Endogenous Serum IgG Antibodies to *Clostridium difficile* Toxin B Are Associated With Protection Against *C. difficile* Infection Recurrence

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BACKGROUND

MODIFY I/II were global Phase 3 trials of the efficacy and safety of bezlotoxumab (bezlo), a fully human monoclonal antibody against *Clostridium difficile* toxin B, and actoxumab, a fully human monoclonal antibody against *C. difficile* toxin A[1]

In both trials, bezlo was found to be superior to placebo at preventing recurrent *C. difficile* infection (rCDI) in participants receiving antibacterials for CDI. The addition of actoxumab did not improve efficacy[1]

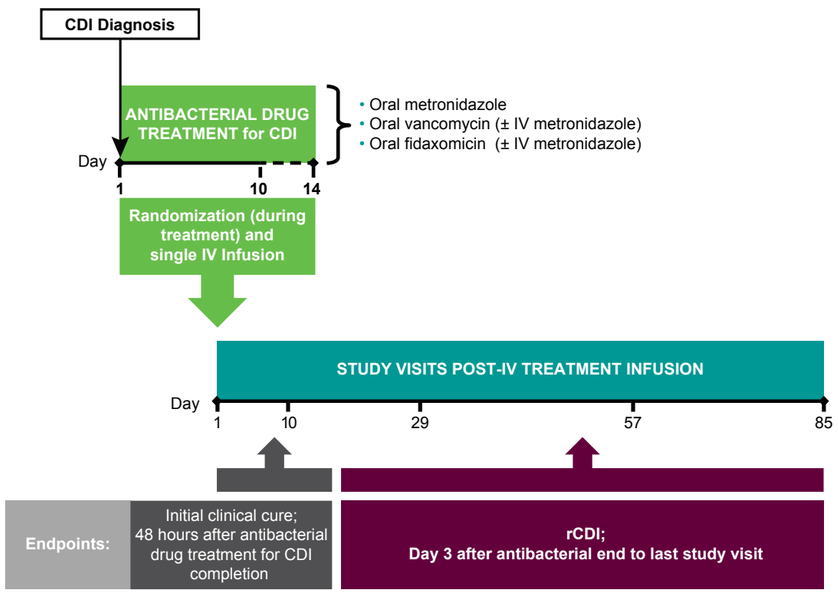
- Individuals with low endogenous IgG antibodies against C. *difficile* toxin A (eAb-A) or toxin B (eAb-B) have been shown to be at increased risk for rCDI[2-4]

Our aim was to explore the correlation of eAb-A and eAb-B with rCDI risk by measuring the titers of these natural antibodies in the participants receiving placebo in the MODIFY trials and to evaluate the utility of assays for these antibodies as potential biomarkers to predict which patients may benefit from monoclonal antibody treatment

METHODS

- MODIFY I (NCT01241552) and MODIFY II (NCT01513239) were randomized, double-blind, placebo-controlled, multicenter, Phase 3 trials that were conducted from November 1, 2011 through May 22, 2015 at 322 sites in 30 countries (**Figure 1**)
- Participants were treated with antibacterial drug treatment for CDI for 10 to 14 days, during which time they received a single infusion of bezlo alone, actoxumab alone (MODIFY I only), bezlo + actoxumab, or placebo
  - Only participants randomized to the placebo groups of MODIFY I or MODIFY II were included in this analysis

Figure 1. Study Design



CDI, *C, difficile* infection; IV, intravenous; rCDI, recurrent CDI.

- Efficacy assessments were performed on the modified-intent-to-treat (mITT) population, which included all randomized participants who received study infusion, had a positive baseline stool test for toxigenic *C. difficile*, and were receiving antibacterial drug treatment for CDI at the time of randomization. mITT participants with serum eAb titer results were included in this analysis
- Initial clinical cure was defined as receipt of a ≤14-day regimen of antibacterial drug treatment for CDI AND no diarrhea during the 2 consecutive days following completion of antibacterial drug treatment for CDI
- rCDI was defined as a new episode of diarrhea associated with a positive stool test for toxigenic *C. difficile* in participants who achieved initial clinical cure of the baseline CDI episode (Clinical Cure population)
- Serum samples were collected on Day 1 and at Week 4 and Week 12
- eAb titers were measured using two sensitive, validated, electrochemiluminescence immunoassays and reported as <1:1000, 1:1000, 1:5000, 1:25000, and ≥1:125000[5]
- Because there is no clearly defined immunological surrogate of efficacy for rCDI tied to a specific eAb-A or eAb-B level, eAb levels were arbitrarily categorized as low (≤1:1000), medium (1:5000), or high (≥1:25000)

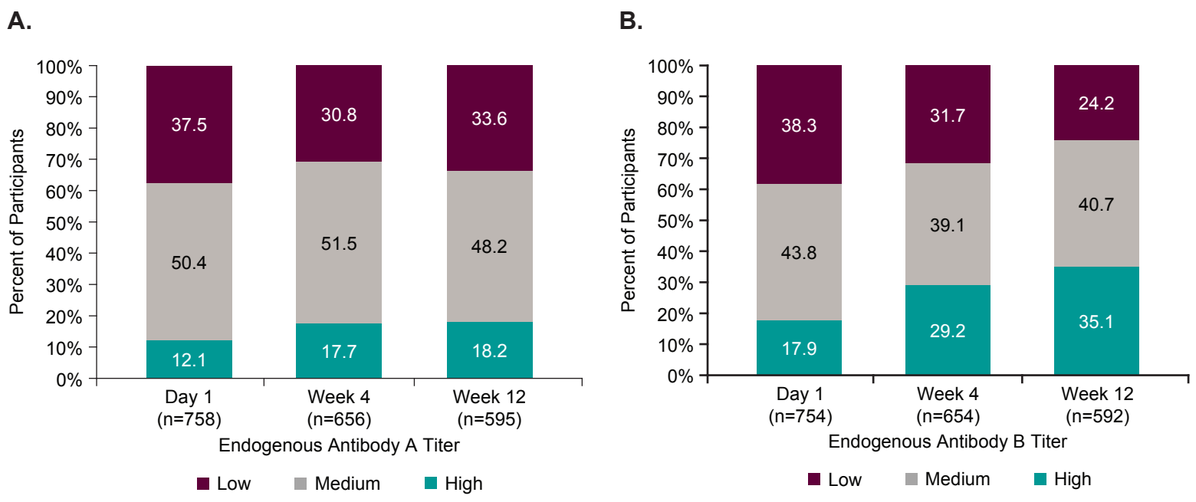
Statistical Analysis

- The proportion of participants with rCDI was summarized by eAb category at Day 1, Week 4, and Week 12 for eAb-A and eAb-B
- A two-sided Cochran-Armitage trend test was performed to evaluate correlations between demographic and clinical characteristics and Day 1 eAb-B titer category

RESULTS

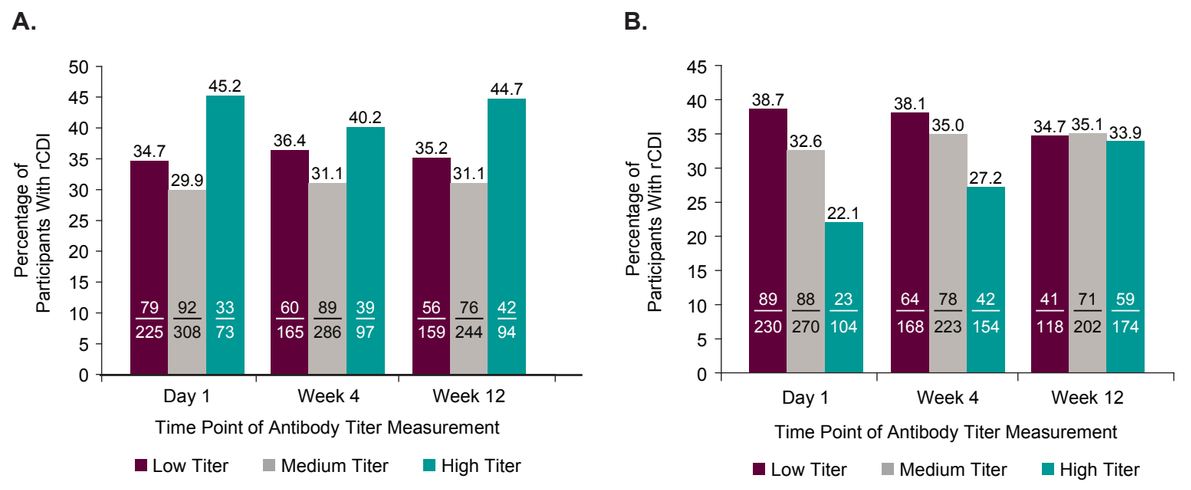
- Approximately 97% of mITT participants were included in the analysis for eAb-A (n=758) and eAb-B (n=754), respectively (**Figure 2**)
- On Day 1, 37.5% of participants had low antibody titers of eAb-A, 38.3% had low antibody titers of eAb-B, and 21.3% of participants had low antibody titers of both antibodies
- The proportion of participants with low eAb-A and eAb-B titers decreased over time; Day 1 was the time point with the highest proportion of participants with low antibody titers

Figure 2. Distribution of Participants by Titer Category at Each Measurement Time Point (A) by eAb-A Titer and (B) by eAb-B Titer (mITT Population)



- There was no evident correlation between eAb-A titers and the proportion of participants experiencing rCDI at any time point (**Figure 3A**)
- A correlation (2-sided Cochran-Armitage trend test *P*=0.001) was observed for the proportion of participants who experienced rCDI and eAb-B titer category, with the highest rate of rCDI observed in participants with low Day 1 eAb-B titers and the lowest rate of rCDI in participants with high Day 1 eAb-B titers (**Figure 3B**)
  - The correlation (2-sided Cochran-Armitage trend test *P*=0.038) was also observed based on Week 4 titers
  - The Week 12 titers were not correlated with the rCDI rate (2-sided Cochran-Armitage trend test *P*=0.937)

Figure 3. Proportion of Participants Experiencing rCDI through Week 12 (A) by eAb-A Titer and (B) by eAb-B Titer Measured on Day 1 and at Weeks 4 and 12 (Clinical Cure Population)



- The characteristics of participants based on baseline eAb-B titer are shown in **Table 1**
  - A higher proportion of immunocompromised participants and participants with a previous CDI episode at some time in the past had low Day 1 eAb-B levels compared with nonimmunocompromised participants and those without a prior history of CDI, respectively
  - Advanced age, a characteristic that is a known risk factor for rCDI, did not appear to be a predictor of low eAb-B titers, as the proportion of participants with low Ab-B titers was similar in participants ≥65 years of age and participants <65 years of age

Table 1. Demographics and Characteristics by Day 1 eAb-B Titer

N	Low 288	Medium 328	High 135	*P* Value*
% of population	38.3%	43.7%	18.0%	
Median age, years	66	66	64	
Female	174 (39.7%)	181 (41.3%)	83 (18.9%)	0.86
Male	114 (36.4%)	147 (47.0%)	52 (16.6%)	
≥65 years of age	154 (39.0%)	176 (44.6%)	65 (16.5%)	0.39
<65 years of age	134 (37.6%)	152 (42.7%)	70 (19.7%)	
≥1 CDI episodes in past 6 mo	65 (30.7%)	106 (50.0%)	41 (19.3%)	0.036†
No CDI episodes in past 6 mo	206 (42.6%)	201 (41.5%)	77 (15.9%)	
Previous CDI episodes ever	217 (41.0%)	220 (41.6%)	92 (17.4%)	0.0011†
No previous CDI episodes ever	75 (29.9%)	122 (48.6%)	54 (21.5%)	
Severe CDI (Zar score ≥2)	60 (48.4%)	43 (34.7%)	21 (16.9%)	0.065
Severe CDI (Zar score <2)	216 (36.3%)	272 (45.7%)	107 (18.0%)	
Immunocompromised	70 (49.6%)	47 (33.3%)	24 (17.0%)	0.025†
Not immunocompromised	218 (35.7%)	281 (46.1%)	111 (18.2%)	
Inpatient	211 (41.9%)	211 (41.9%)	82 (16.3%)	0.0047†
Outpatient	77 (31.2%)	117 (47.4%)	53 (21.5%)	
Charlson Index ≥3	108 (36.7%)	129 (43.9%)	57 (19.4%)	0.36
Charlson Index <3	180 (39.4%)	199 (43.5%)	78 (17.1%)	
Albumin ≤25 g/dL	52 (48.6%)	38 (35.5%)	17 (15.9%)	0.064
Albumin >25 g/dL	232 (36.8%)	284 (45.1%)	114 (18.1%)	

*2-sided Cochran-Armitage trend test significance level *P*<0.05; †*P*<0.05.

Limitations

- The assays employed in this analysis measured polyclonal antibodies to *C. difficile* toxins A and B and did not discriminate between neutralizing antibodies and nonfunctional antibodies. Therefore, some participants categorized in the medium- or high-titer categories may have had a low proportion of neutralizing antibodies

CONCLUSIONS

- The rise in eAb-A and eAb-B titers over time is consistent with a convalescent humoral immune response to toxins A and B following CDI[6]
- The lack of correlation between eAb-A titers and rCDI is consistent with the lack of efficacy of actoxumab in the prevention of rCDI observed in the MODIFY I trial[1]
- Conversely, higher eAb-B titers are associated with lower risk for rCDI, consistent with the efficacy of bezlo[1]
- Although there was an inverse correlation between eAb-B titers and rCDI rate, there was a fairly high proportion of participants with high eAb-B titers on Day 1 who experienced rCDI (22.1%). Therefore, eAb-B titers measured by an electrochemiluminescence immunoassay may have marginal utility as a biomarker for rCDI risk and are not likely to improve predictive value over clinical and demographic characteristics such as advanced age, compromised immunity, and CDI history

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