A Randomised, Controlled, Open-label Phase III Clinical Trial in Patients With Primary or Recurrent Clostridioides Difficile (CD) Infection, to Evaluate the Efficacy and Safety of Capsules of Lyophilised Faecal Microbiota vs Fidaxomicin.

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August 4, 2023, version 7.0

MIKROBIOMIK

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Table of Contents

Table List

Version History

VERSION	DATE	CHANGES
Version 3.0	30-July-2021	Modification of exclusion criteria
Version 4.0	07-December-2021	Modification of inclusion and exclusion criteria Expanding Sites Modified definition of poor evolution Modification of interim analysis
Version 5.0	20-July-2022	Modification of study population Modification of inclusion criteria Change in sample size Expanding Sites
Version 6.0	17-May-2023	Modification of donor exclusion criteria Sample Size Modification
Version 7.0	04-August-2023	Sample size calculation Definition of study variables Premature Termination of Study Safety follow-up (withdrawn patients)

List of Acronyms

ADR: Adverse Drug Reaction

AE: Adverse Event

AEMPS: Spanish Agency for Medicinal Products and Medical Devices (*Agencia Española de Medicamentos y Productos Sanitarios*)

ALT: Alanine transaminase

AR: Adverse Reaction

AST: Aspartate aminotransferase

BMI: Body Mass Index

CD: *Clostridioides difficile*

CDI: *Clostridioides difficile* Infection

CEIm: Clinical Research Ethics Committee (*Comité de Ética de la Investigación con medicamentos*)

CI: Confidence Interval

CRA: Clinical Research Associate

CRP: C-reactive protein

eCRF: electronic Case Report Form

EPEC: Enteropathogenic *Escherichia coli*

ESCMID: European Society of Clinical Microbiology and Infectious Diseases

FDA: US Food and Drug Administration

FMT: Fecal Microbiota Transplantation

GCP: Good Clinical Practice

GDH: Glutamate dehydrogenase

GDPs: Good Distributing Practices

GGTP: Gamma-glutamyltransferase

GMP: Good Manufacturing Practices

HDL: High-Density Lipoprotein

HDPE: High-Density Polyethylene

ICF: Informed Consent Form

ICU: Intensive Care Unit

IDSA: Infectious Diseases Society of America

ITT: Intention-to-treat

LDH: Lactate dehydrogenase

LDL: Low-Density Lipoprotein

OS: Overall Survival

PaLoc: Pathogenicity region

PCR: Polymerase chain reaction

PI: Principal Investigator

PP: per Protocol Population

PPI: Proton-Pump inhibitors

QP: Query Plan

r-CDI: Recurrent *Clostridioides difficile* Infection

SAE: Serious Adverse Event

SAP: Statistical Analysis Plan

SDU: Standard Drink Unit

SEIMC: Spanish Society of Infectious Diseases and Clinical Microbiology (*Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica*)

SOP: Standard Operating Procedure

SP: Safety Population

STEC: Shiga Toxin-producing *Escherichia coli*

SUSAR: Suspected Unexpected Serious Adverse Reaction

00. OVERVIEW

00. 01. Study Title, Protocol ID

Title: A Randomised, Controlled, Open-label Phase III Clinical Trial in Patients With Primary or Recurrent Clostridioides Difficile (CD) Infection, to Evaluate the Efficacy and Safety of Capsules of Lyophilised Faecal Microbiota vs Fidaxomicin.

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00. 02. Sponsor ID

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00. 03. Investigational Product

Drug product consisting of heterologous lyophilized fecal microbiota capsules from healthy donors.

00. 04. Planned Study Sites

Hospital Ramón y Cajal [Ramón y Cajal Hospital] (Madrid)

Hospital Universitario 12 de Octubre [12 de Octubre University Hospital] (Madrid)

Hospital Universitario Puerta de Hierro [Puerta de Hierro University Hospital] (Madrid)

Hospital Universitario de Bellvitge [Bellvitge University Hospital] (Barcelona)

Hospital Clínic [Clinical Hospital] (Barcelona)

Hospital Universitario Reina Sofia [Reina Sofia University Hospital] (Cordoba)

Hospital Universitario Son Espases [Son Espases University Hospital] (Mallorca)

Hospital Universitario Marqués de Valdecilla [Marqués de Valdecilla University Hospital] (Santander)

Hospital Universitario de Cruces [Cruces University Hospital] (Bilbao)

Hospital Universitario de Basurto [University Hospital of Basurto] (Bilbao)

Hospital Universitario de Araba [University Hospital of Araba] (Alava)

Hospital Universitario de Donostia [University Hospital of Donostia] (San Sebastian)

Hospital Clínico Universitario Lozano Blesa [Lozano Blesa University Clinical Hospital] (Zaragoza)

Hospital San Pedro [San Pedro Hospital] (Logroño)

Hospital Universitario y Politécnico La Fe [La Fe University and Polytecnic Hospital] (Valencia)

Hospital General Universitario Gregorio Marañón [Gregorio Marañón University General Hospital] (Madrid)

La Paz University Hospital [Hospital Universitario La Paz] (Madrid)

Hospital General Universitario de Alicante [University General Hospital of Alicante] (Alicante)

Hospital Universitario de Gerona Doctor Josep Trueta [University Hospital of Gerona Doctor Josep Trueta] (Gerona)

Hospital Universitario Quironsalud Madrid [Quironsalud University Hospital Madrid] (Madrid)

Quironsalud Barcelona Hospital [Quironsalud Hospital of Barcelona] (Barcelona)

00.05. Coordinating Investigator

Javier Cobo, MD

Hospital Universitario Ramón y Cajal [Ramón y Cajal University Hospital]

00.06. Clinical Research Ethics Committee

Ethics Committee for Investigation with Medicinal Products of Euskadi

00.07. Study Design

A multi-center, open-label, controlled phase III prospective follow-up clinical trial in a cohort of patients with a diagnosis of CDI (a first episode or subsequent recurrences). The proposed treatment is a single-dose orally administered drug consisting of lyophilized fecal microbiota capsules from healthy donors.

00.08. Study Objectives and Endpoints

Primary objective: to evaluate the efficacy of the investigational medicinal product (MBK-01) compared to the control (fidaxomicin).

Primary variable: no CD diarrhea without need for retreatment (or no recurrence as defined in Section 03.07.02), as assessed by subject interview and physical examination. The primary variable is operated by the probability of recurrence.

Secondary Objectives: Evaluate the safety of the investigational medicinal product and quality

of patients participating in the study.

00.09. Study population

Patients who experience an episode of CD infection (either a first episode or subsequent recurrences).

00.10. Calendar

Run-In Period: August 2021 - December 2023

Estimated follow-up: six months

Estimated End of Study Date: June 2024

01. INTRODUCTION AND RATIONALE FOR THE STUDY

01.01. Disease or disorder under study

Clostridioides difficile (CD) bacteria is the leading pathogen responsible for diarrhea associated with previous antibiotic use in healthcare facilities in developed countries and an increasingly common cause of community diarrhea. Although it usually presents with mild diarrhea, it can also cause severe conditions such as pseudomembranous colitis, toxic megacolon, septic shock and, in extreme cases, death. The epidemiology of *Clostridioides difficile* infection (CDI) has changed dramatically in recent decades and has become a major global health problem. CDI has experienced significant epidemiological changes in the past 15 years, with increased incidence, mortality, and recurrence observed (Dieterle et al., 2019). This is due in part to the appearance of increasingly virulent strains but also to the improvement of available diagnostic methods and the inappropriate use of antibiotics, as well as the aging of a population affected by a larger number of chronic and debilitating diseases (Abreu et al., 2019).

Over the last decade, serious interhospital outbreaks have occurred in North America and Europe, reaching CDI at an incidence of up to 92 per 100,000 population. Data from the EUCLID study estimate that in Europe, the mean number of CDI episodes is 7 per 10,000 patients admitted and an estimated 23% of cases are not detected (Davies et al., 2014). Although there is no national CDI registry in Spain, the incidence of nosocomial-origin CDI is estimated to be 10 episodes per 10,000 hospital stays, while limited descriptive CDI studies show recurrence percentages of 12-18% (Rodriguez-Pardo et al., 2013; Reigadas et al., 2015; Larrainzar-Coghen 2016).

The recurrence of CDI poses a serious problem at both the health and economic levels. Recurrence aggravates the patient's general condition and promotes disease spread to other patients. On the other hand, recurrence increases healthcare expenditure to a greater extent than initial episodes (€4,900 vs. €3,900) (Asensio et al., 2013). CD is a gram-positive, strict anaerobic bacillus of oral-fecal transmission capable of producing spores whose primary pathogenic mechanism are two exotoxins (toxin A and B), which are responsible for the clinical manifestations. Toxins are the major virulence factors of CD; toxin A or enterotoxin (308 kDa) and toxin B or cytotoxin (270 kDa) that are encoded, respectively, by the *tcdA* and *tcdB* genes located in a region of pathogenicity (PaLoc) of the CD chromosome (Oka et al., 2012). Toxins A and B cause inflammation at the level of the large intestine and lead to increased epithelial permeability, cytokine production, neutrophil infiltration, oxygen reactive intermediate production, mast cell activation, substance P production, and direct intestinal mucosal damage (Perez et al., 2013).

Other virulence factors that have been described are binary toxin and surface proteins that mediate CD adherence to host epithelial cells. The presence of these varies in different strains of CD and could influence the pathogen's ability to bind to the colon epithelium, acting synergistically with toxins A and B (Voth et al. 2005; McDonald et al., 2006; Tenover et al., 2011; Penichea et al., 2013). CDI may present with different clinical manifestations

ranging from mild or moderate diarrhea to fulminant pseudomembranous colitis, toxic megacolon, or death (SEIMC, Alcalá-Hernández et al., 2016).

The type of disease and its severity will depend on factors such as the virulence of the infective strain and the host's immune response. Contact with spores of a toxin-producing strain of CD in combination with disruption of the host microbiota allows colonization by CD.

The main risks of CD infection are exposure to antibiotics (especially to clindamycin, cephalosporins, beta-lactams and fluoroquinolones), prolonged hospitalization, admission to an intensive care unit, the physical proximity of infected individuals, advanced age (over 65 years old), the severity of another underlying disease, immunosuppression, a poor immune response to CD toxins, the performance of non-surgical gastrointestinal procedures and the use of antacids (SEIMC, Alcala-Hernández et al. 2016).

Elderly, hospitalized and antibiotic-treated patients are the main risk group for CDI; however, in recent years, an increase of cases – around 25% of total CDI cases – has been observed in patients without traditional risk factors: young subjects who have not had contact with hospital institutions or antibiotic treatment (SEIMC, Alcalá-Hernández et al., 2016).

Patients with clinical symptoms of the disease can be classified according to different severity criteria. Severity classification is important when assessing the need for treatment, type of treatment, or even the need for surgery or admission to an intensive-care unit to control infection (SEIMC, Alcalá-Hernández et al., 2016).

Mild disease is characterized by only diarrhea, with an absence of symptoms and signs of colitis, while patients with moderate disease typically present with diarrhea and evidence of colitis characterized by fever and abdominal colic, usually in the lower quadrants. Laboratory signs of mild-moderate CDI include leukocytosis <15,000 cells/µL and serum creatinine levels below 1.5 times the pre-morbidity levels (SEIMC, Alcalá-Hernández et al., 2016).

Systemic manifestations typically occur in moderate or severe disease. Evidence of severe colitis includes fever (up to 40ºC), abdominal colic, leukocytosis ≥15,000 cells/µL, hypoalbuminemia (with serum albumin levels <2.5 mg/dL), presence of leukocytes in feces, and colon inflammation visualized by endoscopy (for pseudomembranes), or computed tomography (to observe colon wall thickening) (BSC, Alkalah-Hernán et al. 2016).

Fulminant CD disease occurs in less than 5% of patients and is characterized by severe abdominal pain, profound diarrhea (although diarrhea can sometimes be absent), while the patient rapidly progresses to the development of ileus or toxic megacolon (SEIMC, Alcalá-Hernández et al., 2016).

The risk of recurrence ranges from 20% after initial infection to 60% after multiple recurrences, with the recurrence rate being similar in nosocomial or community-acquired infection (SEIMC, Alcalá-Hernández et al., 2016).

Diarrhea is the most common clinical manifestation of CDI and is mediated by toxins A and B. Diarrhea is defined as the presence of loose stools (taken into the shape of the container

or graded between types 5 and 7 on the Bristol scale) accompanied by at least 3 stools in 24 hours or less or the patient's perception of a higher than normal number of stools. These are usually watery, greenish stools with a characteristically bad odor, although they can sometimes contain mucous and are soft. The presence of blood is rare (SEIMC, Alcalá-Hernández et al., 2016).

For the CDI diagnosis to be made, at least two criteria must be met: on the one hand, there must be microbiological detection of the toxin and/or isolation of toxin-producing CD in the absence of another cause for diarrhea, or colonoscopic or histopathological evidence of pseudomembranous colitis, and, on the other hand, the presence of diarrhea (≥3 non-formed stools in 24 hours) or, evidence of ileus or toxic megacolon through imaging tests. Laboratory diagnosis should be made only in symptomatic patients (diarrhea and/or abdominal pain, frequently accompanied by leukocytosis and fever) and in non-formed feces (Bristol scale levels 5-7), with the important exception of cases in which paralytic ileus or toxic megacolon is suspected, diarrhea may not be present and feces may be formed (SEIMC, Alcalá-Hern 2016 et al).

Commercial immunological techniques are available for the detection of toxin A and/or B, the Glutamate dehydrogenase (GDH) enzyme and, also, a combination of both. In addition, nucleic acid amplification testing on the same day as sample receipt allows detection of gene encoding for toxins A and/or B (SEIMC, Alcalá-Hernández et al., 2016) to be performed.

01.02. Current Therapies

Current treatment recommendations vary by country and clinical definition and are primarily linked to the number of episodes and severity of CDI that each patient presents (Bouza et al., 2020). The optimal treatment of patients with recurrence and/or severe complications is not entirely clear and is based on limited clinical evidence (Bouza et al., 2013; McDonald et al., 2018).

01.02.01. Metronidazole and vancomycin

First-line drugs for the treatment of CDI are metronidazole and vancomycin. They have a similar degree of efficacy, with response rates of around 90-97%, and oral administration is recommended.

Oral treatment with metronidazole is generally well tolerated, although systemic effects may occur. In one study conducted over a ten-year period, more than 600 patients with IDC were treated with metronidazole, and only 1% of them had significant adverse effects. These may include nausea, a metallic taste, disulfiram-*like* reactions with alcohol, and the effect of anticoagulants may be potentiated (Gonzalez-García et al., 2005). In addition, when metronidazole is administered for more than four weeks, it may generate neurotoxicity (McDonald et al., 2018). Systemic effects of oral vancomycin are rare. The main drawbacks

are the risk of developing resistant enterococci and the lack of therapeutic activity for CD when administered intravenously (Gonzalez-García et al., 2005).

In cases of severe infection, the use of vancomycin as first-line drug is recommended. This recommendation is based on clinical experiences in which patients appear to respond more quickly to vancomycin than to metronidazole.

The recommended duration of antibiotic treatment (metronidazole or vancomycin) in CD diarrhea is 10-14 days, both in initial treatment and in relapses (McDonald et al., 2018).

Currently, and according to the American Society for Infectious Diseases (IDSA) (McDonald et al., 2018), the use of vancomycin and fidaxomicin over metronidazole is recommended for initial episodes of CDI unless access to them is limiting, in which case metronidazole is recommended only for non-severe cases.

01.02.02. Fidaxomicin

Fidaxomicin is a macrocyclic antibiotic approved for the treatment of CDI in Europe and USA (Johnson et al., 2012; Debast et al., 2014), more respectful of gut microbiota than vancomycin and metronidazole (Louie et al., 2012). There are two completed prospective, randomized, double-blind clinical trials demonstrating that the clinical efficacy observed following oral treatment with fidaxomicin is not inferior to that observed following oral treatment with vancomycin. However, differences in the number of recurrences were observed.

In one of the studies, the percentage of recurrences observed in vancomycin-treated patients was 31%, whereas in those receiving fidaxomicin it was 21% (p=0.14) (Louie et al., 2011). In another study, in which patients from the previous study were compared together with the de novo study, the rate of CDI recurrence was evaluated in a total of 1,164 patients treated with vancomycin vs. fidaxomicin in two phase III clinical trials. Treatment efficacy was similar (>90% cure) in both groups (fidaxomicin-treated patients vs. vancomycin-treated patients) (Cornely et al., 2012).

However, 28-day recurrence reached 35.5% for vancomycin-treated patients and 19.7% for fidaxomicin-treated patients (-15.8% difference; 95% confidence interval, -30.4% to -0.3%; P=0.045). Early recurrence (at 14 days) occurred in 27% of vancomycin-treated patients and 8% of fidaxomicin-treated patients (p=0.003). In patients with a first recurrence of CDI, vancomycin and fidaxomicin were similar in terms of clinical cure at the end of therapy, but fidaxomicin was superior when preventing a second recurrence within the 28-day interval (Cornely et al., 2012). Both trials also showed an overall increase in sustained response over time (Louie et al., 2011; Johnson et al., 2012; Cornely et al., 2012). Oral fidaxomicin is as well tolerated as oral vancomycin is, and both treatments have been shown to have a similar safety profile (Louie et al., 2011; Cornely et al., 2012).

In 2014, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) recommended fidaxomicin for the first episodes of CDI, for the treatment of subjects with first recurrences, in patients at high risk of recurrence, and in patients with multiple

recurrences of CDI (Debast et al., 2014). Currently, the IDSA-published guideline recommends both vancomycin and fidaxomicin as the best initial treatment for CDI (McDonald et al., 2018); fidaxomicin is the recommended treatment for CDI patients at a very high risk of recurrence (Cornely et al., 2014; Bouza et al., 2020). Fidaxomicin is recommended by groups of Spanish and European experts for critically ill, immunocompromised, chronic renal failure, transplant patients and cancer patients (Aguado et al., 2015; Bouza et al., 2019).

Fidaxomicin is a very high-cost treatment and is, therefore, limited in use in many European hospitals (Cornely et al., 2012; Bouza et al., 2020).

01.02.03. Bezlotoxumab

Bezlotoxumab is a recombinant human monoclonal antibody that prevents the binding of toxin B to intestinal cell receptors. This blocks the first step of the cellular intoxication cascade. Currently, the approved indication for bezlotoxumab is prevention of CDI recurrence in adult patients (≥18 years) receiving treatment for CDI and at a high risk for recurrence (IPT 31, 2018).

The efficacy of bezlotoxumab has been evaluated in two randomized, double-blind, placebo-controlled, multicenter (320 centers in 30 countries) phase III studies called MODIFY I and MODIFY II (Wilcox et al., 2017). The results of the comparison show that, among adult patients receiving antimicrobial therapy versus a primary or recurrent episode of CDI, the use of this antimicrobial therapy in combination with bezlotoxumab reduces the number of patients who will have a recurrence of CDI over the next 12 weeks by 37.5%; in other words: a 10% reduction in absolute relapse values in a population with an estimated prevalence of relapse of 26.6%.

Following the good results, in Spain, the Spanish Agency of Medicines and Medical Devices (AEMPS, Agencia Española de Medicamentos y Productos Sanitarios) (IPT, 31. 2018) authorizes the use of bezlotoxumab as adjunctive treatment for the treatment of CDI (to date, it is the only antibody-based product approved for the treatment of recurrences) in patients at high risk of recurrence, including patients ≥65 years of age with prior episodes of CDI in the previous six months, in immunosuppressed patients, in patients with CDI produced by hypervirulent strains (such as 027 and 244), patients with severe CDI (Zar ≥2), and patients with a high probability of recurrence based on externally validated predictor models.

01.02.04. Fecal Microbiota Transplantation

Fecal Microbiota Transplantation (FMT) consists of the infusion of stools from a healthy donor into a patient's intestinal tract to treat a specific disease associated with alteration of the intestinal microbiota (Cammarota et al., 2017), as is the case for CDI. Within this context, FMT has emerged as a clinical procedure demonstrating high efficacy and safety in the treatment of recurrent CD infection (r-CDI) by restoring the gut microbiota and preventing successive recurrences (van Nood et al., 2013). Because of the beneficial effects obtained in

patients with r-CDI, studies have also been conducted to propose FMT as a treatment for the first CD infection and have also yielded favorable efficacy and safety results within this population (Camacho-Ortiz et al., 2017; Ng SCC et al., 2017; Juul et al., 2018). FMT can be administered by colonoscopy, enema, naso-duodenal or naso-jejunal tubes and oral capsules; and is, even after the onset of bezlotoxumab, the preferred non-antimicrobial treatment for treating CDI recurrences (Reigadas et al., 2019). The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) Guidance in 2014 strongly recommended the use of FMT in cases of multiple CDI recurrences (Debast et al., 2014). Moreover, a panel of 28 experts from 10 countries across Europe recently published a European Consensus in which they recommended FMT as a treatment option for refractory CDI and moderate-to-severe CDI recurrences, as well as implementation in clinical practice, based on the quality of evidence (Cammarota et al., 2017).

01.03. Nonclinical Studies

CDI has been extensively studied in different animal models. Distinct aspects of the disease (colonization, pathophysiology, transmission, recurrence, mechanism of action of toxins, etc.) have been investigated in small animal models (hamster, mouse, rabbit, hare, guinea pig, prairie dog, partridge, or zebrafish embryos) as well as in a limited number of large animal studies (foal, pig, and monkey) (Hamm et al., 2006; Lanis et al., 2010; Best et al., 2012; Hutton et al., 2014).

The primary therapy for the treatment of CDI is antibiotics against the pathogen. Once the antibiotic treatment is underway, normal intestinal function is restored. To prevent disease recurrence, the indigenous microbiota must also be restored. Current antibiotic therapy does not favor this. Thus, FMT is an alternative treatment that contributes to the definitive restoration of the indigenous microbiota through the inoculation of a full ecosystem from a healthy donor (Khoruts et al., 2010, Greham et al., 2010).

Different studies in mouse models of r-CDI have evaluated the efficacy of FMT treatment. In a study comparing vancomycin treatment with FMT, vancomycin-only mice were shown to have a recurrence of CDI when treatment was stopped. However, recurrence was prevented in mice treated with FMT (Seekatz et al., 2015). Another study demonstrated that FMT is an effective method for palliating the inflammatory effects of mice with antibiotic-induced CDI, as well as for eliminating the infectious agent and recovery of the gut microbiota (Litchman et al., 2016). Similar results were obtained in other animal (Buffie et al., 2015, Lawley et al., 2012) and human (Seekatz et al., 2014, Hamilton et al., 2013, Song et al., 2013) models, suggesting that FMT is an effective treatment for the restoration of microbiota and may be necessary to prevent CD recurrence in CDI patients.

In a study in mice with antibiotic-induced CDI, the efficacy of fresh, frozen, and lyophilized FMT was evaluated for 2 to 15 months, and so was the stability. The transferred microbiota was of human origin, and the number of days that the mice had diarrhea after being infected with CD strain VPI 10463, toxin A and B was evaluated. The results showed that both frozen and lyophilized products can be stored for up to seven months and that, during that time, they maintained the same clinical effectiveness as the fresh product. However, after seven

months, the products began to lose efficacy *in vivo*. These results demonstrate that FMT products have a limited storage time, even if they are stored at very low temperatures (Jiang et al., 2017b).

Animal FMT studies have shown certain limitations, such as lack of inter-individual variability. In addition, in the case of preclinical studies, the statistic for being able to determine differences is mainly based on the potential of the treatment. When treatment is less effective, a larger study sample size is required. On the other hand, it is difficult to extrapolate the results of FMT in animals to humans, adverse effects and safety are not always adequately assessable in animal models; therefore, the safety profiles of FMT in previous human studies should be analyzed (Staley et al., 2017, Arrieta et al., 2016, Best et al., 2012).

01.04. Previous Clinical Experience

As described above, oral vancomycin and fidaxomicin are the antibiotics of choice for the treatment of CDI, with FMT treatment being reserved for recurrences (Bouza et al., 2020, McDonald et al., 2018). It has been shown that the use of FMT not only protects against infection but also prevents future recurrences (Khoruts et al., 2010, Greham et al., 2010).

On the other hand, there are also clinical trials that have evaluated its potential efficacy and safety for the treatment of patients with primary CD infection. Although antibiotic therapy is recommended for the first episode of CDI, treatment with oral vancomycin and/or metronidazole often leads to significant treatment failure, with recurrences in up to 30-40% of patients experiencing a first episode. In addition, antibiotic treatment does not correct deficiencies in the intestinal microbiota that facilitate CD infection and is associated with the risk of antibiotic-resistant bacteria emerging (Camacho-Ortiz et al., 2017). Because administration of FMT results in faster reconstitution of the underlying intestinal microbiota alterations, as well as eradication of infection, and has been used for the treatment of patients with recurrent CDI with high efficacy and safety even in immunocompromised patients, the following trials have studied FMT administration as a potential treatment for primary CD infection.

Camacho-Ortiz et al. conducted an open-label trial with two treatment arms that patients were randomized to in a 1:1 ratio: oral vancomycin (250 mg every 6 hours for 10-14 days) or FMT (consisting of a mixture of fecal donors). Patients in either arm who did not clinically improve by 72 hours after the first treatment received a second FMT treatment. The study was conducted in adults hospitalized with the first episode of CDI. None of the patients had had a prior CDI or had received prior treatment for their current CDI. Patients with toxic megacolon, documented or suspected intestinal perforation, pregnancy, or concurrent colon neoplasms were excluded.

FMT doses were prepared by collecting three stool samples from each donor, mixing the stools from all donors, resuspending in 0.9% saline and filtering. Finally, glycerol, at a final concentration of 15% (v/v), was added before storing the mixture in 45 mL aliquots and freezing at -80ºC. A 45-mL aliquot was considered a dose. In total, 881.62 g of feces were collected. The final suspension had a concentration of 0.19 g/mL. FMT could be

administered by different routes at the discretion of the physician. FMT was administered by upper endoscopy in all but two cases: one patient received FMT by a nasojejunal tube already in place, and another, by colonoscopy.

A total of 16 patients out of the 19 recruited completed the study. Out of the 9 patients in the vancomycin treatment group, 8 patients experienced resolution of their infectious symptoms (88.9%); in the FMT treatment group, 4 out of 7 patients (57.1%) experienced resolution after the first dose of FMT (P=0.26), and 5 out of 7 patients, after the second dose of FMT (P=0.55). The microbial composition of the participants over time in the vancomycin group was different from the microbiome in the FMT group, confirming the impact oral antibiotics have on the gut microbiota. Regarding treatment safety, no adverse effects related to FMT were observed throughout the study (Camacho-Ortiz et al., 2017).

Juul et al conducted a randomized, blinded Phase 2 clinical trial in patients with primary CD infection (NCT02301000). Twenty-one adult patients with acute *C. difficile* infection who had not had a previous *C. difficile* infection were randomly assigned to receive oral metronidazole (a dose of 400 mg three times daily for 10 days) or FMT (an enema administered by a renal catheter of 60 mL of anaerobically cultured human intestinal microbiota). The primary objective of the study was to evaluate disease resolution without evidence of recurrence of CD infection at Day 70. One patient was excluded, and, out of the 20 eligible patients, 9 were randomly assigned to fecal microbiota transplantation and 11, to metronidazole.

A primary complete response was observed in five patients (56%; 95% CI: 21-86) in the FMT group and in 5 in the metronidazole arm (45%; 95% CI: 17-77) (exact P=1.00). Three of the remaining four patients in the FMT group received antibiotics on day 4 after treatment initiation, and two of them had a secondary complete response. In the metronidazole group, of the remaining six patients, none had a complete secondary response because the infection was refractory or recurrent. Thus, overall treatment response (primary or secondary complete response) was achieved in seven patients in the FMT-treated group (78%; 95% CI: 40-97), compared to five in the metronidazole group (45%; 95% CI: 17-77) (P=0.20). Serious treatment-related adverse events were not observed in either study group (Juul et al, 2018).

Due to the beneficial results obtained in this study, patients are currently being recruited for a multi-center, randomized, controlled, parallel-group, two-arm (FMT and antibiotic) phase III clinical trial (NCT03796650), which is a continuation of the phase II trial for primary *Clostridium difficile* infection. In this trial, patients with CDI and no prior CDI within 12 months prior to enrollment will be randomized in a 1:1 ratio to receive FMT or be treated with vancomycin (10 days, 125 mg, four times daily). The trial is still active, so no results are available as yet.

Another clinical trial (Ng SCC et al., 2019) has been conducted in patients with a primary episode of CDI, which evaluated the efficacy of FMT compared to the standard regimen of vancomycin. This was an open-label, randomized study in which 30 patients were assigned to receive either oral vancomycin (500 mg, four times daily) followed by FMT (a single infusion of stool from donors administered via nasoduodenal tube), or a standard regimen

of oral vancomycin (500 mg four times daily for 10 days). The primary objective was to demonstrate resolution of CDI-associated diarrhea without relapse within 10 weeks of treatment initiation. In addition, 30-day and six-month mortality, 30-day colectomy rates, length of hospital stay, adverse effects, and fecal microbiota alteration after FMT were studied as secondary objectives. Sixty percent of subjects in the vancomycin arm and 47% of subjects in the FMT arm, respectively, had severe CDI. Resolution of *C. difficile* infection occurred in 10 of 15 patients (66.7%) who received vancomycin and in 11 of 15 patients (73.3%) who received FMT (p=1.00). Two deaths occurred in the vancomycin group and none occurred in the FMT group within 30 days. After six months of the study, there were nine (60%) deaths in the vancomycin arm and three (20%) deaths in the FMT arm. Therefore, mortality was higher in the vancomycin-treated group. None of the patients had to have a colectomy. The median length of stay was the same in the two groups (13 vs. 15 days; p=0.95). In patients receiving FMT, a restoration of enriched bacteria from healthy donors was observed in recipients after FMT. Regarding treatment safety, no serious adverse events attributed to FMT were detected.

Therefore, while results still must be proven in studies with a larger sample size, these trials reflect the potential efficacy and safety of FMT for the treatment of patients with primary CD infection.

A meta-analysis published by Madoff and colleagues evaluated all the clinical trials conducted up to February 2018 that analyzed the efficacy and safety of different treatments for the prevention of CDI recurrence. Treatments used were antibiotics, FMT, monoclonal antibodies, probiotics, or other compounds (Madoff et al., 2020). Out of the clinical trials discussed in this review, eight evaluated the efficacy of FMT treatment for the prevention of CDI recurrence in a total of 582 patients. In all the studies evaluating FMT, only mild intervention-related adverse effects such as abdominal distension, cramping, nausea, and vomiting were reported. No serious adverse effects were reported in any case (Van Nood et al., 2013; Youngster et al., 2014a; Kelly et al., 2016; Lee et al. 2016; Hota et al., 2017; Jiang et al., 2017a; Kao et al., 2017).

The first clinical trial demonstrating superiority of FMT treatment versus vancomycin treatment in patients with recurrent CDI was published in 2013. In this clinical trial, patients were randomly assigned to three treatment groups: the first group received four days of treatment with oral vancomycin (500 mg, 4 times daily), followed by bowel lavage and FMT via gavage; the second group was treated with oral vancomycin for 14 days, and a third group received oral vancomycin for 14 days, followed by bowel lavage. In patients receiving FMT, diarrhea resolved more effectively compared with the other two groups of patients who did not receive FMT (p <0.001). The frequency of adverse events was similar in all three groups. Of the 16 patients in the FMT group, 81% (n=13) showed resolution of CD-associated diarrhea after a single transfer. Of the remaining three patients who received a second microbiota transfer, the infection was resolved in two of them. In the vancomycin-treated group, 31% (4/13 patients) resolved the diarrhea caused by CD, and 23% (3/13 patients) in the third group (Van Nood et al., 2013).

Another subsequent study compared FMT treatment by colonoscopy to a standard cycle of oral vancomycin (125 mg 4 times daily, followed by 125-500 mg/day every 2-3 days for at least three weeks). This study demonstrated that FMT treatment is significantly more effective in preventing CDI recurrence than the standard vancomycin regimen ($p < 0.001$). This study ended one year from the start, as 90% (18/20 patients) of those treated with FMT showed clinical resolution of the disease; whereas, in the vancomycin-treated group, only 26% (5/19 patients) experienced resolution; the cure rate was 87% for FMT-treated patients and 55% for vancomycin-treated patients (Cammarota et al., 2015).

Following early studies showing the efficacy of FMT as a treatment for recurrent CDI, other studies emerged that evaluated the differences in efficacy and safety between the different routes of FMT administration, as well as the development of different preparations that could be stored to allow for greater availability and flexibility for treatment application.

In a comparative study between FMT treatment administered by colonoscopy vs. oral liquid suspension capsules, no differences in efficacy were found between both routes of administration (RR 0.98 [95% CI, 0.14-6.71]) (Kao et al., 2017). Another study comparing colonoscopy vs. nasogastric tube administration also found no differences in efficacy (RR 0.20 [95% CI, 0.01-3.70]) (Youngster et al., 2014a).

Jiang et al compared the efficacy of frozen FMT suspension administered via enema versus lyophilized FMT administered orally via capsules in a total of 65 patients with three or more episodes of CDI recurrence. Patients treated with oral FMT received a dose of lyophilized microbiota obtained from 100 g feces (1.5 g microbiota powder/100 g fecal product), which was subsequently increased to 200 g to improve treatment efficacy. Patients treated with FMT via enema were administered a dose of microbiota obtained from 100 g of feces. The efficacy in preventing CDI recurrence 60 days after treatment was 84% for patients treated orally and 88% for patients treated with enema. Normalization of microbiota diversity was observed in all patients, although *Bacteroides* repopulation was lower in orally treated patients. No differences were observed in adverse effects (flatulence, nausea...) found after both administrations (Jiang et al., 2018).

Studies evaluating the efficacy of different routes of administration and different FMT preparations conclude that administration of FMT with previously frozen, colonoscopy-administered microbiota has an efficacy similar to that of fresh FMT suspension (RR 9.36 [95% CI, 0.53-165.03]) (Jiang et al., 2017a). Treatment efficacy was not reduced in cases in which administration of previously frozen FMT was via enema (RR 0.84 [95% CI, 0.54-1.30]) (Lee et al., 2016).

Hecker and colleagues conducted a study comparing the efficacy of lyophilized microbiota oral capsules to frozen microbiota liquid suspension capsules. Twenty patients with recurrent CDI were treated and dosed with 20 to 40 capsules (sized 0 capsules containing 60 mg lyophilized feces, including 00 capsules), obtained from approximately 40 g of feces (total anaerobic concentration/mL lyophilized vs. frozen microbiota was 8.5 ±0.5 and 9.1 ±0.2 $\log_{10}$ colony forming units, respectively). One month after treatment, diarrhea resolved in 85% of patients, and overall resolution amounted to 90% after treatment with a second dose of FMT in patients who had relapsed after the first dose. No serious adverse

effects were reported during the 204-day mean follow-up (range 31-408 days). Therefore, the use of lyophilized microbiota oral capsules was shown to be effective and to allow for the delivery of a high concentration of viable bacteria, with a predominance of anaerobes (Hecker et al., 2016).

Overall, no significant differences in clinical efficacy and safety outcomes were found for the different routes of FMT administration in patients with recurrent CDI, nor were there differences between the different sample preparations.

Numerous studies have evaluated not only the efficacy but also the safety of FMT treatment by oral capsules in patients with CDI (Youngster et al., 2014b, Hirsch et al., 2015, Youngster et al., 2016, Hecker et al., 2016, Staley et al., 2017, Staley et al., 2019, Reigadas et al., 2018, Reigadas et al., 2019).

One of the studies discussing the efficacy and safety of FMT administered as previously frozen oral capsules evaluated treatment in 20 patients with recurrent CDI. Each patient was administered 30 microbiota capsules (15 capsules daily, for two consecutive days); this microbiota was obtained from the processed 48 g of feces, with a mean dose equivalent to 1.6 g of microbiota/capsule (range 1.0-2.05 g/capsule). Resolution of diarrhea was assessed at eight weeks post-treatment, and follow-up was prolonged for six months. Seventy percent of patients had no diarrhea after eight weeks of treatment, and the percentage amounted to 90% after a second dose of FMT. The efficacy obtained in this study corroborates the results of previous studies of this same group in which the treatment applied by the nasogastric route or colonoscopy was evaluated. During the six months of follow-up, 30% of patients reported mild adverse events such as abdominal cramps or bloating that resolved spontaneously at 72 hours. On the other hand, freezing of the microbiota was shown to not diminish treatment efficacy, which allowed for the detection of other types of possible infections in the donor, thus increasing safety in treated patients (Youngster et al., 2014b). Another study in the same group evaluated the efficacy and safety of FMT via frozen microbiota capsules in a total of 180 patients with at least three moderate CDI episodes and at least two severe CDI episodes (requiring hospitalization). Each patient was administered 30 capsules of FMT microbiota obtained from the 48 g of feces processed, 15 per day, for two consecutive days. Again, resolution of diarrhea was assessed for eight weeks following FMT treatment at six months of follow-up. After eight weeks, the primary resolution of diarrhea in such patients was 82%. Following a re-transfer with the same dose of 30 capsules, the overall resolution increased to 91% and then to 93% after a third transfer (Youngster et al., 2016). The efficacy of administration of frozen microbiota capsules was shown to be the same as that observed in previous studies by the same team, in which FMT was administered by liquid preparations via gavage or colonoscopy (Youngster et al., 2014a, Youngster et al., 2016). Only three grade 2 or higher effects (one episode of transient high fever and two newly diagnosed cases of ulcerative colitis) considered to be related or possibly related to FMT were observed during the six months of follow-up. The other cases reported in 84% of patients were grade 1-adverse events, mostly mild intestinal discomfort. (Youngster et al, 2016).

Another study evaluated treatment with liquid microbiota oral capsules to treat patients (n=19) with two or more recurrence episodes (a mean of four recurrences per patient) of CDI. All patients had previously received treatment with metronidazole, vancomycin, and/or fidaxomicin. Each patient was administered 8-12 capsules, each one containing 0.4 mL of liquid suspension at 0.5 g/mL (patients received 1.1 to 8.3 g of input material). A 90-day follow-up was performed, after which 68% of patients had resolved; after a second administration of FMT, the percentage of diarrhea resolution was 89%. In this study, the minimum effective dose was lower than the minimum dose used in other studies, as 70% of patients showed a positive response with a dose less than 3 g of starting material, and with an estimate of 9.7 $x10^{10}$ viable bacteria. No serious adverse effects were recorded during the entire follow-up period. In addition, the capsule presentation was shown to be an easy and safe type of administration for patients of advanced age and/or with comorbidities (Hirsch et al., 2015).

The efficacy of FMT was evaluated in patients in whom treatment with antibiotics (including vancomycin) had failed. Forty-nine patients with 3-4 episodes of CDI (patients with inflammatory bowel disease excluded from the study) were treated with frozen and lyophilized oral capsules. Patients were initially treated with 24-27 capsules (~2.5 $x10^{12}$ bacteria) and, subsequently, due to a limitation on the number of FMT capsules obtained in the development phase, the dose was reduced to 2-3 capsules per patient (~2.1-2.5 $x10^{11}$ bacteria), which were administered as a single dose. Two months after transfer, overall recovery was 87.7%, with no significant differences observed between the highest (78.9% recovery; 15/19 patients) and lowest (93.3% recovery; 28/30 patients) doses. Following treatment, the fecal intestinal microbiota increased in time toward the normalization of the patient's microbiota one month after FMT, with no differences in kinetics or in this increase observed between high and low doses. However, they recorded a delay in this normalization of the microbiota, especially *Bacteroides,* when compared to the team studies in which FMT administration was performed with colonoscopy, although it did not affect clinical response. Administration of these capsules was shown to be safe, as the adverse effects that one third of patients showed consisted of abdominal swelling, flatulence, etc., in the first weeks after FMT (Staley et al., 2017).

This same group performed the assessment of colonization restoration in FMT-treated patients in order to account for the effect of this treatment on the recovery of the indigenous microbiota. The study was conducted in 18 patients with at least two episodes of CDI recurrence who had been previously treated with vancomycin. Treatment consisted of administration of 2 to 4 lyophilized FMT capsules, with the bacterial dose ratio of 2.1 $x10^{11}$ to 5 $x10^{11}$ bacteria/capsule. All patients showed clinical recovery and were free of CDI, although with varying degrees of colonization restoration throughout the year of follow-up. Sixty-one percent of patients had a successful restoration of colonization after one week of FMT, and it was maintained throughout the year. Twenty-two percent of patients had decreased colonization after one month, and 17% of patients, of whom two were taking metformin, had poor colonization. Following these observations, it can be concluded that no more than one dose of FMT is required to achieve clinical recovery in patients. Furthermore, no difference in efficacy was observed between the high and low doses

administered. The results of this study describing the restoration of long-term colonization following FMT treatment also suggest that the numerical similarity of the donor is not strictly related to clinical outcome, and they identify a persistent donor-specific effect on fecal bacterial communities in the patient (Staley et al. 2019).

In line with the studies described above, Hospital Universitario Gregorio Marañón has been conducting several cohort studies in 2014 in which patients with recurrent CDI are treated with FMT. One of the studies conducted by this group was in 13 patients with 2 or more episodes of CDI recurrence in which FMT is applied via three different routes of administration: liquid-containing oral capsules, colonoscopy, and nasojejunal tube. Some of the patients included in the study had other comorbidities such as hematological diseases or cirrhosis. Overall efficacy with a single dose of FMT was 83.3%; when patients who received more than one dose were included, efficacy reached 91.7%. Oral capsule use was found to be as effective as treatment given by colonoscopy or nasojejunal route. Only one adverse effect was reported in one of the patients who experienced bacteremia. This study demonstrated that the use of FMT is safe and effective, even in patients with different comorbidities (Reigadas et al., 2018). Another study conducted by the same group in which 32 patients with recurrent CDI were treated (a mean of two relapses/patient) and supplied with 4-5 lyophilized fecal content capsules at a single dose (final bacterial dose range per patient was 2.1 to 2.5 $x10^{11}$) has recently been published, with the minimum administered dose of 2.1 $x10^{11}$. The primary efficacy was 81.3% and, after a second transfer, the overall efficacy amounted to 87.5%. During the follow-up time, no adverse effects were observed in patients, and the capsules were well tolerated (Reigadas et al., 2019).

Overall, a high recovery rate is observed for patients with recurrent CDI treated with FMT with similar percentages across studies. It has been seen that in cases where the first FMT transfer is not effective, the administration of a second dose is effective. One of the most recent meta-analyses evaluating the efficacy of FMT in recurrent CDI is 92% for both controlled and uncontrolled studies (Quraishi et al., 2017).

On the other hand, with respect to the optimal dose of FMT there is a disparity between the different studies. There is considerable heterogeneity among published studies with respect to the dose to be used, both in the treatment with liquid FMT (administered by colonoscopy, nasogastric tube or enema) and in the form of capsules. In addition, not all studies adequately detail the dose that has been used; most papers using liquid FMT with non-oral or oral gavage administration say they use a stool dose of ≥50g. There are some studies reaching 152 g ±32 g of donor stool (Cammarota et al. 2015) with 90% resolution, and another study or so using a mean dose of 62 g, not as high as the previous one, that shows a recurrence of 9.1% (Kelly et al. 2016, Madoff et al. 2020). Other studies use a dose of 100 g via enema, with a recurrence of CDI of 12%, somewhat higher than that reported with lower doses, although the difference is not significant (Jiang et al., 2018).

In addition, when performing FMT via capsules across studies, the effectiveness and efficacy of FMT is similar from one study to another, even if different doses are applied. Using lyophilized formulations facilitates bacteria concentration, leverages donor samples better,

and decreases the number of capsules the patient needs to swallow (Hirsch et al., 2015, Hecker et al., 2016, Reigadas et al., 2019).

The few studies that have evaluated the minimum bacterial doses indicate that FMT containing bacterial doses of 9.7 $x10^{10}$ for liquid capsules and 2.1-2.5 $x10^{11}$ for lyophilized capsules were effective in the treatment of CDI. Dosing in the range of 2.1-5.7 $x10^{11}$ makes it possible to administer a low number of capsules and obtain an effective treatment outcome (Reigadas et al., 2019).

In terms of assessing the safety of FMT in patients with CDI recurrence, treatment has been shown to have a high level of safety in all previous studies, with a low number of adverse effects reported, mostly moderate and self-limited. In a review of the safety of FMT, 50 studies were analyzed and it was concluded that the safety profile of FMT is positive, given that patients have no serious adverse effects, with only mild or moderate adverse effects having been reported, such as nausea, vomiting, swelling, abdominal pain or cramps or flatulence, all self-limited episodes with a short duration occurring shortly after FMT (Youngster et al 2014a, Youngster et al. 2016, Staley et al. 2017, Cheng et al. 2019, Madoff et al. 2020). In addition, the type or severity of adverse effects was shown not to differ depending on the route of administration, although patient perception is more positive when capsules are administered by oral administration (Kao et al., 2017, Hirsch et al., 2015, Staley et al., 2017, Jiang et al., 2018, Cheng et al., 2019).

The short-term safety of treatment has been demonstrated in all studies. Long-term safety was assessed in one of the studies, which follows patients treated with FMT for one year. This study reports that patients had no gastrointestinal adverse effects (Staley et al., 2019).

The safety of FMT has been demonstrated even in immunocompromised patients who are at increased risk of CDI. The safety of FMT was evaluated in a study of 80 immunocompromised patients (75 adults and 5 pediatric) with relapsed, refractory, or severe CDI treated with FMT who were followed up on during a mean of 11 months (3 to 46 months of follow-up). Overall treatment efficacy was 89%. In addition, the safety of FMT was demonstrated in these patients, who had the mild adverse effects described above. None of the patients had infectious complications (Kelly C.R. et al., 2014). The safety of this treatment has been evaluated in another immunocompromised population: patients with a history of solid organ transplants and, therefore, also immunocompromised. In these patients the treatment was administered by colonoscopy and enema. After three months, 58.7% of single-transfer patients recovered, and this increased to 91.3% after repeat transfer (repeating administration up to 2, 3, 4 or more times). The recovery ratio with a single transfer was lower for patients with severe or fulminant CDI, although it was also observed that the mean immunosuppressive medication was higher in the patients in whom the transfer failed. Twenty-two percent of patients showed the adverse effects described above, no cases of bacteremia (3.2%) were due to FMT, and only 14% of cases with underlying CMV (three patients) had reactivation. They concluded that treatment with FMT is safe for immunocompromised patients (Cheng et al., 2019).

No severe adverse effects due to transmission of such organisms have been seen in more than 45,000 FMT treatments provided by OpenBiome, a not-for-profit stool bank (founded

in 2012 in Cambridge, MA, USA) or in randomized controlled trials for other indications (Kuijper 2019). This transmission is also absent in both the systematic review of more than 50 articles (Wang et al. 2016) and the retrospective analysis of the 80 immunocompromised patients treated with FMT (Kelly C.R. et al. 2014). In conclusion, the safety of FMT is high, and no serious adverse effects resulting from this treatment have been reported. However, it should be noted that further analyses are needed to verify long-term safety (Tan et al. 2019).

However, despite the positive safety results reported in the FMT studies, in July 2019 the US Food and Drug Administration (FDA) issued a safety alert regarding serious adverse reactions caused by the transfer of multidrug-resistant organisms via FMT; concretely, in a case in which there was transmission of a β-lactamase-producing strain of *Escherichia coli* from the feces of one donor to two immunocompromised patients. Subsequently, six additional cases of CDI patients with enteropathogenic *E. coli* or CTE infection (two immunocompromised patients) and Shiga toxin-producing *E. coli* or STEC infection (four patients) were reported in March 2020 after receiving an FMT provided by OpenBiome. Out of these six cases, four were hospitalized and subsequently discharged, and two were treated as outpatients (important safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse reactions due to transmission of multi-drug resistant organisms, FDA 2019, Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Events Likely Due to Transmission of Pathogenic Organisms, FDA 2020). Moreover, in August 2022, the FDA issued a safety alert on the potential risk of transmission of monkey smallpox via FMT. Although the risk of transmitting this virus via FMT is not yet determined, there are studies that have documented the presence of this DNA in rectal swabs and/or stool samples of infected individuals. In one study, virus DNA was detected in rectal swabs from three individuals who reported no symptoms of monkey smallpox disease, and in two of them the virus was isolated from the viable monkey smallpox in the rectal swabs. ("Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Additional Safety Protections Pertaining to Monkeypox Virus", FDA 2022).

In relation to these potential risks, Mikrobiomik performs selective cultures to detect enteropathogenic *E. coli* and multi-resistant strains. All donors in which this type of bacteria is detected will be discarded, thus minimizing the risk of transmission of these organisms via FMT. With regard to monkey smallpox, the questionnaire that is administered to donors raises the question as to whether the patient is infected with this virus or has been in contact with someone with this disease. In addition, the presence of the monkey smallpox virus by polymerase chain reaction (PCR) is determined in the analysis performed on the feces.

In conclusion, the short-term efficacy and safety of FMT treatment of patients with recurrent CDI is well demonstrated in several clinical studies. This treatment represents an effective and safe way to prevent CDI recurrence and is an effective alternative treatment in patients who have failed antibiotic treatment (Bouza et al., 2020) or have multiple CDI recurrences.

02. STUDY OBJECTIVES

02.01. Primary Objective

The primary objective of the study is to evaluate the efficacy of the investigational medication (MBK-01) compared to the control (fidaxomicin) at eight weeks after treatment initiation.

02.02. Secondary Objectives

The secondary objectives are to assess the safety of the investigational medication and the quality of life of patients participating in the study.

03. TYPE OF CLINICAL STUDY AND DESIGN

03.01. Type of study. Global Design

Phase III randomized, controlled, open-label clinical trial with 2 treatment arms.

03.02. Randomization Process

Since the study case report form is electronic (eCRF), the eCRF's own platform has a randomization tool that will allow this process to be performed.

03.03. Type of Control and Design

I. Experimental group: single-dose treatment of four oral capsules of lyophilized fecal microbiota (MBK-01).
II. Control group: treatment with fidaxomicin, 200 mg/12 hours for 10 days.

03.04. Blinding Techniques

There are no blinding techniques in this study; it is an open-label study.

03.05. Study Plan

Once the patient is considered for participation in the study due to him or her experiencing an episode of diarrhea (≥3 stools/24 hours), and the presence of CD is confirmed with the prescribed tests, the inclusion and exclusion criteria will be assessed.

Treatment of the diarrhea episode with 3-5 days of vancomycin is permitted as long as a subsequent 24-hour washout is performed prior to randomization and study medication administration. The patient will be enrolled in the study by signing the informed consent (ICF) at the time the 24-hour washout period begins.

For patients who have been started on fidaxomicin but have not completed the first day of antibiotic treatment, treatment with fidaxomicin may be stopped and treatment of the diarrhea episode started with 3-5 days of vancomycin, provided that a subsequent 24-hour washout, prior to randomization and administration of study medication, as in the previous case, is performed. The patient will be included in the study upon signing the ICF, once the 24-hour washout period begins.

If the patient, upon enrolling in the study, is on antibiotic treatment for a medical indication (other than the episode of infection being studied), he/she should discontinue antibiotic treatment before enrolling in the study and undergo a 24-hour washout period. The patient will be included in the study upon signing the ICF, once the 24-hour washout period begins.

Accordingly, in the event that patients have received previous treatment for the episode with vancomycin (3-5 days) and/or antibiotic treatment for any other indication, it is necessary that, after signing the ICF, they undergo a 24-hour washout period prior to randomization.

Finally, subjects will be randomized and assigned to one of the two treatment arms. The patient will then be administered the study medication. Subsequently, follow-up will be performed for six months after treatment until the study close-out visit.

03.06 Early Trial Termination

This study may be terminated prematurely if, in the opinion of the Sponsor, there is sufficient cause. The investigator will receive written notification that the cessation party documents the reason for the suspension and provides instructions for the follow-up of active patients.

In case of premature termination of the trial, the investigator will be responsible for ensuring the patient's medical care, per standard of care or compassionate use.

03.07. Treatment Efficacy Assessments

03.07.01. Definition of Study Endpoints

03.07.01.A. Efficacy Variables

Primary variable: The absence of CD diarrhea without the need for re-treatment (or absence of recurrence as defined in Section 03.07.02), as assessed by the subject interview and physical examination. The primary variable is operated by the probability of recurrence.

Other efficacy variables:
Good/poor patient progress: The definition of good/bad evolution is detailed in Section 03.07.02.

Time to Recurrence Based on Randomization Groups: The definition of recurrence is detailed in Section 03.07.02.
Overall survival (OS): Percentage of patients still alive after a defined period of time since treatment was started.

03.07.01.B. Safety Variables

- Number of Adverse Events by randomization group.
- Number of patients within each Adverse Event by randomization group.
- Type of Adverse Events by randomization group.
- Number of Serious Adverse Events by randomization group.
- Type of Serious Adverse Events by randomization group.
- Treatment-Related Adverse Events.
- Severity of Adverse Events.
- Adverse Events related to CDI.
- Mortality associated with CDI.
- Intensive Care Unit (ICU) admissions.
- Adverse Events of Special Interest:

Common Treatment-Related:

Abdominal cramps, bloating and pain
Flatulence
Nausea
Vomiting
Transient fever (>38ºC)
C-reactive protein increased (>20 mg/L)
Fatigue
Weight gain
Headache
Anorexia
Constipation
Diarrhea

Uncommon (less than or equal to 1%):

Ulcerative colitis
Bacteremia
Inflammatory Bowel Disease

Rare or very rare or not known:

Enteropathogenic infections

03.07.01.C. Quality of life variables

Quality of life will be assessed using the indicators obtained by the SF-36 test, according to the design described in the literature (Ware et al., 1993; Alonso et al., 2003; Vilagut et al. 2005; Vilagut et al, 2008). These measurements will be performed on all patients enrolled in the study, and data will be collected at baseline, eight weeks and six months post-treatment.

03.07.02. Definitions

Mild to moderate CDI

Episode of CDI with WBC count ≤15,000 cells/µL and serum creatinine less than 1.5 times baseline.

Severe CDI

CDI cases with a WBC count >15,000 cells/µL and/or a serum creatinine level equal to or greater than 1.5 times the baseline level.

Complicated severe CDI

CDI cases presenting with septic shock, ileus or megacolon, or requiring intensive care unit admission within seven days of episode diagnosis or death attributable to CDI.

Episode of diarrhea

Presence of ≥3 stools/24 hours.

Resolution/response to treatment

Resolution of diarrhea as <3 stools/24 hours for at least two consecutive days after the end of drug treatment.

Mortality associated with CDI

Cases in which there is clinical suspicion and, at the discretion of the treating physician, the death can be attributed to CDI and cannot be clearly attributed to other non-CDI causes, and it occurs within 10 days after the CDI episode and/or due to well-known complications of CDI (dehydration, renal failure, septic shock or megacolon, among others).

Poor evolution

The detection of poor progression 48-72 hours after starting treatment (MBK-01 or fidaxomicin) is given by a:

1. **Worsening of the diarrhea episode** (at least one bowel movement more than at baseline*, with the start of study treatment (fidaxomicin or MBK-01) as baseline).

And at least one of the following factors:

1. **C-reactive protein (CRP) elevation** (>5% of baseline value*)
2. **Leukocyte absolute elevation** (>5% of baseline value*)
3. **Progression to sepsis:** hypotension or organ failure with no other apparent cause.

**NOTE: Baseline is considered the time of start of study treatment (fidaxomicin/investigational medication).*

Primary CD infection

First episode of laboratory-confirmed CD infection.

Recurrence

Recurrence is the reappearance of clinical manifestations of a new episode of CDI that recurs within eight weeks after the onset of symptoms from a previous episode, provided that the symptoms of the previous episode had resolved after completion of treatment (Debast et al., 2014).

04. STUDY POPULATION. PATIENT SELECTION

The clinical trial will be conducted in patients who experience an episode of CD infection (either the first episode or subsequent recurrences).

04.01. Inclusion Criteria

1. Male and female patients, 18 years of age and older.
2. Patients who experience an episode of CD infection (either a first episode or subsequent recurrences).
3. Presence of an episode of diarrhea defined as ≥3 stools/24 hours, at the onset of the episode.
4. Confirmation of the presence of CD toxin A and/or B in feces, by a direct toxin detection test or by PCR for detection of toxin-producing genes, at the beginning of the episode to be treated in the clinical trial (toxin test must be positive within seven days prior to patient's inclusion in the trial).

04.02. Exclusion Criteria

1. Prior fecal microbiota transfer.
2. Transplanted patients, except for those with solid organ transplant over two years, with good organ function.
3. Absolute neutrophil count <500 cells/µL at the time of study inclusion.
4. Pregnancy, breast-feeding, or intent to become pregnant during the course of the study.
5. Active treatment with bile acid sequestrants (for example: cholestyramine).
6. Human immunodeficiency virus (HIV)-positive subjects except those with CD4 T-cell count >200 cells/µL and viral load less than 20 copies.
7. Dysfunction when swallowing or deficit of oral-motor coordination.
8. Patient admitted to ICU, or expected to be admitted to ICU, for severe illness.
9. History of significant medical conditions that, in the opinion of the investigator, would not allow for adequate assessment or follow-up of the patient.

04.03. Expected number of patients

Some 104 patients (52 in the control group treated with fidaxomicin and 52 patients in the experimental group treated with MBK-01).

04.04. Withdrawal criteria and planned analysis of withdrawals

Patients are free to withdraw from the study at any time for any reason, without any consequence.

The primary reason for withdrawal from the study will be recorded on the end of study form. Patients who decide to withdraw will be encouraged to complete an early termination telephone visit to assess adverse events. Patients who withdraw will be discontinued from all study procedures.

In addition, the investigator may withdraw the subject from the study for any reason, including the following:

1. Lost to follow-up.
2. Adverse event (AE) or serious adverse event (SAE).
3. When the patient does not cooperate or comply with the requirements of the study, this will be considered protocol violation/non-compliance.
4. Subject's decision to withdraw consent.
5. Clinical conditions of the patient that prevent continuity.
6. Investigator decision to terminate the study.
7. Recurrence by CD as defined in Section 03.07.02.

04.05. Withdrawal criteria for lack of efficacy

A patient's lack of efficacy is defined as the occurrence of a new recurrence of CD infection. Patients who experience a new recurrence will be withdrawn from the study and treated with rescue medication, which will be antibiotic treatment (vancomycin or fidaxomicin).

04.05.01. Discontinuation from treatment

Patients who are considered lost before receiving the experimental treatment or antibiotic do not require follow-up within the clinical trial.

Patients who are considered lost after receiving the experimental treatment or antibiotic will be followed until completion of the study visits (six months), or until ≥30 days after the start of treatment, if they discontinue their follow-up in the study. In the latter case, information relevant to the assessment of safety (adverse events and concomitant treatments) will be collected at a minimum.

04.06. Duration of participation

Patients will be followed for six months from the start of study treatment.

05. DESCRIPTION OF STUDY TREATMENT

05.01. Definition of treatment

The investigational product (MBK-01) is a capsule-containing medicinal product with a heterologous, healthy donor-derived, oral fecal microbiota lyophilisate.

05.02. Investigational Product

05.02.01. Description of the manufacturing technique(s) of the proposed therapy(ies)

The starting samples used to perform FMT are feces from healthy donors previously tested by clinicians, and validated after passing microbiological screening in blood and feces, including tests for COVID-19, monkey smallpox virus, and enteropathogenic microorganisms and toxins (Youngster et al., 2014). The selection and exclusion criteria for donors are described in **Table 1** and **Table 2**.

***Table 1**. Donor Inclusion Criteria.*

Inclusion Criteria
Age 18-50 years (50-60 if screening for negative colorectal cancer)
Body mass index (BMI) 18-30 kg/m^2
Ability to donate 2 to 5 times a week
Good overall health

***Table 2.** Donor exclusion criteria.*

Exclusion Criteria
Infectious
History of infection or exposure to HIV, hepatitis B, or hepatitis C, syphilis, primate T-lymphotropic viruses (PTLVs), malaria, or tuberculosis
At-risk sexual contacts or behaviors or partner change in the past three months
Sexual contact, in the past 12 months, with someone who has used needles for drug or steroid use or for anything else that has not been prescribed by a doctor
Sexual contact, within the past 12 months, with someone with HIV, Hepatitis B, or Hepatitis C, with hemophilia, or receiving clotting factor concentrates. Contacting someone with hepatitis

Exclusion Criteria
Infection and/or treatment of any sexually transmitted disease within the past 12 months
Illicit drug use within the past three months
Previous organ or tissue transplantation
Have been in contact, within the past six months, with blood from another person, or have been punctured/cut with material that could be contaminated with blood or fluids from another person
Tattoos, piercings, acupuncture, or accidental needle stick within the past 12 months
Risk factors for Creutzfelt-Jakob disease
Recent gastrointestinal infection (Rotavirus, *Giardia lamblia,* etc.)
Travel in the past 12 months to tropical countries with endemic diarrheal illnesses or high-risk of traveler's diarrhea
Vaccination within the last 12 months with live attenuated vaccines or any injection
Healthcare personnel or workers from hospitals and healthcare institutions (risk of transmission of multi-resistant microorganisms) or if working with animals (risk of transmission of zoonoses)
Those who have been in a prison or reformatory facility within the past 12 months or have been under arrest for more than 72 hours
Those who have experienced malaria, Chagas disease, or babesiosis or trypanosomasis
Those who have suffered, or have anyone in their environment who has suffered, COVID-19
If you have been exposed to or are infected with monkey smallpox virus
Fever, frequent cough, or feeling short of breath in the past two weeks
Working with animals (to avoid the risk of transmission of zoonoses)
Gastrointestinal comorbidities
Inflammatory bowel disease
Irritable bowel syndrome, chronic functional constipation, or chronic functional diarrhea (per ROME IV)
Celiac disease and other chronic digestive disorders
History or high risk for gastrointestinal cancer or polyposis
A family member diagnosed with Crohn's disease or ulcerative colitis (parents, siblings or children)
Family history of colon cancer (parents, siblings or children)
Diarrhea, blood in stool, abdominal pain, or other significant digestive symptomatology within the past three months, or routinely
Consumption of drugs that may alter gut microbiota
Use of antibiotics, antifungals, or antivirals within the past three months
Use of Proton-pump inhibitors (PPIs) in the past three months

Exclusion Criteria
Use of systemic anti-cancer agents within the past three months
Taking medications related to gastric reflux, heartburn, within the past three months
Use of immunosuppressive medication or chemotherapy within the past three months
Other reasons
Legal inability to give consent
Active cancer process or cancer history in the past 10 years
Consumer of >28 Alcohol Standard Drink Unit (SDU) per week
Have had surgery for something in the past three months
Gastroscopy or colonoscopy within the past three months
Prior major digestive system surgery (excluding appendectomy) (parents, siblings or children)
Have had a transplant within the last 12 months
Evolved liver disease
Chronic kidney disease
Autoimmune and systemic diseases
Chronic or autoimmune systemic diseases with GI involvement
Diabetes mellitus
Metabolic syndrome
Neurological, neurodegenerative or psychiatric diseases
Symptoms of eczema or psoriasis in the past eight weeks
Food, seasonal, animal, medication, latex, dust, other allergies
Asthma in the past 12 months
Experienced symptoms or taken treatment in the past 12 months for attention deficit, hyperactivity, anxiety, and/or depression
Pregnancy or delivery or termination of pregnancy in the last six months
Vaccinations not correctly received per the vaccine schedule
Significant vascular disease

In addition, due to the incubation window of potential infection-causing agents, all samples received will pass a quarantine period and, upon donor re-screening, will be used in the production of gut microbiota capsules or microbiota suspension.

Once validated by screening testing, the donor will visit our facilities to perform the stool in situ. The donor performs the stool in a specific stool collection container that can be

attached to the toilet. Once the sample is obtained, it will be processed in less than six hours after evacuation, during which time it will be stored refrigerated.

05.02.02. Pharmacokinetics

Depending on the extent of microbiota alteration, and subsequent bacterial exposure, the microbiota composition could be restored to a similar state or assume a new steady state consisting of different species of bacteria (Costello et al., 2012). This disruption in the bacterial community therefore gives rise to an opportunity for externally supplied bacteria to be established due to reduced competition or elimination of resident species occupying the same niche that need the same nutrients (Lee et al., 2013; Maldonado-Gómez et al., 2016).

The transplanted microbiota restores the receptor's gut microbiota by reintroduction of bacterial toxins that were absent from the receptor prior to FMT, supporting expansion of the receptor's own commensal microbiota and restoring, over time, an optimal microbiota community (Browne et al., 2017).

To date, there is no literature describing or showing data suggesting that the transferred microbiota leaves the intestinal environment and colonizes other areas.

05.02.02.A. Bioavailability of the investigational medication

There are no studies evaluating the bioavailability of the investigational medication or other FMT. Most bacteria that constitute the gut microbiota reside in the large intestine, while a minority of bacteria are found in the small intestine and stomach (Guarner, 2011; Lawley et al., 2013; Clarke et al. 2019). The stomach and duodenum harbor a small number of microorganisms that adhere to the mucosal surface or are in transit, usually less than 10^3 bacteria per gram of content. Acidic, biliary, and pancreatic secretions destroy most of the ingested microorganisms, and propulsive motor activity prevents stable colonization of the lumen of the small intestine. The number of bacteria increases progressively throughout the jejunum and ileum, from about 10^4 in the jejunum to 10^7 colony-forming units per gram of content at the ileal end. The colon is densely populated with anaerobes, and bacterial counts reach densities of about 10^{11} colony-forming units per gram of content (Guarner et al., 2011). The hypromellose capsules of the investigational medication MBK-01 do not dissolve in the stomach but in the small intestine and, incrementally, into the large intestine, the area of the gastrointestinal tract richest in microbiota. For that reason, the microbiota (which constitutes the active ingredient in MBK-01) will reach this area of the body, it is estimated that each bacterial type could access its specific niches, to reconstitute the intestinal flora.

05.02.03. Adverse effects

FMT has been shown to have a high safety profile for the different administrations in all studies with patients with primary CD infection and with r-CDI in which it has been evaluated, reporting a low number of effects, mostly moderate and self-limiting and occurring shortly after administration (Youngster et al 2014a, Youngster et al. 2016, Staley et al. 2017, Camacho-Ortiz et al. 2017, Ng SCC et al. 2017, Juul et al. 2018, Cheng et al. 2019, Madoff et al. 2020). In addition, the type or severity of adverse effects was shown not to

differ depending on the route of administration, although patient perception is more positive when capsules are administered by oral administration (Kao et al., 2017, Hirsch et al., 2015, Staley et al., 2017, Jiang et al., 2018, Cheng et al., 2019). Even in two of these studies using lyophilized microbiota capsules, no adverse effects were reported (Reigadas et al., 2019; Staley et al., 2019). The short-term safety of treatment has been demonstrated in all studies. Long-term safety was assessed in one of the studies, which follows patients treated with FMT for one year. This study reports that patients had no gastrointestinal adverse effects (Staley et al., 2019).

05.02.04. Presentation and instructions for proper administration

The investigational drug substance MBK-01 is contained in white hypromellose capsules. These capsules will be contained, in fours, in 35 mL white high-density polyethylene (HDPE) bottles with a tamper evident stopper and a built-in desiccant. The bottle will be properly labeled.

The four capsules contained in each bottle correspond to the dosage established for each patient.

05.02.05. Storage and receipt of study product

Each site will be supplied with a sufficient amount of MBK-01 for the treatment of patients. Where applicable, the drug product will be shipped to the appropriately refrigerated administration site at 5 ±3°C. All finished product shipments are performed through a Good Distribution Practices (GDPs) certified company.

Upon receipt of the investigational medication, it should be stored refrigerated at a temperature of 5 ±3°C until administration to the patient.

05.02.06. Administration and Dosing Schedule

The dosing regimen is four capsules in a single dose (≥2.1-2.5 x10^{11} microorganisms). The decision on timing of intake should be left to the discretion of the PI and will be swallowed orally, in a single intake. The investigational medication MBK-01 should be taken in a fasting state. In case the patient has difficulty swallowing, a gel or gel water may help to facilitate the swallowing process.

05.02.07. Supportive measures during treatment

Measures will be taken to ensure the safety of patients participating in the trial at all times.

Use of more timely supportive measures (e.g., hydration-monitoring of patient's electrolytes and antipyretics in case of fever (>38ºC)) will be permitted and as deemed by the treating medical team.

05.02.08. Dose modification

A second dose of FMT is not planned to be provided during the course of this clinical trial, and re-administration of the same dose will not be evaluated for a patient who has already received it.

05.03. Fidaxomicin

Fidaxomicin is an antibiotic that belongs to the group of macrocyclic antibacterials, is bactericidal, and inhibits RNA synthesis by bacterial RNA polymerase. Interferes with RNA polymerase at a site other than rifampicin. Inhibition of CD RNA polymerase occurs at a concentration twenty-fold lower than that of the *E. coli* enzyme (1 µM vs. 20 µM), partially explaining the significant specificity of fidaxomicin activity. Fidaxomicin has been shown to inhibit CD sporulation *in vitro*.

It is indicated for the treatment of CDI in adults and in pediatric patients weighing at least 12.5 kg.

The dose administered is 200 mg (one tablet), given twice daily (every 12 hours) for 10 days.

The most common adverse reactions are vomiting (1.2%), nausea (2.7%), and constipation (1.2%).

The supply and shipment of fidaxomicin will be performed by an external company with Good Distribution Practices (GDPs) certificate. Fidaxomicin 200 mg tablets does not require any special storage conditions.

05.04. Permitted and Prohibited Concomitant Treatments

Subjects should not take any systemic antibiotics for the duration of the study.

Antiemetics and antidiarrheals are not recommended as supportive measures. There are no specific dietary restrictions, except those listed in medical order.

Apart from the listed types of medication, there is no drug contraindicated or not to be used concurrently with the study drug.

06. STUDY ASSESSMENTS

06.01. Medical History

The patient's medical history will be collected and will be part of the study documentation.

06.02. Physical examination

A comprehensive physical examination will be performed including major systems (cardiovascular, abdominal, neurological [sensory and motor], musculoskeletal, and limb systems). This examination will be performed at the baseline visit (before and after treatment administration) and at the in-person follow-up visits. The physical examination

that will be performed after treatment will be: measurement of your temperature and blood pressure.

06.03. Laboratory tests

06.03.01. Stool samples

Stool samples will be collected at the Start-of-Treatment visit, and at Visit 4 (eight weeks after end of treatment). The Baseline and Visit 4 stool sample will be used for subsequent studies and will therefore be stored in a freezer (-20ºC - -80ºC).

In case of an episode of diarrhea throughout the study, a new stool sample may be taken to confirm the presence of CD and rule out other pathologies.

Tests to be performed may include:

- Confirmation of CD with direct detection of GDH and/or A/B toxins.
- Confirmation of CD with molecular techniques (PCR).

06.03.02. Other laboratory tests

- Blood count.
- Thyroid profile.
- Lipid profile: High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), total cholesterol, and triglycerides.
- Liver profile tests: Alanine transaminase (ALT), Aspartate aminotransferase (AST), Gamma-glutamyltransferase (GGTP), Lactate dehydrogenase (LDH), alkaline phosphatase, bilirubin, total protein and albumin.
- Renal panel tests: urea, creatinine, sodium, potassium, chloride, uric acid, total protein, serum albumin, blood glomerular filtrate, urine quantitative protein, urine creatinine, basic urine examination, and urine sediments.
- C-reactive protein (CRP).

06.04. Follow-up Assessments: Study Visits

Visit 1. Baseline Visit (Randomization and Treatment)

When a candidate patient presents with a clinical presentation consistent with an episode of CD infection (either first episode or subsequent recurrences), he/she will be routinely evaluated for CD in stool to confirm suspicion, as well as laboratory tests and a physical examination. In parallel, a member of the research team will review the patient's medical

history and, if all preliminary eligibility and none of the exclusion criteria are met, the investigator will ask the patient and/or his/her legal representative to participate in the study, explaining the nature of the study, its rationale, risks and benefits, and answering any questions the patient asks about the study. Patients will be provided with an information sheet and a signed consent form. If the legal representative and/or the patient agree to participate in the study and sign the consent form, the patient may participate in the study.

Once the preliminary eligibility of the patient has been determined and the above documents signed, study procedures will be performed.

After the patient has given written consent to participate, he/she will undergo a 24-hour washout period (in case he/she has been treated in the current episode with vancomycin 3-5 days and/or antibiotic for another indication) and will then be randomized into one of the two treatment groups. The subject will be assigned a four-digit screening number (first two digits for the site and the other two digits for the subject) in the order of their screening within the clinical trial. The number will be assigned by a system integrated into the eCRF. The site number and subject screening number will be included on all study forms and documentation. The subject withdrawn from the clinical trial retains his/her number. New subjects should always be assigned a new screening and randomization number.

It must be confirmed that the patient meets all inclusion and none of the exclusion criteria. Only patients who meet all of the inclusion criteria and none of the exclusion criteria may be randomly assigned and receive the clinical trial treatment.

The following procedures will be performed during this visit:

- Obtain informed consent (ICF).
- Evaluation of inclusion and exclusion criteria.
- Pregnancy test (for women of childbearing potential).
- In the event that the patient has been treated with antibiotics for the current episode of CD infection (either the first episode or subsequent recurrences) and/or for another medical indication, it should be confirmed that a 24-hour washout period is performed prior to randomization.
- Physical examination: weight, height, BMI, blood pressure.
- Assessment of patient's quality of life using the SF-36 Test.
- Assessment of concomitant medication.
- Confirmation of the presence of CD in feces by direct detection test of CD toxins or by PCR. Tests performed within seven days prior to patient enrollment in the clinical trial will be valid.
- Analytical Study: Blood count, lipid profile, thyroid profile, liver and kidney function. Tests performed within 24 hours prior to patient enrollment in the clinical trial will be valid.
- Stool sample collection for subsequent studies.
- Stool sample collection to confirm CDI (tests performed within seven days prior to patient enrollment in the clinical trial are valid).

At this visit the patient will be started on treatment.

The duration of treatment will be different depending on the assignment:

- Patients in the experimental arm will receive MBK-01: a single dose of four capsules in a single intake.
- Patients in the control group will receive fidaxomicin: a dose of 200 mg (one tablet), administered twice a day (every 12 hours) for 10 days.

The duration of the baseline visit has a maximum of 24 hours (from day -1 to day 0), in the event that patients need to undergo a 24-hour washout period prior to randomization and treatment. If patients do not have to undergo a washout period, the duration of this visit may be shorter.

Visit 2. Clinical course assessment (f2f): 72 hours ±1 day after treatment initiation.

The following procedures will be performed during this visit:

- Physical examination: weight, height, BMI, blood pressure
- Evaluation of the evolution of the patient's clinical symptomatology
- Assessment of concomitant medication
- Assessment of potential adverse events
- Patient's assessment of good/bad progress
- Laboratory tests for good/bad progress: CRP, and absolute leukocyte value
- Assessing Patient Adherence

Visit 3. Post-Treatment Assessment (F2F): three weeks ±4 days after treatment initiation.

The following procedures will be performed during this visit:

- Physical examination: weight, height, BMI, blood pressure
- Evaluation of the evolution of the patient's clinical symptomatology
- Assessment of concomitant medication
- Assessment of potential adverse events
- Assessing Patient Adherence

Visit 4. Post-Treatment Assessment (F2F): eight weeks ±4 days after treatment initiation.

The following procedures will be performed during this visit:

- Physical examination: weight, height, BMI, blood pressure
- Evaluation of the evolution of the patient's clinical symptomatology
- Assessment of concomitant medication
- Evaluation of potential adverse events
- Quality of life assessment using SF-36 Test

- Analytical study: Blood count, lipid profile, thyroid profile, liver and kidney function.
- Stool sample collection for subsequent studies.

Visit 5. Early follow-up (telephone call): three months ±6 days after treatment start.

The following procedures will be performed during this visit:

- Evaluation of potential adverse events
- Evaluation of the evolution of the patient's clinical symptomatology
- Assessment of concomitant medication

Visit 6 (end of study). Late follow-up (phone call): six months ±6 days after treatment initiation

The following procedures will be performed during this visit:

- Evaluation of the evolution of the patient's clinical symptomatology
- Assessment of potential adverse events
- Assessment of concomitant medication
- Quality of life assessment using the SF-36 Test

All patients who discontinue from the study and/or are discontinued prior to completing the planned follow-up should have an end of study visit ≥30 days after the start of treatment, where, at the very least, information relevant to the safety assessment (adverse events and concomitant treatments) will be collected.

Unscheduled Visits

In the event of an episode of diarrhea during the follow-up period (especially with suspected recurrence due to CD) and/or other clinical situation of significance at the discretion of the PI and the research team, unscheduled visits may be performed. All tests performed during these visits as well as their results should be detailed.

07. MONITORING

Monitoring shall mean the act of monitoring the progress of this clinical trial, ensuring that it is performed, recorded and reported in accordance with:

- The protocol,
- Standard Operating Procedures (SOPs)
- Good Clinical Practice (GCP)
- And applicable regulatory requirements.

In this study, monitoring tasks will be performed on behalf of the Sponsor by Sermes CRO.

08. ADVERSE EVENTS

08.01. General Considerations

All adverse events (AEs) will be recorded in the eCRFs. All adverse events will be collected from the first dose of experimental or antibiotic treatment until the patient's last follow-up visit, or until 30 days after the start of treatment, if they discontinue follow-up in the study.

08.02. Adverse Event (AE)

Any untoward health occurrence in a patient or clinical investigation subject treated with a medicinal product, even if it does not necessarily have a causal relationship with this treatment, is considered an AE.

08.03. Adverse Drug Reaction (ADR)

An adverse drug reaction (ADR) is any untoward and unintended reaction to an investigational medicinal product, regardless of the dose administered.

08.04. Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is defined as any untoward experience involving a patient, whether or not considered related to protocol treatment. Serious adverse events are those that result in:

- Death
- An event that threatens the patient's life
- Hospitalization or prolongation of hospitalization
- Persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- Any other condition deemed clinically relevant

08.05. Suspected Unexpected Serious Adverse Reaction (SUSAR)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious adverse reaction whose nature, severity, or outcome is not related to the reference safety information.

It is the Investigator's obligation to report serious and/or unexpected adverse reactions to the Sponsor within 24 hours of awareness.

08.06. Causality assessment

The causality of the events will be discussed both in relation to treatment and pathology under the clinical judgment of the treating physicians.

Causality is defined by the modified Karch-Lasagna algorithm (Karch et al., 1997):

Probability of Causality:

- ***Definitive –*** The adverse reaction (AR) appears in a reasonable temporal sequence after administration of the drug. The effect had already been mentioned previously or is an expected response to the study medication, with an improvement confirmed after the reduction or discontinuation of the medication and a reappearance of the effect after re-administration of the study medication, with no other apparent etiological alternative.
- ***Probable –*** RA appears with a reasonable temporal sequence after drug administration. The effect had already been referred to previously or is an expected response for the study medication. Improvement occurs after treatment reduction or discontinuation, and any other etiology is unlikely or less likely. Information on rechallenge is not required.
- ***Possible –*** RA appears in a reasonable temporal sequence after drug administration. The effect had been previously referred to or is an expected response for the study medication. There is a possible etiological alternative that may be responsible for RA. No information is necessary on the suspension of the medication or it may be unclear.
- ***Unlikely/doubtful –*** RA does not appear in a temporal sequence after drug administration, or if it exists, it is remote. The effect is not an expected or known response to the study medication. There is a possible etiological alternative that may be responsible for RA.
- ***Unrelated –*** RA is due to an underlying or concurrent disease or the effect of another medication and does not meet any of the above definitions.

09. ETHICAL ASPECTS

09.01. General Considerations. Approvals

The study will be conducted in accordance with the requirements expressed in the Declaration of Helsinki (review of the 64th General Assembly, Fortaleza, Brazil, October 2013) and following the recommendations of Good Clinical Practice (CPMP/ICH/135/95) and the current regulation establishing the requirements for the conduct of clinical trials with medicinal products, Royal Decree 1090/2015 of December 4 and European Regulation 536/2014 of April 16.

The IRB/IEC will review this protocol and all study documentation to ensure that the rights, safety, and well-being of the patient are protected, and only those sites approved by the IRB/IEC may conduct the study.

09.02. Informed Consent Form (ICF)

Subjects who wish to participate in the study will be given a written document called the "Patient Information Sheet", which will include information on the most important aspects of the study. The Informed Consent document will detail that the subject freely and voluntarily grants his/her informed consent before being included in the study and that he/she is informed about the nature, significance, implications and risk of the study.

The investigator will be responsible for obtaining informed consent for this study, directly from the subject or otherwise from a legal representative. The subject participating in this study or his/her representative may revoke his/her consent at any time, without a pronouncement of cause, and this situation will not result in any liability or harm.

09.03. Confidentiality

The processing, communication and transfer of personal data of all participating subjects will be in accordance with the provisions of Organic Law 3/2018, of December 5, on the Protection of Personal Data and guarantee of digital rights.

The data collected for the study will be identified by a code, so that no information that can identify the patient is included, and only the study doctor/collaborators will be able to relate this data to the patient and to his/her medical history.

All documents generated during the study, as well as the contents of the case report forms, will be considered strictly confidential and not be disclosed to third parties.

09.04. Data Integrity and Quality Assurance

All data collected in the study, including data obtained from the eCRFs, as well as documents prepared during the study, will be preserved from uses not permitted by persons outside the research and, therefore, will be strictly estimated confidential and not be exposed to third parties.

Clinical data management defined for this study follows applicable laws and regulations.

10. STATISTICAL ANALYSIS

10.01. General Statistical Considerations

A Statistical Analysis Plan (SAP) will be developed during the execution of the study and prior to database lock, which will describe in detail the statistical methods to be employed, the strategy to be followed for missing values, and the tables and figures to be included in the statistical report.

As an overview of the statistical analysis, data will be presented as absolute frequencies and percentages for qualitative variables. For quantitative variables, central tendency statistics, such as mean and median, and scatter statistics, such as standard deviation and interquartile range, minimum and maximum values, may be provided as indicators of the shape of the distribution such as asymmetry and kurtosis indices, will be used. For ordinal variables, depending on the number of categories, one form or another of description will be used. In all cases, a column should be added to the data presentation table that includes the number of patients with available data. The analyses will be done divided by randomization group.

A descriptive analysis of all relevant variables collected at baseline will be performed, and this analysis will be divided by randomization groups.

10.02. Determination of sample size

For the sample size calculation, the information presented throughout the protocol was taken into account. The sample size calculation was performed considering:

The confidence level (97.06%)

Statistical power (80%)

The effect size (η_*^2=0.02 for quantitative outcomes; Odds ratio=2.5 for probability analysis)

The study design: number of visits, study groups and primary variable.

Potential losses (50%), including patients withdrawn (5%), death (5%), patients not evaluable (40%).

The estimated total of patients is 104, and considering the sample size per group, 52 patients should be recruited in each group.

The sample size calculation was performed with the software G*Power (version 3.1.9.7).

10.03. Randomization

Randomization will occur as follows:

1. **Arm identification (randomization groups):** in this case the arms will be:
    - **Experimental group:** single-dose treatment of four oral capsules of lyophilized fecal microbiota.
    - **Control group:** treatment with fidaxomicin, 200 mg/12 hours for 10 days.
2. **Randomization process:** each patient will be individually and independently randomized with equal chances of assignment between the different arms of the

study. If the study has two arms, this type of randomization would be equivalent to the toss of a coin to decide on the face of the resulting coin.

10.04. Study Populations

The **Intent-to-Treat (ITT) population** consists of all patients who have been included in this study, meet or fail to meet the eligibility criteria and have been randomized. In addition, the following ITT subpopulations will be considered for the analysis of probability of recurrence and time to recurrence:

- ITT Subpopulation 1: It will consist of all ITT patients who have data on whether or not recurrence has occurred through Visit 4 or later.
- ITT Subpopulation 2: It will consist of all ITT patients who have been withdrawn shortly before Visit 4 and are aware of whether recurrence has occurred or not.

The **Per Protocol population (PP)** includes all patients who have been recruited for the present study, meet all inclusion and none of the exclusion criteria, and have remained in the study without any major protocol deviations.

The **Safety Population (SP)** consists of all patients who have been randomized to either of the two randomization groups and who have taken at least one dose of the corresponding treatment.

10.05. Efficacy Analyses

The final efficacy analysis will be performed on the ITT Population as well as the PP Population.

Inferential analysis of efficacy variables will be performed as follows:

- Probability of recurrence based on randomization group will be studied using binary logistic regression analysis. The odds recurrence ratio will be reported, using the MBK-01 group as a reference. In addition, the confidence interval, Nagelkerke's R-squared and Yule's Q-coefficient will be included, as well as the critical level. The number of diarrhea episodes by visit and group will be analyzed, if data are available, using mixed models of analysis of variance or relevant robust/non-parametric models.
- Time to recurrence (weeks) by group will be analyzed using Cox regression. An event is defined as a recurrence, censoring cases without recurrence.
- Good/bad patient evolution will be analyzed using logistic regression procedures and incorporating the same indicators as described for the analysis of the probability of recurrence.
- Overall survival in weeks will be analyzed by Cox regression including randomization group as an independent variable.

Due to the trial characteristics, the hypotheses may be hypothesized as either non-inferiority or superiority. Since the trial is Phase III, both hypotheses can be tested in the same clinical trial as long as the α risk is controlled; or, alternatively, it is confirmed that there are significant differences when, in fact, at the population level, there are no such differences. It is important to remember that, in order to avoid false positives, it is required that the type I or α error be distributed across the different hypotheses, resulting in a lower value of the critical level or p to find statistically significant results (Ferreira-Gonzalez, 2014).

Although the overall significance level will be alpha=0.05, because more than one data analysis will be performed, it will be adjusted to 0.0294 to avoid inflation of the type I error probability (Geller and Pocock, 1987). If multiple pairwise comparison analyses are used, the statistical report will describe the procedure used. Outcome effect size measures will be incorporated. Detailed adjustments and procedures for each statistical analysis as well as treatment of missing values and sensitivity analysis will be described in detail in the SAP.

10.06. Safety Analyses

Safety variables are operated with the number of adverse events documented and classified by frequency, severity, seriousness and causality as assessed by the patient interview and physical examination.

For the analysis of Adverse Events, a descriptive analysis (frequency and percentages) of them will be performed throughout the study, and a list of them will be presented in which they will be grouped according to gravity and severity. These events will be listed by patient and randomization group. Recording of adverse reactions will be done using Verbatim described in the eCRF.

Adverse Events and concomitant medication will be coded using a standardization system. For Adverse Events, the most current version of the MedDRA system will be used, and the ATC code will be used for concomitant medication.

The analysis of physical examination variables will be done by frequencies and percentages and divided by randomization group.

The safety analysis, both interim and final, will be conducted on the Safety Population (SP).

10.07. Variables and Subgroups

The table presented below (**Table 3**) lists the different variables and subgroups to be considered for statistical analysis.

Table 3. *Study variables and subgroups to be considered in the statistical analysis.*

Efficacy Variables	Safety Variables	Quality of life variables	Subgroups*
Primary variable: Probability of recurrence.	Number of Adverse Events by Randomization Group	SF-36 Dimension Score	Primary episode of CDI.
	Number of patients within each Adverse Event by randomization group.		
Good/Poor patient progress.	Type of Adverse Events by Randomization Group		Recurrent episodes of CDI.
Time to recurrence	Number of Serious Adverse Events by Randomization Group		Patients without prior vancomycin.
	Type of Serious Adverse Events by Randomization Group		
Overall survival	Relationship to treatment		
	Severity of the Adverse Event		
	Relationship to CDI		
	Mortality associated with CDI		
	Adverse Events of Special Interest (AESI) – See Section 03.07.01.B.		
	Suspected Unexpected Serious Adverse Reaction (SUSARs)		

**These subgroups will be included for the analysis of the probability of recurrence, time to recurrence, and the analysis of the patient's good/bad outcome.*

10.08. Interim Statistical Analysis

Two interim analyses are planned in this study.

1. The first interim analysis will be performed **when approximately 50% of patients have completed treatment.**
2. The second interim analysis will be performed **when approximately 80% of patients have completed V4.**

All interim analyses will incorporate a descriptive analysis of patient baseline characteristics, an efficacy analysis, and a safety analysis as described above.

Analysis of baseline and efficacy and safety variables will be performed by comparing the randomization groups.

The interim statistical analysis will be performed on the ITT population for efficacy and on the PS in the safety analyses section.

The results of the interim statistical analysis could lead to early discontinuation of the study if large differences between the two randomization groups are revealed after the study, or obvious futility is shown. In addition to saving time and resources, this could reduce the exposure of study participants to a treatment that is inferior or shows no difference from the comparator.

It should be noted that, in the event that large and clear differences between the randomization groups in the interim statistical analysis or a clear futility are observed, early study discontinuation should be assessed. The study of the differences between the randomization groups should be accompanied by measures of effect size, confidence intervals, and not just statistical significance.

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ANNEX 1: SUMMARY OF ICD-01 PROTOCOL AMENDMENTS

Summary and justification of Substantial Modification No.01

(07/30/2021)

➢ **Substantial modification of the clinical trial protocol:**

Changes have been performed to the **Exclusion Criteria of the Clinical Trial** (see the section "Study population. Patient selection. 2. Exclusion criteria" of the Protocol) that have a direct impact on the recruitment capacity of the study. The modification of the exclusion criteria was motivated by the following causes:

1. The modification or the elimination of some of the initially proposed exclusion criteria significantly promotes the recruitment capacity of the centers that are involved in the study.
2. The proposed modifications in the exclusion criteria do not affect the fulfillment of the objectives (primary and secondary) of the study. The increase in the recruitment capacity of the study promotes the fulfillment of the study objectives within the established period.
3. The sample of treated patients in the clinical trial is more heterogeneous, and therefore more representative of the population that is intended to be treated with MBK-01.

In conclusion, the changes contained in this substantial modification would help to achieve the objectives of the clinical trial within the established period.

Summary and justification of Substantial Modification No.02

(11/18/2021)

Substantial Modification No. 02 of November 18, 2021, consists of the addition of the following participating centers:

- Hospital General Universitario de Alicante – Dra. Esperanza Merino de Lucas
- Hospital Universitario La Paz – Dra. Alicia Rico Nieto
- Hospital General Universitario Gregorio Marañón – Dra. Patricia Muñoz García
- Hospital Universitari de Girona Dr. Josep Trueta – Dr. Antoni Castro Guardiola

The number of centers has been increased to expand the recruitment options so that the number of patients under study can be reached in the estimated time.

Summary and justification of Substantial Modification No.03

(12/20/2021)

- **Substantial modification of the clinical trial protocol:**

Modifications have been performed to the Inclusion and Exclusion Criteria of the clinical trial (see the section "Study population. Patient selection"). These changes have a direct impact on some aspects of the study design: by allowing the use prior antibiotic treatment for the patients included in the study, it is necessary to consider a washout period prior to the treatment.

The modification of the inclusion and exclusion criteria was motivated by the following causes:

1. Due to the low recruitment rate, and because the modification of the inclusion and exclusion criteria directly impacts the recruitment capacity, it is intended to significantly promote the recruitment capacity of the involved centers.
2. The proposed modifications do not affect the fulfillment of the objectives of the study. The higher recruiting capacity will favor the fulfillment of objectives within the established period.
3. The sample to be treated in the study is more representative of the population that is intended to be treated with MBK-01.

In addition, the timing of the interim analysis of the study has been modified, so that it will be performed when half of the patients have completed treatment. Thus, it is intended to know beforehand the partial efficacy results of the study.

In conclusion, for everything stated above, the changes contained in this substantial modification would help to achieve the objectives of the clinical trial within the established period.

- **Patient Information Sheet – Informed consent form**

The changes produced in the new V4.0 protocol version results in the update of the Patient Information Sheet-Informed Consent form, then generating the new 3.0 version of December 17, 2021.

- **Study schedule**

Due to the low recruitment rate achieved so far, and as stated in the new version of the protocol, the inclusion period has been changed from August-December 2021 to August 2021 – June 2022.

Summary and justification of Substantial Modification No.04

(04/18/2022)

The substantial modification No.04 of April 18, 2022 consists of the addition of the following participating centers:

- Hospital Universitario Quirónsalud Madrid – Dr. Daniel Carnevali
- Hospital Quirónsalud Barcelona – Dr. Fernando Cereto Castro

The number of centers has been increased to expand the recruitment options so that the number of patients under study can be reached in the estimated time.

Summary and justification of Substantial Modification No.05

(08/05/2022)

➢ **Substantial modification of the clinical trial protocol:**

The clinical trial population has been expanded, and the study inclusion criteria have been modified (see the section "Study population. Patient selection"). This modification is motivated by the following causes:

1. Due to the low recruitment rate, and because the modification of the inclusion criteria directly impacts the recruitment capacity, it is intended to significantly promote the recruitment capacity of the centers involved.
2. The proposed modifications do not affect the fulfillment of the objectives of the study. The increase in the recruiting capacity will promote the fulfillment of objectives within the established period.
3. The changes allow an additional group of patients with *Clostridioides difficile* (CD) infection to have access to a potentially effective treatment, while still using the same patient population as before, without compromising the data that has already been collected in the study.
4. The extension of the period of detection of the *Clostridioides difficile* toxin within 7 days prior to the inclusion of the patient in the trial has been carried out to take into consideration the duration of treatment with vancomycin and the washout period that some participants may require.

The statistical analysis has been adapted to include the evaluation of an additional secondary safety variable, which will provide more information on the safety of the investigational medicinal product.

The sample size has been adapted, without this change affecting the fulfillment of the study objectives.

Some aspects of the study design have been modified, allowing the inclusion in the trial of patients who were previously treated with fidaxomicin, if the duration of treatment is not therapeutic (see the "Study plan" section).

In addition, it has been included that the interim analysis of the study could lead to the early interruption of the study with the aim of maintaining the safety of the participants.

In conclusion, for everything stated above, the changes contained in this substantial modification would help to achieve the objectives of the clinical trial within the established period.

- **Patient Information Sheet – Informed consent form**

The changes produced in the new V5.0 protocol version result in the update of the Patient Information Sheet-Informed Consent form, generating the new 5.0 version on July 29, 2022.

- **Investigator´s brochure (IB)**

The changes produced in the new V5.0 protocol version result in the update of the Investigator´s brochure, generating the new 2.0 version on July 29, 2022.

- **Study schedule**

Due to the low recruitment rate achieved so far, and as stated in the new version of the protocol, the inclusion period has varied from August 2021 - June 2022 to August 2021 - December 2022, so that the total duration of the study will last approximately 22 months.

Summary and justification of Substantial Modification No.06

(06/14/2023)

- **Substantial modification of the clinical trial protocol:**

The procedures adopted in October 2022 have been included in the protocol, following the recommendations of the guidance document "Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Additional Safety Protections Pertaining to Monkeypox Virus", issued by the FDA in 2022, and which were notified to the AEMPS through an Urgent Security Notification on 10/21/2022.

The donor inclusion criteria have been modified in accordance with the procedures above mentioned (see the section "Product under investigation. Description of the manufacturing technique(s) of the proposed therapy(s)").

The sample size has been adapted from 66 to 82 patients to adjust the expected number of patients recruited to the actual observed dropout rate from the study, without this change affecting the fulfillment of the study objectives.

A new planned study schedule has been established, taking into consideration the changes performed to the number of patients to be recruited.

Some non-relevant explanatory editions have been made with the aim of facilitating the understanding and application of the protocol.

- **Patient Information Sheet – Informed consent form**

The changes produced in the new V6.0 protocol version result in the update of the Patient Information Sheet-Informed Consent form, then generating the new 6.0 version of May 18, 2023.

- **Non-substantial modification of the IB and Investigational Medicinal Product Dossier (IMPD):**

Both documents have been modified to include the above explained safety procedures for the prevention of monkeypox transmission at the IB and IMPD.

The IMPD annexes have been modified to adapt the structure to the new regulation, and incorporating the recommendations made by the AEMPS in tests with the same product in clinical research phase for other indications, regarding the quality section.

Summary and justification of Substantial Modification No.07

(08/07/2023)

- **Substantial modification of the clinical trial protocol:**

Reformulation of main and secondary variables, and adaptation of the statistical plan

Revision to the definition of the study variables to add the use of odds ratios and logistic regression, in addition to the mixed ANOVA, in the analysis of the main efficacy variable, thus allowing to perform the study of the probability of recurrence, in addition to the number of diarrhea episodes. Given that if the patient has a recurrence he or she is removed from the study, and it is not possible to model recurrences using ANOVA, but it is possible to model recurrences using binary logistic regression.

Furthermore, the variable "Duration of hospitalization" is eliminated given that many patients receive outpatient treatment or the reason for admission was not CD infection. The variable "Duration of treatment" is also removed given that the duration of treatment with MBK-01 is equal to one day for all patients, and in the case of Fidaxomicin the duration of the treatment is 10 days, except in cases of withdrawal of the study prior to the completion.

Complementary ITT subpopulations are added for the analysis of the probability of recurrence and time to recurrence (ITT-1: allows patients withdrawn before V4 to be considered; ITT-2: allows increasing the precision of recurrence monitoring).

The covariate analysis is removed since no information has been collected on CD infection severity, and information on the number of previous recurrences in some patients is not available or is 0 in primary episodes. Furthermore, it is of interest to the promoter to study the added subgroups.

Additionally, a clarifying and non-substantive revision of the definition of CD infection recurrence was included.

Review of sample size calculation

The reformulation of the main variable results in the need to review the statistical calculation of the sample size, which will be based on a confidence level of 97.06%, Odds Ratio of 2.5, statistical power of 80% and loss rate of 50%, then generating a sample size of 104 subjects (52 in each group). The dropout rate remains at a value close to that observed to date (50%).

Premature trial termination

Criteria that allow premature termination of the clinical trial are defined, including unforeseen risks, changes in product development plans, difficulties in recruitment, or significant and evident interim results of superiority/futility of the investigational drug compared to the control.

The possibility of a premature termination could provide advantages such as minimizing risks to subjects, a higher efficiency in the management of research resources, and/or, where appropriate, accelerating the development of the product and its access to the market.

Interim analyses

The possibility of a premature termination of the trial makes it necessary to plan a second interim statistical analysis to evaluate trends in efficacy and safety results. In addition, the variables that will enter in these interim analyses are reviewed and completed.

Safety monitoring (withdrawn patients)

The high rate of follow-up loss, as well as the possibility of a premature trial termination, make it necessary to define a strategy for the follow-up of patients withdrawn before completing the scheduled follow-up. Thus, it is defined that all patients withdrawn before completing the planned follow-up must undergo an end-of-study visit ≥30 days after starting treatment, in which, at least, relevant safety information (events) will be collected (adverse events and concomitant treatments).

The results of the first interim analysis of the trial show a low-risk safety profile of MBK-01, with a frequency of treatment-related adverse events (AEs) of 0% (0/19 in MBK-01 and 0/14 in the Fidaxomicin). The most frequent AEs were diarrhea and elevated C-reactive protein. On July 17, after a review of the safety information, it was observed that there are no serious adverse events (SAEs) related to the treatments (0% for both the MBK-01 group and the group receiving Fidaxomicin). In both the MBK-01 group and the Fidaxomicin

group, the most frequent adverse event was diarrhea, with this happening in all cases before 8 weeks after treatment.

In addition, small explanatory and non-relevant format editions have been made with the aim of facilitating the understanding and application of the protocol.

- **Patient Information Sheet – Informed consent form**

The changes produced in the new V7.0 protocol version result in the update of the Patient Information Sheet-Informed Consent form, then generating the new 7.0 version on August 4, 2023.

- **The IB and IMPD of the study have not been modified**