Results from a Randomized Placebo-Controlled Clinical Trial of a RBX2660—a Microbiota-based Drug for the Prevention of Recurrent *Clostridium difficile* Infection

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Running Title: RBX2660 microbiota-based therapy for CDI

Summary: In this randomized, double-blinded, placebo-controlled Phase 2B trial, two doses of RBX2660 did not meet the primary endpoint of superiority to placebo for preventing recurrent *Clostridium difficile* infection. However, RBX2660 was safe, and a single dose was superior to placebo.

Abstract

Background: Despite advancements, recurrent *Clostridium difficile* infections (CDI) remain an urgent public health threat with insufficient response rates to currently-approved antibiotic therapies. Microbiota-based treatments appear effective, but rigorous clinical trials are required to optimize dosing strategies and substantiate long-term safety.

Methods: This randomized, double-blind, placebo-controlled Phase 2B trial (NCT02299570) enrolled adult patients with two or more CDI recurrences to receive: two doses of RBX2660, a standardized microbiota-based drug (Group A); two doses of placebo (Group B); or one dose of RBX2660 followed by one dose of placebo (Group C). Efficacy was defined as prevention of rCDI for 8 weeks following treatment. Participants who recurred within 8 weeks were eligible to receive up to two open-label RBX2660 doses. The primary endpoint was efficacy for Group A compared to Group B. Secondary endpoints included the efficacy of Group C compared to Group B, combined efficacy in the blinded and open-label phases, and safety for 24 months.

Results: The efficacy for Groups A, B, and C were 61%, 45%, and 67%, respectively. The primary endpoint was not met (*P*=.152). One RBX2660 dose (Group C) was superior to placebo (Group B; *P*=.048), and the overall efficacy (including open-label response) for RBX2660-treated participants was 88.8%. Importantly, the proportion of adverse or serious adverse events did not differ significantly among treatment groups.

Conclusions: One but not two doses of RBX2660 was superior to placebo in this randomized, placebo-controlled trial. These data provide important insights for a larger Phase 3 trial and continued clinical development of RBX2660.

Keywords: Clostridium difficile infection, recurrence, microbiota-based therapy, placebo, clinical trial

Introduction

Clostridium difficile infection (CDI) is the most common health-care associated infection in the US, an urgent public health threat [1-4], and causes significant morbidity, mortality, and healthcare costs [4, 5]. The incidence of recurrent CDI (rCDI) is increasing disproportionately to the incidence of primary CDI [6], with significantly worse outcomes in patients who develop rCDI [7-10]. In general, antibiotics (including CDI antibiotics) disrupt and decrease the diversity of the intestinal microbiota, creating a dysbiotic environment in which *C. difficile* can colonize, proliferate, and produce toxins responsible for symptomatic rCDI [11-13]. Accordingly, restoring the composition and diversity of the intestinal microbiota protects against recurrence [11,14,15].

Recognition of the role of the intestinal microbiota in health and disease has prompted interest in microbiota-based approaches for treating rCDI. Several studies have indicated that fecal microbiota transplantation (FMT) can help prevent rCDI, with relatively few adverse events (AEs) [16-19], but these treatments lack standardization of process and composition. Moreover, definitions and procedures for AE reporting, follow-up, and optimal dosing are not clearly established. Several standardized microbiota-based rCDI therapeutics are in clinical development [20, 21], but to date there are no published randomized, blinded, placebo-controlled FDA-registration trials of standardized microbiota therapeutics for preventing rCDI. The trial described herein evaluated the efficacy and safety of the microbiota-based drug candidate RBX2660 compared to placebo to prevent future rCDI among patients with a history of rCDI.

Methods

Study Design and Treatment

This international multicenter, randomized, doubled-blind, placebo-controlled Phase 2B study (NCT02299570) aimed to demonstrate the efficacy and safety of one or two doses of RBX2660 to prevent rCDI among patients with a history of multiple rCDI. Participants were randomly assigned to receive two blinded treatments by enema as follows: Group A-two doses of RBX2660, Group B-two doses of placebo, Group C-one dose of RBX2660 followed by one dose of placebo. Administration of the first dose commenced 24-48 hours following completion of CDI treatment antibiotics, with the second dose administered 7±2 days thereafter, although the second enema treatment could be administered earlier than the 7-day interval if necessary to control suspected CDI recurrence. Participants who failed both blinded treatments were eligible to receive up to two open-label doses of RBX2660 7±2 days apart, which could be administered after CDI antibiotics at the discretion of the investigator. Additional details are included in the online Supplementary Material.

Study Population

The study population included participants at least 18 years old with a diagnosis of rCDI and either a) two or more documented recurrences of CDI after a primary episode, or b) two or more documented episodes of severe CDI resulting in hospitalization. CDI was defined as the presence of diarrhea (three or more unformed stools in 24 hours for at least two consecutive days) and a positive stool test for *C. difficile* or its toxins, performed according to the enrolling physician's standard procedure. Recurrent CDI was defined as a diagnosis of CDI that began less than 8 weeks after completion of treatment for a previous episode. Participants were required to have a positive stool test within 60 days prior to enrolment for the qualifying rCDI episode; to have completed at least two courses of standard-of-care

oral antibiotics; and to have rCDI symptoms controlled before treatment. Major exclusion criteria included: ongoing or anticipated antibiotic therapy for a condition other than CDI, known or suspected causes of diarrhea other than CDI, a compromised immune system, a history of inflammatory bowel disease, or pregnancy. A detailed list of inclusion and exclusion criteria is provided in the Supplementary Material.

Study participants were enrolled at 21 centers in the United States and Canada from December 10, 2014 through November 13, 2015. The study protocol received Institutional Review Board approval at each center. All participants provided written informed consent. A medical monitor examined the trial on an ongoing case-by-case basis along with a Data Safety Monitoring Board (DSMB) as needed.

Randomization and Blinding

Participants were randomized using permuted blocks within three strata based on the antibiotic regimen for the enrolling episode (vancomycin, fidaxomicin, or metronidazole) and assigned one of three treatments (Group A, B or C) at a 1:1:1 ratio. There was no stratification by site; each drew from the same set of blocks. The randomization code, date, and time were captured in the clinical database. Study participants, investigators, and site personnel who performed follow-up procedures were blinded to the assignment and study drug administration, and the enema administrator was not involved in study follow-up. RBX2660 and placebo were shipped in a ready-to-use enema bag shrouded in an opaque brown sleeve, and the tubing set was shrouded by the enema administrator during the procedure and disposed in an opaque biohazard container after administration.

RBX2660 Preparation

RBX2660 is a microbiota suspension prepared from human stool. Donor selection and screening, and RBX2660 preparation were as previously described [20,22] and included a total of 75 doses from 17 donors. Each dose consisted of a 150 mL suspension containing $\geq 10^7$ live organisms/mL in a single-dose ready-to-use enema bag. The placebo consisted of normal saline and formulation solution in the same proportions found in RBX2660. Each unit of RBX2660 and placebo was identified by a unique batch number and was traceable to a specific donor and recipient. Drug and placebo were stored frozen at −80°C in a secure location at the manufacturer and shipped frozen to the site in a temperature-controlled container. Products were thawed in a refrigerator for 24 hours and administered within 48 hours after thawing.

Study End Points

The primary endpoint was prevention of recurrence (treatment success) after two doses of RBX2660 compared with two doses of placebo. Success was defined as the absence of diarrhea and no retreatment for CDI, any time after the first dose until 8 weeks after the second dose of assigned study treatment. Treatment failure was defined as meeting all four of the following criteria at <8 weeks after completion of both assigned blinded study treatments: diarrhea, a positive laboratory diagnosis for *C. difficile* or its toxins as conducted and reported by the study investigator, a need for retreatment for CDI, and no other cause for CDI symptoms. An independent DSMB reviewed each participant for final determination of treatment success or failure while blinded to the randomization. Some participants were declared treatment failures by the study investigator due to suspected CDI recurrence, even though all four criteria were not met. These were categorized by the DSMB as having an indeterminate response and considered treatment failures for efficacy analyses. In addition, some participants were

declared failures and offered open-label treatment after only one blinded study treatment. These were recorded as protocol deviations but were classified as failures for efficacy analysis.

Secondary end points included safety and the following efficacy assessments: one dose of RBX2660 and one dose of placebo (Group C) compared to either two doses of placebo (Group B) or two doses of RBX2660 (Group A); and the efficacy of up to two open-label RBX2660 doses administered to participants who failed blinded treatment. Participants recorded solicited AEs and their symptom severity daily through 7 days after the final blinded dose. AEs were assessed at in-office visits at 1, 4, and 8 weeks after completing the assigned study treatment. Telephone assessments occurred weekly during weeks 2, 3 and 5-7 and will continue through 3, 6, 12 and 24 months.

Statistical Analysis

To achieve a power of 90% with a two-sided level of significance of 0.05 for an estimated 80% success in Group A vs. 40% success in Group B, 105 participants were required for a 1:1:1 randomization ratio. An additional 12 enrollments were included to account for 10% attrition, for a total of approximately 39 participants per group. After the first five participants were enrolled, a protocol modification was made to stratify participants according to the antibiotic they received for CDI treatment prior to study enrollment. Accordingly, the randomization schedule was recreated and the sample size was increased to 44 participants per group. Enrolled participants who withdrew prior to randomization were replaced without counting toward the size cap. All analyses were performed on participants in the intent-to-treat (ITT) population who received at least one assigned blinded treatment. Participants who were classified by the DSMB as having an indeterminate response to blinded treatment were analyzed as failures.

The primary efficacy endpoint was analyzed with the Pearson's chi-square test. Descriptive statistics were used for safety assessments. All analyses were performed using Version 9.3 or later of SAS, Cary, NC.

Results

Participants

A total of 150 participants at 21 centers in the U.S. and Canada were enrolled. Seventeen were screen failures and exited prior to randomization, and 133 were randomly assigned to receive either two doses of RBX2660 (Group A, n=45); two doses of placebo (Group B, n=44); or one dose of RBX2660 followed by one dose of placebo (Group C, n=44). Three withdrew prior to treatment, one was withdrawn by the investigator prior to treatment, and one died before receiving treatment, leaving 128 participants in the safety population. One participant withdrew after experiencing anxiety during the first attempted enema which was not completed, leaving 127 participants for the ITT efficacy analysis (Figure 1, Table 1). Positive *C. difficile* laboratory diagnosis prior to enrolment was established as follows: Nucleic acid amplification tests (NAAT) including PCR or loop-mediated amplification (n=103), enzyme immunoassay (EIA, n=23), and not specified (n=1). The three treatment groups included similar proportions of participants diagnosed by each method.

Primary Outcome

The efficacy for each blinded treatment group in the ITT analysis was as follows: Group A=61% (25/41), Group B=45% (20/44), and Group C=67% (28/42) (Figure 2, Table 2). There was no significant difference between Group A and Group B (*P*>.05), thus the primary study endpoint was not met. There was no association between outcome and which antibiotic was being administered at screening (*P*=.15, Cochran-Mantel-Haenszel). Laboratory *C. difficile* diagnosis for failure determination was by NAAT (n=33) or EIA (n=7), with the remaining 14 participants classified as treatment failures by the site investigator despite not having a positive *C. difficile* laboratory diagnosis (n=3, 6, and 5 in Groups A, B, and C, respectively). Eleven of these were never tested and three tested negative (EIA). These 14 participants were adjudicated by the DSMB as having an indeterminate response but were included in the ITT analyses as failures.

Secondary Outcomes

Participants that received one RBX2660 dose followed by one placebo dose (Group C) showed superior response relative to participants that received two placebo doses (Group B, *P*=.049). Likewise, the response among participants that received at least one blinded dose of RBX2660 (Groups A and C combined, 64%, 53/83) was significantly higher than Group B participants that received placebo (*P*=.047).

All participants that were classified as treatment failures during the blinded phase of the study (n=54) opted to receive one (n=5) or two (n=49) open-label RBX2660 treatments, with a median time of 8 days (range 0 to 50 days) between failure determination and open-label RBX2660 treatment. Thirteen of these were offered open-label treatment after only a single blinded treatment (n=5, 3, and 5 in Groups A, B, and C, respectively).

Treatment success rates were 80% (4/5) and 78% (38/49) for participants who received one or two open-label RBX2660 treatments, respectively, with a combined success rate of 78% (42/54). Open-label treatment success rates according to blinded-phase study groups were: 69% (11/16), 88% (21/24), and 71% (10/14), for Groups A, B, and C, respectively. The overall success rate for participants who received at least one dose of RBX2660 in the blinded and/or open-label phases was 89% (95/107; median number of doses per participant=2).

Safety

In total, 379 AEs were reported in 82 (64.1%) participants during the blinded treatment phase (Table 3). There were no differences in the number or rate of AEs among blinded treatment groups. The most common AEs were gastrointestinal disorders, followed by general disorders, infections, and nervous system disorders. Safety follow up beyond the blinded treatment phase is ongoing (mean of 8.3 months post-treatment, range 0.1 to 15.9 months) and will continue to 24 months. As of this interim analysis, 580 AEs have been reported, with a similar distribution and type as observed in the blinded treatment phase (Supplementary Table S1).

Including the follow up period, 45 serious adverse events (SAEs) have been reported (Table 4). As determined by the site investigator and DSMB, none were related to the enema procedure, 31.1% were related to CDI, and 77.8% were related to a pre-existing condition. Three of the SAEs were adjudged possibly related to the blinded study drug: one participant developed recurrent acute myeloid leukemia, another reported abdominal cramping and pain, and a third experienced constipation requiring hospitalization. Among the SAEs were six participant deaths, all of which were determined by the site investigator and medical monitor to be unrelated to RBX2660 or study procedures. The median time to

death from the last enema administration was 67 days (range 28-156 days). The reported causes of death included general decline of health (n=2), renal failure (n=2), respiratory failure, and MRSA bacteremia. Four of the six deaths were adjudged to be possibly, probably, or definitely related to a pre-existing condition.

Discussion

To address the need for rigorous safety and efficacy data for microbiota-based therapeutics, we report a randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of RBX2660 for preventing rCDI. To our knowledge, this is the first trial of its type for a microbiota-based therapeutic.

We examined several possible reasons why the primary objective was not met (*P*>.05). The 61% response rate observed for Group A is consistent with the previously reported activity of RBX2660 in a Phase 2 trial: 51% of participants responded to a single RBX2660 dose [20]. The present results are also consistent with reported responses to single colonoscopic administration of FMT [10, 23-27]. Thus, the activity of RBX2660 was not unexpectedly low.

Next, we assessed the trial design. Based on our previous data for RBX2660, this trial was designed to test a regimen of two RBX2660 doses spaced one week apart (Group A). Even though the protocol allowed for administration of the second enema after less than seven days, thirteen participants were declared failures by the site investigator and offered open-label RBX2660 after only a single blinded treatment (Group A=5, Group B=3, Group C=5). This may have affected the power of the trial to demonstrate the primary objective, because some might have responded had they received the full per-protocol treatment. This compliance finding and its potential impact on the outcome suggest a single-dose study design could provide a clearer analysis of efficacy. Moreover, the efficacy of Group C was significantly better than placebo (*P*=.049), which suggests a single-dose RBX2669 regimen is sufficient.

The higher than expected response rate among placebo-treated participants also contributed to lack of significance. This trial was powered with a conservative expectation of 40% placebo response based on a 30% placebo response observed in the only randomized FMT trial available at the time [23]. Consequently, the 46% response rate for Group B did diminish the power of the trial. Since 2016, three additional trials have reported 43% to 58% response rates among placebo-treated cohorts [28-30], which is in line with our results and likely reflects a basal recurrence-free rate after standard-of-care antibiotics [31, 32]. Thus, our results, and now the literature, teach that rCDI trials should be powered to account for higher response rates in placebo-treated cohorts.

The heterogeneity of CDI laboratory diagnostic practices, which is a known challenge for the field [33], also may have contributed to the statistical outcome. Despite the protocol definition of treatment failure, positive laboratory diagnosis was established in only 40 of 54 investigator-designated failures. These 14 participants were included in the ITT analysis as failures because an alternative diagnosis could not be established *post hoc*—that is, CDI recurrence could not be ruled out. This could have biased the study toward the null if some were not bona fide recurrences. Although the "optimal" method to diagnose CDI remains elusive, some analyses suggest that detection of *C. difficile* toxin in stool is more specific for CDI [34, 35], whereas NAAT testing alone leads to over-diagnosis [36]. As such, the fact that the majority of enrolled subjects were diagnosed by NAAT may have also biased the study toward the null.

This study will add significant long-term safety data for microbiota-based rCDI therapies. The overall safety profile was favorable through a mean follow-up of 8.3 months, with 90.8% of reported AEs being mild to moderate, consistent with the open-label Phase 2 trial of RBX2660 [20]. Safety follow-up is ongoing and will continue until 24-months post-treatment. This study also underscores the safety of

enema administration, which is important given some reports associating morbidity and mortality with duodenal or colonoscopic FMT [37, 38].

This study demonstrated that one dose of RBX2660 (Group C) was more effective than placebo ($P<.05$). The observation that a second dose within seven days did not provide additional benefit ($P>.59$, Group C compared to Group A) was somewhat surprising, based on prior open-label results for RBX2660 [20]. However, the second dose in that trial was only administered to participants who recurred—a distinctly different population. The present data suggest that a single RBX2660 dose is sufficient to elicit maximal benefit after each episode of rCDI. Consistent among both trials is the conclusion that patients who recur after a single dose can benefit from a subsequent dose if needed. Indeed, the overall response rate of 88.8% among participants who received at least one RBX2660 dose highlights the potential clinical benefit of repeated doses of RBX2660.

Conclusions

In this double-blinded, randomized, placebo-controlled trial, two RBX2660 doses spaced one week apart were not superior to placebo, but a single dose of RBX2660 was significantly better than placebo. Participants who recurred after blinded study treatment benefitted from open-label RBX2660. RBX2660 was safe and well tolerated. Future clinical evaluation is warranted to confirm long-term RBX2660 benefit and safety.

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Potential Conflicts of Interest

E.D., D.G., G.H., C.L., and R.O. serve on the Rebiotix Physician Advisory Board. S.K. has received research support and consulting fees from Rebiotix.

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Table 1. Baseline Demographics and Clinical Characteristics of Participants in the Intent-to-Treat Population.

CHARACTERISTIC	GROUP A 2 Doses of RBX2660	GROUP B 2 Doses of Placebo	GROUP C 1 Dose of RBX2660 & 1 Dose of Placebo
n	41	44	42
Median age – years (range)	66 (24-89)	62 (19-92)	63 (18-88)
Female sex- no. (%)	25 (61)	30 (68)	24 (57)
Race-White – no. (%)	39 (95)	42 (96)	40 (95)
Antibiotic used at screening – no. (%)			
Vancomycin	38 (93)	40 (91)	37 (88)
Fidaxomicin	1 (2)	2 (5)	2 (5)
Metronidazole	2 (5)	2 (5)	2 (5)
None			1 (2)
Median number of CDI episodes (range)	4 (3-11)	3 (2-11)	4 (2-14)
Median duration of CDI episode, days (range)[1]	15 (1-74)	15 (1-98)	14 (1-71)

[1]As reported by enrolling physician.

Table 2: RBX2660 Efficacy in Blinded and Open-Label Arms of PUNCH CD 2 Study

	Group A	Group B	Group C	Total
BLINDED ARM				
Treatment	*2 doses RBX2660*	*2 doses placebo*	*1 dose RBX2660, 1 dose placebo*	
n	41	44	42	127
Success n (%)	25 (61)	20 (45)	28 (67)	
OPEN-LABEL ARM				
n	16	24	14	54
Success n (%)	11 (69)	21 (88)	10 (71)	42 (78)
OVERALL RBX2660 EFFICACY				
n	41	24	42	107
Success n (%)	36 (82)	21 (88)[1]	38 (90)	95 (89)[1]

[1]Overall RBX2660 efficacy analysis does not include participants who responded in Group B of the blinded phase analysis.

Table 3: Overall adverse events and by-organ-class adverse events reported in at least 5% of the Safety population during the blinded treatment phase.

	Events/participants (% of participants)			
	Group A (n=41)	**Group B** (n=44)	**Group C**[1] (n=43)	**Total** (N=128)
Overall	169/25 (61.0)	105/26 (59.1)	105/31 (72.1)	379/82 (64.1)
Gastrointestinal disorders	78/21 (51.2)	56/16 (36.4)	49/20 (46.5)	183/57 (44.5)
General disorders	12/8 (19.5)	15/7 (15.9)	14/9 (20.9)	41/24 (18.8)
Infections	8/6 (14.6)	8/6 (13.6)	5/5 (11.6)	21/17 (13.3)
Nervous system disorders	5/3 (7.3)	8/6 (13.6)	1/1 (2.3)	14/10 (7.8)
Renal and urinary disorders	6/5 (12.2)	2/2 (4.5)	2/2 (4.7)	10/9 (7.0)
Skin and subcutaneous tissue disorders	5/4 (9.8)	3/2 (4.5)	3/3 (7.0)	11/9 (7.0)
Musculoskeletal and connective tissue disorders	5/4 (9.8)	2/2 (4.5)	2/2 (4.7)	9/8 (6.3)
Injury, poisoning, procedural	3/2 (2.4)	0	10/5 (11.6)	13/7 (5.5)

complications				
Metabolism and nutrition disorders	7/4 (9.8)	1/1 (2.3)	2/2 (4.7)	10/7 (5.5)
Respiratory, thoracic, and mediastinal disorders	11/4 (9.8)	1/1 (2.3)	2/2 (4.7)	14/7 (5.5)

[1]The safety population included one participant in Group C that was not in the ITT population due to withdrawal before completing a blinded study treatment.

Table 4. Serious adverse events and relatedness reported during blinded and open-label arms to a mean follow up of 8.3 months, range 0.1 to 15.9 months.

	Events/participants (% of participants)			
	Group A (n=41)	**Group B** (n=44)	**Group C**[1] (n=43)	**Total** (N=128)
Overall SAE	19/13 (31.7)	8/6 (13.6)	18/7 (16.3)	45/26 (20.3)
Related to RBX2660[2]	3/3 (7.3)	0	0	3/3 (2.3)
Related to procedure	0	0	0	0
Related to *C. difficile* disease	8/5 (12.2)	1/1 (2.3)	5/3 (7.0)	14/9 (7.0)
Related to a pre-existing condition	16/10 (24.4)	6/4 (9.1)	13/7 (16.3)	35/21 (16.4)
Deaths[3]	3 (7.3)[3]	0	3 (7.0)	6 (4.6)
Related to RBX2660	0	0	0	0
Related to procedure	0	0	0	0
Related to *C. difficile* disease	1 (2.4)	0	0	1 (0.8)
Related to a pre-	1 (2.4)	0	3 (7.0)	4 (3.1)

existing condition

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[1]The safety population included one participant in Group C that was not in the ITT population due to withdrawal before completing a blinded study treatment.

[2]Three SAEs were adjudged possibly related to the blinded study drug: one participant developed recurrent acute myeloid leukemia, another reported abdominal cramping and pain, and a third experienced constipation requiring hospitalization.

[3]One participant death was due to MRSA bacteremia and was adjudged to be unrelated to the treatment, procedure, or pre-existing condition.

Figure Legends

Figure 1. Flow of Participants in Multi-Center, Double-Blind, Placebo-Controlled Randomized Trial of Microbiota-Based Drug RBX2660 for Prevention of Recurrent *C. difficile* Infection

Figure 2. Efficacy of Microbiota-Based Drug RBX2660 or Placebo Following Blinded Treatment. The proportions of participants in the blinded phase who responded to treatment with two doses of RBX2660 (Group A); two doses of placebo (Group B); and one dose of RBX2660 followed by one dose of placebo (Group C).

Figure 1

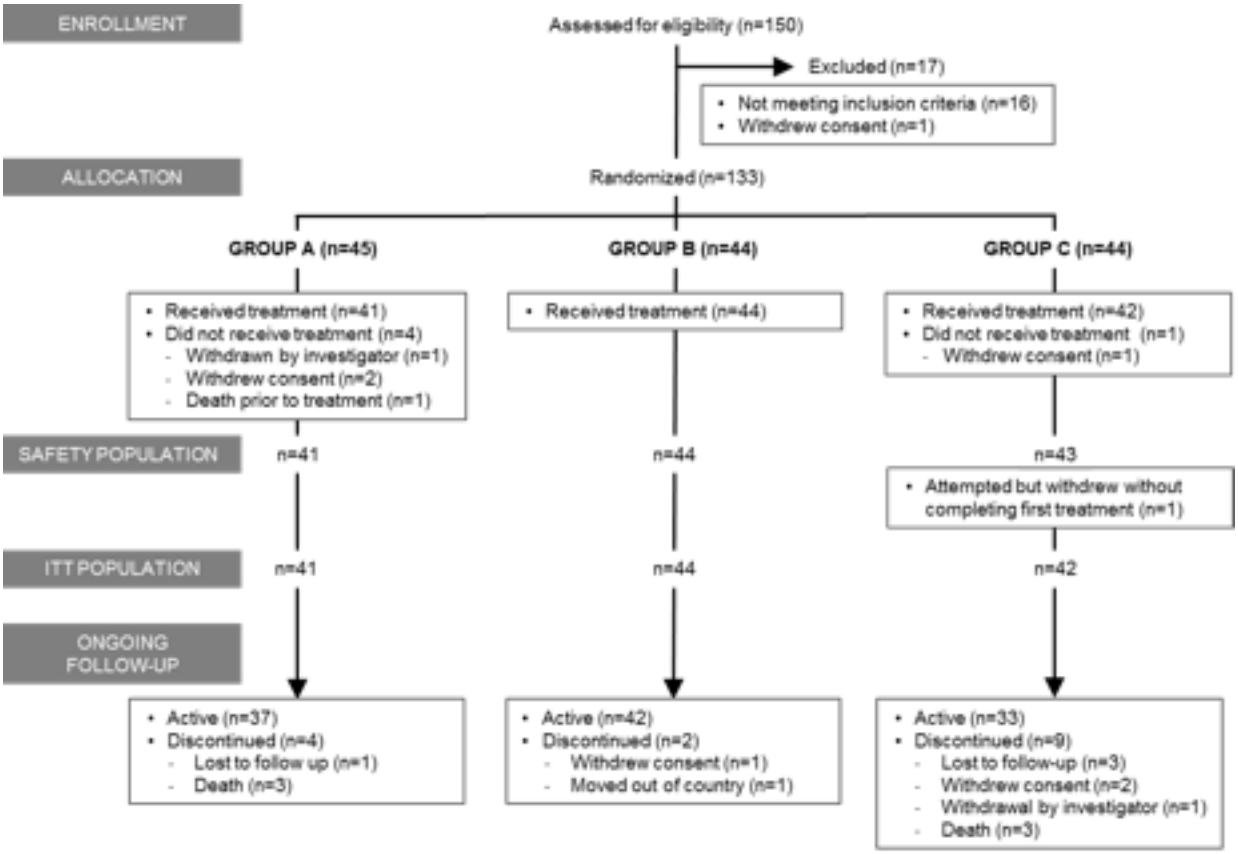

Figure 2