Impact of RBX2660 on the Intestinal Microbiota of Patients with Recurrent *Clostridium difficile* Infection Enrolled in the Randomized, Placebo-Controlled PUNCH CD 2 Trial

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**Background**

• Perturbation of the intestinal microbiota, primarily by antibiotics, is a risk factor for *Clostridium difficile* infection (CDI).
• Restoration of a variable intestinal microbiota protects against recurrence of CDI.
• RBX2660 is a microbiota-based drug under study for the prevention of recurrent CDI.

We evaluated the impact of RBX2660 treatment on the intestinal microbiota of patients enrolled in the PUNCH CD 2 trial, a multicenter, randomized, double-blinded, placebo-controlled phase 2b study.

**Methods**

• Patients in the 3-arm PUNCH CD 2 trial were randomized 1:1:1 to receive either: 2 doses of RBX2660 (Group A); 2 doses of placebo (Group B); or 1 dose of RBX2660 and 1 dose of placebo (Group C). Success was defined as the absence of *Clostridium difficile*-associated diarrhea at 8 weeks following completion of the last treatment.
• Failure was defined as recurrence of CDI symptoms; a positive stool test; a need for CDI retreatment; and no other cause for CDI symptoms within 8 weeks.
• Longitudinal 16s rRNA analysis using the Illumina MiSeq platform was performed on a total of 120 stool samples from patients in the 3 arms of the study (Group A: n=23; Group B: n=22; Group C: n=22) at baseline, 7 days and 30 days after the second blinded dose.
• The variable region V4 was targeted to identify the operational taxonomic units (OTUs) in each sample.

**Results**

• At baseline, the microbiota profiles were similar across all samples.
• OTU analysis demonstrated higher diversity between baseline and follow-up time points for all patients who responded successfully to their assigned treatment.
• The higher diversity at follow-up points was also true for placebo responders (12 patients in Group B who received two doses of placebo and did not experience recurrent CDI symptoms prior to 8-week follow-up).
• Specifically, successful Group A patients had an increase in *Akkermansia* and *Bacteroides* from baseline; successful Group B and C patients had a decreased abundance of *Enterobacter* (Figures 1-3).
• Of the three groups of patients, those in Group A (two doses of active treatment) showed the greatest increase in bacterial diversity (number of OTUs) and abundance of taxa between baseline and all follow-up points (Figure 4).

**Conclusions**

In this randomized, placebo-controlled study of RBX2660 for recurrent CDI, 16s rRNA analysis found that patients who succeeded with their assigned therapy had a more diverse intestinal microbiota at 7 and 30 days compared with baseline.

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