Infect Dis Ther (2023) 12:703–709
https://doi.org/10.1007/s40121-022-00744-3

BRIEF REPORT

Final Results from a Phase 2b Randomized, Placebo-Controlled Clinical Trial of RBX2660: A Microbiota-Based Drug for the Prevention of Recurrent *Clostridioides difficile* Infection

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Received: October 14, 2022 / Accepted: December 1, 2022 / Published online: December 21, 2022

ABSTRACT

Introduction: Effective treatments for recurrent *Clostridioides difficile* infection (rCDI) are urgently needed. RBX2660 is an investigational microbiota-based live biotherapeutic to reduce CDI recurrence following standard-of-care antibiotic treatment in individuals with rCDI. Here we report the final safety data through 24 months of follow-up as well as final efficacy data, reflecting alignment of the pre-specified statistical analysis plan definitions with the data presented.

Methods: The PUNCH CD2 clinical trial was a prospective, multicenter, randomized, double-blinded, placebo-controlled, three-arm phase 2b study conducted to evaluate the efficacy and safety of RBX2660 for the reduction of rCDI compared to placebo. Eligible patients were at least 18 years of age and had at least three episodes of CDI and at least two rounds of standard antibiotic treatment or had at least two episodes of severe CDI resulting in hospitalization. Patients were randomized 1:1:1 to group A, two doses of RBX2660; group B, two doses of placebo; or group C, one dose of RBX2660 and one dose of placebo; all administered 7 ± 2 days apart. Treatment success was prevention of recurrence, defined as absence of diarrhea and no re-treatment for CDI any time after the first dose until 8 weeks after the second dose of the study treatment. Safety was assessed by reports of adverse events and symptoms. The final efficacy and safety are reported for data available through 24 months.

Results: For the primary endpoint, treatment success at 8 weeks, 56.8% (25/45) of participants who received one dose of RBX2660 + one dose of placebo, 55.6% (25/45) of participants who received two doses of RBX2660, and 43.2% (19/44) of participants who received two doses of placebo in the final intention-to-treat (ITT) population were responders (both $p = 0.2$ vs placebo). In the per-protocol population, 87.5% (21/24) of participants who received one dose of RBX2660 + one dose of placebo and 58.1% (18/31) of those who received two doses of placebo had treatment success ($p = 0.017$; treatment difference, 29.4 [95% CI 7.6, 51.3]); 75.0%

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(21/28) of participants in the PP population who received two doses of RBX2660 were responders ($p = 0.17$ vs placebo). The safety profile of RBX2660, whether delivered as one or two doses, was similar to the placebo group.
Conclusion: While the phase 2b PUNCH CD2 clinical trial did not meet its pre-defined primary endpoint of treatment success at 8 weeks after two doses of RBX2660 vs two doses of placebo, clinically meaningful data were obtained to justify proceeding with the single dose regimen in the phase 3 clinical trial, PUNCH CD3, now complete. To date, the cumulative data for RBX2660 demonstrate consistent efficacy and safety outcomes for reduction of CDI recurrence in adults.
Clinical Trial Registration: ClinicalTrials.gov: NCT02299570.

Keywords: Live biotherapeutic product; RBX2660; *Clostridioides difficile*; *Clostridium difficile*; Microbiota; Recurrent CDI

Key Summary Points

RBX2660 is an investigational microbiota-based live biotherapeutic designed to reduce *Clostridioides difficile* infection (CDI) recurrence following standard-of-care antibiotic treatment in individuals with recurrent CDI.

Preliminary efficacy analysis at 8 weeks showed no significant improvement in participants who received the two-dose RBX2660 regimen compared to placebo.

Clinically significant improvement in efficacy was demonstrated in participants receiving one dose of RBX2660 vs placebo, providing meaningful data to conduct the phase 3 trial of RBX2660 with a single-dose treatment regimen.

Safety data for RBX2660 demonstrated 87.6% of treatment-emergent adverse events were mild to moderate in severity and primarily gastrointestinal in nature.

INTRODUCTION

Effective treatments for recurrent *Clostridioides difficile* infection (rCDI) are urgently needed. CDI is recognized as a public health threat and is associated with significant morbidity, mortality, and cost [1–4]. Treatment of CDI with antibiotics is the current standard of care, but often fails to eradicate the infection, resulting in high rates of recurrence [5–8]. Microbiota-based therapies have been shown to be effective for reducing recurrent CDI, but the lack of standardization of fecal microbiota transplantation has raised concerns about safety [9–11].

RBX2660 is an investigational microbiota-based live biotherapeutic designed to reduce CDI recurrence following standard-of-care antibiotic treatment in individuals with rCDI [12]. RBX2660 has undergone extensive evaluation through five prospective clinical trials, with three completed phase 2 trials, the completed PUNCH CD3 phase 3 trial [13], one ongoing phase 3 trial (NCT03931941), and one retrospective study, with more than 1000 patients in total treated to date [12, 14–16].

The PUNCH CD2 study was a phase 2b trial examining the efficacy and safety of RBX2660 for the treatment of recurrent *C. difficile* infection compared to placebo. A report on the 8-week efficacy data and preliminary safety data was previously published [15], while the 24-month safety follow-up was in progress. The preliminary report was based on the interim clinical study report. In developing the final clinical study report after the 24-month safety follow-up was complete, all data analyses and prospectively defined study populations were reviewed, to ensure consistency with the statistical analysis plan (SAP). During this review, some corrections were made to population definitions and analyses used in the preliminary report, to ensure the definitions and analyses were aligned with the SAP.

Here we report the final efficacy and safety analysis of data available through 24 months from the PUNCH CD2 study of RBX2660 vs placebo in recurrent CDI.

METHODS

The PUNCH CD2 phase 2b clinical trial was conducted to test the efficacy and safety of RBX2660 for the treatment of recurrent *C. difficile* infection compared to placebo (NCT02299570). The trial was conducted in the USA and Canada according to the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and requirements of publicly registered clinical trials. The protocol was approved by an institutional review board prior to the trial commencing. All participants provided written informed consent. Full details of the study design were described in the published preliminary report [15]. The trial was designed with an 8-week database lock for efficacy assessment, followed by up to a 24-month safety evaluation at the conclusion of the treatment.

To ascertain the most appropriate RBX2660 treatment regimen, three groups were evaluated in the trial using different treatment regimen. Group A received two doses of RBX2660, group B received two doses of placebo, and group C received one dose of RBX2660 and one dose of placebo, with the second dose given to each group 7 ± 2 days after the first dose. Participants in groups A and C who failed blinded treatment were offered open-label treatment with RBX2660.

All analyses presented in this report were prespecified in the study protocol. Treatment success was prevention of recurrence, defined as the absence of diarrhea and no re-treatment for CDI any time after the first dose until 8 weeks after the second dose of the study treatment. Treatment failure was defined as meeting all four of the following criteria at less than 8 weeks after completion of both study treatments: *C. difficile*-associated diarrhea, a positive laboratory diagnosis for *C. difficile* or its toxins as conducted and reported by the study investigator, a need for re-treatment for CDI, and no other cause for CDI symptoms. An independent data safety monitoring board (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 because of 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 [15]. Safety was assessed by reports of symptom severity daily through 7 days after the final assigned dose, and assessments for adverse events (AEs) in person during visits at weeks 1, 4, and 8, and by telephone at weeks 2, 3, 5, 6, and 7, and months 3, 6, 12, and 24. In addition, at 24 months, serious AEs and new onset of chronic diseases were assessed by telephone call. Investigators could classify an adverse event as related to the study drug at any time during the 24-month study.

For the preliminary analysis conducted at the 8-week efficacy database lock, the definition of intention-to-treat (ITT) was limited to only participants who completed at least one dose of RBX2660. Six participants were randomized but not treated with RBX2660. Consistent with the SAP, in the final data analysis participants with missing data were recorded as treatment failures, whereas in the preliminary analysis, participants with missing data were recorded as treatment successes.

Final efficacy data were analyzed for the ITT population (all randomized participants), modified ITT population (mITT; participants who received treatment, excluding those who discontinued prior to outcome evaluation or had eligibility deviations), and the per-protocol population (PP; all randomized participants who successfully received both treatment doses and were evaluable for outcome, excluding participants for predefined reasons such as major protocol deviations). Final safety data are reported for all participants who were exposed to an RBX2660 dose, presented by actual treatment received.

Table 1 Final and interim results for treatment success in the PUNCH CD2 clinical trial

	Interim ITT *N* = 127		Final ITT *N* = 133		Final mITT* *N* = 121		Interim PP *N* = 94		Final PP *N* = 83	
	% (*n*/*N*)	*p* value[#]	% (*n*/*N*)	*p* value[#]	% (*n*/*N*)	*p* value[#]	% (*n*/*N*)	*p* value[#]	% (*n*/*N*)	*p* value[#]
Group A (2 × RBX2660)	61.0 (25/41)	0.152	55.6 (25/45)	0.243	62.5 (25/40)	0.095	71.0 (22/31)	0.136	75.0 (21/28)	0.170
Group B (2 × placebo)	45.5 (20/44)	–	43.2 (19/44)	–	44.2 (19/43)	–	52.9 (18/34)	–	58.1 (18/31)	–
Group C (1 × RBX2660, 1 × placebo)	66.7 (28/42)	0.048	56.8 (25/44)	0.201	65.8 (25/38)	0.051	79.3 (23/29)	0.029	87.5 (21/24)	0.017

ITT (intention-to-treat) population consisted of all participants who were randomized, including those who were not treated, as well as participants with an "indeterminate" treatment outcome, who were also conservatively categorized as treatment failures. mITT (modified intention-to-treat) population consisted of participants who received treatment; excludes participants who discontinued prior to outcome evaluation or had eligibility deviations. PP (per protocol) population defined as all ITT participants who received randomized treatment and were evaluable for outcome; excludes participants for predefined reasons

*mITT population was not calculated for interim analysis; final mITT population results are compared with interim ITT results

[#]*p* value from chi-square test for the difference between group A or group C vs group B with respect to % of treatment successes

RESULTS

Here we report the final efficacy and safety analysis of data available through 24 months of the PUNCH CD2 study of two doses of RBX2660 vs placebo, and one dose of RBX2660 plus one dose of placebo vs two doses of placebo in recurrent CDI (as reported on ClinicalTrials.gov listing NCT02299570, accessed September 20, 2022).

Efficacy

In the final analysis for the primary endpoint, treatment success at 8 weeks, 55.6% (25/45) who received two doses of RBX2660, 56.8% (25/45) of those who received one dose of RBX2660 plus one dose of placebo, and 43.2% (19/44) of those who received two doses of placebo in the ITT population were responders (both $p = 0.2$ vs placebo; Table 1). In the per-protocol population, 75.0% (21/28) of participants who received two doses of RBX2660 and 58.1% (18/31) of those who received two doses of placebo had treatment success ($p = 0.17$; treatment difference, 16.9 [95% CI − 6.7, 40.6]), while 87.5% (21/24) of participants who received one dose of RBX2660 plus one dose of placebo had treatment success ($p = 0.017$; treatment difference, 29.4 [95% CI 7.6, 51.3]).

Safety

In the final safety analysis, treatment-related AEs (TEAEs) were reported by 82.0% (105/128) of all participants, with similar rates of TEAEs across the three treatment groups (Table 2). Most (87.6%; 496/566 events) of the TEAEs were mild or moderate in severity, and primarily related to gastrointestinal disorders (32.5%; 184/566 events). Three (2.3%) serious AEs were reported as possibly related to RBX2660 (constipation; recurrent acute myeloid leukemia; abdominal pain). None of the 16 deaths reported were related to RBX2660 or the rectal administration procedure. The safety profile of RBX2660, whether delivered as one or two doses, was similar to the placebo group.

Table 2 Final safety results: treatment-emergent adverse events in the PUNCH CD2 clinical trial, safety population

Events/participants, (% of participants)	Group A (2 × RBX2660) *N* = 42	Group B (2 × placebo) *N* = 44	Group C (1 × RBX2660, 1 × placebo) *N* = 42	Total *N* = 128
Any TEAE	313/34 (81.0)	290/38 (86.4)	238/33 (78.6)	841/105 (82.0)
TEAE related to study drug	55/14 (33.3)	33/15 (34.1)	14/8 (19.0)	102/37 (28.9)
TEAE related to rectal administration procedure	40/10 (23.8)	30/17 (38.6)	14/7 (16.7)	84/34 (26.6)
TEAE related to CDI	73/17 (40.5)	59/16 (36.4)	50/15 (35.7)	182/48 (37.5)
TEAE related to pre-existing condition	159/27 (64.3)	98/27 (61.4)	105/21 (50.0)	362/75 (58.6)

CDI C. difficile infection, *TEAE* treatment-emergent adverse event

DISCUSSION

Interim vs Final Efficacy and Safety Results: What Changed?

This final report of the phase 2b PUNCH CD2 trial of RBX2660 in recurrent CDI provides the final efficacy and safety outcomes for all participants. A preliminary analysis of the efficacy and safety data from the phase 2b PUNCH CD2 trial that were available at the 8-week database lock was published [15]. The preliminary analysis showed no statistically significant treatment response to two doses of RBX2660 compared to two doses of placebo ($p = 0.152$). The preliminary study results did show significant improvement in efficacy of one dose of RBX2660 plus one dose of placebo vs two doses of placebo ($p = 0.048$ in the ITT population) [15]. For the final analysis presented here, the definition of the ITT population was modified slightly to ensure alignment with the pre-specified statistical analysis plan, which resulted in more participants being included in the final analysis. Additionally, missing data were handled differently in the preliminary vs the final SAP-aligned analysis, which resulted in a few participants being deemed treatment failures due to missing data in the final analysis.

CONCLUSION

While the PUNCH CD2 clinical trial did not meet its pre-defined primary efficacy endpoint of treatment success at 8 weeks after two doses of RBX2660 vs two doses of placebo in the ITT population, meaningful data were obtained from the single dose regimen in the mITT and PP populations to justify moving forward with the phase 3 clinical trial, PUNCH CD3, using a single dose regimen [13].

Notably, adverse events in the PUNCH CD2 trial did not differ significantly among treatment groups [15]. The final efficacy and safety outcomes from the PUNCH CD2 study reported here are consistent with, but slightly different from, the preliminary findings reported by Dubberke et al. [15].

To date, the cumulative data for RBX2660 demonstrate meaningful efficacy and safety outcomes for reduction of CDI recurrence in adults.

ACKNOWLEDGEMENTS

Funding. The original study was funded by Rebiotix Inc, a Ferring Company, and Rapid Service fees were funded by Ferring Pharmaceuticals Inc. (Parsippany, NJ).

Medical Writing and/or Editorial Assistance. Editorial assistance (copyediting, proofreading, submission) was provided by Agnella Izzo Matic, PhD, CMPP (AIM Biomedical, LLC, Fairfield, CT) and was funded by Ferring Pharmaceuticals Inc.

Author Contributions. Drs. Dubberke, Orenstein, Khanna, and Lee – Investigation; Writing – Original draft; Writing – Review & Editing. Beth Guthmueller – Methodology; Formal analysis; Writing – Original draft; Writing – Review & Editing; Supervision.

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Disclosures. Dr Dubberke serves on the Rebiotix Advisory Board, and is a consultant for Merck, Pfizer, Abbott, Seres and GSK, receiving research funding from Rebiotix/Ferring and Synthetic Biologics. Dr Orenstein reports being an investigator for studies sponsored by Finch Therapeutics and Rebiotix; an advisor for Rebiotix; and serves on the speaker's bureau for Ferring Pharmaceuticals Inc. Dr Khanna has received grants or contracts from Rebiotix (a Ferring company), Finch Therapeutics, Seres Therapeutics, and Vedanta BioSciences, consulting fees from Niche Pharmaceuticals and Immuron Limited, participated on Advisory or Data Safety monitoring boards for Ferring Pharmaceuticals, and has stock options with Jetson Probiotics. Dr Lee has received grants or contracts from Rebiotix, Inc, Seres Therapeutics, and Summit Therapeutic as well as participated on Advisory or Data Safety monitoring boards for Ferring Pharmaceuticals and Pfizer. Beth Guthmueller is an employee of Rebiotix, Inc.

Compliance with Ethics Guidelines. The original study was approved by local institutional review boards at the study sites and complied with the principles set forth in the Declaration of Helsinki and in compliance with ICH-GCP standards. All participants provided written informed consent.

Data Availability. The data reported here are available at ClinicalTrials.gov, registration number NCT02299570.

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