Efficacy of bezlotoxumab in preventing recurrence of Clostridioides difficile infection: an Italian multicenter cohort study

Marianna Meschiari , Alessandro Cozzi-Lepri , Adriana Cervo , Guido Granata , Carlotta Rogati , Erica Franceschini , Stefania Casolari , Paola Tatarelli , Daniele Roberto Giacobbe , Matteo Bassetti , Simone Mornese Pinna , Francesco Giuseppe De Rosa , Francesco Barchiesi , Benedetta Canovari , Carolina Lorusso , Giuseppe Russo , Giovanni Cenderello , Antonio Cascio , Nicola Petrosillo , Cristina Mussini

PII: S1201-9712(23)00131-5
DOI: https://doi.org/10.1016/j.ijid.2023.04.004
Reference: IJID 6699

To appear in: *International Journal of Infectious Diseases*

Received date: 21 November 2022
Revised date: 15 March 2023
Accepted date: 3 April 2023

Please cite this article as: Marianna Meschiari , Alessandro Cozzi-Lepri , Adriana Cervo , Guido Granata , Carlotta Rogati , Erica Franceschini , Stefania Casolari , Paola Tatarelli , Daniele Roberto Giacobbe , Matteo Bassetti , Simone Mornese Pinna , Francesco Giuseppe De Rosa , Francesco Barchiesi , Benedetta Canovari , Carolina Lorusso , Giuseppe Russo , Giovanni Cenderello , Antonio Cascio , Nicola Petrosillo , Cristina Mussini , Efficacy of bezlotoxumab in preventing recurrence of Clostridioides difficile infection: an Italian multicenter cohort study, *International Journal of Infectious Diseases* (2023), doi: https://doi.org/10.1016/j.ijid.2023.04.004

Efficacy of bezlotoxumab in preventing recurrence of *Clostridioides difficile* infection: an Italian multicenter cohort study

Marianna Meschiari[1], Alessandro Cozzi-Lepri [2], Adriana Cervo[1], Guido Granata [3], Carlotta Rogati[1], Erica Franceschini[1], Stefania Casolari[4], Paola Tatarelli[4], Daniele Roberto Giacobbe[5,6], Matteo Bassetti[5,6], Simone Mornese Pinna[7], Francesco Giuseppe De Rosa[7], Francesco Barchiesi[8], Benedetta Canovari[9], Carolina Lorusso[10], Giuseppe Russo[10], Giovanni Cenderello[11], Antonio Cascio[12], Nicola Petrosillo[13], Cristina Mussini[1]

Corresponding author:

Marianna Meschiari[1]

[1]*Department of Infectious Diseases, Azienda Ospedaliero-Universitaria di Modena, Policlinico di Modena, University of Modena and Reggio Emilia – Modena (Italy);* *mariannameschiari1209@gmail.com*; *mmeschiari@unimore.it*

+39 059 4225830

Alessandro Cozzi-Lepri [2]

[2]*Infection and Population Health, UCL Institute for Global Health, London (UK);* *a.cozzi-lepri@ucl.ac.uk*

Adriana Cervo[1]

[1]*Department of Infectious Diseases, Azienda Ospedaliero-Universitaria di Modena, Policlinico di Modena, University of Modena and Reggio Emilia – Modena (Italy);* *adriana.cervo@gmail.com*

Guido Granata [3]

[3]*Clinical and Research Department for Infectious Diseases, National Institute for Infectious Diseases L. Spallanzani IRCCS, Roma (Italy);* *guido.granata@inmi.it*

Erica Franceschini[1]

[1]*Department of Infectious Diseases, Azienda Ospedaliero-Universitaria di Modena, Policlinico di Modena, University of Modena and Reggio Emilia – Modena (Italy);* *ericafranceschini0901@gmail.com*

Stefania Casolari[4]

[4]*Infectious Diseases clinic, Hospital of Ravenna – Ravenna (Italy);* *casolaristefania@hotmail.com*

Paola Tatarelli[4]

[4]*Infectious Diseases clinic, Hospital of Ravenna – Ravenna (Italy);* *paolatatarelli@gmail.com*

Daniele Roberto Giacobbe[5,6]

[5]*Department of Health Sciences, University of Genoa, Genoa (Italy)*

[6]*IRCCS Ospedale Policlinico San Martino, Genoa (Italy);* *danieleroberto.giacobbe@unige.it*

Matteo Bassetti[5,6]

[5]*Department of Health Sciences, University of Genoa, Genoa (Italy)*

[6]*IRCCS Ospedale Policlinico San Martino, Genoa (Italy);* *matteo.bassetti@unige.it*

Simone Mornese Pinna[7]

[7]*Department of Medical Sciences, Infectious Diseases, University of Turin, A.O.U. Città della Salute e della Scienza di Torino – Torino (Italy);* *simonemornese87@gmail.com*

Francesco Giuseppe De Rosa[7]

[7]*Department of Medical Sciences, Infectious Diseases, University of Turin, A.O.U. Città della Salute e della Scienza di Torino – Torino (Italy);* *francescogiuseppe.derosa@unito.it*

Francesco Barchiesi[8]

[8]*Dipartimento di Scienze Biomediche e Sanità Pubblica, Università Politecnica delle Marche, Ancona; Malattie Infettive, Azienda Ospedaliera – Ospedali Riuniti Marche Nord, Pesaro (Italy);* *f.barchiesi@staff.univpm.it*

Benedetta Canovari[9]

[9]*Malattie Infettive, Azienda Ospedaliera – Ospedali Riuniti Marche Nord, Pesaro (Italy);* *benedetta.canovari@ospedalimarchenord.it*

Carolina Lorusso[10]

[10]*Department of Mental Health and Addiction -Local Health Unit 4-LIGURIA, Genoa (Italy);* *carolina.lorusso@asl4.liguria.it*

Giuseppe Russo[10]

[10]*Department of Mental Health and Addiction -Local Health Unit 4-LIGURIA, Genoa (Italy);* *giuseppe.russo@asl4.liguria.it*

Giovanni Cenderello[11]

[11]*Infectious Diseases Unit ASL1 Imperiese, Sanremo (Italy);* *g.cenderello@asl1.liguria.it*

Antonio Cascio[12]

[12]*Infectious and Tropical Diseases Unit- Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties "G D'Alessandro," University of Palermo, Palermo, Italy;* *antonio.cascio03@unipa.it*

Nicola Petrosillo[13]

[13]*Fondazione Policlinico Universitario Campus Biomedico, Roma (Italy);* *n.petrosillo@unicampus.it*

Cristina Mussini

[1]*Department of Infectious Diseases, Azienda Ospedaliero-Universitaria di Modena, Policlinico di Modena, University of Modena and Reggio Emilia – Modena (Italy);* *cristina.mussini@unimore.it*

Abstract

Objectives: Bezlotoxumab (BEZ) is a promising tool for preventing recurrence of *Clostridioides difficile infection* (rCDI). The aim of the study was to emulate, in a real-world setting, the MODIFY trials in a cohort of participants with multiple risk factors for rCDI treated with BEZ in addition to standard of care (SoC) vs. SoC alone.

Methods: A multicenter cohort study was conducted including 442 patients with CDI from 2018 to 2022 collected from 18 Italian centers. The main outcome was the 30-days occurrence of rCDI. Secondary outcomes were: (i) all-cause mortality at 30 days (ii) composite outcome (30-day recurrence and/or all-cause death).

Results*:* rCDI at day 30 occurred in 54 (12%): 11 in the BEZ+SoC group and 43 treated with SoC alone (8% vs. 14%, OR=0.58, 95%CI:0.31-1.09, p=0.09). The difference between BEZ+SoC vs. SoC was statistically significant after controlling for confounding factors (aOR=0.40, 95%CI:018-0.88, p=0.02) and even more using the composite outcome (aOR=0.35, 95%CI:0.17-0.73, p=0.005).

Conclusion: Our study confirms the efficacy of BEZ+SoC for the prevention of rCDI and death in a real-world setting. BEZ should be routinely considered among participants at high risk of rCDI regardless of age, type of CDI therapy (vancomycin vs. fidaxomicin) and number of risk factors.

Keywords: *Clostridioides difficile* infection, recurrence, bezlotoxumab

Introduction

Clostridioides difficile (CD) is the main pathogen responsible for community and healthcare-associated bacterial infectious colitis and hospital outbreaks worldwide[1]. In Europe, *Clostridioides difficile* infection (CDI) accounts for 4% of care-related infections with an incidence rate of 4 per 10,000 patient-days and mortality ranging from 8 to 31% [2,3]. The same results were confirmed in the FADOI-PRACTICE observational study involving more than 40 different Italian Internal Medicine Units, reporting an overall CDI incidence rate of 5.3 per 10,000 patient-days over a 4-month period from October 2013 to January 2014 [4]. The clinical manifestations of CDI are extremely variable, ranging from mild symptoms such as simple enteritis to potentially lethal forms such as toxic megacolon, shock and intestinal perforation. Complications mainly occur in elderly, immunocompromised individuals and in the context of infection with epidemic ribotypes such as 027 [5]. Among these specific populations at risk, together with appropriate antimicrobial therapy tailored to the severity of the disease, preventing recurrence of CDI (rCDI) is becoming increasingly crucial. Indeed, the reported recurrence rate of CDI varies from 10 to 25% in the first episode and increase from 30 to 65% in cases of subsequent recurrences (up to 50% over the age of 65 years) [6,7]. A recent prospective study enrolled 309 hospitalized participants from 15 Italian hospitals showed that rCDI occurred in 21% of participants with an incidence rate of 72/10,000 patient-days and an all-cause mortality rate of 10.7%[8]. Moreover, rCDI is associated with a higher risk of death, decrease quality of life and higher hospitalization costs and hospital readmissions [9,10]. In this ever-increasing scenario, the prevention of rCDI represents the main challenge in the clinical management of participants with CDI. Bezlotoxumab (BEZ), a novel fully humanized monoclonal antibody directed against the binding domains of Toxin B produced by CD, that is given as a one-time infusion in addition to a standard of care (SoC) antimicrobial, fits in as a promising tool at our disposal to breaking the cycle of recurrence [11]. The main advantage of this innovative strategy is that it does not affect the effectiveness of the antibacterial agents used to treat CDI and, on the

contrary, could reduce the need for them, thus minimizing further intestinal micro-perturbation that predisposes to subsequent recurrences.

Two randomized, placebo-controlled, phase 3 trials, the MODIFY I and MODIFY II studies, showed a substantial lower rate of recurrent infection than placebo with a comparable safety profile [12]. One limitation of these trials was the fact that the target population was a selected sample of participants with low prevalence of multiple risk factors for recurrence and that several of these factors, including immunodeficiency, have been loosely defined on clinical criteria.

Nevertheless, similar results were observed in a number of more recent observational studies of real-world populations conducted in Europe as well as in the USA [13–15]. The majority of these were retrospective cohorts including only participants treated with BEZ with no control group. The most recent study conducted in Colorado was a standard of care (SoC)-controlled trial emulation which also confirmed the difference in risk seen in the trials and extended these findings to a population enriched with participants with multiple risk factors [16]. These studies led to update in 2021 the most recent European and American guidelines, that recommend the use of Bezlotoxumab in addition to SoC in case of: (i) a first CDI episode with high risk of recurrence; (ii) a first CDI recurrence when fidaxomicin was used to manage the initial CDI episode; (iii) second or multiple CDI recurrences [7,17].

However, despite this growing data evidence supporting the use of BEZ to prevent rCDI, its use in Italy, as in many other European Countries, is still limited and restricted to participants who experienced previous relapses. This might be mainly explained by direct drug cost of BEZ which is higher than available standard of care treatments.

Here we aimed to emulate, in a real-world setting, the MODIFY trials in a multi-center cohort of participants treated with BEZ in addition to SoC vs. SoC alone seen for care in several tertiary care hospitals across Italy.

Material and methods

Study design and clinical definitions

Our study design is that of a multicentre cohort enrolling participants from 18 Italian hospitals, including academic or tertiary referral hospitals (see full detailed list in Supplementary Table S1). All adult participants (age > 18 years) admitted to these participating sites over the period January 2018 to January 2022 had at least an episode of CDI and i) ≥1 risk factor for rCDI, ii) at least ≥30 days of documented follow-up after the end of antimicrobial treatment for CDI episode in question (baseline), and iii) were treated with either BEZ+SoC or only SoC.

The SoC cohort was an historical comparator group of participants included in the ReCloDi (Recurrence of *Clostridioides difficile* Infection) Study Group cohort, over the period from January 2018 to March 2020 [8]. The BEZ cohort was a newly recruited group from a subset of the sites participating in ReCloDi and three others sites over the more contemporary period of September 2018 to January 2022.

An incident CDI episode was defined on the basis of the new onset of the following conditions: a clinically significant diarrhea (≥3 stools of Bristol type 5, 6, or 7 in a 24-hour period) accompanied by a positive diagnostic test result (e.g. toxin enzyme immunoassay (EIA) and nucleic acid amplification test (NAAT)). A Zar-Score ≥2 was used to define a severe CDI episode [18].

In all participants the CDI was successfully treated until resolution of all CDI-defining conditions described above and they were followed-up until the development of the primary outcome of a CDI recurrence (rCDI) or at least 30 days from baseline.

rCDI was defined as the reappearance of the CDI-defining conditions within 30 days from baseline, which resulted again in pharmaceutical intervention, with or without positive stool test for toxigenic CD [7,19]. rCDI was assessed by physician follow-up visit, patient records or telephone interview with the patient or caregiver who were not blind to treatment allocation.

Data collection

Data collection from medical records included patient demographics, inpatient departments, prior hospitalization, and origin from long-term care facilities within 12 weeks of the current CDI episode, comorbidity burden assessed using Charlson Comorbidity Index (CCI), history of previous CDIs, risk factors for rCDI, severity of the current episode and CDI treatment and duration.

Risk factors for rCDI were considered as age > 65 years, compromised immunity (defined as use of immune-suppressive medication and/or presence of underlying disease such as onco-haematological conditions, solid organ transplant, chemotherapy), renal impairment, hepatic impairment, inflammatory bowel disease, HIV infection, use of pump proton inhibitors (PPI), concomitant antibiotic treatment at the CDI diagnosis and previous antibiotic exposure within 12 weeks and previous CDI episodes, according to the current literature [11,20].

SoC included vancomycin (VAN) alone or in association with iv metronidazole, fidaxomicin (FDX), iv metronidazole in monotherapy. VAN was prescribed at the standard fixed dosage or in taper regimes[21]. BEZ (10 mg/ kg) was administered as a single intravenous infusion over 60 minutes during or at the end of CDI treatment with SoC [12].

The investigation was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki. The study was approved by the Clinical Research Ethics Committee from the coordinating center (reference number CE n. 86/2021/OSS/AOUMO). Written informed consent was provided by all participants.

Outcome

The main outcome was the binary outcome indicating the occurrence of a rCDI at 30 days after the completion of CDI treatment [7,19].

Secondary outcomes were the alternative binary outcomes: (i) all-cause mortality at 30 days (ii) composite outcome (30-day recurrence or all-cause death).

Infusion-related adverse reactions and serious adverse events (SAE) that could potentially be related to BEZ were also assessed.

Statistical analysis

Descriptive statistics of the main characteristics of participants at study entry have been calculated. The $\chi 2$ and Fisher exact tests were utilized to compare categorical variables by treatment group, whereas continuous variables were analysed via Wilcoxon rank sum test as appropriate.

To control for potential confounding bias while aiming to emulate a randomised controlled trial, we fitted a marginal structural logistic regression model by means of inverse probability of treatment weighting (IPTW) of potential confounding factors. Our assumptions regarding underlying causal structure of the data is described in Supplementary Figure S1 through the visual aid of a direct acyclic graph (DAG). According to our assumptions, controlling for age, Zar-score, immuno-suppression, ≥1 CDI episodes within 8 weeks (all fitted as time-fixed covariates) is sufficient to block all backdoor confounding pathways from treatment to outcomes. In an alternative adjustment we have used the number of previous CDI episodes fitted as continuous instead of the indicator for ≥1 CDI episodes within 8 weeks. In order to assess the robustness of the results against potential unmeasured confounding bias, the e-value was calculated on the basis of the predictor showing the strongest association with the outcome [22]. We performed another adjusted analysis not considering patients treated with metronidazole iv alone, that is not considered anymore as optimal choice in CDI treatment among standard of care regimens[7].

Because of the larger number of events observed when using the composite outcome, to maximise the statistical power, subgroup analysis was planned for this secondary outcome by stratification by a number of *a priori* identified predictors: age (binary with a threshold of 70 years), type of CDI therapy (VAN vs. FDX) and the number of risk factors for rCDI (binary with threshold of 5 risk factors). Formal interaction test was performed to evaluate whether the difference in risk of outcomes might vary by strata.

Given the small number of participants and events, a couple of unadjusted sensitivity analyses were conducted: the first after restricting the analysis to the 3 clinical sites contributing data to both

treatment groups (Modena, Palermo and Genova); the second after restricting to the participants who never experienced previous CDI episodes.

The level of statistical significance was generally set at 0.05 or 0.05/3 for the interactions test to correct for inflation of type I error (Bonferroni correction). All analyses were conducted using SAS version 9.4 (Carey North Carolina USA).

Results

Overall, 442 participants with CDI were included in this analysis: 135 (31%) were treated with BEZ in combination with standard of care (SoC) therapy, 307 (69%) were treated with SoC alone. Demographic, clinical characteristics and treatments of the study participants are shown in Tables 1-3**.** The median age of patients was 73 (IQR 61, 81), 210 (48%) were female and the median Charlson score at time of treatment initiation was 5 (IQR 4, 7). BEZ was infused in the outpatient setting only in 10 (2%) participants, during or at the end of the treatment with SoC antibiotics.

Patients treated with SoC alone were all at their first CDI episode, while more than two third (n=95, 71%) of participants who received BEZ+SoC had experienced ≥1 previous CDI episodes; in particular, 56 (42%) and 39 (29%) were at the second or later episode, respectively. Sixty-five (48%) of these 95 participants treated with BEZ+SoC had a previous episode which occurred within 8 weeks of the date of treatment initiation, then treated for a recurrence.

The CDI episode was severe (Zar-score ≥ 2) in 152 (34%) individuals and there was little evidence for a difference by treatment group (BEZ+SoC vs. SoC alone, 39% vs. 32%, p=0.153).

Overall, the study population included patients at high risk of recurrence, however those in the BEZ+SoC group had a slightly higher number of risk factors for rCDI than those in SoC alone group (p=0.005) and were more likely to have ≥2 risk factors (99.3% vs. 95.7%, p=0.05). Regarding comorbidities, intestinal bowel disease was more frequent in individuals treated with BEZ+SoC (4%

vs. 0.3%, p=0.005); participants in BEZ+SoC group were also more likely to have in general an immunocompromising condition (58% vs. 39%, p<0.001).

There was no evidence for a difference by treatment group in previous antibiotic use, while concomitant antibiotic use was higher in the SoC alone group (62% vs. 47%, p=0.003) with similar data regardless of specific antibiotic class.

With regard to CDI therapy, vancomycin was the most frequently used drug, adopted in fixed dose (65%), in tapered regimen (4%) and in association with metronidazole (9%). As expected, the tapered regimen was mostly used in participants treated with BEZ+SoC (11% vs. 1%, p>0.001). Fidaxomicin was used mostly in participants of the BEZ+SoC group than in those treated with SoC alone (25% vs. 5%, p<0.001).

BEZ was well tolerated in all participants. No adverse events were reported even mild hypersensitivity reactions due to infusion.

Cure was obtained in 94% of participants, without any difference by treatment group (BEZ+SoC 91% vs. SoC alone 96%).

rCDI at day 30 occurred in 54 (12%) participants while all-cause death at 30 days occurred in 16 (3.6%) patients (Supplementary Table S2). Unadjusted and adjusted 30-day effectiveness outcomes are shown in Table 4. Among 54 participants who experienced rCDI, 11 were in the BEZ+SoC group and 43 were treated with SoC alone (8.1% vs. 14.0%, OR=0.58, 95% CI:0.31-1.09, p=0.09). This difference was more marked and statistically significant after controlling for confounding factors (aOR=0.40, 95% CI:0.18-0.88, p=0.02). Results were similar after controlling for total number of previous CDI episodes (fitted as a continuous covariate, Supplementary Table S3). Of note, with an observed odds ratio of 0.40 and an incidence of outcome of <15%, an unmeasured confounder that was associated with both the outcome and the treatment by a RR=4.4-fold each could explain away the estimate, but weaker confounding could not. Similarly, to move the confidence interval to include the null, an unmeasured confounder that was associated with the

outcome and the treatment by a risk ratio of 1.53-fold each could do so, but weaker confounding could not.

All-cause mortality within 30 days occurred less frequently in participants treated with BEZ+SoC than in those treated with SoC alone (0.7% vs. 4.9%, p=0.03). Using the composite outcome (recurrence and/or all-cause death at 30 days) there was even greater evidence for a benefit for participants treated with BEZ+SoC vs. SoC alone (aOR=0.35, 95% CI:0.17, 0.73, p=0.005) (Table 4). The benefit of BEZ+SoC vs SoC alone was strongly confirmed also in another supplemental analysis performed excluding patients treated with metronidazole intravenously and belonging only to SoC group (Table S4).

In the sensitivity analyses (unadjusted estimates only) results were also similar to those of the main analysis. After restricting to 141 participants enrolled in sites contributing both BEZ+SoC and SoC alone treated patients, the risk of rCDI was 5/72 (7%) in participants treated with BEZ+SoC vs. 11/69 (16%) in those treated with SoC alone (unadjusted OR 0.39, 95% CI: 0.10-1.32, p=0.09). Similarly, after restricting the analysis to 347 participants who were at their first CDI episode, 1/40 (3%) in the BEZ+SoC vs. 43/307 (14%) experienced a rCDI (unadjusted OR 0.16, 95% CI: 0.004-.99, p=0.04).

Finally, the forest plot in Figure 1 shows the estimated aOR in subsets of the study population for the secondary outcome of rCDI and/or death at day 30. Overall, there was no evidence for effect measure modification considering age, type of CDI therapy and number of risk factors. In particular, the aOR was similar regardless of the number of risk factors and similar to that of the main analysis (68-70% reduction in risk, p=0.79). Although not reaching statistical significance, the benefit of BEZ+SoC on the composite outcome appeared to be attenuated in participants aged under 70 years (p=0.61) and in those who received fidaxomicin (p=0.71). Follow-up up to 90 days was available for 127 of the 135 participants treated with BEZ+SoC (95%) and, among these, only one experienced a recurrence in the window 31-90 days from end of CDI treatment; therefore, the estimated 90-day risk

of rCDI in the BEZ+SoC group was 9.4% (Supplementary Table S5). No infusion-related reactions or SAE have been observed in the BEZ+SoC treated subset.

Discussion

To our knowledge, ours is the analysis of the largest real-world dataset to date, comparing BEZ plus SoC to SoC alone for prevention of rCDI. Our results are consistent with those of randomised trials showing a marked efficacy of BEZ when used in combination with SoC in rCDI prevention, reducing the risk of recurrence by 60% in the multiplicative scale (and 6% using the risk difference as the estimand) after controlling for key confounding factors. Importantly, we showed an even more significant reduction in the risk of developing a composite outcome (30-day recurrence and death) associated with the administration of BEZ+SoC.

Another recent trial emulation using observational study has been conducted in the USA showing similar results, although suggesting an even large effect of BEZ vs. SoC for the risk of rCDI (86% risk reduction by 90 days) [16]. In this study 53 participants also received BEZ between 2015 and 2019, in addition to SoC, were compared to 53 historical controls, receiving SoC alone, in the 2 years immediately prior to BEZ use [16]. As compared to the USA setting, access to care in Italy is universal and therefore it is important to show reproducibility (direct and conceptual) of these previous findings in a distinct geographical area with a national health system. In addition, although follow-up was shorter, sample size of our cohort is 4-fold bigger than the recent trial emulation conducted in the USA and the cohort of unexposed participants treated with SOC alone is a more contemporary group seen for care over 2018-2020 (vs. 2015-2016 in the study by Johnson et al), thus reducing one possible source of confounding [16].

The largest randomized studies comparing these same strategies are the MODIFY trials which also found similar efficacy of BEZ showing a risk difference vs. placebo for rCDI ranging between 10% and 16%, again slightly larger than the magnitude that we found, although the timing of the endpoint

was also 90 days [12]. Importantly, compared to these trials and more recent real-word European cohorts treated with BEZ, our study population has a larger proportion of hospitalized participants, more immunocompromised and a higher proportion with multiple rCDI risk factors (Supplementary Table S5) [8,12–16]. Indeed, when restricting to the subset of participants who received BEZ, most of our participants (71%) had ≥1 previous CDI episode pre-BEZ, 95% of participants had ≥2 risk factors for rCDI, and 63% an age > 65 years. In addition, multiple comorbidities were present at baseline, as shown by a mean CCI of 4.6. Despite these differences at baseline in comparison to other studies, the CDI recurrence rate of 8.1% in our participants who received BEZ+SoC by day 30 is entirely consistent with those reported by others (Supplementary Table S5). If anything, our risk of rCDI was slightly higher, possibly reflecting the fact that ours was a more difficult to treat population and/or because of other potential effect modifiers.

Unfortunately, although our study population included a large proportion of participants treated with fidaxomicin as part of SoC, was not powered to evaluate whether the benefit of BEZ might vary according to fidaxomicin use. Interestingly, subgroup analysis from MODIFY I/II showed effect measure modification by fidaxomicin use which, however, was not confirmed by our analysis and by others in the observational setting [23]. Although without reaching statistical significance, our results however indicate that the efficacy of BEZ+SoC in preventing recurrences might be even greater in participants aged 70+ and in those treated with vancomycin as SoC. These results are important to identify participants who are at risk for recurrent CDI and may best benefit from receiving this new promising therapeutic strategy in addiction to SoC.

In addition, our results for the first time show a larger beneficial effect of BEZ+SoC in preventing not only rCDI but also death. Indeed, although Spanish colleagues in their study including only patients treated with BEZ with no control group have shown that death is not directly related to CDI, it has been equally demonstrated how rCDI is independently associated with further nosocomial bloodstream infections (BSIs) and these increased significantly mortality attributable to primary BSI.

Moreover, innovative strategies to restore microbiome such as fecal microbiota transplantation increase overall survival by 30% [24]. The protective role of BEZ towards death could justify the reason why the 2021 ESCMID guidelines placed greater emphasis on the importance of preventing rCDI despite the higher costs of these innovative therapeutic strategies.

Our study has several limitations. First, the design of the study has potential pitfalls as it includes an historical control with only a few clinical sites contributing data for both strategies and none of the participants who received SoC alone had previously experienced ≥1 episode. However, the latter is a potential conservative bias and results were similar in sensitivity analyses after restricting to more comparable populations. Second, it is not a randomized study and although the analysis was conducted under transparent assumptions regarding the underlying causal structure of the data, unmeasured confounding cannot be ruled out (e.g. the exact clostridium ribotype). Data on CD strain type was also missing in Johnson's study; however previous studies suggested BEZ efficacy is not impacted by ribotype [25]. Nevertheless, several important confounders have been accounted for and our sensitivity analysis (e-values) shows that results are very robust to potential unmeasured confounding bias. Moreover, the presence of patients treated with suboptimal metronidazole iv only in the SoC group could influence the occurrence of the outcome in favor of SoC+BEZ group; however, the supplemental analysis conducted excluding those patients confirmed the benefit of the use of BEZ together with SoC in preventing rCDI.

In addition, most of the other studies reported the incidence of rCDI at day 90 while our follow-up ends at day 30 and therefore the overall incidence rates are difficult to compare. However, for the participants treated with BEZ+SoC alone we also provided the risk of rCDI by 90 days and our estimate is similar to that of other real-words studies of similar populations treated with BEZ (<10%). Moreover, the 30 day-period after the end of anti-CDI treatment corresponds to the time frame in which most of the rCDIs tend to occur (<30% of participants in MODIFY and <1% in our study experienced the event beyond 4 weeks of observation) and by extending the follow-up to 90

days, re-infections can also be included which complicates the interpretation. Finally, although the target population is likely to be representative of the Italian population, our results may not be applicable to other epidemiological contexts.

In conclusion, our results show a higher efficacy of BEZ+SoC vs. SoC alone for the prevention of rCDI confirming those seen in randomized studies and a similar previous trial emulation performed using observational data. A benefit of using BEZ+SoC vs. SoC alone was seen regardless of age, concomitant use of vancomycin vs. fidaxomicin and number of risk factors. Overall, these results support the updated clinical practice guidelines indicating that BEZ effectively and safely prevents rCDI and should be routinely considered among participants at high risk of rCDI regardless of their age and concomitant use of other CDI drugs.

Further studies are needed to assess the potential benefit associated with the use of fidaxomicin treatment concomitantly with BEZ. One of the main obstacles to more universal use of BEZ in routine practice is its high cost. A more precise selection of CDI treatments, based on independent cost-benefit analysis of health-economic studies in different settings and populations, is also required.

Transparency declaration

Conflict of interest

All authors declare no competing interests.

Funding

No external funding was received to complete this study.

Acknowledgement

All the participants to ReCloDi (Recurrence of *Clostridioides difficile* Infection) Study Group cohort.

Author contributions

MMes, CM and AC-L conceptualised and designed the study. AC, AC-L, MMes, CR and GG wrote and revised the manuscript. AC-L, Mmes, GG, and NP supervised the final version of the manuscript. AC-L did the statistical analysis. MMen and all the author participants contributed to data collection, clinical management of the patients and data interpretation. MMes is also the author responsible for the overall content as the guarantor.

Ethical Considerations

The study was approved by the local Institutional Review Board, that waived the need for the participants to sign the informed consent. The study was approved by Local ethical committee of University of Modena and Reggio Emilia. Reference number 0019510/21 of 06/23/2021.

References

[1] Balsells E, Shi T, Leese C, Lyell I, Burrows J, Wiuff C, et al. Global burden of Clostridium difficile infections: a systematic review and meta-analysis. J Glob Health 2019;9:010407. https://doi.org/10.7189/jogh.09.010407.

[2] European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals :2011 2012. LU: Publications Office; 2013.

[3] Evans CT, Safdar N. Current Trends in the Epidemiology and Outcomes of Clostridium difficile Infection. Clin Infect Dis 2015;60 Suppl 2:S66-71. https://doi.org/10.1093/cid/civ140.

[4] Cioni G, Viale P, Frasson S, Cipollini F, Menichetti F, Petrosillo N, et al. Epidemiology and outcome of Clostridium difficile infections in patients hospitalized in Internal Medicine: findings from the nationwide FADOI-PRACTICE study. BMC Infect Dis 2016;16:656. https://doi.org/10.1186/s12879-016-1961-9.

[5] Chilton CH, Pickering DS, Freeman J. Microbiologic factors affecting Clostridium difficile recurrence. Clin Microbiol Infect 2018;24:476–82. https://doi.org/10.1016/j.cmi.2017.11.017.

[6] Sheitoyan-Pesant C, Abou Chakra CN, Pépin J, Marcil-Héguy A, Nault V, Valiquette L. Clinical and Healthcare Burden of Multiple Recurrences of Clostridium difficile Infection. Clin Infect Dis 2016;62:574–80. https://doi.org/10.1093/cid/civ958.

[7] van Prehn J, Reigadas E, Vogelzang EH, Bouza E, Hristea A, Guery B, et al. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. Clin Microbiol Infect 2021;27 Suppl 2:S1–21. https://doi.org/10.1016/j.cmi.2021.09.038.

[8] Granata G, Petrosillo N, Adamoli L, Bartoletti M, Bartoloni A, Basile G, et al. Prospective Study on Incidence, Risk Factors and Outcome of Recurrent Clostridioides difficile Infections. J Clin Med 2021;10:1127. https://doi.org/10.3390/jcm10051127.

[9] Bouza E, Cornely OA, Ramos-Martinez A, Plesniak R, Ellison MC, Hanson ME, et al. Analysis of C. difficile infection-related outcomes in European participants in the bezlotoxumab MODIFY I and II trials. Eur J Clin Microbiol Infect Dis 2020;39:1933–9. https://doi.org/10.1007/s10096-020-03935-3.

[10] Olsen MA, Yan Y, Reske KA, Zilberberg MD, Dubberke ER. Recurrent Clostridium difficile infection is associated with increased mortality. Clin Microbiol Infect 2015;21:164–70. https://doi.org/10.1016/j.cmi.2014.08.017.

[11] Gerding DN, Kelly CP, Rahav G, Lee C, Dubberke ER, Kumar PN, et al. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection in Patients at Increased Risk for Recurrence. Clin Infect Dis 2018;67:649–56. https://doi.org/10.1093/cid/ciy171.

[12] Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection. N Engl J Med 2017;376:305–17. https://doi.org/10.1056/NEJMoa1602615.

[13] Escudero-Sánchez R, Ruíz-Ruizgómez M, Fernández-Fradejas J, García Fernández S, Olmedo Samperio M, Cano Yuste A, et al. Real-World Experience with Bezlotoxumab for Prevention of Recurrence of Clostridioides difficile Infection. J Clin Med 2020;10:E2. https://doi.org/10.3390/jcm10010002.

[14] Hengel RL, Ritter TE, Nathan RV, Van Anglen LJ, Schroeder CP, Dillon RJ, et al. Real-world Experience of Bezlotoxumab for Prevention of Clostridioides difficile Infection: A Retrospective Multicenter Cohort Study. Open Forum Infect Dis 2020;7:ofaa097. https://doi.org/10.1093/ofid/ofaa097.

[15] Oksi J, Aalto A, Säilä P, Partanen T, Anttila V-J, Mattila E. Real-world efficacy of bezlotoxumab for prevention of recurrent Clostridium difficile infection: a retrospective study of 46 patients in five university hospitals in Finland. Eur J Clin Microbiol Infect Dis 2019;38:1947–52. https://doi.org/10.1007/s10096-019-03630-y.

[16] Johnson TM, Molina KC, Howard AH, Schwarz K, Allen L, Huang M, et al. Real-World Comparison of Bezlotoxumab to Standard of Care Therapy for Prevention of Recurrent Clostridioides difficile Infection in Patients at High Risk for Recurrence. Clinical Infectious Diseases 2022;74:1572–8. https://doi.org/10.1093/cid/ciab674.

[17] Johnson S, Lavergne V, Skinner AM, Gonzales-Luna AJ, Garey KW, Kelly CP, et al. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis 2021;73:e1029–44. https://doi.org/10.1093/cid/ciab549.

[18] Zar FA, Bakkanagari SR, Moorthi KMLST, Davis MB. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. Clin Infect Dis 2007;45:302–7. https://doi.org/10.1086/519265.

[19] Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol 2013;108:478–98; quiz 499. https://doi.org/10.1038/ajg.2013.4.

[20] Deshpande A, Pasupuleti V, Thota P, Pant C, Rolston DDK, Hernandez AV, et al. Risk factors for recurrent Clostridium difficile infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol 2015;36:452–60. https://doi.org/10.1017/ice.2014.88.

[21] Hota SS, Sales V, Tomlinson G, Salpeter MJ, McGeer A, Coburn B, et al. Oral Vancomycin Followed by Fecal Transplantation Versus Tapering Oral Vancomycin Treatment for Recurrent Clostridium difficile Infection: An Open-Label, Randomized Controlled Trial. Clin Infect Dis 2017;64:265–71. https://doi.org/10.1093/cid/ciw731.

[22] VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. Ann Intern Med 2017;167:268–74. https://doi.org/10.7326/M16-2607.

[23] Dubberke ER, Gerding DN, Kelly CP, Garey KW, Rahav G, Mosley A, et al. Efficacy of Bezlotoxumab in Participants Receiving Metronidazole, Vancomycin, or Fidaxomicin for Treatment of Clostridioides (Clostridium) difficile Infection. Open Forum Infect Dis 2020;7:ofaa157. https://doi.org/10.1093/ofid/ofaa157.

[24] Falcone M, Russo A, Iraci F, Carfagna P, Goldoni P, Vullo V, et al. Risk Factors and Outcomes for Bloodstream Infections Secondary to Clostridium difficile Infection. Antimicrob Agents Chemother 2016;60:252–7. https://doi.org/10.1128/AAC.01927-15.

[25] Johnson S, Citron DM, Gerding DN, Wilcox MH, Goldstein EJC, Sambol SP, et al. Efficacy of Bezlotoxumab in Trial Participants Infected With Clostridioides difficile Strain BI Associated With Poor Outcomes. Clin Infect Dis 2021;73:e2616–24. https://doi.org/10.1093/cid/ciaa1035.

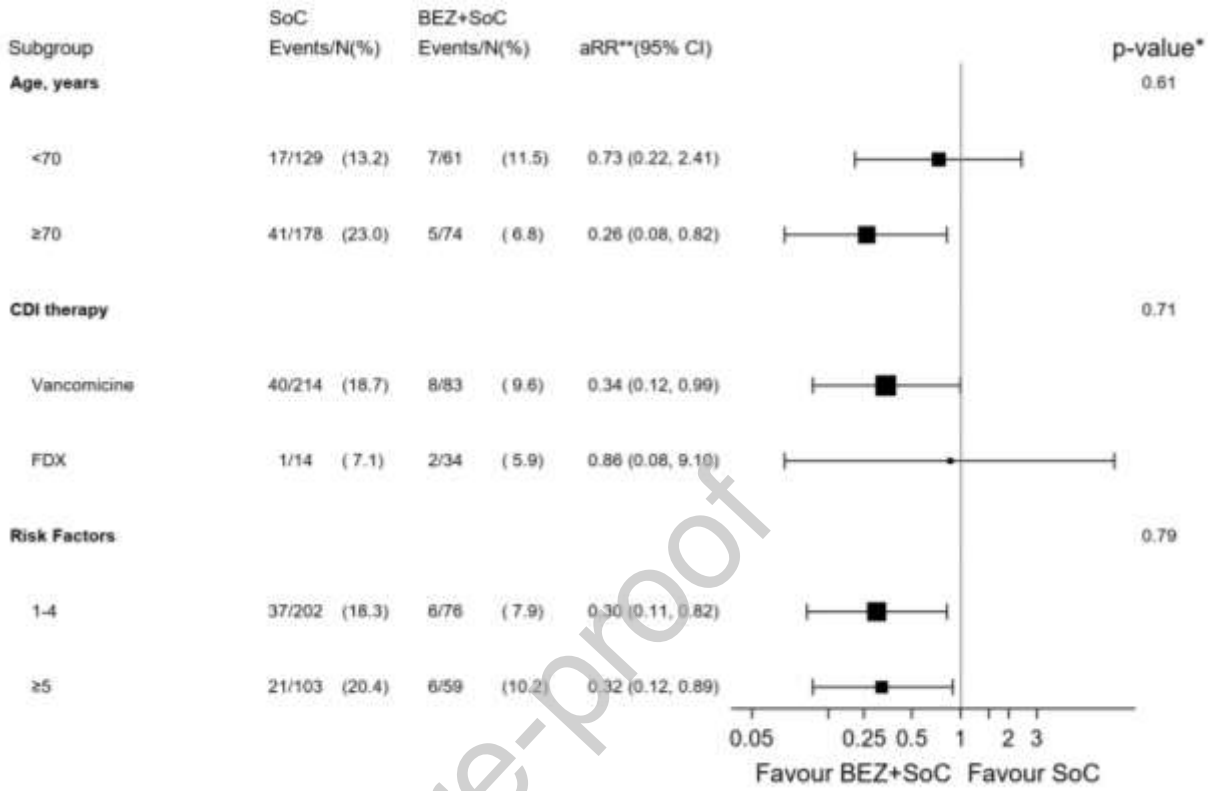

Figure 1. Forest plot of subsets analysis by secondary endpoint (CDI recurrence or death at day 30)

Subgroup analysis was conducted for the secondary outcome (rCDI or death at day 30) by stratification by a number of *a priori* identified predictors: age (binary with a threshold of 70 years), risk factors). Formal interaction test was performed to evaluate whether the difference in risk of outcomes might vary by strata. type of CDI therapy (VAN vs. FDX) and the number of risk factors for rCDI (binary with threshold of 5

*p-value corresponds to the test for interaction between intervention (BEZ+SoC vs SoC alone) and each subgroup unadjusted for multiplicity; **aRR from fitting a standard logistic regression analysis adjusted for age, immunosuppression, zar score and previous CDI episode within 8 weeks.

Abbreviations: aRR, adjusted relative risk; BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; CI, confidence interval; FDX, fidaxomicin; SoC, standard of care.

402

403 **Table 1. Key baseline factors by intervention: standard of care (SoC) treatment for *Clostridioides difficile***

404 **infections vs SoC + Bezlotoxumab**

405

	Intervention			
Characteristics	**SoC +BEZ**	**SoC**	**p-value***	**Total**
	N = 135	N = 307		N = 442
Age, years			0.6004	
Median (IQR)	72 (62,	73 (60,		73 (61,

	80)	82)		81)
Gender, n(%)			0.814	
Female	63 (46.7%)	147 (49.7%)		210 (47.5%)
Long term facility over prior 3 months, n(%)			0.501	
Yes	20	38		58

	(14.8%)	(12.5%)	(13.2%)
Hospitalization over prior 3 months, n(%)			<.001
	107	178	285
Yes	(79.3%)	(58.0%)	(64.5%)
Admission ward, n(%)			<.001

	115	236	351
Medical area	(87.1%)	(76.9%)	(80.0%)
	9	43	52
Surgical area	(6.8%)	(14.0%)	(11.8%)
	0	10	10
Outpatient	(0.0%)	(3.3%)	(2.3%)

Emergency	0 (0.0%)	18 (5.9%)	18 (4.1%)
ICU	8 (6.1%)	0 (0.0%)	8 (1.8%)
Previous CDI episodes, n(%)		<.001	
Yes	95 (70.9)	0 (0.0%)	95 (21.5)

	%)			%)
CDI episodes over prior 8 weeks, n(%)			< .001	
Yes	65 (48.1%)	0 (0.0%)		65 (14.7%)
Year of starting			< .001	
Median (IQR)	2020 (2019 (	2020 (2019 (		2020 (2019 (

	2 0 1 9 , 2 0 2 1)	2 0 1 8 , 2 0 1 9)	2 0 1 8 , 2 0 2 0)
Duration of treatment, days			0 . 8 2 5
Median (IQR)	1 0 (1 0 , 1 4)	1 2 (1 0 , 1 5)	1 1 (1 0 , 1 4)

*Chi-square or Mann-Whitney test as appropriate

Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; ICU, intensive care unit; IQR, interquartile range; SoC, standard of care.

410
411 **Table 2 . Comorbidities by intervention: standard of care (SoC) treatment for *Clostridioides difficile* infections vs**
412 **SoC + Bezlotoxumab**

	Intervention			
Characteristics	**SoC+BEZ**	**SoC**	**p-value***	**Total**
	N=135	N=306		N=441
Charlson comorbidity index			0.312	
Median (IQR)	5 (4, 7)	5 (4, 7)		5 (4, 7)
No FDR for CDI			0.005	

Median (IQR)	4 (4, 5)	4 (3 , 5)	4 (3, 5)
FDR=1, n(%)	1 (0.7 %)	13 (4 . 2 %)	14 (3. 2 %)
FDR=2, n(%)	8 (5.9 %)	40 (13 . 0 %)	48 (10. 9 %)
FDR=3, n(%)	23 (17. 0%)	57 (18 . 6	80 (18. 1 %

		%		)
		)		
FDR=4,	44	9		1
n(%)	(32.	2		3
	6%	(		6
	)	3		(
		0		3
		.		0.
		0		8
		%		%
		)		)
FDR>=5,	59	1		1
n(%)	(43.	0		6
	7%	3		2
	)	(		(
		3		3
		3		6.
		.		7
		6		%
		%		)
		)		
Zar Score	53	9	0	1
>=2, n(%)	(39.	9	.	5
	3%	(	1	2
	)	3	5	(
		2	3	3
		.		4.
		2		4
		%		%
		)		)

Comorbidities, n(%)				
Chronic kidney disease	26 (19.3%)	61 (19.9%)	0.882	87 (19.7%)
Cirrhosis/hepatopathy	11 (8.1%)	29 (9.4%)	0.662	40 (9.0%)
IBD	5 (3.7%)	1 (0.3%)	0.005	6 (1.4%)
HIV	1 (0.7%)	1 (0.5	0.55	2 (0.5

		3%)	0	%)
Immunosuppression	78 (57.8%)	12 1 (39.4%)	<.001	19 9 (45.0%)
Solid organ transplant	11 (8.1%)	.(.%)		11 (8.1%)
Haematological disease	18 (13.3%)	24 (7.8%)	0.069	42 (9.5%)
Chemotherapy	3 (2.2	.(.		3 (

	%)	%)	2. 2 %)

*Chi-square or Mann-Whitney test as appropriate

Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; FDR, risk factor; HIV, human immunodeficiency virus, IBD, intestinal bowel disease; IQR, interquartile range; SoC, standard of care.

418

419 **Table 3. Antibiotic therapies by intervention: standard of care (SoC) treatment for *Clostridioides difficile***

420 **infections vs SoC + Bezlotoxumab**

	Intervention			
Therapies	**SoC+BEZ**	**SoC**	**p-value***	**Total**
	N=135	N=306		N=441
Antibiotic use within 3 months	99 (73.3%)	218 (71.2%)	0.653	317 (71.9%)

Penicillines	53 (39.3%)	108 (36.2%)	0.548	161 (37.2%)
Cephalosporines	45 (33.3%)	81 (27.2%)	0.192	126 (29.1%)
Fluoroquinolones	16 (11.9%)	51 (17.1%)	0.161	67 (15.5%)

		%)		%)
Concomitant use of antibiotic	63 (47.0 %)	19 (62.1 %)	0.003	25 (57.5 %)
Penicillines	30 (22.4 %)	6 (21.3 %)	0.018	9 (21.6 %)
Cephalosporines	14 (10.4 %)	5 (17.2 %)	0.075	6 (15.6 %)

		0 %)		0 %)
Fluoroquinolones	5 (3.7%)	20 (6.6%)	0.240	25 (5.7%)
Carbapenems	8 (6.0%)	34 (11.1%)	0.090	42 (9.6%)
Glycopeptides	4 (3.0%)	15 (4.9%	0.360	19 (4.3%

		)		)
Use of PPI	108 (80.6%)	214 (69.7%)	0.016	322 (73.0%)
CDI treatment				
Vancomycin	76 (57.1%)	210 (69.1%)	0.016	286 (65.4%)
Vancomycin tapered	15 (11.1%)	41 (13.3%)	<.001	19 (4.3%)

	.4%)	%)		4%)
Fidaxomicin	34 (25.6%)	14 (4.6%)	<.001	48 (11.0%)
Metronidazole	0 (0.0%)	37 (12.2%)	<.001	37 (8.5%)
Vancomycin+ Metronidazole	8 (6.0	39 (12	0.035	47 (10

	%	.	.
	)	8	8
		%	%
		)	)

*Chi-square or Mann-Whitney test as appropriate

421

422 Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; PPI, pump

423 proton inhibitor; SoC, standard of care.

424

425

Table 4. Effectiveness of Bezlotoxumab (BEZ) associated with standard of care (SoC) versus SOC alone by primary (recurrence of CDI) and secondary (rCDI or death) endpoint at 30 days of follow-up.

	Unweighted and weighted marginal relative risk			
	Unweighted RR (95% CI)	**p-value**	**Weighted* RR (95% CI)**	**p-value**
	All patients			
Primary endpoint (rCDI at day 30)				
SoC	1.00		1.00	
SoC+ BEZ	0.58 (0.31, 1.09)	0.092	0.40 (0.18, 0.88)	0.023

Secondary endpoint (rCDI or death at day 30)				
SoC	1.00		1.00	
SoC+ BEZ	0.47 (0.26, 0.85)	0.012	0.35 (0.17, 0.73)	0.005

*adjusted for age, Zar Score, immuno-suppression, CDI episodes within 8 weeks using IPW

Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; CI, confidence interval; IPW, inverse probability weighting; rCDI, *Clostridioides difficile* infection recurrence; RR, relative risk; SoC, standard of care.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: