Article:
Zeng, Z, Zhao, H, Dorr, MB et al. (6 more authors) (2020) Bezlotoxumab for prevention of Clostridium difficile infection recurrence: Distinguishing relapse from reinfection with whole genome sequencing. Anaerobe, 61. 102137. ISSN 1075-9964

https://doi.org/10.1016/j.anaerobe.2019.102137

Bezlotoxumab for prevention of *Clostridium difficile* infection recurrence: distinguishing relapse from reinfection with whole genome sequencing

Zhen Zeng[1,a], Hailong Zhao[2,3,4,a], Mary Beth Dorr[1], Judong Shen[1], Mark H Wilcox[5], Ian R Poxton[6], Dalya Guris[1], Junhua Li[2,3,7], Peter M Shaw[1]

[a]Contributed equally to this work

[1]Merck & Co., Inc., Kenilworth, NJ, USA; [2]BGI-Shenzhen, Shenzhen, China; [3]China National GeneBank, BGI-Shenzhen, Shenzhen, China; [4]Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, 2100 Copenhagen Ø, Denmark; [5]Leeds Teaching Hospitals & University of Leeds, Leeds, UK; [6]University of Edinburgh, Edinburgh, UK; [7]School of Bioscience and Biotechnology, South China University of Technology, Guangzhou, China

Running title: Bezlotoxumab for relapse and reinfection

Conflict of interest statement: ZZ, MBD, JS, DG, and PMS are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and may own stock and/or stock options in the company. MHW has received consulting fees from Actelion, Astellas, bioMerieux, Cambimune, Da Volterra, MedImmune, Menarini, MSD, Meridian, Pfizer Inc., Qiagen, Sanofi-Pasteur, Seres, Summit, Synthetic Biologics, Inc., and Valneva; lecture fees from Alere, Astellas, MSD, and Pfizer Inc.; and grant support from Actelion, Astellas, bioMerieux, Da Volterra, MSD, Sanofi-Pasteur, Seres, and Summit.
HZ and JL are employees of BGI-Shenzhen, Shenzhen, China.
IRP has received consulting fees from Astellas and a research grant from ViroPharma.

Funding statement: This work was supported by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and grants from the National Natural Science Foundation of China (Grant Number: 31601073) and the National Major Science and

Technology Project of the Ministry of Science and Technology of China (Grant Number: 2017ZX10303406).

Previously presented: The data included in this manuscript were previously presented at IDWeek; San Diego, CA, USA; October 4–8, 2017; Poster #1271

Corresponding authors and contact details:

Peter M. Shaw

Genetics and Pharmacogenomics

Head of Clinical Pharmacogenetics

Merck & Co., Inc.

PO Box 4

770 Sumneytown Pike

WP53B-120

West Point, PA 19486, USA

Phone: +1-215-652-5713

Email: peter_shaw3@merck.com

Abstract:

Background: Bezlotoxumab has been shown to prevent *Clostridium difficile* infection recurrence (rCDI) in high-risk patients.

Methods: We used whole genome sequencing to estimate the impact of bezlotoxumab on same-strain relapse or new-strain reinfection in MODIFY I/II trials. Reinfection with a new strain and relapse with the same strain were differentiated by the comparison of ribotype (RT) and pair-wise single-nucleotide whole genome sequencing (WGS) variations (PWSNV). Relapse was assigned if the baseline RT and the RT isolated during rCDI were the same, and if PWSNVs were ≤ 2. Reinfection was assigned if the baseline RT and the RT isolated during rCDI were different, or if the RT was the same but PWSNVs were > 10. Unknown status was assigned if the RT was the same but PWSNVs were 3 - 10.

Results: 259 rCDI events were evaluable (50 [19.3%] reinfection; 198 [76.4%] relapse). The proportion of relapses was higher for ribotype 027 (84.5%) compared with other ribotypes (74.1%). Cumulative incidence of relapse was significantly lower for bezlotoxumab versus no bezlotoxumab ($p < 0.0001$), with a non-significant trend towards reduction for reinfection ($p = 0.14$).

Conclusion: Bezlotoxumab treatment significantly reduced the rate of CDI relapse versus a regimen without bezlotoxumab. (NCT01241552/NCT01513239)

Suggested keywords: Bezlotoxumab; *Clostridium difficile* infection; recurrence; reinfection; relapse; single nucleotide polymorphism; whole genome sequencing

Background

Clostridium difficile infection (CDI) causes considerable economic burden [1], and is a significant cause of morbidity and mortality worldwide [2]. Although antibacterial treatment for CDI is often successful, the rate of CDI recurrence (rCDI) following primary infection is between 15-25%, depending on treatment [3, 4]. Moreover, following a first recurrence, there is ~40% probability of a second recurrence [5]. While the majority of rCDI episodes are due to relapse with the same strain of *C. difficile* that caused the initial infection, 13–50% of recurrences are due to infection with a different strain [6, 7].

Bezlotoxumab, a fully human monoclonal antibody against *C. difficile* toxin B, has been shown to prevent rCDI in adults who are receiving antibacterial treatment for CDI and are at high risk of rCDI[8]. In two Phase 3 clinical trials, MODIFY I and II, bezlotoxumab was shown to reduce rCDI over 12 weeks compared with placebo [9]. A *post-hoc* analysis in participants with ≥1 risk factor for rCDI found that bezlotoxumab treatment resulted in a 16% absolute (43% relative) reduction in rCDI [10]. Another *post-hoc* analysis showed that bezlotoxumab reduced CDI-associated 30-day hospital readmission rates versus placebo, potentially reducing the costs related to CDI-associated readmissions [11]. Previous analyses had not evaluated whether benefits with bezlotoxumab result from preventing relapse of the same infection and/or preventing reinfection with a different strain of *C. difficile*.

Multiple methods can distinguish relapse from reinfection with a new strain of *C. difficile*; however, conventional typing methods such as multilocus sequencing and ribotyping are not sensitive enough to identify diversity present at a whole genome level, and may underestimate reinfection rates [12]. Alternatively, bacterial whole genome sequencing (WGS) allows for increased resolution and accuracy compared with traditional typing methods, and can distinguish between cases of relapse and reinfection [12, 13].

The objective of this *post-hoc* analysis was to estimate the impact of bezlotoxumab on rates of same-strain (relapse) or new strain (reinfection) rCDI using pooled data from the MODIFY I/II trials, along with WGS.

Methods

Study design

MODIFY I (NCT01241552) and MODIFY II (NCT01513239) were randomized, double-blind, placebo-controlled, multicenter Phase 3 trials conducted across 322 sites in 30 countries from November 1, 2011 to May 22, 2015 [9]. Participants were adults diagnosed with primary or recurrent CDI prior to study entry, who were receiving antibacterial treatment for CDI (metronidazole, vancomycin, or fidaxomicin, chosen by the treating physician) prescribed for 10–14 days. Participants were randomized 1:1:1:1 to receive a single 60-minute infusion (on Day 1) of bezlotoxumab alone (10 mg/kg of body weight), actoxumab and bezlotoxumab (10 mg/kg each), actoxumab alone (10 mg/kg; MODIFY I only), or placebo (0.9% saline), while they continued to receive antibacterial treatment.

Both trials were conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from all participants.

Endpoints

rCDI was defined as a new episode of diarrhea (≥3 unformed stools in 24 hours) associated with a positive stool test for toxigenic *C. difficile* within 12 weeks of randomization, in participants who had achieved initial clinical cure (ICC). ICC was defined as no diarrhea for 2 consecutive days following completion of antibacterial treatment administered for ≤16 days.

In this *post-hoc* analysis, only participants who experienced rCDI during the 12-week follow-up period were included. As the proportion of participants experiencing rCDI was not different for the bezlotoxumab and the bezlotoxumab + actoxumab groups, to increase power and estimation precision, the participants randomized to these groups were pooled and referred to as the 'bezlotoxumab' group. Similarly, because rCDI rates were not different for the actoxumab alone and placebo groups, these groups were pooled and referred to as the 'no bezlotoxumab' group.

Whole-genome sequencing and quality control.

Polymerase chain reaction (PCR)-free index libraries with an average insert size of 350 bp were prepared for each isolate. Genome sequencing was performed using the Illumina® HiSeq™ 2000 platform to generate 100-bp paired-end (PE) or 90-bp PE reads (Illumina, Inc., San Diego, CA). Raw reads were filtered by SOAPnuke (https://github.com/BGI-flexlab/SOAPnuke) to remove sequencing adapters and low-quality reads, including those containing more than three unknown (denoted 'N') bases, those in which > 50% of the bases scored < Q20, and those with read length < 30 bp. After the quality control process, high-quality reads with an average of 686 Mb were generated, which has a coverage of 170-fold across whole genome (~4Mb).

Whole-genome single nucleotide polymorphism (SNP) calling

High-quality reads were mapped against reference genome CD196 (NC_013315) by Burrows-Wheeler Aligner (BWA v0.6.2).[14] SNPs were identified using SAMtools v0.1.19.[15] An SNP was only considered to be valid if align qual ≥100, depth ≥ 10, and alt rate ≥ 0.8 (found in ≥ 80% of reads), otherwise it was treated as missing.

Repetitive regions in the reference genome sequence were identified using an in-house pipeline. Briefly, the reference genome was fragmented *in silico* into 20-bp reads using a sliding window approach; generated reads were then mapped back to the reference, and

reads mapping to multiple locations of the genome were identified. SNPs falling within these repetitive regions were excluded from all subsequent analyses. The remaining SNPs were used to constructed whole-genome SNP matrix. PWSNV was calculated as the number of pair-wise single nucleotide variants between any two given isolates based on the SNP matrix, ignoring the positions missing in any of the two isolates compared.

Distinguishing between relapse and reinfection

C. difficile isolates from participant stool samples were typed by polymerase chain reaction (PCR) ribotyping. In addition, single nucleotide polymorphism (SNP) data were generated using PCR free library construction and Illumina WGS, followed by bioinformatic processing comparing individual strains against a reference strain (*C. difficile* 196), with masking of several repetitive regions.

For participants who experienced rCDI, reinfection with a new strain and relapse with the same strain were differentiated by the comparison of ribotype (RT) and pair-wise single-nucleotide WGS variations (PWSNV), as described previously [12, 13]. Relapse was assigned if the baseline RT and the RT isolated during rCDI were the same, and if PWSNVs were ≤ 2. Reinfection was assigned if the baseline RT and the RT isolated during rCDI were different, or if the RT was the same but PWSNVs were > 10. Unknown status was assigned if the RT was the same but PWSNVs were 3 - 10.

Statistical methods

The analysis population was the modified intent-to-treat (mITT) population, which included all participants in the overall trial populations who received study infusion, had a positive test

155 at baseline for toxigenic *C. difficile*, and began receiving antibacterial treatment for CDI
156 before or within one day after the study infusion.

157 The cumulative incidence of relapse and reinfection by treatment group and the time to
158 rCDI event by treatment group are presented in this analysis. Since observing a relapse
159 means that a reinfection can no longer be observed, and vice versa, Fine & Gray's competing
160 risks survival analysis model was used to estimate the effect of antibiotic treatment and
161 hospitalization status (the randomization stratification factors) on the cumulative incidence of
162 the different types of rCDI [16]. All data were analyzed using the R statistical computing
163 program (https://www.R-project.org/). The "cmprsk" R package at CRAN (https://cran.r-
164 project.org/web/packages/cmprsk/) was used for competing risks analyses.

165 **Results**

166 ***Participants***

167 The mITT population from MODIFY I/II consisted of 2,559 participants (bezlotoxumab
168 N=1,193 and no bezlotoxumab N=790 among clinical cure population); 514 participants
169 experienced rCDI (bezlotoxumab n=247 [20.7%]; no bezlotoxumab n=267 [33.8%]; Table
170 1). Among these, 259 (50.4%) participants had both a baseline and post-baseline *C. difficile*
171 isolate and were evaluable for analysis.

172 Analysis was conducted to identify any potential systematic difference or sampling bias
173 between the evaluable rCDI events and those that were not evaluable (Supplementary Table
174 1). rCDI events were similarly distributed across the treatment groups regardless of whether
175 or not they were evaluable for analysis (two-sided Fisher Exact Test p=0.644 and p=0.925 in
176 MODIFY I and II respectively). Therefore, although approximately half of rCDI events were
177 not evaluable, any inference about treatment on type of recurrence should still be valid. In
178 addition, no significant difference was observed between participants with evaluable events

and those with non-evaluable events in terms of stratification factors, demographics, rCDI risk factors, and days to rCDI (Supplementary Table 1).

Of the 259 participants with evaluable data, a higher number of participants experienced a relapse compared with reinfection (198 [76.4%] versus 50 [19.3%] participants, respectively; Table 1). In total, 11 participants (4.2%) had a recurrence status assigned as unknown (PWSNVs 3–10). Among the 50 participants who had a recurrence classified as a reinfection, only two participants had a reinfection with a strain that was the same RT.

The proportions of reinfections and relapses among rCDI cases were similar between different treatment arms, with 74.0% and 78.7% of cases classified as relapse, and 21.1% and 17.6% of cases classified as reinfection in the bezlotoxumab and no bezlotoxumab groups, respectively (p=0.53; Table 1). The proportion of relapses was higher for RT 027 (commonly referred to as a 'hypervirulent' strain) [17], compared with all other RTs (49/58 [84.5%] versus 149/201 [74.1%], respectively); although the study was not powered to show a statistically significant difference in this subgroup, there was a strong trend towards a difference (p = 0.13; Table 2).

Compared with participants who experienced a reinfection, greater proportions of participants who experienced a relapse were ≥ 65 years of age, had a high number of comorbid conditions (Charlson Comorbidity Index ≥ 3), had hypoalbuminemia, had ≥ 2 risk factors for CDI, had severe CDI (Zar score ≥ 2), and were immunocompromised (Table 2). Conversely, a greater proportion of participants characterized as having reinfection had no pre-specified risk factors for rCDI compared with participants who experienced a relapse (Table 2).

Relapse/reinfection competing risk

A significant reduction in crude relative cumulative incidence of relapse ($p < 0.0001$) for the bezlotoxumab group compared with the no bezlotoxumab group was seen (Figure 1). A trend for a reduction in reinfection was observed in the bezlotoxumab group, although these results were not statistically significant ($p = 0.14$; Figure 1). In sensitivity analyses, the trend for reduction in rCDI with bezlotoxumab was observed regardless of the unknown recurrence cases classification (data not shown). Similar results were observed when data from each study was analyzed separately (Supplementary Figure 1).

Plotting of time to event for rCDI revealed a similar distribution of reinfection and relapse events in both treatment groups over time (Figure 2). A larger proportion of events that occurred after Week 8 were classified as reinfections (29.4%) compared with the first 8 weeks (19.5%) across both treatment groups; however, the number of relapses still exceeded the number of reinfections throughout the 12-week follow-up period.

The competing risk regression analysis of the pooled data for relapse revealed that treatment with bezlotoxumab resulted in a 50% reduction in the hazard of relapse (hazard ratio 0.5; $p < 0.0001$). Outpatients were shown to have a higher relapse hazard than inpatients (hazard ratio 1.54; $p = 0.0028$), and there was no significant difference in the effect of the antibiotic treatments for primary CDI on relapse hazard. The competing risk for the reinfection was not analyzed due to its sample size.

Discussion

To our knowledge, this is the largest analysis performed to differentiate CDI relapse and reinfection rates using WGS, thus providing more accurate estimates of the proportion for each event type. The results confirm previous findings, whereby cases of rCDI were largely due to a relapse of the baseline strain [12]. In the current study, 198/259 (76%) of evaluable rCDI cases were classified as a relapse, versus 50/259 (19%) as a reinfection. Based on the

same criteria of ≤ 2 PWSNVs for relapse and > 10 PWSNVs for reinfection, the corresponding rates in the fidaxomicin Phase 3 data were similar: 74/93 (80%) of participants with rCDI classified as relapse and 16/93 (17%) classified as reinfection [12].

This analysis provides evidence that bezlotoxumab-induced reduction in rCDI largely reflects prevention of same strain relapses. A non-significant reduction in the number of reinfections in the bezlotoxumab treatment arm compared with the no bezlotoxumab arm was observed and was likely due to the smaller number of reinfection cases in our study. Competing risk regression for relapse also confirmed the effectiveness of bezlotoxumab on relapse incidence, and indicated a significant role of outpatient status, suggesting that hospital care may reduce the risk of CDI relapse.

While the proportions of reinfection and relapse were similar between the bezlotoxumab and no bezlotoxumab groups overall, compared with relapses, recurrences that were classified as reinfections tended to occur in younger participants who did not have risk factors for rCDI and had fewer comorbidities. Compared with other RTs, we observed a higher proportion of relapses versus reinfections for participants with baseline pathogen RT 027, although the trend did not reach statistical significance.

Possible limitations of this *post-hoc* analysis are that only ~50% of participants who experienced rCDI in the MODIFY I/II trials had both baseline and post-baseline WGS data; therefore, the true proportion of relapses versus reinfections may be obscured. However, it is unlikely that the isolates available would have biased the overall conclusions here. Additionally, in 11 cases the number of PWSNVs was 3 - 10; thus, these samples could not be characterized as a relapse or reinfection using the classification criteria. Furthermore, as only one *C. difficile* colony was selected from each culture plate for typing, misclassification as relapse or reinfection cannot be excluded, as a mixed infection has been shown to occur in up to 10% of cases [13].

In conclusion, participants with rCDI in the MODIFY I/II trials were more likely to experience relapse with the same strain of *C. difficile* than reinfection with a new strain. Participants who received bezlotoxumab-containing regimens had a significantly reduced rate of relapse compared with participants who did not receive bezlotoxumab. Bezlotoxumab treatment also appeared to reduce the rate of reinfections, but the difference was not statistically significant.

Funding

This work was supported by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and grants from the National Natural Science Foundation of China (Grant Number: 31601073) and the National Major Science and Technology Project of the Ministry of Science and Technology of China (Grant Number: 2017ZX10303406).

Conflict of Interests

ZZ, MBD, JS, DG, and PMS are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (MSD), and may own stock and/or stock options in Merck & Co., Inc., Kenilworth, NJ, USA.

MHW has received consulting fees from Actelion, Astellas, bioMerieux, Cambimune, Da Volterra, MedImmune, Menarini, MSD, Meridian, Pfizer Inc., Qiagen, Sanofi-Pasteur, Seres, Summit, Synthetic Biologics, Inc., and Valneva; lecture fees from Alere, Astellas, MSD, and Pfizer Inc.; and grant support from Actelion, Astellas, bioMerieux, Da Volterra, MSD, Sanofi-Pasteur, Seres, and Summit.

HZ and JL are employees of BGI-Shenzhen, Shenzhen, China.

IRP has received consulting fees from Astellas and a research grant from ViroPharma.

Author contributions

ZZ, MBD, JS, IRP, JL, and PMS were involved in the design of this analysis. ZZ, HZ, MBD, JS, JL, and PMS were involved in the analysis of data. MBD was involved in the trial oversight. MHW, IRP, and DG were involved in the interpretation of the results. All authors were involved in drafting and revising this manuscript, and provided final approval of the version to be published. All authors vouch for the accuracy of the content included in the final manuscript.

Acknowledgments

We gratefully acknowledge the MODIFY I/II trial investigators and especially the individuals who consented to participate. We are also grateful for the helpful discussions with colleagues at Merck Sharp & Dohme Corp. and BGI-Shenzen.

Medical writing support, under the direction of the authors, was provided by Ceilidh McConnachie, MSc, of CMC AFFINITY, a division of McCann Health Medical Communications Ltd, Glasgow, UK and Fiona Scott, PhD, on behalf of CMC AFFINITY, in accordance with Good Publication Practice (GPP3) guidelines. This assistance was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Mary E. Hanson, PhD, provided writing support and Carol Zecca, BS, provided editorial support. Both are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

References

1. Desai, K., et al., *Epidemiological and economic burden of Clostridium difficile in the United States: estimates from a modeling approach.* BMC. Infect. Dis, 2016. **16**: p. 303.
2. Burke, K.E. and J.T. Lamont, *Clostridium difficile infection: a worldwide disease.* Gut Liver, 2014. **8**(1): p. 1-6.
3. Johnson, S., et al., *Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials.* Clin Infect Dis, 2014. **59**(3): p. 345-54.
4. Louie, T.J., et al., *Fidaxomicin versus vancomycin for Clostridium difficile infection.* N. Engl. J. Med, 2011. **364**(5): p. 422-431.
5. Sheitoyan-Pesant, C., et al., *Clinical and Healthcare Burden of Multiple Recurrences of Clostridium difficile Infection.* Clin. Infect. Dis, 2016. **62**(5): p. 574-580.
6. Figueroa, I., et al., *Relapse versus reinfection: recurrent Clostridium difficile infection following treatment with fidaxomicin or vancomycin.* Clin. Infect. Dis, 2012. **55 Suppl 2**: p. S104-S109.
7. Barbut, F., et al., *Epidemiology of recurrences or reinfections of Clostridium difficile-associated diarrhea.* J. Clin. Microbiol, 2000. **38**(6): p. 2386-2388.
8. Gerding, D.N., et al., *Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection in Patients at Increased Risk for Recurrence.* Clin Infect Dis, 2018. **67**(5): p. 649-656.
9. Wilcox, M.H., et al., *Bezlotoxumab for Prevention of Recurrent Clostridium difficile Infection.* N. Engl. J. Med, 2017. **376**(4): p. 305-317.
10. Gerding, D.N., et al., *Bezlotoxumab for prevention of recurrent Clostridium difficile infection in patients at increased risk for recurrence.* Clin Infect Dis, 2018. **67**(5p): p. 649-656.
11. Prabhu, V.S., et al., *Thirty-Day Readmissions in Hospitalized Patients Who Received Bezlotoxumab With Antibacterial Drug Treatment for Clostridium difficile Infection.* Clin Infect Dis, 2017. **65**(7): p. 1218-1221.
12. Eyre, D.W., et al., *Whole-genome sequencing demonstrates that fidaxomicin is superior to vancomycin for preventing reinfection and relapse of infection with Clostridium difficile.* J Infect Dis, 2014. **209**(9): p. 1446-51.
13. Eyre, D.W., et al., *Diverse sources of C. difficile infection identified on whole-genome sequencing.* N. Engl. J. Med, 2013. **369**(13): p. 1195-1205.
14. Li, H. and R. Durbin, *Fast and accurate short read alignment with Burrows-Wheeler transform.* Bioinformatics, 2009. **25**(14): p. 1754-60.
15. Li, H., et al., *The Sequence Alignment/Map format and SAMtools.* Bioinformatics, 2009. **25**(16): p. 2078-9.
16. Fine, J.P. and R.J. Gray, *A proportional hazards model for the subdistribution of a competing risk.* Journal of the American Statistical Association, 1999. **94**(446): p. 496-509.
17. Kuijper, E.J., et al., *Update of Clostridium difficile infection due to PCR ribotype 027 in Europe, 2008.* Euro. Surveill, 2008. **13**(31).

Table 1. Proportion of rCDI cases designated as relapse or reinfection by treatment group in MODIFY I/II (Clinical Cure Population)

	Bezlotoxumab N=1193	No bezlotoxumab N=790
Recurrence, N (%)	247 (20.7)	267 (33.8)
Baseline and recurrence isolates available, N (% of total recurrences)	123 (49.8)	136 (50.9)
Relapse, n (%)	91 (74.0)	107 (78.7)
Reinfection, n (%)	26 (21.1)	24 (17.6)
Unknown, n (%)	6 (4.9)	5 (3.7)
p-value for relapse vs reinfection	0.53	

CDI, *Clostridium difficile* infection; rCDI, CDI recurrence.

336 **Table 2.** Clinical characteristics and risk factors for rCDI in participants characterized as having
337 relapse or reinfection during the 12-week follow-up period across all treatment groups

Characteristic	Relapse N=198	Reinfectior N=50	Unknowı N=11	P-value (relaı vs reinfectior
Clinical characteristic, n (%)				
≥65 years of age	122 (61.6	23 (46.0)	6 (54.5)	0.054
≥1 CDI episodes in past 6 months	72 (36.4)	18 (36.0)	6 (54.5)	1.000
Severe CDI (Zar score[a] ≥2)	34 (17.2)	3 (6.0)	1 (9.1)	0.048
Immunocompromised[b]	40 (20.2)	6 (12.0)	1 (9.1)	0.224
Charlson Comorbidity Index ≥3	75 (37.9)	9 (18.0)	2 (18.2)	0.008
Albumin <2.5 g/dL	26 (13.1)	2 (4.0)	0 (0.0)	0.081
Hospitalization status, n (%)				
Outpatient	85 (42.9)	25 (50.0)	3 (27.3)	0.427
Inpatient	113 (57.1	25 (50.0)	8 (72.7)	
Antibiotic treatment for CDI, n (%)				
Vancomycin	98 (49.5)	26 (52.0)	5 (45.5)	0.343

Metronidazole	95 (48.0)	21 (42.0)	6 (54.5)	
Fidaxomicin	5 (2.5)	3 (6.0)	0 (0.0)	
Number of pre-specified risk factors for rCDI[c], n (%)				
0 risk factors	31 (15.7)	19 (38.0)	1 (9.1)	
1 risk factor	58 (29.3)	14 (28.0)	5 (45.5)	0.002
≥2 risk factors	109 (55.1	17 (34.0)	5 (45.5)	
Ribotype, n (%)				
RT 027	49 (24.7)	7 (14.0)	2 (18.2)	0.130
Other RT	149 (75.3	43 (86.0)	9 (81.8)	

[a]Based on the following: (1) age > 60 years (1 point); (2) body temperature > 38.3°C (> 100°F) (1 point); (3) albumin level < 2.5 g/dL (1 point); (4) peripheral WBC count > 15,000 cells/mm^3 within 48 hours (1 point); (5) endoscopic evidence of pseudomembranous colitis (2 points); and (6) treatment in an intensive care unit (2 points); [b]defined on the basis of a participant's medical history or use of immunosuppressive therapy; [c]pre-specified risk factors: CDI history in the past 6 months, severe CDI at baseline (Zar score ≥ 2), age ≥ 65 years, CDI due to hypervirulent strain (ribotypes 027, 078, or 244) and/or immunocompromised; [d]from Fisher's Exact Test.

CDI, *Clostridium difficile* infection; rCDI, CDI recurrence; RT, ribotype; WBC, white blood cell.

346 **Figure Legends**

347 **Figure 1.** Cumulative incidence for *C. difficile* infection relapse and reinfection by treatment

348 group in MODIFY I/II

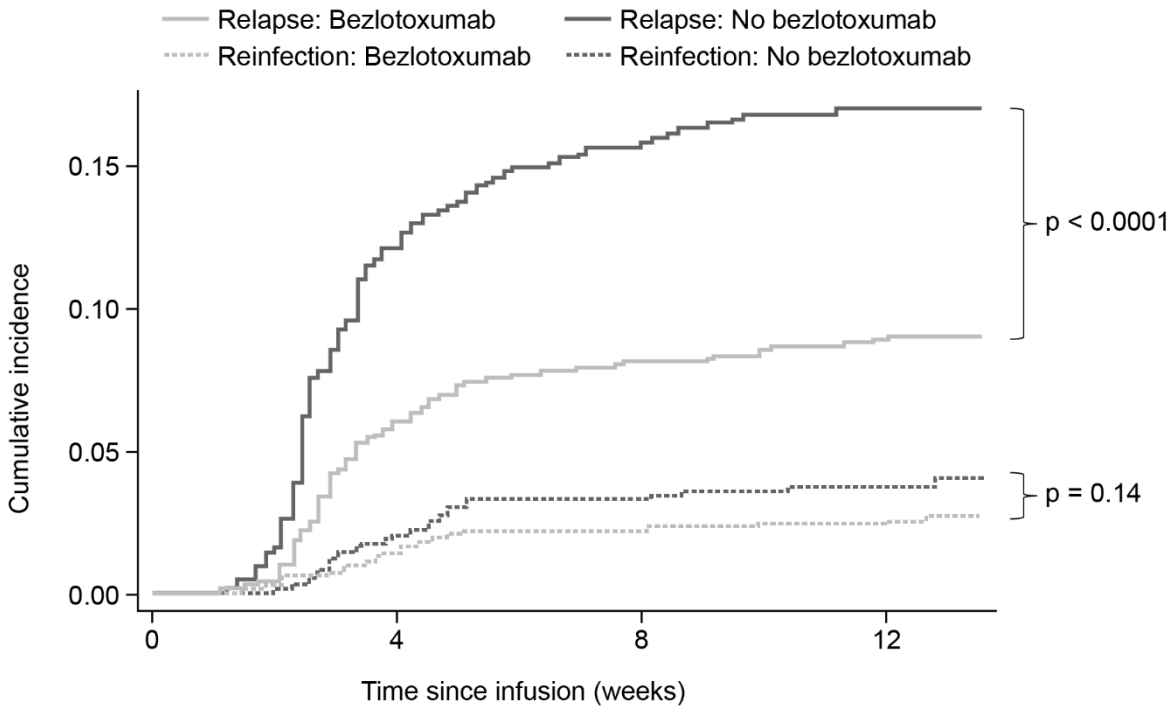
349 **Figure 2.** Distribution of time to event for rCDI and classification as relapse or reinfection by

350 treatment group ([A] no bezlotoxumab; [B] bezlotoxumab) in MODIFY I/II evaluable

351 population

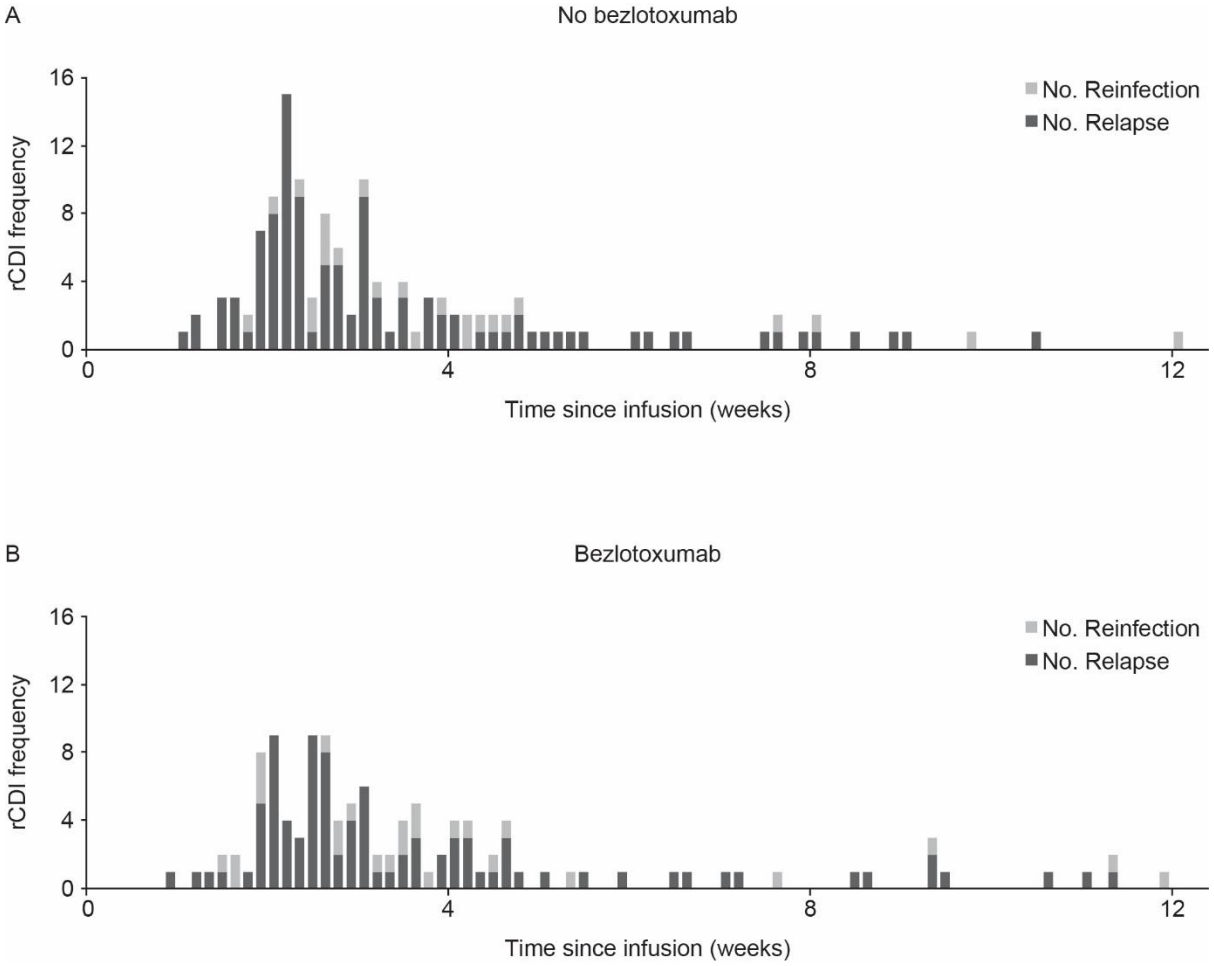
352 **Figure 1.** Cumulative incidence for *C. difficile* infection relapse and reinfection by treatment

353 group in MODIFY I/II



354

Figure 2. Distribution of time to event for rCDI and classification as relapse or reinfection by treatment group ([A] no bezlotoxumab; [B] bezlotoxumab) in MODIFY I/II evaluable population



rCDI, *Clostridium difficile* infection recurrence.

Supplementary Materials

Supplementary Table 1. Distribution of type of rCDI event and treatment group for evaluable events versus all CDI recurrence events by trial

	MODIFY I (N=1,396)		MODIFY II (N=1,163)	
All rCDI events, n (%)	297 (100)		217 (100)	
Evaluable rCDI events, n (%)	155 (52)		104 (48)	
Type of rCDI event in evaluable participants, n (%)[a]				
Reinfection	29 (19)		21 (20)	
Relapse	119 (77)		79 (76)	
Unknown	7 (5)		4 (4)	
	Evaluable (n=155)	**Non-evaluable (n=142)**	**Evaluable (n=104)**	**Non-evaluable (n=113)**
Treatment groups, n (%)[b]				
Bezlotoxumab	38 (58)	28 (42)	29 (47)	33 (53)
Bezlotoxumab + actoxumab	29 (48)	32 (52)	27 (47)	31 (53)
Actoxumab	33 (55)	27 (45)	–	–
Placebo	55 (50)	55 (50)	48 (49)	49 (51)
Two-sided Fisher's Exact Test p-value	0.644		0.925	
Hospitalization status, n (%)				
Outpatient	67 (58)	48 (42)	46 (48)	49 (52)

Inpatient	88 (48)	94 (52)	58 (48)	64 (52)
Two-sided Fisher's Exact Test p-value	0.672		0.965	
Antibiotic treatment for CDI, n (%)				
Vancomycin	80 (49)	82 (51)	49 (42)	67 (58)
Metronidazole	69 (56)	55 (44)	53 (56)	41 (44)
Fidaxomicin	6 (55)	5 (45)	2 (29)	5 (71)
Two-sided Fisher's Exact Test p-value	0.565		0.066	
Geographical region, n (%)				
Africa	0 (0)	1 (100)	–	–
Asia-Pacific	8 (80)	2 (20)	20 (53)	18 (47)
Europe	43 (45)	53 (55)	40 (48)	43 (52)
Latin America	7 (58)	5 (42)	1 (33)	2 (67)
North America	97 (54)	81 (46)	43 (46)	50 (54)
Two-sided Fisher's Exact Test p-value	0.119		0.860	
Gender, n (%)				
Female	95 (51)	91 (49)	62 (48)	66 (52)
Male	60 (54)	51 (46)	42 (47)	47 (53)
Two-sided Fisher's Exact Test p-value	0.633		0.891	
Clinical characteristic, n (%)				
≥65 years of age	82 (51)	78 (49)	69 (51)	66 (49)
Two-sided Fisher's Exact Test p-value	0.729		0.263	

Severe CDI (Zar score ≥2)	23 (61)		15 (39)	15 (54)		13 (46)
Two-sided Fisher's Exact Test p-value		0.533			0.273	
≥1 CDI episodes in past 6 months	58 (50)		59 (50)	39 (41)		56 (59)
Two-sided Fisher's Exact Test p-value		0.592			0.224	
Immunocompromised	30 (50)		30 (50)	17 (46)		20 (54)
Two-sided Fisher's Exact Test p-value		0.773			0.858	
Charlson Comorbidity Index ≥3	55 (50)		55 (50)	31 (44)		40 (56)
Two-sided Fisher's Exact Test p-value		0.631			0.390	
Days to rCDI, median (interquartile range)	22 (16 – 31)		21 (16 – 34)	19.5 (16 – 29)		21 (16 – 31)
Two-sided Rank Sum Test p-value		0.860			0.265	

362 [a]Percentages calculated based on total number of evaluable rCDI events; [b]percentages calculated based on total number of participants with rCDI

363 in each treatment group (evaluable vs non-evaluable). CDI, *Clostridium difficile* infection; rCDI; CDI recurrence.

Supplementary Figure 1. Cumulative incidence for *C. difficile* infection relapse and reinfection by treatment group in (A) MODIFY I and (B) MODIFY II.