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

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ABSTRACT

Objectives: Bezlotoxumab (BEZ) is a promising tool for preventing the 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 the standard of care (SoC) versus SoC alone.

Methods: A multicenter cohort study was conducted including 442 patients with *Clostridioides difficile* infection from 2018 to 2022, collected from 18 Italian centers. The main outcome was the 30-day occurrence of rCDI. The secondary outcomes were (i) all-cause mortality at 30 days (ii) and the 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%, odds ratio [OR] = 0.58, 95% confidence interval [CI]: 0.31-1.09, $P = 0.09$). The difference between BEZ + SoC versus SoC was statistically significant after controlling for confounding factors (adjusted OR = 0.40, 95% CI: 018-0.88, $P = 0.02$) and even more using the composite outcome (adjusted OR = 0.35, 95% CI: 0.17-0.73, $P = 0.005$).

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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 *Clostridioides difficile* infection therapy (vancomycin vs fidaxomicin), and number of risk factors.

Introduction

Clostridioides difficile (CD) is the main pathogen responsible for community and health care-associated bacterial infectious colitis and hospital outbreaks worldwide [1]. In Europe, CD 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 the 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 that 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, decreased 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 microperturbation that predisposes to subsequent recurrences.

Two randomized, placebo-controlled phase III 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 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 United States [13–15]. The majority of these were retrospective cohorts that included only participants treated with BEZ, with no control group. The most recent study conducted in Colorado was a 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 the updates in 2021 of the most recent European and American guidelines that recommend the use of BEZ in addition to SoC in case of: (i) a first CDI episode with a high risk of recurrence, (ii) a first CDI recurrence when fidaxomicin was used to manage the initial CDI episode, and (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 the available SoC treatments.

Here, we aimed to emulate, in a real-world setting, the MODIFY trials in a multicenter cohort of participants treated with BEZ in addition to SoC versus 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 multicenter cohort, enrolling participants from 18 Italian hospitals, including academic or tertiary referral hospitals (see full detailed list in Supplementary Table S1). All adult participants (aged >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 CDI) 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 based on 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 and nucleic acid amplification test). A Zar score ≥2 was used to define a severe CDI episode [18].

In all participants, the CDI was successfully treated until the resolution of all CDI-defining conditions described previously and they were followed up until the development of the primary outcome of an 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 a 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 blinded to the treatment allocation.

Data collection

Data collection from medical records included patient demographics, inpatient departments, previous hospitalization, and origin from six care facilities within 12 weeks of the current CDI episode, comorbidity burden assessed using the Charlson comorbidity index, history of previous CDIs, risk factors for rCDI, severity of the current episode, and CDI treatment and duration.

The risk factors for rCDI were considered as age >65 years, compromised immunity (defined as the use of immunosuppressive medication and/or presence of underlying disease, such as oncohematologic conditions, solid organ transplant, chemotherapy), renal impairment, hepatic impairment, inflammatory bowel disease, HIV infection, use of pump proton inhibitors, 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 intravenous metronidazole, fidaxomicin (FDX), and 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 an rCDI at 30 days after the completion of CDI treatment [7,19].

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

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

Statistical analysis

The descriptive statistics of the main characteristics of the participants at study entry have been calculated. The χ^2 and Fisher's exact tests were used to compare categorical variables by treatment group, whereas continuous variables were analyzed using the Wilcoxon rank sum test, as appropriate.

To control for potential confounding bias while aiming to emulate a randomized controlled trial, we fitted a marginal structural logistic regression model by means of inverse probability of treatment weighting of potential confounding factors. Our assumptions regarding the underlying causal structure of the data is described in Supplementary Figure S1 through the visual aid of a direct acyclic graph. According to our assumptions, controlling for age, Zar score, immunosuppression, and ≥1 CDI episodes within 8 weeks (all fitted as time-fixed covariates) are sufficient to block all backdoor confounding pathways from treatment to outcomes. In an alternative adjustment, we used the number of previous CDI episodes fitted as continuous instead of the indicator for ≥1 CDI episodes within 8 weeks. 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, which is no longer considered as the optimal choice in CDI treatment among SoC regimens [7].

Because of the larger number of events observed when using the composite outcome, to maximize the statistical power, a subgroup analysis was planned for this secondary outcome through 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 five risk factors). A 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 three 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 (Cary, NC, USA).

Results

Overall, 442 participants with CDI were included in this analysis: 135 (31%) were treated with BEZ in combination with SoC therapy, and 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 years (interquartile range 61, 81), 210 (48%) were female, and the median Charlson score at time of treatment initiation was 5 (interquartile range 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, whereas more than two-thirds (n = 95, 71%) of the 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. A total of 65 (48%) of these 95 participants treated with BEZ + SoC had a previous episode, which occurred within 8 weeks of the date of treatment initiation and was then treated for a recurrence.

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

Overall, the study population included patients at a high risk of recurrence; however, those in the BEZ + SoC group had a slightly more risk factors for rCDI than those in the 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 the BEZ + SoC group were also more likely to have, in general, an immunocompromising condition (58% vs 39%, $P < 0.001$).

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

Regarding 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%, $P < 0.001$).

Table 1
Key baseline factors by intervention: SoC treatment for CDI versus SoC + BEZ.

Characteristics	Intervention			
	SoC+BEZ N = 135	SoC N = 307	*P*-value[a]	Total N = 442
Age, years			0.604	
Median (IQR)	72 (62, 80)	73 (60, 82)		73 (61, 81)
Gender, n(%)			0.814	
Female	63 (46.7%)	147 (47.9%)		210 (47.5%)
Long-term facility over prior 3 months, n(%)			0.501	
Yes	20 (14.8%)	38 (12.5%)		58 (13.2%)
Hospitalization over prior 3 months, n(%)			<.001	
Yes	107 (79.3%)	178 (58.0%)		285 (64.5%)
Admission ward, n(%)			<.001	
Medical area	115 (87.1%)	236 (76.9%)		351 (80.0%)
Surgical area	9 (6.8%)	43 (14.0%)		52 (11.8%)
Outpatient	0 (0.0%)	10 (3.3%)		10 (2.3%)
Emergency	0 (0.0%)	18 (5.9%)		18 (4.1%)
Intensive care unit	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, 2021)	2019 (2018, 2019)		2019 (2018, 2020)
Duration of treatment, days			0.825	
Median (IQR)	10 (10, 14)	12 (10, 15)		11 (10, 14)

[a] Chi-square or Mann-Whitney test as appropriate.Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; IQR, interquartile range; SoC, standard of care.

Table 2
Comorbidities by intervention: SoC treatment for CDI versus SoC + BEZ.

Characteristics	Intervention			
	SoC+BEZ N = 135	SoC N = 306	*P*-value[a]	Total 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, n(%)	44 (32.6%)	92 (30.0%)		136 (30.8%)
FDR ≥ 5, n(%)	59 (43.7%)	103 (33.6%)		162 (36.7%)
Zar score ≥ 2, n(%)	53 (39.3%)	99 (32.2%)	0.153	152 (34.4%)
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%)
Intestinal bowel disease	5 (3.7%)	1 (0.3%)	0.005	6 (1.4%)
HIV	1 (0.7%)	1 (0.3%)	0.550	2 (0.5%)
Immunosuppression	78 (57.8%)	121 (39.4%)	<.001	199 (45.0%)
Solid organ transplant	11 (8.1%)	. (.%)		11 (8.1%)
Hematologic disease	18 (13.3%)	24 (7.8%)	0.069	42 (9.5%)
Chemotherapy	3 (2.2%)	. (.%)		3 (2.2%)

[a] Chi-square or Mann-Whitney test as appropriate.Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; FDR, risk factor; IQR, interquartile range; SoC, standard of care.

BEZ was well tolerated in all participants. No adverse events were reported, not 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, whereas all-cause death at 30 days occurred in 16 (3.6%) patients (Supplementary Table S2). The 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%, odds ratio [OR] = 0.58, 95% confidence interval [CI]: 0.31-1.09, $P = 0.09$). This difference was more marked and statistically significant after controlling for confounding factors (adjusted OR = 0.40, 95% CI: 0.18-0.88, $P = 0.02$). The results were similar after controlling for the total number of previous CDI episodes (fitted as a continuous covariate; Supplementary Table S3). Of note, with an observed OR 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 relative risk of 4.4-fold each could explain the estimate but weaker confounding could not. Similarly, to move the CI 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 (adjusted OR = 0.35, 95% CI: 0.17-0.73, $P = 0.005$) (Table 4). The benefit of BEZ + SoC versus SoC alone was strongly

Table 3
Antibiotic therapies by intervention: SoC treatment for CDI versus SoC + BEZ.

Therapies	Intervention			
	SoC+BEZ N = 135	SoC N = 306	*P*-value[a]	Total 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%)	190 (62.1%)	0.003	253 (57.5%)
Penicillines	30 (22.4%)	65 (21.3%)	0.801	95 (21.6%)
Cephalosporines	14 (10.4%)	52 (17.0%)	0.075	66 (15.0%)
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 pump proton inhibitor	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 (11.4%)	4 (1.3%)	<.001	19 (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 (12.8%)	0.035	47 (10.8%)

[a] Chi-square or Mann-Whitney test as appropriate.Abbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; SoC, standard of care.

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

	Unweighted and weighted marginal relative risk			
	Unweighted RR (95% CI)	*P*-value	Weighted RR (95% CI)[a]	*P*-value
	All patients			
Primary end point (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 end point (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

[a] Adjusted for age, Zar score, immunosuppression, CDI episodes within 8 weeks using inverse probability weightingAbbreviations: BEZ, bezlotoxumab; CDI, *Clostridioides difficile* infection; CI, confidence interval; rCDI, CDI recurrence; RR, relative risk; SoC, standard of care.

confirmed also in another supplemental analysis performed excluding patients treated with metronidazole intravenously and belonging only to the SoC group (Table S4).

In the sensitivity analyses (unadjusted estimates only), the 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 versus 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 versus 43/307 (14%) experienced an rCDI (unadjusted OR 0.16, 95% CI: 0.004-.99, $P = 0.04$).

Finally, the forest plot in Figure 1 shows the estimated adjusted odds ratio (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 the effect measure modification considering age, type of CDI therapy, and number of risk factors. In particular, the adjusted odds ratio (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 <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 of 31-90 days from the 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 serious adverse events have been observed in the BEZ + SoC-treated subset.

Discussion

To the best of our knowledge, ours is the analysis of the largest real-world dataset to date, comparing BEZ + SoC to SoC alone for the prevention of rCDI. Our results are consistent with those of randomized 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 United States showing similar results, although suggesting an even large effect of BEZ versus SoC for the risk of rCDI (86% risk reduction by 90 days) [16]. In this study, 53 participants who 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 before BEZ use [16]. Compared with the US setting, access to care in Italy is universal; 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 the follow-up was shorter, the sample size of our cohort is 4-fold bigger than the recent trial emulation conducted in the United States, and the

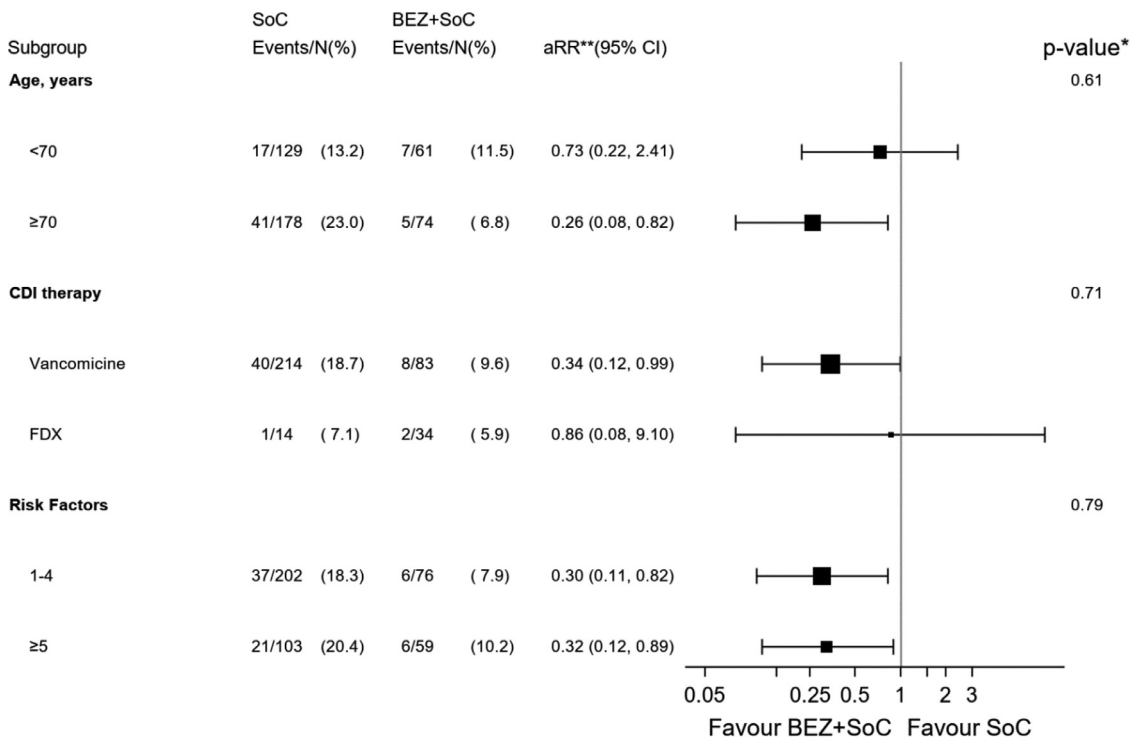

Figure 1. Forest plot of subsets analysis by secondary end point (CDI recurrence or death at day 30). Subgroup analysis was conducted for the secondary outcome (recurrence CDI or death at day 30) by stratification by a number of *a priori* identified predictors: age (binary with a threshold of 70 years), risk factors. Formal interaction test was performed to evaluate whether the difference in risk of outcomes might vary by strata. Type of CDI therapy (vancomycin vs FDX) and the number of risk factors for recurrence CDI (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.

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.* [16]), 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 versus placebo for rCDI ranging from 10% to 16%, again, slightly larger than the magnitude that we found, although the timing of the end point was also 90 days [12]. Importantly, compared with these trials and the more recent real-word European cohorts treated with BEZ, our study population has a larger proportion of hospitalized participants, more immunocompromised participants, and a higher proportion of participants with multiple rCDI risk factors (Supplementary Table S5) [8,12–16]. Indeed, when restricting to the subset of participants who received BEZ, most (71%) of our participants had ≥1 previous CDI episode pre-BEZ, 95% of participants had ≥2 risk factors for rCDI, and 63% were aged >65 years. In addition, multiple comorbidities were present at baseline, as shown by a mean Charlson comorbidity index of 4.6. Despite these differences at baseline compared with 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 that our population was more difficult to treat 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 the SoC, it was not powered to evaluate whether the benefit of BEZ might vary according to fidaxomicin use. Interestingly, the 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+ years and in those treated with vancomycin as the SoC. These results are important to identify participants who are at a risk for rCDI and may best benefit from receiving this new promising therapeutic strategy in addition to the 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 that included 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 and these increased significantly the mortality attributable to primary bloodstream infections. Moreover, innovative strategies to restore microbiome, such as fecal microbiota transplantation, increase the overall survival by 30% [24]. The protective role of BEZ toward death could justify the reason why the 2021 European Society of Clinical Microbiology and Infectious Diseases (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 because 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 conserva-

tive bias, and the results were similar in the 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 that BEZ efficacy is not impacted by ribotype [25]. Nevertheless, several important confounders have been accounted for, and our sensitivity analysis (e-values) shows that the 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, whereas our follow-up ends at day 30; 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 the 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 versus 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 versus SoC alone was seen regardless of age, concomitant use of vancomycin versus 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 a 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 a 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.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical considerations

The study was approved by the local institutional review board that waived the need for the participants to sign written informed consent forms. The study was approved by local ethical committee of University of Modena and Reggio Emilia. Reference number 0019510/21 of 06/23/2021.

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Author contributions

MMes, CM, and AC-L conceptualized 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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.004.

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