appendix) and randomly assigned these patients to groups: 207 (47·8%) patients were assigned to receive ceftriaxone plus ribaxamase and 206 (47·6%) patients were assigned to receive ceftriaxone plus placebo.

However, one (0·5%) patient in the ribaxamase group withdrew consent and was not treated with ribaxamase. Therefore 206 patients in each group were included in the modified intention-to-treat population and were assessed for the primary outcome. Study demographics are shown in table 1. 350 patients (172 [83·4%] patients in the ribaxamase group and 178 [86·4%] patients in the placebo group) remained in the study until the 6-week follow-up phone call, and 63 patients (34 [16·5%] patients in the ribaxamase group and 29 [14·1%] patients in the placebo group) discontinued the study before the 6-week follow-up phone call. The main reasons for discontinuation were adverse events (17 [8·3%] patients in the ribaxamase group and 15 [7·3%] patients in the placebo group) and patient withdrawal (seven [3·4%] patients in the ribaxamase group and eight [3·9%] patients in the placebo group).

Patients received study drug for a median and mean duration of 11·0 days (in both groups), which ranged from 1 to 17 days for those in the ribaxamase group and from 2 to 18 days for those in the placebo group. Dosages of ceftriaxone ranged from 1 g once a day to 2 g twice a day, and 264 (64%) of the patients received 2 g once a day. Patients received a median of ten infusions of ceftriaxone (in both treatment groups), with a mean of 11·6 infusions in the ribaxamase group and 10·6 infusions in the placebo group for a median of 8 days of treatment (in both groups). The mean total dosage of ceftriaxone was 18·9 g for the ribaxamase group and 17·7 g for the placebo group. Per infusion, the mean dose was 1·71 g for the ribaxamase group and 1·75 g for the placebo group (appendix). About half of the patients in each group received other antibiotics in addition to ceftriaxone for variable durations, depending on the patient. Most of these antibiotics were macrolides: 76 (36·9%) patients in the ribaxamase group and 72 (35·0%) patients in the placebo group received macrolides. These antibiotics were administered before the 4-week follow-up visit. Of patients who were positive for *C difficile* infection, four of seven patients with locally diagnosed *C difficile* infections in the placebo group and one of two patients with locally diagnosed infections in the ribaxamase group also received macrolides before diagnosis with a *C difficile* infection. Ten (4·9%) patients in the ribaxamase group and 14 (6·8%) patients in the placebo group received probiotics during the study. 20 probiotic regimens were administered prophylactically and four probiotic regimens were for treatment of diarrhoea (of which two were for *C difficile* infection in the placebo group and one in each group was for diarrhoea that was not associated with a *C difficile* infection). None of the patients who received prophylactic probiotics developed a *C difficile* infection.

The original statistical analysis plan considered local laboratory-confirmed *C difficile* infection plus any discontinuations from the study before the 4-week follow-up visit as presumed treatment failures, and 33 patients in the ribaxamase group and 27 patients in the placebo group met this criterion. With a prespecified sensitivity analysis that was incorporated as an amendment to the statistical analysis plan and protocol, which defined the primary endpoint as local laboratory-confirmed *C difficile* infection and no longer considered patients lost to follow-up to be treatment failures, we found that two (1·0%) patients in the ribaxamase group and seven (3·4%) patients in the placebo group met the criteria for *C difficile* infection at or before the 4-week follow-up visit (table 2). This finding represented 2·4% risk reduction (95% CI –0·6 to 5·9) in the incidence of *C difficile* infection (p=0·045).

	Placebo group (n=206)	Ribaxamase group (n=206)
Age, years		
Mean (SD)	69·7 (9·4)	68·8 (9·4)
Median	68·0	68·0
Minimum, maximum	50, 93	50, 93
Sex, n (%)		
Female	80 (39%)	73 (35%)
Male	126 (61%)	133 (65%)
Race, n (%)		
Black	1 (<1%)	0
White	205 (>99%)	206 (100%)
Height, cm		
Mean (SD)	166·67 (9·2)	168·18 (9·8)
Median	167·0	169·0
Minimum, maximum	140·0, 198·1	145·0, 192·0
Weight, kg		
Mean (SD)	74·68 (16·1)	75·58 (18·6)
Median	73·0	75·0
Minimum, maximum	37·0, 129·0	37·5, 168·0
Body-mass index, kg/m²		
Mean (SD)	26·87 (5·4)	26·54 (5·3)
Median	26·3	26·0
Minimum, maximum	14·8, 48·9	16·9, 50·2

Table 1: **Baseline characteristics**

Part of all faecal samples from patients with diarrhoea (including those from patients with *C difficile* infections and other diarrhoea not caused by *C difficile*) was expected to be sent to the central laboratory for confirmatory testing for *C difficile* infection. Eight of the nine faecal samples from patients that tested positive for *C difficile* in the local laboratories, were sent to the central laboratory for confirmatory testing by toxin ELISA and all were also found to be positive at the central laboratory. One of the faecal samples (from a patient locally diagnosed with a *C difficile* infection in the ribaxamase group) was not sent to the central laboratory because of an administrative error at the clinical site. The central laboratory identified two additional *C difficile*-positive samples, thereby finding eight samples in the placebo group and two samples in the ribaxamase group that were positive for *C difficile* toxins by ELISA (p=0·027), and nine of these ten samples were also

	Placebo (N=206)	Ribaxamase (N=206)
Local laboratory-confirmed *C difficile* infections		
Number of patients (%)	7 (3·4%)	2 (1·0%)
Risk reduction (95% CI)	..	2·4% (–0·6 to 5·9)*
One-sided p value†	..	0·045
Central laboratory-confirmed *C difficile* infections		
Number of patients (%)	8 (3·9%)	2 (1·0%)
Risk reduction (95% CI)	..	2·9% (–0·2 to 6·6)
p value	..	0·027
Patients receiving treatment for *C difficile* infections‡		
Number of patients (%)	6 (2·9%)	1 (0·5%)
Risk reduction (95% CI)	..	2·4% (–0·3 to 5·8)
p value	..	0·028

Data are until the 4-week follow-up. *Represents a 71% reduction in the relative risk of *C difficile* infection; CI is based on the Newcombe-Wilson CI. †Based on the pre-specified Z test. ‡Received treatment with oral vancomycin, metronidazole, fidaxomicin, or a combination, specifically to treat a *C difficile* infection.

Table 2: **Number of patients with *Clostridium difficile* infections and being actively treated for these infections, and the risk reduction associated with ribaxamase treatment**

	Placebo	Ribaxamase	p value
Clostridium difficile			
Screening	5 (2%)	3 (1%)	0·239
End of treatment period 2	14 (8%)	7 (4%)	0·059
4-week follow-up visit	18 (9%)	11 (6%)	0·088
Vancomycin-resistant enterococci			
Screening	8 (4%)	5 (2%)	0·198
End of treatment period 2	69 (37%)	36 (19%)	0·0001
4-week follow-up visit	71 (36%)	40 (20%)	0·0002
Extended-spectrum, β-lactamase-producing Gram-negative bacilli			
Screening	46 (22%)	37 (18%)	0·134
End of treatment period 2	30 (16%)	31 (17%)	0·565
4-week follow-up visit	44 (22%)	49 (25%)	0·714

The number of patients with samples taken per treatment group varied by collection time: at screening, samples were provided by 206 patients from each group; at the end of treatment period 2 (72 h after the last dose of ceftriaxone), samples were provided by 186 patients in the placebo group and 185 patients in the ribaxamase group; and at the 4-week follow-up visit, samples were provided by 197 patients in the placebo group and 198 patients in the ribaxamase group. p values are one-sided and based on the prespecified Z test. Data from the end of treatment period 2 and the follow-up visit were cumulative data for new colonisation (ie, patients with a negative faecal sample at screening, then a positive result at these timepoints).

Table 3: **Number of patients positive for faecal colonisation by opportunistic pathogens at prespecified collection times**

culture-positive for *C difficile* (the exception being one sample from a patient in the ribaxamase group).

A secondary efficacy analysis looked at *C difficile* infections at or before the 2-week follow-up visit, one (0·5%) patient in the ribaxamase group and six (2·9%) patients in the placebo group were found to be positive for a *C difficile* infection by use of local laboratory results. One (0·5%) patient in the ribaxamase group and six (2·9%) patients in the placebo group (not the same patients) received treatment with oral antibiotics for *C difficile* infection (p=0·028; table 2). No additional cases of *C difficile* infection were identified after the 4-week follow-up visit. None of the patients diagnosed with *C difficile* infection received laxatives during the study, and no patients reported previous *C difficile* infections. We found 32 cases of all-cause diarrhoea (13 patients in the ribaxamase group and 19 patients in the placebo group), including *C difficile* infection, which did not significantly differ between groups. Of these, 23 cases (11 patients in the ribaxamase group and 12 patients in the placebo group) were protocol-defined antibiotic-associated diarrhoea in which the faecal sample was negative for *C difficile* toxin.

We found no significant difference in new colonisation with *C difficile* in the ribaxamase versus placebo groups at the end of treatment period 2 (p=0·059) or at the end of the 4-week follow-up (p=0·088; table 3). None of the patients diagnosed with *C difficile* infection (by local or central laboratories; all of whom submitted faecal samples at screening) showed colonisation by *C difficile* at screening. There was a significant reduction in the risk of new colonisation by VRE in the ribaxamase versus placebo groups at the end of treatment period 2 (p=0·0001) and at the 4-week follow-up visit (p=0·0002). There was a relatively high overall risk of colonisation by extended-spectrum β-lactamase producing Gram-negative bacilli at screening (about 20%), which remained fairly constant across the study timepoints and was similar between the two groups at all three collection timepoints.

206 patients from each group who received at least one dose of study drug were included in the safety analysis. Haematology, serum chemistry, quantitative urinalysis, vital signs, and electrocardiogram results, and their respective changes from baseline, were similar between treatment groups. 84 (40·8%) patients in the ribaxamase group and 91 (44·2%) patients in the placebo group reported adverse events, and most were of mild or moderate intensity (table 4). Severe adverse events were recorded for 18 (8·7%) patients in the ribaxamase group and 15 (7·3%) in the placebo group. Most of the events (169 of 175 events) were considered unrelated to the study drug before unmasking of the groups. However, five (2·4% of patients) adverse events in the ribaxamase group and one (0·5%) adverse event in the placebo group were considered possibly or probably related to the study drug by the study investigators. The most common adverse events were gastrointestinal disorders, infections, and respiratory, thoracic, and mediastinal disorders, and the frequency of these events was similar between groups.

Serious adverse events were observed in 33 (16·0%) patients in the ribaxamase group and 21 (10·2%) patients in the placebo group, and fatal adverse events were recorded for 11 (5·3%) patients in the ribaxamase group and five (2·4%) patients in the placebo group (table 4). 11 (5·3%) patients in the

ribaxamase group and ten (4·9%) patients in the placebo group had adverse events that led to discontinuation of the study drug, which was a similar proportion between the two treatment groups. The number and type of non-fatal serious adverse events was also similar between the two groups, and most of these prolonged the patient's hospital stay and were resolved during the study. Serious adverse events, fatal adverse events, or adverse events leading to discontinuation were not considered to be drug-related by the investigators, medical monitors or a non-affiliated, third-party reviewer, and instead, most serious adverse events appeared to be related to the patient's medical history, an identified malignancy, or progression of the original respiratory infection.

Among the 11 deaths in the ribaxamase group and five deaths in the placebo group, there were two deaths from cancer in each group (from existing or newly diagnosed lung tumours or leukaemia) and three deaths in the ribaxamase group and two deaths in the placebo group due to respiratory failure (respiratory insufficiency, worsening pneumonia, or fibrosis; table 5). The imbalance in deaths between the groups appeared to be associated with more cardiac-associated causes of death in the ribaxamase group (in six [2·9%] patients), compared with one associated death (0·5% of patients) in the placebo group, all of which were considered to be unrelated to the study drug. As would be expected for this patient population, most of the patients who died of cardiac-associated causes had pre-existing cardiopulmonary pathology, putting them at increased risk for cardiac-associated fatal events.

The imbalance in cardiac-related deaths between the groups was investigated with a post-hoc comprehensive retrospective analysis of patient characteristics to determine whether imbalances in baseline factors might suggest a differential risk of death between the groups. This analysis identified numerical disparities in certain comorbidities and laboratory abnormalities between the two groups, suggesting that more patients with cardiac-associated comorbidities that could lead to death were enrolled in the ribaxamase group relative to the placebo group (figure 2). Almost all patients who died of a cardiac-associated disorder entered the study with at least one of three key risk factors: chronic obstructive pulmonary disease (COPD), increased plasma glucose, or increased blood urea nitrogen (BUN). Nine (82%) of 11 patients in the ribaxamase group who died, and all five (100%) patients in the placebo group who died had at least one of these three risk factors. The percentage of patients with all three risk factors (ie, those at highest risk) was 2·3 times higher in the ribaxamase group (23 [11·2%] patients) compared with that in the placebo group (ten [4·9%] patients), and the combination of all three risk factors was observed in four (1·9% of all patients) of six patients who died from a cardiac-associated cause in the ribaxamase group.

There also appeared to be an imbalance in the number of patients with specific combinations of risk factors and evidence of poor oxygenation (such as cor pulmonale or COPD) between the groups: more of these patients had been randomly assigned to the ribaxamase group compared with the placebo group (appendix). Of the seven (3·4%) patients in the ribaxamase group with COPD, diabetes, and a previous myocardial infarction, three patients died, whereas there were only two (1·0%) such patients in the placebo group and neither died during the study. The three patients in the ribaxamase group who died who had these three conditions had additional comorbidities, including a previous stroke, cor pulmonale, or two previous myocardial infarctions. There were also two (1·0%) patients in the ribaxamase group with COPD, diabetes, heart failure, and cor pulmonale, of whom one (50%) died, whereas there was only one (0·5%) such patient in the placebo group. Although changes in the electrocardiogram readings relative to baseline were similar between the two groups, at enrolment, nine (4·4%) patients in the ribaxamase group had a QRS interval of at least 120 ms, four (44%) of whom had fatal cardiac-associated events, whereas only one (0·5%) patient in the placebo group met this criterion. The

	Placebo group (N=206)	Ribaxamase group (N=206)
Overall adverse events	91 (44%)	84 (41%)
Mild adverse event*	55 (27%)	38 (18%)
Moderate adverse event*	21 (10%)	28 (14%)
Severe adverse event*	15 (7%)	18 (9%)
Drug-related adverse event†	1 (<1%)	5 (2%)
Serious adverse event	21 (10%)	33 (16%)
Drug-related serious adverse event†	0	0
Fatal adverse event	5 (2%)	11 (5%)
Adverse event leading to permanent discontinuation of study drug	10 (5%)	11 (5%)

Data are the number of patients with at least one event (%). Mild events were easily tolerated by the patient, caused minimal discomfort, and did not interfere with everyday activities. Moderate events were sufficiently uncomfortable to interfere with normal everyday activities and sometimes required intervention. Severe events prevented normal everyday activities and typically required treatment or another intervention. *Patients are counted only once in the category of their adverse event with the most severe intensity. †As assessed by the principal investigator.

Table 4: **Adverse events from the safety analysis dataset**

	Placebo (n=5)	Ribaxamase (n=11)
Cancer	2 (40%)	2 (18%)
Respiratory failure	2 (40%)	3 (27%)
Worsening pneumonia	1 (20%)	1 (9%)
Respiratory insufficiency	1 (20%)	1 (9%)
Pulmonary fibrosis	0	1 (9%)
Cardiac	1 (20%)	6 (55%)*

Data are the number of events (%). *Includes one patient who died on day 1 of the study and one patient with a previously undiagnosed and untreated atrial fibrillation who died 27 days after the last dose of study drug from worsening of atrial fibrillation.

Table 5: **Fatal adverse events**

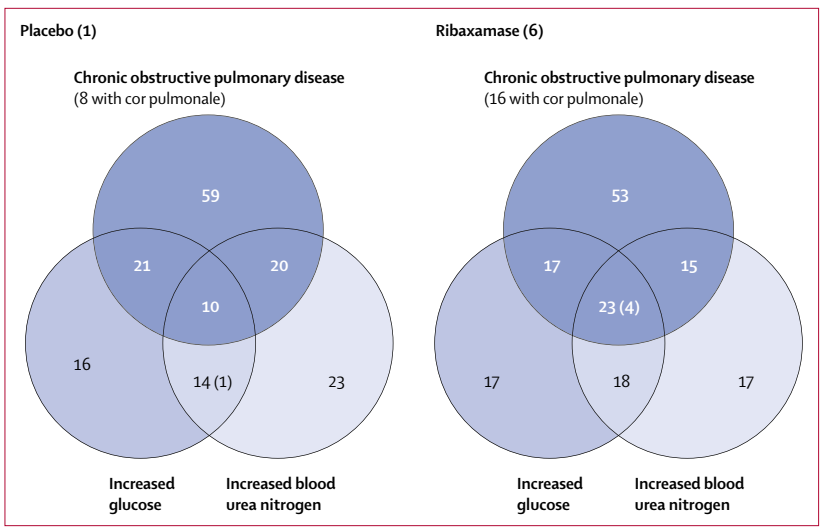

Figure 2: **Prevalence of baseline factors that could confer a risk of cardiac-associated death by treatment group**
Data are the number of patients in each treatment group with cardiac risk factors. Numbers in brackets indicate the patients in each treatment group who died of cardiac-associated causes. One patient in the ribaxamase group had none of these comorbidities. Increased blood glucose was defined as more than 7·8 mmol/L and increased blood urea nitrogen was defined as more than 8·2 mmol/L, which are indicative of end organ failure.

other electrocardiogram findings were similar between the groups.

A similar number of patients in each group received medication for stomach acid-related disorders: 80 (38·9%) patients in the ribaxamase group and 73 (35·4%) patients in the placebo group, including one patient with a *C difficile* infection in the ribaxamase group and three such patients in the placebo group. Only 5% of patients in each group received any drugs for constipation during the study, none of whom had a *C difficile* infection. The number of patients in whom the lower respiratory tract infection was adequately treated was similar between the groups at all three assessment timepoints including at the 2-week follow-up visit, at which the infection had been resolved in 175 (98·9%) of 177 patients in the ribaxamase group and 180 (99·4%) of 181 patients in the placebo group.

Discussion

Although the mechanism of action of ribaxamase was confirmed in previous studies,[13] our phase 2b study was designed to provide preliminary evidence that the addition of ribaxamase to treatment with β-lactam antibiotics, such as ceftriaxone, could reduce the incidence of *C difficile* infection, thus supporting its continued development. Our study design originally assumed an incidence of *C difficile* infection of 10% in the placebo group and 3% in the ribaxamase group, in accordance with the increased frequencies reported during the regional epidemic *C difficile* outbreak in the early 2000s.[1] In agreement with the original study design, however, ribaxamase reduced the incidence of *C difficile* infection in patients being treated with ceftriaxone, as confirmed by local laboratories in our study, by the same extent as originally anticipated: we found an incidence of 3·4% in the ribaxamase group versus 1·0% in the placebo group, corresponding to a 2·4% risk reduction (a 71% relative risk reduction), but the overall incidence of *C difficile* infection was lower than anticipated. Although infection with *C difficile* remains one of the most common hospital-acquired infections,[2] its incidence now ranges from 1–4%.[2,5,21]

The inclusion criteria for our study, including older age,[22] a longer hospital admission,[21] and the extended use of ceftriaxone (of at least 5 days), were meant to enrich for a population that was at increased risk of *C difficile* infection, and these criteria appeared to be successful, showing an incidence of 3·4% in the placebo group. This finding was consistent with the higher end of incidence of *C difficile* infection in the absence of an outbreak. The difference between the ribaxamase and placebo groups in incidence of *C difficile* infection, the primary endpoint of the study, was found to be significant at a prespecified one-sided p value of less than 0·05, which supports continued development of ribaxamase to prevent *C difficile* infection.

The statistical analysis plan originally considered all patients who discontinued the study before the 6-week follow-up telephone call as treatment failures, but this did not account for the study population being older and having more comorbidities than the general population and incorrectly assumed that all patients lost to follow-up should be considered to have developed *C difficile* infection. Although the incidence of *C difficile* of 3·4% in the placebo group was fairly high relative to previously reported incidences, to assume that all patients who discontinued the study would have developed *C difficile* infection was not supported by the nature of this infection or its reported incidence. This assertion was supported by the absence of reports of any additional cases of *C difficile* infection in patients who discontinued the study and also supported the primary study endpoint of laboratory-confirmed *C difficile* infection without discontinuations being counted as treatment failures. Before unmasking of the study groups, the protocol and statistical analysis plan were amended to incorporate a sensitivity analysis that did not consider patients who discontinued the study early as treatment failures in the assessment of the primary endpoint.

Although ribaxamase has the capacity to degrade most penicillins and cephalosporins, ceftriaxone was chosen for these studies because of its high concentration in the bile and its strong association with *C difficile* infection.[10–12] It was reasoned that, if ribaxamase could protect patients being treated with ceftriaxone against *C difficile* infection, then it could possibly also protect against *C difficile* infection caused by other β-lactam antibiotics.

Of the patients who developed *C difficile* infection, none reported having a *C difficile* infection previously, suggesting that these cases were all new onset and not recurrence of existing disease. Furthermore, none of the *C difficile*

infection patients received laxatives during the study which can, in some cases, account for diarrhoea. Seven of the patients with a *C difficile* infection (six patients in the placebo group and one patient in the ribaxamase group) received oral antibiotics as treatment for their *C difficile* infection, whereas no other patients received these antibiotics, further supporting the outcome of the study.

Disruption of the gut microbiome allows opportunistic pathogens such as *C difficile* and VRE to colonise the gastrointestinal tract;[6,23,24] we found a significant reduction in new colonisation by VRE in the ribaxamase group relative to the placebo group. Although colonisation with VRE is an important risk factor for VRE infection,[25] no VRE infections were reported during the 6-week follow-up period. These results were consistent with ribaxamase functioning to protect the integrity of the gut microbiome and thus preventing new colonisation by opportunistic pathogens.

Ribaxamase appeared to be generally well tolerated, and the resolution of the lower respiratory tract infection was similar between the two treatment groups, indicating that ribaxamase did not reduce the efficacy of ceftriaxone for treatment of the lower respiratory tract infection. There was, however, an imbalance in the number of cardiac-associated deaths between the groups. Most of these deaths appeared to be related to the patient's medical history. On the basis of risk analysis studies,[26] we would expect that 25–75% of high-risk patients with lower respiratory tract infections could have a fatal outcome. The difference in cardiac-associated deaths between the study groups was consistent with more patients with severe baseline conditions and at highest risk of death from lower respiratory tract infections being randomly assigned to receive ribaxamase relative to those assigned to receive placebo. The electrocardiogram results at baseline also indicated that more of the patients in the ribaxamase group had QRS intervals of at least 120 ms, which is a known risk factor for cardiovascular mortality.[27] These findings were consistent with the safety findings in earlier studies[13,18] that found that ribaxamase was well tolerated and not systemically absorbed. Further supporting this conclusion, ribaxamase is an enzyme that remains in the intestinal lumen to degrade residual β-lactams but does not affect systemic concentrations of antibiotics,[13] and it did not reduce the resolution of lower respiratory tract infections by ceftriaxone in our study.

To date, the only products licensed for treatment of *C difficile* infection are antibiotics such as oral vancomycin, metronidazole and fidaxomicin, or bezlotoxumab (which is indicated only for reducing recurrence of *C difficile* infection in adults receiving antibacterial treatment for *C difficile* infection).[4,28] Development is ongoing for other strategies for prevention and treatment of *C difficile* infection but, to date, none of these treatments have been approved.[4,29] Our study supports the continued clinical development of ribaxamase for prevention of *C difficile* infection in at-risk patients treated with intravenous β-lactam antibiotics, especially those being treated for extended durations for serious infections[11] or who have other underlying risk factors for *C difficile* infection.[21]

Contributors

JFK-K drafted the report. JFK-K, TR, OC, CL, HW, RS, VJW, and JS designed and supervised the study. JF-K, TR, OC, CL, HW, RS, VJW, and JS analysed the data. TR, OC, CL, HW, RS, VJW, and JS reviewed the report. TR, HW, and JS also supervised the clinical operations, and CL provided statistical analysis.

Declaration of interests

All authors are employees or paid consultants of Synthetic Biologics. We declare no additional competing interests.

Data sharing

The data will not be freely available because they are supporting evidence for the development of a commercial product.

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