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Bezlotoxumab for the prevention of recurrent *Clostridioides difficile* infection: 12-month observational data from the randomized Phase 3 trial MODIFY II

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Abstract (50/50 words)

From MODIFY II (NCT01513239), no participants (n=0/69) who achieved sustained clinical cure through 12-weeks following infusion with bezlototxumab experienced recurrent *Clostridium difficile* (rCDI) during a subsequent 9 months (versus actoxumab + bezlotoxumab, n=2/65; placebo, n=1/34). Bezlotoxumab efficacy appears to be due to prevention rather than delay in onset of rCDI.

Introduction

Immune responses against *Clostridioides difficile* toxins A and B, are associated with a lower rate of *C. difficile* recurrence (rCDI) [1, 2]. Bezlotoxumab and actoxumab, fully human monoclonal antibodies (mAbs), neutralize *C. difficile* toxins B and A, respectively [3-5]. In MODIFY I/II (NCT01241552/NCT01513239), actoxumab + bezlotoxumab, or bezlotoxumab were superior to placebo in rCDI prevention through 12 weeks ($p<0.001$) [5]. We summarize results from MODIFY II participants who were followed for 12 months to assess the long-term rates of rCDI and *C. difficile* colonization following antitoxin mAb infusion.

Methods

In MODIFY II, participants receiving oral antibacterials for primary or recurrent CDI (metronidazole, vancomycin, or fidaxomicin, chosen by the treating physician) were randomized 1:1:1 to actoxumab + bezlotoxumab (10 mg/kg each), bezlotoxumab (10 mg/kg), or placebo [5]. Randomization was stratified according to antibacterial and inpatient/outpatient status. mAb or placebo was administered by a 60-minute intravenous infusion on day 1. Participants who completed the core 12-week follow-up could participate in the 9-month extension (**Supplementary Figure 1**).

Twelve weeks (85–95 days) following core study infusion, loose stool counts were recorded daily; samples were tested for toxigenic *C. difficile* for participants with a new diarrheal episode. At the end of the core study, those participating in the 9-month extension were contacted monthly about new diarrheal episodes. In-person visits occurred at Months 6, 9, and 12, where stool samples or rectal swabs were collected to assess *C. difficile* colonization.

The primary endpoint of the core MODIFY II trial was the proportion of participants with rCDI through 12 weeks of follow-up in the modified intent-to-treat (mITT) population: randomized participants who received treatment, had a positive stool test for toxigenic *C. difficile* and were receiving a CDI antibacterial.

Initial clinical cure (ICC) was assessed in the mITT population and based on daily loose stool counts and duration of antibacterial therapy, and was defined as no diarrhea for 2 consecutive days after completion of ≤16 days of CDI antibacterial therapy. rCDI was defined as a new episode of diarrhea (≥3 loose stools in ≤24 hours) associated with a positive stool test for toxigenic *C. difficile* in participants who had achieved ICC (clinical cure population). Participants achieving sustained clinical cure (SCC), defined as ICC, and no rCDI through Week 12 in the core mITT population were included in the extension phase rCDI analysis. Participants who provided stool samples at Months 6, 9, and/or 12 were included in the *C. difficile* colonization analysis.

Rates of *C. difficile* colonization at Months 6, 9, and 12 were assessed based on whether a toxigenic *C. difficile* strain was isolated in collected samples. For participants with a positive sample during the extension phase, the ribotype of the *C. difficile* isolate was compared with the baseline ribotype and that of any previous isolate, when available.

Deaths and treatment-related serious adverse events (SAEs) were recorded throughout the extension in the 'all patients as treated' population, including participants who received the infusion.

The R.M. Alden Research Laboratory (Culver City, CA, USA) performed anaerobic culture of *C. difficile* and tested isolates using the TechLab *C. difficile* Tox A/B enzyme immunoassay kit. PCR ribotyping was performed at the University of Leeds, Leeds, UK.

MODIFY II was conducted in conformance with Good Clinical Practice requirements and applicable country and/or local statutes and regulations regarding ethical committee review, and the protection of human subjects participating in biomedical research. All participants gave written, informed consent prior to study enrollment.

Results

Of 1203 participants randomized in MODIFY II, 1163 were included in the mITT population; 966 completed the core 12-week study and 295 entered the 9-month extension phase. Of these, 293 were included in the mITT population of the core study, 242 provided ≥1 sample for colonization analyses;

168 had achieved SCC at Week 12 and were included in the 12-month rCDI analysis. Twenty-five (8.5%) participants prematurely discontinued (**Supplementary Figure 1**).

At baseline, compared with those followed for 12 weeks, extension cohort participants had fewer prognostic risk factors for rCDI (**Supplementary Table 1**). The extension cohort included a higher proportion of females (60.8% vs 56.4%), and was younger (46.4% vs 53.1% ≥65 years of age) than the 12-week cohort. The extension cohort had lower incidence of clinically severe CDI at study entry (7.8% vs 17.2%), fewer had ribotype 027 (7.5% vs 12.6%), and fewer were immunocompromised (12.6% vs 18.1%), compared with the 12-week cohort. A lower proportion of participants in the extension cohort had Charlson Comorbidity Index (CCI) ≥3 (31.4% vs 41.9%) compared with the 12-week cohort.

A higher proportion of placebo-treated participants who entered the extension phase were female (64.6% vs 59.2%), aged ≥65 years (50.0% vs 45.0%) and had ribotype 027 (9.8% vs 6.6%) compared with the active treatment groups (**Supplementary Table 1**). More participants in the actoxumab + bezlotoxumab and bezlotoxumab groups had clinically severe CDI (Zar score ≥2) (9.5% vs 3.7%, respectively), compromised immunity (14.2% vs 8.5%, respectively), and a CCI ≥3 (33.6% vs 25.6%, respectively) compared with the placebo group. At Months 6, 9, and 12, missed samples were 23 each for the actoxumab + bezlotoxumab group; 17, 19, and 22 for the bezlotoxumab group; and 11, 13, and 16 for the placebo group, respectively.

rCDI incidence during the core study in participants enrolled in the extension phase was 25.3%, 18.8%, and 50.0% in the actoxumab + bezlotoxumab, bezlotoxumab, and placebo groups, respectively. Among extension cohort participants who achieved SCC at Week 12, only 3 (actoxumab + bezlotoxumab, n=2; placebo, n=1) experienced rCDI during the extension phase (all occurred during Months 9–12, **Table 1**). The 12-month incidence of rCDI for the actoxumab + bezlotoxumab, bezlotoxumab, and placebo groups was 27.6%, 18.8%, and 51.5%, respectively. In total, 26 extension phase participants had an episode of diarrhea associated with a positive stool test, this included the 3

who achieved SCC during the core phase and 23 who failed antibiotic therapy or had rCDI during the core study (actoxumab + bezlotoxumab, n=8; bezlotoxumab, n=8; placebo, n=7).

Overall, 130/293 participants (44%) in the extension cohort analysis had toxigenic *C. difficile* isolated from ≥1 stool sample collected during Months 4 through 12. *C. difficile* colonization rates among participants who provided a stool sample were similar across treatment groups at each follow-up visit and was 18–25% in the actoxumab + bezlotoxumab group, 16–24% in the bezlotoxumab group, and 19–32% in the placebo group.

Isolates were the same ribotype as the baseline isolate in ~40% of paired stool samples at each time point, and the same as any previous ribotype in 44.4%, 63.9%, 61.0% of positive stool samples collected at Months 6, 9, and 12, respectively (**Supplementary Table 2**).

One drug-related SAE (osteoporotic fracture) occurred during the extension phase in a bezlotoxumab-treated participant. During the extension phase, 2/112 (1.8%) participants in the actoxumab + bezlotoxumab group, 5/100 (5.1%) in the bezlotoxumab group, and 2/83 (2.4%) in the placebo group experienced AEs with a fatal outcome; none were considered treatment-related.

Discussion

In the 9-month extension cohort of MODIFY II, toxigenic *C. difficile* colonization rates ranged from 16–32% at Months 6, 9, and 12 post-infusion. Colonization rates were broadly similar across treatment groups. Participants who were colonized during the extension phase and had a baseline pathogen isolated, ~40% were colonized with the same strain as the baseline strain, suggesting persistence of spores in the environment or within the gastrointestinal tract. Despite persistence of the same strain or colonization with a new toxigenic *C. difficile* strain, rCDI was observed in <2% of participants exhibiting SCC through 12 weeks post-infusion. Among bezlotoxumab-treated participants, there were no cases of rCDI in the 9-month extension period.

This suggests that the efficacy of bezlotoxumab seen in the core study was due to rCDI prevention rather than a delay in rCDI onset after antibody concentrations were diminished. Bezlotoxumab has an

elimination half-life of ~19 days and was detectable in serum at clinically important concentrations up to 6 months after treatment [6]. As low levels of antibodies against toxins A and/or B are associated with increased recurrence risk, [1, 2] the concentration of bezlotoxumab remaining in serum 6 months after a single dose [6] may be sufficiently high to neutralize *C. difficile* toxin B for ≥6 months [7].

The relatively small sample size and high percentage of missing surveillance samples at each follow-up is a limitation. The sample was also biased towards younger participants with less severe initial disease and fewer comorbidities. Additionally, since extension phase participants were contacted less frequently than in the core study, episodes of CDI may have been missed. As this is an extension of a Phase 3 study, the results may not be representative of a real-world population.

The mortality rate and SAEs reported during the extension phase were consistent for the medical condition studied, baseline comorbidities, and the study population age.

In conclusion, rCDI is rare after 12 weeks, justifying the typical follow-up period for studied interventions to treat or prevent CDI. SCC observed following bezlotoxumab appears to be due to prevention, rather than delay in rCDI onset. Bezlotoxumab should be considered for patients with CDI who are at increased risk of rCDI, in particular those with multiple risk factors (e.g. aged ≥65 years, immunosuppression, prior history of CDI and severe CDI) and/or those who remain at risk for prolonged periods of time. Bezlotoxumab reduces the risk of rCDI for 3 months and so may be suitable for patients undergoing chemotherapy with possible repeat antibiotic exposure.

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Conflicts of Interest

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

EJCG is an advisory board member for Bayer Pharmaceuticals, Bio-K+, Daiichi Sankyo, Kindred Healthcare Inc., MSD, Novartis, Rempex, Sanofi-Aventis, and Summit Therapeutics plc. He is on the speakers' bureau for Bayer Pharmaceuticals and MSD. He receives research grants from Amicrobe, Inc., Astellas, AvidBiotics Corp, Cerexa, Clinical Microbiology Institute, Durata Therapeutics, Inc., Forest Laboratories, Genzyme, GlaxoSmithKline, GLSynthesis Inc, Gynuity Health Projects, Immunome Inc, Impax Laboratories, MSD, NanoPacific Holdings, Novartis, Pfizer Inc, Rempex, Romark Laboratories, Salix Pharmaceuticals Inc., Summit Therapeutics plc, Symbiomix Therapeutics, The Medicines Company, Theravance Biopharma, Toltec Pharmaceuticals, ViroXis Corp., and Warner Chilcott.

DMC was contracted to perform portions of this work.

DNG holds patents for the treatment and prevention of CDI, is an advisory board member for Actelion Pharmaceuticals US, Inc, MSD, Rebiotix, Inc., and Summit Therapeutics plc, and he is a consultant for Da Volterra, Medpace, MGB Biopharma, Pfizer Inc, and Sanofi Pasteur. He holds research grants from the Centers for Disease Control and Prevention, the Department of Veterans Affairs Research Service, and Seres Therapeutics.

MHW has received consulting fees from Actelion, Astellas, bioMérieux, MedImmune, Merck & Co., Inc., Pfizer Inc, Sanofi Pasteur, Seres Therapeutics, Summit Therapeutics plc, Synthetic Biologics, and Valneva. He receives lecture fees from Alere, Astellas, Merck & Co., Inc., and Pfizer Inc; as well as grant support from Actelion, Astellas, bioMérieux, Da Volterra, Merck & Co., Inc., and Summit Therapeutics plc.

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Table 1. Outcomes by treatment group for the extension cohort and cohort of participants that did not not enter the extension phase; mITT population

	Actoxumab + Bezlotoxumab (n=390)		**Bezlotoxumab (n=395)**		**Placebo (n=378)**	
	Entered extension (n=112)	**Did not enter extension (n=278)**	**Entered extension (n=99)**	**Did not enter extension (n=296)**	**Entered extension (n=82)**	**Did not enter extension (n=296)**
Endpoint results during core study (through 12 weeks), n/N[a] (%)						
Initial clinical cure (mITT population)	87/112 (77.7)	195/278 (70.1)	85/99 (85.9)	241/296 (81.4)	68/82 (82.9)	226/296 (76.4)
CDI recurrence (clinical cure population)	22/87 (25.3)	36/195 (18.5)	16/85 (18.8)	46/241 (19.1)	34/68 (50.0)	63/226 (27.9)
Sustained clinical cure (mITT population)	65/112 (58.0)	159/278 (57.2)	69/99 (69.7)	195/296 (65.9)	34/82 (41.5)	163/296 (55.1)
Endpoint results during the extension phase, n/N[a] (%)						
[b]CDI recurrence months 4 to 12	2/65 (3.1)[c]	-	0/69 (0.0)	-	1/34 (2.9)[d]	-
CDI recurrence months 1 to 12 (clinical cure population)	24/87 (27.6)	-	16/85 (18.8)	-	35/68 (51.5)	-

Colonization rates at Month 6	21/89 (23.6)	-	20/82 (24.4)	-	23/71 (32.4)	-
Colonization rates at Month 9	16/89 (18.0)	-	13/80 (16.3)	-	13/69 (18.8)	-
Colonization rates at Month 12	22/89 (24.7)	-	13/77 (16.9)	-	14/66 (21.2)	-

[a]N is the number of participants evaluable for the endpoint. [b]Participants who met the definition of CDI recurrence during the extension, i.e. participants who had sustained clinical cure during the core study and experienced rCDI during the extension phase. [c]Subject 1: recurrence occurred 6 months after the baseline episode and the ribotype of the recurrent episode was 490 and baseline ribotype was unknown; Subject 2: recurrence occurred 12 months after the baseline episode and the ribotype of the baseline and recurrent episode was 052. [d]Recurrence occurred 12 months after the baseline episode and the ribotype of the recurrent episode was 012 and the baseline ribotype was unknown.

CDI, *Clostridium difficile* infection; mITT, modified intent-to-treat; rCDI, *Clostridium difficile* infection recurrence.

Supplementary Table 1. Baseline characteristics and prognostic risk factors for rCDI by treatment group for the extension cohort and cohort of participants that did not enter the extension phase; mITT population

	Actoxumab + Bezlotoxumab (n=390)		Bezlotoxumab (n=395)		Placebo (n=378)	
	Entered extension (n=112)	Did not enter extension (n=278)	Entered extension (n=99)	Did not enter extension (n=296)	Entered extension (n=82)	Did not enter extension (n=296)
Baseline characteristics, n (%)						
Female	66 (58.9)	146 (52.5)	59 (59.6)	154 (52.0)	53 (64.6)	173 (58.4)
Age ≥65 years	59 (52.7)	182 (65.5)	36 (36.4)	169 (57.1)	41 (50.0)	165 (55.7)
History of CDI in past 6 months	28 (25.0)	76 (27.3)	32 (32.3)	81 (27.4)	28 (34.1)	82 (27.7)
≥2 CDI episodes in the past (ever)	19 (17.0)	36 (12.9)	16 (16.2)	41 (13.9)	13 (15.9)	40 (13.5)
Clinically severe CDI (Zar score ≥2)	10 (8.9)	70 (25.2)	10 (10.1)	45 (15.2)	3 (3.7)	62 (20.9)
Ribotype 027	8 (7.1)	31 (11.2)	6 (6.1)	37 (12.5)	8 (9.8)	56 (18.9)
Ribotype 027, 078, or 244	11 (9.8)	35 (12.6)	8 (14.8)	43 (14.5)	10 (12.2)	61 (20.6)
Compromised immunity	15 (13.4)	60 (21.6)	15 (15.2)	67 (22.6)	7 (8.5)	46 (15.5)

Charlson Comorbidity Index ≥3	33 (29.5)	132 (47.5)	38 (38.4)	133 (44.9)	21 (25.6)	130 (43.9)
[a]Renal impairment	10 (8.9)	37 (13.3)	10 (10.1)	58 (19.6)	7 (8.5)	42 (14.2)
[b]Hepatic impairment	2 (1.8)	25 (9.0)	5 (5.1)	21 (7.1)	0 (0.0)	20 (6.8)
Region of enrollment/geographical distribution, n (%)						
Asia-Pacific	12 (10.7)	51 (18.3)	10 (10.1)	49 (16.6)	7 (8.5)	47 (15.9)
Latin America	1 (0.9)	7 (2.5)	1 (1.0)	3 (1.0)	2 (2.4)	3 (1.0)
Europe	38 (33.9)	123 (44.2)	37 (37.4)	137 (46.3)	22 (26.8)	139 (47.0)
North America	61 (54.5)	97 (34.9)	51 (51.5)	107 (36.1)	51 (62.2)	107 (36.1)

[a]Serum creatinine ≥1.5 mg/dL. [b]Hepatic impairment defined by two or more of the following: (a) albumin ≤3.1 g/dL, (b) ALT ≥2x ULN, (c) total bilirubin ≥1.3x ULN, or (d) mild, moderate, or severe liver disease (as reported on the Charlson Comorbidity Index).

ALT, alanine aminotransferase; CDI, *Clostridium difficile* infection; mITT, modified intent-to-treat; rCDI, *Clostridium difficile* infection recurrence; ULN, upper limit of normal.

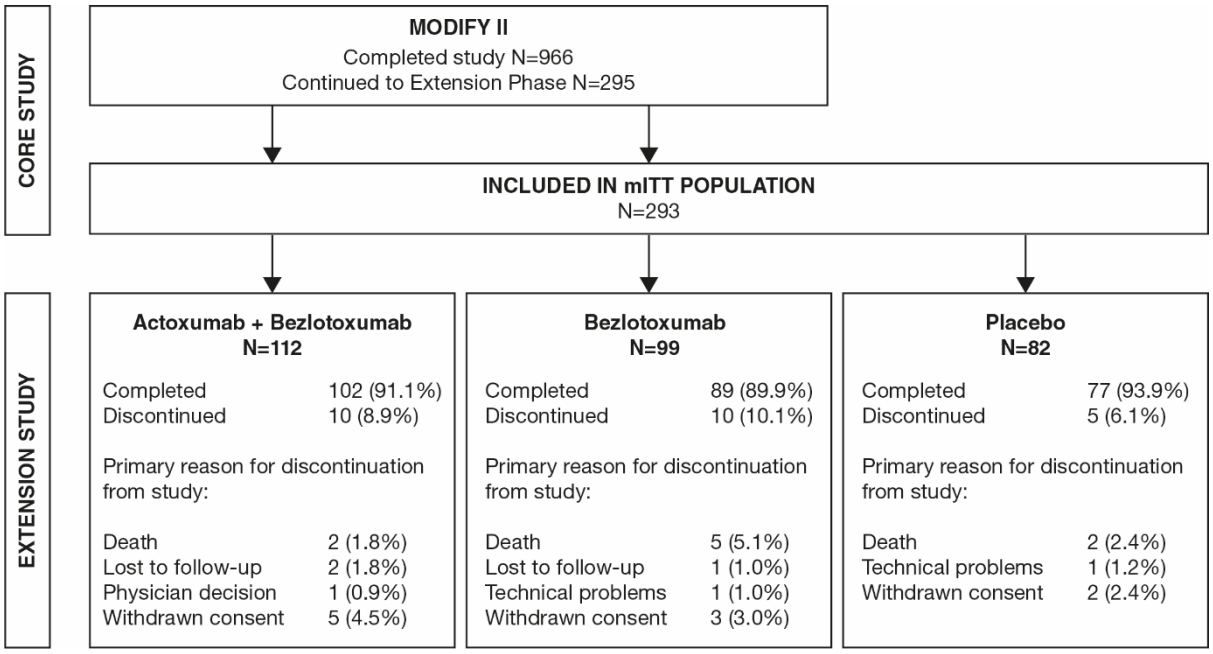
Supplementary Table 2. Comparison of ribotypes in paired positive stool samples across all treatment groups

	Month 6	Month 9	Month 12
Number of participants with paired samples at each time point			
Ribotype data at time point, N	62	42	47
Ribotype data at baseline, n/N (%)	38/62 (61.3)	22/42 (52.4)	31/47 (66.0)
Ribotype data at any previous time point, n/N (%)	45/62 (72.6)	36/42 (85.7)	41/47 (87.2)
Ribotype comparisons			
Same ribotype as baseline, n/N[a] (%)	16/38 (42.1)	9/22 (40.9)	13/31 (41.9)
Same ribotype as any previous time point, n/N[b] (%)	20/45 (44.4)	23/36 (63.9)	25/41 (61.0)

[a]N is number of participants with known baseline ribotype and ribotype data at respective visit.

[b]N is number of participants with known ribotype at any previous time point and ribotype data at respective visit.

Supplementary Figure 1. MODIFY II study design and participant disposition



mITT, modified intent-to-treat; SCC, sustained clinical cure, rCDI, recurrence of *Clostridium difficile* infection