Bezlotoxumab for Preventing Recurrent *Clostridioides difficile* Infection: A Narrative Review from Pathophysiology to Clinical Studies

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ABSTRACT

Clostridioides difficile infection (CDI) and recurrent CDI (rCDI) remain associated with a reduction in the patients' quality of life and with increased healthcare costs. Bezlotoxumab is a monoclonal antibody against toxin B of *C. difficile*, approved for prevention of rCDI. In this narrative review, we briefly discuss the pathophysiology of CDI and the mechanism of action of bezlotoxumab, as well as the available evidence from investigational and observational studies in terms of efficacy, effectiveness, and safety of bezlotoxumab for the prevention of rCDI. Overall, bezlotoxumab has proved efficacious in reducing the burden of rCDI, thereby providing clinicians with an important novel strategy to achieve sustained cure. Nonetheless, experiences outside randomized controlled trials (RCTs) remain scant, and mostly represented by case series without a control group. Along with the conduction of RCTs to directly compare bezlotoxumab with faecal microbiota transplantation (or to precisely evaluate the role of their combined use), further widening our post-marketing experience remains paramount to firmly guide the use of bezlotoxumab outside RCTs, and to clearly identify those real-life settings where its preventive benefits can be exploited most.

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Key Summary Points

Clostridioides difficile infection (CDI) is a frequent cause of antibiotic-associated diarrhea, which mainly affects elderly patients exposed to broad-spectrum antimicrobials.

About 25% of patients with CDI are at risk of developing a recurrent CDI (rCDI) after resolution of the first episode. After the first rCDI, the risk of multiple recurrences increases to 40%.

Bezlotoxumab has proved efficacious in reducing the burden of rCDI, thereby providing clinicians with an important novel strategy to achieve sustained cure in patients with CDI.

However, published experiences outside randomized controlled trials remain scant, and mostly represented by case series without a control group.

Further widening our post-marketing experience remains paramount to firmly guide the use of bezlotoxumab in real-life, and to clearly identify those clinical settings where its preventive benefits can be exploited most.

INTRODUCTION

Clostridioides difficile infection (CDI) is a frequent cause of antibiotic-associated diarrhoea, which mainly affects elderly patients exposed to broad-spectrum antimicrobials [1]. Both advanced age and antibiotics, in fact, may lead to an imbalance in intestinal microbiota with consequent disruption of its barrier effect [2–4]. In addition, about 25% of patients with CDI are at risk of developing a recurrent CDI (rCDI) after resolution of the first episode. Then, after the first rCDI, the risk of multiple recurrences increases to 40% [5].

Recurrent CDI (rCDI) is defined as a CDI episode occurring within 8 weeks after a previous episode resolved with treatment, whereas sustained cure is defined as no recurrence of symptoms up to 12 weeks after the previous episode [6]. Therapy of CDI usually relies on oral vancomycin or fidaxomicin, depending on the severity and type of episode (first or recurrent episode) [7, 8], and on stopping the administration of non-necessary parenteral antibiotics.

In the last few years, another option for reducing the impact of rCDI on patients' health has become available. Bezlotoxumab, a monoclonal antibody against toxin B of *C. difficile*, has been approved for prevention of rCDI. Bezlotoxumab is administered as a single intravenous dose during the course of oral antibiotic therapy for CDI in patients at high risk of rCDI [9–12]. In this narrative review, we briefly discuss the pathophysiology of CDI and the mechanism of action of bezlotoxumab, as well as the available evidence from investigational and observational studies in terms of efficacy, effectiveness, and safety of bezlotoxumab for the prevention of rCDI.

METHODS

In February 2019, the authors were separately assigned different topics to address through inductive PubMed searches: (1) pathophysiology of CDI; (2) chemistry and mechanism of action of bezlotoxumab; (3) pharmacology of bezlotoxumab; (4) efficacy of bezlotoxumab in phase 3 randomized controlled trials (RCTs); (5) bezlotoxumab in observational studies; and (6) safety of bezlotoxumab in clinical studies. Then, they were asked to prepare separated drafts related to their assigned research topic. Eventually, the drafts were merged into a complete manuscript to be reviewed and approved by all the authors.

Pathophysiology of CDI

After being ingested, the spores of *C. difficile* resist the gastric acid and pass through the stomach, ultimately reaching the gut. Once
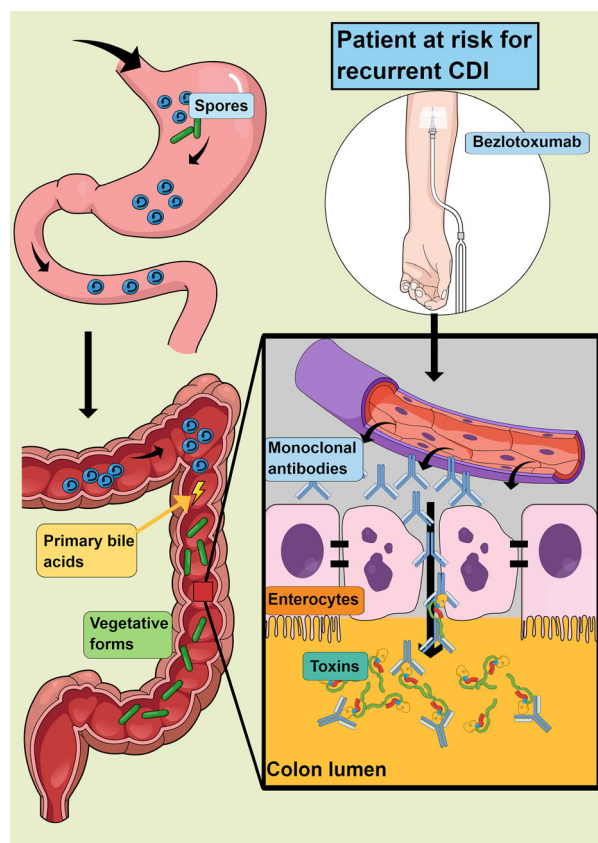
Fig. 1 Pathophysiology of CDI and mechanism of action of bezlotoxumab

there, *C. difficile* can persist as spores or germinate into vegetative forms. Germination is dependent on sensing primary bile acids from the liver, recognized by the germinant receptor CspC, and is inhibited by secondary bile acids in the colon [13]. In principle, while the "healthy" gut microbiota converts primary bile acids into secondary bile acids (which inhibit *C. difficile* germination), a disrupted microbiota following broad-spectrum antibiotic therapy, deficient of primary bile acid converters, may facilitate *C. difficile* germination and overgrowth. Once germinated, the vegetative forms of *C. difficile* are capable of producing toxins, the eventual mediators of the biologic damage (Fig. 1).

The pathophysiology of *C. difficile* relies mainly on the effects of toxin A and toxin B. These are two large proteins that contain a common multi-modular domain structure described as the ABCD model (A: biological activity; B: binding; C: cutting; D: delivery) [14]. The crystal structure of toxin A and toxin B has recently been elucidated and reported [15, 16]. The toxins are encoded by the *tcdA* and *tcdB* genes, respectively, located within a region known as the pathogenicity locus or PaLoc, a chromosomally integrated DNA sequence. The PaLoc also contains three other genes: (1) *tcdR*, encoding an alternative RNA polymerase sigma factor that is responsible for *tcdA* and *tcdB* expression; (2) *tcdE*, encoding a putative holin-like protein necessary for the extracellular release of both toxins; and (3) *tcdC*, which negatively regulates TcdA and TcdB synthesis [17]. PaLoc can be horizontally transferred to non-pathogenic strains characterized by the lack of tcdA and tcdB, converting them into a pathogenic strains producer [18].

Toxin A and toxin B bind to receptors on the surface of target cells. The main candidate receptors are glycosphingolipids containing the Galβ1-4GlcNAc motif for toxin A, and chondroitin sulfate proteoglycan 4, poliovirus receptor-like 3, and Wnt receptor frizzled proteins for toxin B [19]. After surface binding, the toxins are internalized through a receptor-mediated endocytosis and hence translocate into the cytosol through a pore-forming mechanism. Once in the cytosol, the toxins undergo an inositol hexakisphosphate-dependent autocatalytic cleavage, with the consequent release of the glucosyltransferase domain (region A), which finally targets Rho proteins. Members of the Rho family of guanosine triphosphatases are hence inactivated, thereby producing cytopathic effects, cytotoxic effects, induction of the programmed cell death, and activation of the inflammasome. Overall, this leads to colonocyte death, loss of intestinal barrier function, and development of neutrophilic colitis [17, 20]. Some bacterial strains may produce a binary toxin called *C. difficile* transferase. Binary toxin causes depolymerization of *F*-actin and rearrangement of the actin cytoskeleton, thereby disturbing the dynamic balance between actin and microtubules in target cells [21]. The pathogenic role of the binary toxin is still debated, but several studies have reported an association between binary toxin production and worse outcomes [22]. It has still not been

fully elucidated why *C. difficile* disposes of two similar toxins to exert its pathogenic effects. However, it now seems clear that toxin B, apart from being several-fold more potent than toxin A, is the one more strongly related to CDI pathogenesis [23]. Finally, in addition to the well-known toxin-mediated effects on the gut, attention has recently also been given to the possible extra-intestinal effects of toxins and toxaemia, that are likely implied in systemic manifestations of the disease. For example, cardiotoxic effects of toxins have been described in animal models [24].

Other factors that significantly contribute to pathogenesis of CDI are: (1) flagellar expression [25], that is variable among *C. difficile* strains and contributes to colonization efficiency; (2) the expression of type IV pili [26] that interact with the intestinal epithelium contributing to *C. difficile* aggregation and biofilm formation; and (3) the combined action of proteins, such as the adhesin fibronectin-binding protein A, cell wall proteins (e.g. Cwp84), Sl-layer protein A, and its modifying protease Cwp84, which contributes to *C. difficile* adherence, which have a role in biofilm formation, ensuring an "ecological niche" to the bacterium [27].

Peripheral leucocytosis is common, especially in severe CDI episodes. Neutrophils are the primary cells that respond to *C. difficile* invasion, and neutrophil inflammation is the hallmark of CDI. *C. difficile* toxins (mainly toxin B) activate neutrophils through formyl peptide receptor-1, and generate bactericidal concentrations of reactive oxygen species [28]. Neutrophils can also ingest complement or anti-*C. difficile* antibody-coated bacteria. However, although useful, these concerted mechanisms also need to be balanced, since they can also fuel tissue damage, and the boundary between "friend or foe" can be narrow [29]. Finally, hypoalbuminemia and hypogammaglobulinemia may be implicated in the pathogenesis of CDI. Indeed, it has been recently been shown that human serum albumin is capable of binding *C. difficile* toxins, impairing their internalization into the host cells thus reducing the toxin-dependent glycosylation of Rho proteins [30]. In clinical studies, hypoalbuminemia has been associated with mortality and recurrent CDI [31, 32]. With regard to hypogammaglobulinemia, humoral immunity is a major protective mechanism against CDI, and it has been demonstrated that lower antibody titres against toxins predisposes to disease development [33, 34].

Chemistry and Mechanism of Action of Bezlotoxumab

Bezlotoxumab (molecular weight 148.2 kDa) is a fully human IgG1 monoclonal antibody against *C. difficile* toxin B [35]. It was developed using mice transgenic for human immunoglobulin genes, and exposed to various antigens and adjuvant for 6–12 weeks [36]. Whole toxin A and toxin B toxoids and a recombinant C-terminal fragment of toxin B were used as immunogens and the splenic fusions were performed on mice with potent immune responses. Distinct toxin-reactive hybridomas were then screened according to in vitro and in vivo toxicity assays. Bezlotoxumab was the human monoclonal antibody derived from recombinant C-terminal toxin B fragment immunization [36].

Bezlotoxumab has been shown to bind and neutralize toxin B. Hernandez et al. assessed bezlotoxumab neutralization potency, as measured in a cell growth/survival assay with purified toxins from various *C. difficile* strains [37]. The authors showed that bezlotoxumab is active against toxins from all *C. difficile* strains, although toxins of ribotypes 027 and 078 were bound with lower affinities resulting in lower neutralization potency. The precise mechanism for different affinities is unknown, but it has been speculated that in these ribotypes the Fab region of bezlotoxumab binds to a single epitope, while in other strains it binds two epitopes. Nevertheless, even in 027 and 078 ribotypes, nearly complete toxin neutralization was achieved at concentrations of antibody that were still below plasma concentrations measured in CDI patients, thus the lower affinity against toxins from hypervirulent strains is likely irrelevant [37].

In 2014, both the mechanism of action of bezlotoxumab and the toxin B epitopes

involved in binding the monoclonal antibody were elucidated. Orth et al. demonstrated that bezlotoxumab binds to two epitopes existing in distinct regions within the N-terminal half of the combined repetitive oligopeptide (CROP) domain of toxin B, causing partial obstruction of two of the four putative carbohydrate-binding pockets involved in colonocytes binding [10]. The stoichiometry of bezlotoxumab to toxin B combined repetitive oligopeptide domain is 1:1, suggesting a direct toxin neutralization mechanism, more than a system mediated by large immune complexes [10]. This hypothesis was confirmed 1 year later by Yang et al. through experiments using multiple murine models of CDI [38]. In addition, in 2017, Gupta and colleagues showed that bezlotoxumab binding to the toxin B CROP domain prevented the host receptor (chondroitin sulfate proteoglycan 4) in mammalian host cells from toxin binding [39].

The transport of bezlotoxumab from the basolateral to the luminal compartment of colonocytes take place through the paracellular path after toxin disruption of the epithelial cells and the intercellular junctions [40] (Fig. 1). Basically, this observation support the hypothesis that bezlotoxumab could be more effective in patients with severe CDI episodes [12], since an increased disruption of colonocytes may allow more monoclonal antibodies to reach the gut lumen.

PK/PD of Bezlotoxumab

Currently, bezlotoxumab is approved for the prevention of rCDI in adult patients at high risk for rCDI. The product must be administered during the active CDI antibacterial treatment and is available as 1000 mg/40 mL single-dose vials. Reconstituted vials should be diluted in 0.9% sodium chloride or 5% dextrose to a final concentration between 1 and 10 mg/mL [41, 42]. The recommended dosage is based on the patient body weight, with 10 mg/kg intravenously over 60 min in a single administration [11, 41].

Like other intravenously administered monoclonal antibodies, bezlotoxumab possess a limited extravascular distribution [43]. In patients with CDI receiving a single 10 mg/kg intravenous dose, bezlotoxumab mean volume of distribution was 7.33 L; the geometric mean AUC_{0-INF} was 53,000 mcg per h/mL and the C_{max} was 185 mcg/mL [41, 43]. Age, gender, ethnicity, and co-morbid conditions, which typically have only a limited effect on the exposure of therapeutic antibodies, are not expected to affect the exposure of bezlotoxumab [41, 44]. Moreover, no clinically meaningful differences in bezlotoxumab exposure have been observed in patients with renal or hepatic impairment, and therefore no dose adjustment is recommended for patients with renal or hepatic disease [41]. Bezlotoxumab elimination occurs primarily by protein catabolism. The drug undergoes catabolism into smaller peptides, with a mean elimination half-life of 19 days [41]. In a phase 2 randomized, double-blind, placebo-controlled trial on the efficacy of a combination of actoxumab (a monoclonal antibody against toxin A) plus bezlotoxumab in preventing rCDI, after the initial infusion CDI patients showed detectable serum levels of bezlotoxumab for 22 ± 13 days [45]. Bezlotoxumab clearance increases with patient body weight, and the resulting exposure differences are addressed by the administration of a weight-based dose [41, 46].

Like other monoclonal antibodies, bezlotoxumab is eliminated via catabolic pathways, including proteolysis by the liver and reticuloendothelial system, target-mediated elimination, and non-specific endocytosis [47]. Therefore, bezlotoxumab differs from other traditional drugs eliminated through non-catabolic pathways, i.e. liver enzyme-systems like the cytochrome P450 and renal and biliary excretion. Considering the bezlotoxumab elimination by protein catabolism, drug–drug interactions with traditional drugs are not expected. So far, there is no in vivo or in vitro evidence of any drug–drug interaction [41].

The clinical phase 3 trials, MODIFY I and II, randomized adult patients with recurrent CDI under anti-CDI antimicrobial treatment to receive the addition of actoxumab–bezlotoxumab versus placebo (for details on efficacy

endpoints, see the next section) [11]. These clinical trials provided data on pharmacokinetic sampling of a large, diverse population, and Yee and colleagues analysed these data, adopting a population pharmacokinetic modelling approach to assess covariate effects on bezlotoxumab pharmacokinetic [43]. In total, bezlotoxumab concentrations from 1587 participants who received either bezlotoxumab alone or bezlotoxumab in combination with actoxumab were included in the population pharmacokinetic modelling analysis [43]. The study confirmed that co-administration with actoxumab, age, ethnicity, hepatic function, ongoing anti-C. difficile antibiotic treatment, and concomitant proton pump inhibitor use do not significantly alter bezlotoxumab exposure [43].

Interestingly, Yee and colleagues also estimated albumin levels to positively correlate with bezlotoxumab exposure. The estimated bezlotoxumab exposures was up to 33% lower in patients with albumin levels of < 3.5 g/dL than in patients with normal albumin levels [AUC_{0-INF} geometric mean ratio: 0.67; 90% confidence interval (CI): 0.65–0.69] [43]. Several mechanisms have been proposed to explain the interaction between albumin levels and the clearance of monoclonal antibodies, including the protective effect from lysosomal degradation exerted by the neonatal Fc receptor (FcRn) [43, 48–50]. According to the ability of FcRn to rescue both albumin and immunoglobulins from early degradation, factors that affect the recycling capacity of FcRn, i.e. low albumin levels, may influence the pharmacokinetic of monoclonal antibodies, including bezlotoxumab [43]. However, at present, there is no definite evidence that low albumin levels reduce bezlotoxumab exposure to a clinically meaningful extent, and no dose adjustments of bezlotoxumab are recommended in the presence of hypoalbuminemia [43].

Finally, the required bezlotoxumab gut lumen concentration to effectively inactivate toxin B is not yet known [51]. It is nonetheless of note that a higher bezlotoxumab concentration was observed in a CDI animal model of intestinal lumen toxin-damaged hamster, in comparison to controls with normal intestinal lumen [40].

Efficacy of Bezlotoxumab in Phase 3 RCTs

MODIFY I and II were two multicentre, double-blind phase 3 RCTs. Adults (≥ 18 years old) with first episode or recurrent CDI and receiving 10–14 days of standard of care antibiotic therapy for CDI (metronidazole, vancomycin, or fidaxomicin) were enrolled. Patients treated with vancomycin or fidaxomicin could also receive intravenous metronidazole [11]. Participants were assigned in a 1:1:1:1 ratio to receive placebo (0.9% saline), actoxumab 10 mg/kg alone (only in MODIFY I), actoxumab 10 mg/kg plus bezlotoxumab 10 mg/kg, or bezlotoxumab 10 mg/kg single dose, respectively. Enrolled patients received a single intravenous infusion of monoclonal antibody or placebo during the treatment period of standard of care for CDI. The primary endpoint of the two studies was the proportion of rCDI during 12 weeks of follow-up in the modified intent-to-treat (mITT) population. The two MODIFY RCTs were independent, and both were adequately powered to assess the primary efficacy endpoint. The design of MODIFY I was adaptive (enrollment in bezlotoxumab or actoxumab arms could be discontinued in the case of inferiority vs. the combined arm in an interim analysis). In fact, this allowed discontinuation of enrollment in the actoxumab arm [11]. As reported above, the actoxumab arm was not included in MODIFY II.

Overall, of 2655 randomized patients, 2559 (96%) were included in the mITT population (1396 in MODIFY I and 1163 in MODIFY II). In MODIFY I, the proportion of patients developing rCDI was lower in the bezlotoxumab (17%, 67/386) than in the placebo arms (28%, 109/395), with an adjusted difference of − 10.1% (95% CI − 15.9 to − 4.3). The same result was observed in MODIFY II [16% (62/395) vs. 26% (97/378), with an adjusted difference of − 9.9%, 95% CI − 15.5 to − 4.3]. The proportion of rCDI was conversely similar when comparing bezlotoxumab plus actoxumab versus bezlotoxumab alone. Indeed, in MODIFY I, the adjusted difference was − 1.4% with 95% CI − 6.7 to 3.9 [15.9% (61/383) vs. 17.4% (67/386), respectively], whereas, in MODIFY II, it was − 0.8% with 95% CI − 5.9 to 4.2 [14.9% (58/390) vs. 15.7% (62/395), respectively]. Similar

results were observed in the pooled analysis of the two trials, overall supporting the efficacy of bezlotoxumab for the prevention of rCDI, whereas actoxumab was not efficacious and did not provide any additional benefit when combined with bezlotoxumab. Of note, most rCDI (71%) occurred within 4 weeks. Another aspect worth noting is that 77% of participants had at least one risk factor for rCDI or for a CDI-related adverse outcome. In most of subgroups stratified according to such risk factors (e.g. ≥ 65 years of age, previous CDI episodes, immunocompromised status, severe CDI according to Zar score ≥ 2), , the protective effect of bezlotoxumab was confirmed, whereas the 95% CI crossed the zero in participants with CDI due to ribotype 027 and in those with CDI due to ribotypes 027, 078, or 244, although the direction of the effect was in favour of a protective effect of bezlotoxumab. Only in these latter two subgroups, was the protective effect of bezlotoxumab plus actoxumab possibly increased compared with that of bezlotoxumab alone, although the small subgroup samples preclude definite conclusions [11].

Several pre-planned/post hoc analyses of the MODIFY RCTs were conducted. An important necessary premise is that several had limited power, which may imply a non-negligible risk of type II error in some of them. In patients at high risk of rCDI (age ≥ 65 years, previous CDI episodes, immunocompromised status, severe CDI according to Zar score ≥ 2, and/or infection by ribotypes 027, 078, or 244), a post hoc analysis confirmed the protective effect of bezlotoxumab versus placebo in patients with a least one risk factor for rCDI, with the greater reduction in risk being observed in patients with at least 3 concomitant risk factors [12]. An increased protective effect of bezlotoxumab in patients at higher risk of rCDI was also suggested in another analysis [52]. In another study, participants in the MODIFY trials with sustained clinical cure at 12 weeks were shown not to develop any rCDI after other 9 months of follow-up (0/69, 0%) versus 2/65 (3%) and 1/34 (3%) in the bezlotoxumab plus actoxumab and placebo groups, respectively [53]. Using whole-genome sequencing, Zeng and colleagues differentiated recurrences due to new infection by

a different ribotype (50/259 evaluable patients, 19%) from recurrences due to relapse of infection by the same ribotype of the index CDI episode (198/259 evaluable patients, 76%) [54]. Unknown categorization of the type of recurrence was reported in 11 cases. The authors found that the cumulative incidence of relapses (assessed by means of a competing risk model) was lower in patients receiving bezlotoxumab versus non-bezlotoxumab (actoxumab or placebo), [54]. Compared with placebo, in another post hoc analysis the use of bezlotoxumab was also associated with reduced CDI-associated hospital readmissions in patients at high risk of rCDI [5.1% (27/530) vs. 11.2% (58/520), with difference − 6.1%, 95% CI − 9.5 to − 2.8] [55]. In a cost-effectiveness model based on pooled data from the MODIFY trials, the administration of bezlotoxumab led to a gain of 0.12 quality-adjusted life-years (QALYs) compared with placebo, and seemed cost-effective in terms of the prevention of rCDI in the entire study population, showing an incremental cost-effectiveness ratio of US$19 824/QALY gained [56]. Favourable results were also observed when adapting the cost-effectiveness model to a Spanish setting [57]. In patients enrolled in the MODIFY trials and receiving placebo, endogenous serum antibodies against toxin B were protective against rCDI, whereas endogenous serum antibodies against toxin A were not, this being in line with the protective effect observed for bezlotoxumab but not for actoxumab [58]. Staying on the topic of endogenous antibiotics, the immunogenicity potential of bezlotoxumab has been shown to be low, and no development of treatment-emergent anti-bezlotoxumab antibodies was observed in patients enrolled in registrative studies [59]. With regard to the timing of bezlotoxumab administration, efficacy in preventing rCDI was not influenced by the time of administration with respect to the onset of antibiotic therapy (i.e. 0–2, 3–4 and ≥ 5 days after onset) [60].

In a post hoc analysis of 44 MODIFY I/II participants with inflammatory bowel disease, treatment with bezlotoxumab showed a trend toward a protective effect when compared with placebo, although the wide 95% CI does not allow for firm interpretations before the

conducting of more powered studies on this topic [26.7% (4/15) vs. 53.8% (7/13), with difference − 27.2%, 95% CI − 57.9 to 9.6] [61]. In 382 MODIFY I/II participants with cancer, the proportion of patients developing rCDI was lower in the bezlotoxumab (26/146, 17.8%) than the placebo arms (42/138, 30.4%), with an absolute difference of − 12.6%, 95% CI − 22.5 to − 2.7 [62]. As shown in another analysis, the mean cumulative inpatient-days were lower in the bezlotoxumab (12.1 days) than the placebo arms (14.1 days), with a mean difference of − 2.1 days (95% CI − 3.7 to − 0.4) [63]. An exploratory study investigated if human genetic variations are able to influence the effect of bezlotoxumab in patients enrolled in the MODIFY trials. The single nucleotide polymorphism *rs2516513* and the human leukocyte antigen alleles *HLA-DRB1*07:01* and *HLA-DQA1*02:01*, which are located in the extended major histocompatibility complex on chromosome 6, showed an association with a reduced risk of rCDI in patients treated with bezlotoxumab. Notably, the same was not observed in patients receiving placebo [64]. Finally, the protective effect of bezlotoxumab was confirmed in a subgroup analysis of Japanese patients enrolled in the MODIFY trials [65].

Available meta-analyses also support the use of bezlotoxumab for preventing rCDI. In this regard, Madoff and colleagues evaluated 38 RCTs of different treatments [antibiotics, faecal microbiota transplantation (FMT), monoclonal antibodies, and various prebiotics and probiotics] for the prevention of rCDI [66]. They observed a greater risk reduction with FMT or monoclonal antibodies therapy, although any extrapolation about the relative efficacy of the different interventions should be made with caution because of the very different comparators employed in the included studies. In a Bayesian network meta-analyses of RCTs, a similar protective effect of bezlotoxumab versus (indirect comparison) single or multiple FMT was suggested, although FMT was possibly associated with a higher rate of non-serious diarrhoea as an adverse event (no differences were noticed for other adverse events) [67].

Bezlotoxumab in Observational Studies

So far, only a few observational studies on the use of bezlotoxumab in real life have been performed (see Table 1). A retrospective, multicentre case series was conducted in the US [68]. Among 200 patients with CDI receiving bezlotoxumab in addition to oral antibiotic therapy, 15.9% developed rCDI within 90 days. The median age of patients was 77 years, and the median Charlson Comorbidity Index was 5. A higher risk of rCDI was observed in patients with ≥ 2 previous recurrences before receiving bezlotoxumab (hazard ratio 2.77, 95% CI 1.14–6.76, p = 0.025) [68]. Another multicentre, retrospective case series was conducted in five university hospitals in Finland [69]. The study included 46 CDI patients who received a standard dose of bezlotoxumab. Their mean age was 66 years (range 15–97 years). Based on a Zar score, 18/46 (39%) had severe CDI. In addition, 28/46 (61%) were immunocompromised. As many as 36/46 (78%) had ≥ 3 risk factors for rCDI. Notably, 28/46 (61%) received concomitant antibacterial treatment for infections other than CDI. Two patients died before 3 months of follow-up after bezlotoxumab infusion. At the end of the 3-month follow-up, 32/44 (73%) patients remained free of rCDI. A similar result was observed in immunocompromised patients (71%). In severe CDI cases, the proportion of patients with no rCDI at 3 months was 63% [69]. Of note, eight were waiting for faecal microbiota transplantation but all remained free of recurrence and did not need the procedure. A prospective observational study assessing the impact of bezlotoxumab on rCDI rate in patients at high risk of recurrence is currently ongoing in five different hospitals in Spain (NCT04075422) [70].

Finally, successful prevention of rCDI with a combination of bezlotoxumab administration with FMT has been described in a patient with two previous FMT procedures that were unable to prevent rCDI [71].

Safety of Bezlotoxumab in Clinical Studies

In the MODIFY RCTs, infusion-specific reactions (i.e. adverse events occurring within 24 h

Table 1 Results of observational studies on the use of bezlotoxumab outside RCTs

References	Study design and study period	Country	Number and characteristic of included patients	Active antimicrobial anti-CDI treatment in association with bezlotoxumab	Primary outcome	Results	Bezlotoxumab-associated adverse events	Study limitations	Conclusions
Hengel RL et al. 2020 [68]	Retrospective, multicentre cohort study April 2017–December 2018	US	200 CDI patients treated with bezlotoxumab At baseline, 173/200 (86%) patients had prior CDI episodes and 158/200 (79%) patients had ≥ 2 risk factors for rCDI Median age: 70 years (range 21–98) Median Charlson comorbidity index: 5 (range 3–9).	Vancomycin fixed dose (76/200 patients, 38%), vancomycin tapered regimen (61/200 patients, 30.5%), fidaxomicin (60/200 patients, 30%), and metronidazole (3/200 patients, 1.5%)	rCDI in the 3 months after bezlotoxumab infusion	Within 3 months after bezlotoxumab infusion, 31/195 (15.9%) patients experienced rCDI	No infusion-related reactions were reported for patients receiving bezlotoxumab	rCDI was clinically defined as recurrence of diarrhoea for ≥ 2 days, and confirmatory diagnostic C. difficile tests were not consistently performed. This could have resulted in a greater proportion of diarrhoea recurrences classified as rCDI	The study observed successful prevention of rCDI with bezlotoxumab comparable to clinical trial results. Multiple prior rCDI were associated with a higher risk of subsequent rCDI
Oksi J et al. 2019 [69]	Retrospective study April 2017–December 2017	Finland	46 CDI patients treated with bezlotoxumab. Mean age: 66 years (range 15–97) Mean number of CDI previous episodes: 2.74 (range 1–7) 28/46 (61%) were immunocompromised and 36/46 (78%) had three or more risk factors for rCDI	Vancomycin in 37/46 patients, metronidazole in 9/46, fidaxomicin in 7/46, and tigecycline in 2/46	rCDI in the 3 months after bezlotoxumab infusion	Overall, 73% of the patients remained free of rCDI in the following 3 months after bezlotoxumab infusion, 71% of the immunocompromised patients remained free of rCDI during the follow-up, 63% of the severe CDI cases remained free of rCDI during the follow-up	Two possible infusion-related adverse reactions: one patient experienced startling sensations after the infusion; one patient presented with fever in the following day after the infusion	Detection of CD toxin gene by polymerase chain reaction was the only method used to detect CDI	Bezlotoxumab infusion as an adjunctive treatment to anti-CD antimicrobial treatment prevented rCDI in a major proportion of enrolled patients, including immunocompromised individuals

CD Clostridioides difficile, CDI Clostridioides difficile infection, rCDI recurrent Clostridioides difficile infection, RCTs randomized controlled trials

of the infusion) were observed in 10.3%, 11.1%, 8.0%, and 7.6% of patients receiving bezlotoxumab, actoxumab, bezlotoxumab plus actoxumab, or placebo, respectively [11]. The most frequent infusion-specific reactions were headache (2%), nausea (2%), fatigue (1%), pyrexia (1%), and dizziness (1%), with equally distributed rates across the study arms. Of note, there was a drug-related discontinuation of bezlotoxumab because of ventricular tachyarrhythmia occurring approximately 36 min after the start of bezlotoxumab infusion. Bezlotoxumab was discontinued and the adverse event resolved [11, 72].

During the 4 weeks after infusion, one or more adverse events, mostly gastrointestinal disorders, were registered in 61.7%, 67.2%, 58.6%, and 61.2% of patients receiving bezlotoxumab, actoxumab, bezlotoxumab plus actoxumab, or placebo, respectively. The rates of drug-related adverse events (with causality being assessed by the blinded investigator) were 7.5%, 7.2%, 6.4%, and 5.9% in patients receiving bezlotoxumab, actoxumab, bezlotoxumab plus actoxumab, or placebo, respectively. Serious drug-related adverse events were observed in 0.5%, 1.3%, 0.6%, and 0.3% in patients receiving bezlotoxumab, actoxumab, bezlotoxumab plus actoxumab, or placebo, respectively. Death during the 12 weeks after infusion (mostly related to infectious events, followed by cardiac disorders) occurred in 7.1%, 11.5%, 6.6%, and 7.6% of patients receiving bezlotoxumab, actoxumab, bezlotoxumab plus actoxumab, or placebo, respectively. Of note, the number of patients with baseline cardiac failure experiencing adverse events, severe adverse events, or death was higher in the bezlotoxumab than the actoxumab plus bezlotoxumab or placebo arms [72].

In the previously described multicentre, observational case series conducted in Finland, possible bezlotoxumab infusion-related adverse reactions were observed in two patients [69]. One experienced startling sensations after the infusion, and the other one presented with fever the day after the infusion [69]. No infusion-related reactions were observed in the US case series by Hengel and colleagues [68]. Two

deaths, conceivably unrelated to bezlotoxumab infusion, occurred during follow-up [68].

CONCLUSIONS

CDI and rCDI remain associated with reduction in the patients' quality of life and with increased healthcare costs. Bezlotoxumab has proved efficacious in reducing the burden of rCDI, thereby providing clinicians with an important novel strategy to achieve sustained cure in patients with CDI. Nonetheless, experiences outside RTCs remain scant, and are mostly represented by case series without a control group. Along with the conduction of RCTs to directly compare bezlotoxumab with FMT (or to precisely evaluate the role of their combined use), further widening our post-marketing experience remains paramount to firmly guide the use of bezlotoxumab in real life, and to clearly identify those clinical settings where its preventive benefits can be exploited most.

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REFERENCES

1. Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to *Clostridium difficile*, *Clostridium perfringens*, and *Staphylococcus aureus*. J Clin Microbiol. 2006;44: 2785–91.

2. Allegranzi B, Bagheri Nejad S, Combescure C, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011;377:228–41.

3. Kociolek LK, Gerding DN. Clinical utility of laboratory detection of *Clostridium difficile* strain BI/NAP1/027. J Clin Microbiol. 2016;54:19–24.

4. Loo VG, Bourgault AM, Poirier L, et al. Host and pathogen factors for *Clostridium difficile* infection and colonization. N Engl J Med. 2011;365: 1693–703.

5. Sheitoyan-Pesant C, Abou Chakra CN, Pepin J, et al. Clinical and healthcare burden of multiple recurrences of *Clostridium difficile* infection. Clin Infect Dis. 2016;62:574–80.

6. Tschudin-Sutter S, Kuijper EJ, Durovic A, et al. Guidance document for prevention of *Clostridium difficile* infection in acute healthcare settings. Clin Microbiol Infect. 2018;24:1051–4.

7. Cornely OA, Nathwani D, Ivanescu C, et al. Clinical efficacy of fidaxomicin compared with vancomycin and metronidazole in *Clostridium difficile* infections: a meta-analysis and indirect treatment comparison. J Antimicrob Chemother. 2014;69:2892–900.

8. Nelson RL, Suda KJ, Evans CT. Antibiotic treatment for *Clostridium difficile*-associated diarrhoea in adults. Cochrane Database Syst Rev. 2017;3: CD004610.

9. Bezlotoxumab MA. First global approval. Drugs. 2016;76:1793–8.

10. Orth P, Xiao L, Hernandez LD, et al. Mechanism of action and epitopes of *Clostridium difficile* toxin B-neutralizing antibody bezlotoxumab revealed by X-ray crystallography. J Biol Chem. 2014;289: 18008–21.

11. Wilcox MH, Gerding DN, Poxton IR, et al. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection. N Engl J Med. 2017;376:305–17.

12. Gerding DN, Kelly CP, Rahav G, et al. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection in patients at increased risk for recurrence. Clin Infect Dis. 2018;67:649–56.

13. Paredes-Sabja D, Shen A, Sorg JA. *Clostridium difficile* spore biology: sporulation, germination, and spore structural proteins. Trends Microbiol. 2014;22: 406–16.

14. Jank T, Aktories K. Structure and mode of action of clostridial glucosylating toxins: the ABCD model. Trends Microbiol. 2008;16:222–9.

15. Chen P, Lam KH, Liu Z, et al. Structure of the full-length *Clostridium difficile* toxin B. Nat Struct Mol Biol. 2019;26:712–9.

16. Chumbler NM, Rutherford SA, Zhang Z, et al. Crystal structure of *Clostridium difficile* toxin A. Nat Microbiol. 2016;1:15002.

17. Di Bella S, Ascenzi P, Siarakas S, et al. *Clostridium difficile* toxins A and B: insights into pathogenic properties and extraintestinal effects. Toxins (Basel). 2016;8:134.

18. Brouwer MS, Roberts AP, Hussain H, et al. Horizontal gene transfer converts non-toxigenic *Clostridium difficile* strains into toxin producers. Nat Commun. 2013;4:2601.

19. Tao L, Tian S, Zhang J, et al. Sulfated glycosaminoglycans and low-density lipoprotein receptor contribute to *Clostridium difficile* toxin A entry into cells. Nat Microbiol. 2019;4:1760–9.

20. Leffler DA, Lamont JT. *Clostridium difficile* Infection. N Engl J Med. 2015;373:287–8.

21. Aktories K, Papatheodorou P, Schwan C. Binary *Clostridium difficile* toxin (CDT)—a virulence factor disturbing the cytoskeleton. Anaerobe. 2018;53: 21–9.

22. Gerding DN, Johnson S, Rupnik M, Aktories K. *Clostridium difficile* binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut Microbes. 2014;5:15–27.

23. Steele J, Mukherjee J, Parry N, Tzipori S. Antibody against TcdB, but not TcdA, prevents development of gastrointestinal and systemic *Clostridium difficile* disease. J Infect Dis. 2013;207:323–30.

24. Hamm EE, Voth DE, Ballard JD. Identification of *Clostridium difficile* toxin B cardiotoxicity using a zebrafish embryo model of intoxication. Proc Natl Acad Sci U S A. 2006;103:14176–81.

25. Baban ST, Kuehne SA, Barketi-Klai A, et al. The role of flagella in *Clostridium difficile* pathogenesis: comparison between a non-epidemic and an epidemic strain. PLoS ONE. 2013;8:e73026.

26. Purcell EB, McKee RW, Bordeleau E, et al. Regulation of type IV pili contributes to surface behaviors of historical and epidemic strains of *Clostridium difficile*. J Bacteriol. 2016;198:565–77.

27. Abt MC, McKenney PT, Pamer EG. *Clostridium difficile* colitis: pathogenesis and host defence. Nat Rev Microbiol. 2016;14:609–20.

28. Goy SD, Olling A, Neumann D, et al. Human neutrophils are activated by a peptide fragment of *Clostridium difficile* toxin B presumably via formyl peptide receptor. Cell Microbiol. 2015;17:893–909.

29. Jose S, Madan R. Neutrophil-mediated inflammation in the pathogenesis of *Clostridium difficile* infections. Anaerobe. 2016;41:85–90.

30. di Masi A, Leboffe L, Polticelli F, et al. Human serum albumin is an essential component of the host defense mechanism against *Clostridium difficile* intoxication. J Infect Dis. 2018;218:1424–35.

31. Butt E, Foster JA, Keedwell E, et al. Derivation and validation of a simple, accurate and robust prediction rule for risk of mortality in patients with *Clostridium difficile* infection. BMC Infect Dis. 2013;13:316.

32. Knafl D, Vossen MG, Gerges C, et al. Hypoalbuminemia as predictor of recurrence of *Clostridium difficile* infection. Wien Klin Wochenschr. 2019;131:68–74.

33. Islam J, Taylor AL, Rao K, et al. The role of the humoral immune response to *Clostridium difficile* toxins A and B in susceptibility to *C. difficile* infection: a case-control study. Anaerobe. 2014;27:82–6.

34. Leav BA, Blair B, Leney M, et al. Serum anti-toxin B antibody correlates with protection from recurrent *Clostridium difficile* infection (CDI). Vaccine. 2010;28:965–9.

35. Johnson S, Gerding DN. Bezlotoxumab. Clin Infect Dis. 2019;68:699–704.

36. Babcock GJ, Broering TJ, Hernandez HJ, et al. Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamsters. Infect Immun. 2006;74: 6339–477.

37. Hernandez LD, Racine F, Xiao L, et al. Broad coverage of genetically diverse strains of *Clostridium difficile* by actoxumab and bezlotoxumab predicted by in vitro neutralization and epitope modeling. Antimicrob Agents Chemother. 2015;59:1052–60.

38. Yang Z, Ramsey J, Hamza T, et al. Mechanisms of protection against *Clostridium difficile* infection by the monoclonal antitoxin antibodies actoxumab and bezlotoxumab. Infect Immun. 2015;83:822–31.

39. Gupta P, Zhang Z, Sugiman-Marangos SN, et al. Functional defects in *Clostridium difficile* TcdB toxin uptake identify CSPG4 receptor-binding determinants. J Biol Chem. 2017;292:17290–301.

40. Zhang Z, Chen X, Hernandez LD, et al. Toxin-mediated paracellular transport of antitoxin antibodies facilitates protection against *Clostridium difficile* infection. Infect Immun. 2015;83:405–16.

41. Zinplava (bezlotoxumab) [prescribing information]. Whitehouse Station: Merck, 2016.

42. Chapin RW, Lee T, McCoy C, et al. Bezlotoxumab: could this be the answer for *Clostridium difficile* recurrence? Ann Pharmacother. 2017;51:804–10.

43. Yee KL, Kleijn HJ, Kerbusch T, et al. Population pharmacokinetics and pharmacodynamics of bezlotoxumab in adults with primary and recurrent *Clostridium difficile* infection. Antimicrob Agents Chemother. 2019;63:e01971–18.

44. Zhou H, Mascelli MA. Mechanisms of monoclonal antibody-drug interactions. Annu Rev Pharmacol Toxicol. 2011;51:359–72.

45. Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. N Engl J Med. 2010;362:197–205.

46. Dostalek M, Gardner I, Gurbaxani BM, et al. Pharmacokinetics, pharmacodynamics and physiologically-based pharmacokinetic modelling of monoclonal antibodies. Clin Pharmacokinet. 2013;52:83–124.

47. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet. 2010;49: 493–507.

48. Kim J, Hayton WL, Robinson JM, Anderson CL. Kinetics of FcRn-mediated recycling of IgG and albumin in human: pathophysiology and therapeutic implications using a simplified mechanism-based model. Clin Immunol. 2007;122:146–55.

49. Ober RJ, Martinez C, Vaccaro C, et al. Visualizing the site and dynamics of IgG salvage by the MHC class I-related receptor. FcRn J Immunol. 2004;172: 2021–9.

50. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol. 2007;7: 715–25.

51. Navalkele BD, Chopra T. Bezlotoxumab: an emerging monoclonal antibody therapy for prevention of recurrent *Clostridium difficile* infection. Biologics. 2018;12:11–21.

52. Wilcox MH, Rahav G, Dubberke ER, et al. Influence of diagnostic method on outcomes in phase 3 clinical trials of bezlotoxumab for the prevention of recurrent *Clostridioides difficile* infection: a post hoc analysis of MODIFY I/II. Open Forum Infect Dis. 2019;6:ofz293.

53. Goldstein EJC, Citron DM, Gerding DN, et al. Bezlotoxumab for the prevention of recurrent *Clostridioides difficile* infection: 12-month observational data from the randomized phase III Trial. MODIFY II. Clin Infect Dis. 2019. https://doi.org/10.1093/cid/ciz1151.

54. Zeng Z, Zhao H, Dorr MB, et al. Bezlotoxumab for prevention of *Clostridium difficile* infection recurrence: distinguishing relapse from reinfection with whole genome sequencing. Anaerobe. 2020;61: 102137.

55. Prabhu VS, Cornely OA, Golan Y, et al. Thirty-day readmissions in hospitalized patients who received bezlotoxumab with antibacterial drug treatment for *Clostridium difficile* infection. Clin Infect Dis. 2017;65:1218–21.

56. Prabhu VS, Dubberke ER, Dorr MB, et al. Cost-effectiveness of bezlotoxumab compared with placebo for the prevention of recurrent *Clostridium difficile* infection. Clin Infect Dis. 2018;66:355–62.

57. Salavert M, Cobo J, Pascual A, et al. Cost-effectiveness analysis of bezlotoxumab added to standard of care versus standard of care alone for the prevention of recurrent *Clostridium difficile* infection in high-risk patients in Spain. Adv Ther. 2018;35: 1920–34.

58. Kelly CP, Poxton IR, Shen J, et al. Effect of endogenous *Clostridioides difficile* toxin antibodies on recurrence of *C. difficile* infection. Clin Infect Dis. 2020;71:81–6.

59. Montgomery DL, Matthews RP, Yee KL, et al. Assessment of bezlotoxumab Immunogenicity. Clin Pharmacol Drug Dev. 2020;9:330–40.

60. Birch T, Golan Y, Rizzardini G, et al. Efficacy of bezlotoxumab based on timing of administration relative to start of antibacterial therapy for *Clostridium difficile* infection. J Antimicrob Chemother. 2018;73:2524–8.

61. Kelly CP, Wilcox MH, Glerup H, et al. Bezlotoxumab for *Clostridium difficile* infection complicating inflammatory bowel disease. Gastroenterology. 2018;155:1270–1.

62. Cornely OA, Mullane KM, Birch T, et al. Exploratory evaluation of bezlotoxumab on outcomes associated with *Clostridioides difficile* infection in MODIFY I/II participants with cancer. Open Forum Infect Dis. 2020;7:ofaa038.

63. Basu A, Prabhu VS, Dorr MB, et al. Bezlotoxumab is associated with a reduction in cumulative inpatient-days: analysis of the hospitalization data from the MODIFY I and II clinical trials. Open Forum Infect Dis. 2018;5:ofy218.

64. Shen J, Mehrotra DV, Dorr MB, et al. Genetic Association reveals protection against recurrence of *Clostridium difficile* infection with bezlotoxumab treatment. mSphere. 2020;5:e00232–20.

65. Mikamo H, Aoyama N, Sawata M, et al. The effect of bezlotoxumab for prevention of recurrent *Clostridium difficile* infection (CDI) in Japanese patients. J Infect Chemother. 2018;24:123–9.

66. Madoff SE, Urquiaga M, Alonso CD, Kelly CP. Prevention of recurrent *Clostridioides difficile* infection: a systematic review of randomized controlled trials. Anaerobe. 2020;61:102098.

67. Alhifany AA, Almutairi AR, Almangour TA, et al. Comparing the efficacy and safety of faecal microbiota transplantation with bezlotoxumab in reducing the risk of recurrent *Clostridium difficile* infections: a systematic review and Bayesian network meta-analysis of randomised controlled trials. BMJ Open. 2019;9:e031145.

68. Hengel RL, Ritter TE, Nathan RV, et al. Real-world experience of bezlotoxumab for prevention of *Clostridioides difficile* infection: a retrospective multicenter cohort study. Open Forum Infect Dis. 2020;7:ofaa097.

69. Oksi J, Aalto A, Saila P, et al. Real-world efficacy of bezlotoxumab for prevention of recurrent *Clostridium difficile* infection: a retrospective study of 46 patients in five university hospitals in Finland. Eur J Clin Microbiol Infect Dis. 2019;38:1947–52.

70. Available at: https://clinicaltrials.gov/ct2/show/NCT04075422. Last accessed 05 Mar 2020.

71. Kaako A, Al-Amer M, Abdeen Y. Bezlotoxumab use as adjunctive therapy with the third fecal microbiota transplant in refractory recurrent *Clostridium difficile* colitis; a case report and concise literature review. Anaerobe. 2019;55:112–6.

72. Federal Drug Administration. FDA briefing document. Bezlotoxumab injection. Meeting of the Antimicrobial Drugs Advisory Committee (AMDAC); 2016. Available from: https://www.fda.gov/media/98708/download. Accessed 9 May 2020.