Poster# 1267

Successful Response to Microbiota-Based Drug RBX2660 in Patients with Recurrent *Clostridium difficile* Infection is Associated with More Pronounced Alterations in Microbiome Profile

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Background

- Recurrent *Clostridium difficile* infections (rCDI) are associated with decreased taxonomic diversity in patients' intestinal microbiome compared to healthy microbiomes
- RBX2660 is a standardized microbiota-based drug designed to rehabilitate a patient's intestinal microbiome
- The effect of RBX2660 on rCDI patient microbiomes was evaluated by comparing pre- and post-treatment samples collected from PUNCH CD 2—a multicenter, randomized, double-blind, placebo-controlled study
- Microbiome profiles from RBX2660 responders (Successes) were compared to those from non-responders (Failures)

Methods

- Patients with rCDI were enrolled in the PUNCH CD2 trial and randomized to receive blinded treatment by enema of 2 doses of RBX2660 (Group A), 2 doses of placebo (Group B), or 1 dose of RBX2660 and 1 dose of placebo(Group C), with doses 7 days apart (Figure 1)
- Success was defined as the absence of CDI at 8 weeks following completion of the last treatment. Patients were classified as a treatment failure if all four(4) of the following criteria were met: recurrence of diarrhea less than 8 weeks after administration of the last assigned study enema, a positive laboratory diagnosis of *C. difficile* as conducted and reported by the study investigator, a need for retreatment for CDI, and no other cause for diarrhea
- Patients voluntarily submitted stool samples at baseline (pre-treatment) 7, 30, and 60 days after treatment
- 16s rRNA analysis using the Illumina MiSeq platform was performed on stool samples collected from patients who received at least one dose of RBX2660 (Groups A & C)
- The variable region V4 was targeted to identify the operational taxonomic units (OTUs) in each sample
- Relative abundance data from subject samples were grouped longitudinally and compared among groups using a Bray-Curtis dissimilarity calculation with non-metric multi-dimensional scaling. Additional analyses were performed based on the Dirichlet-Multinomial distribution to compare group mean relative taxonomic abundances (pi) and within group over dispersion (theta)
- The microbial diversity was analyzed based on Shannon and Simpson indices
- Differentiation between sample communities were visualized using a Kullback-Leibler (KL) divergence analysis model (BioRankings, St. Louis, MO), a measure of the difference between microbial diversity at different time points or between different samples

Figure 1: PUNCH™ CD2 Trial Design

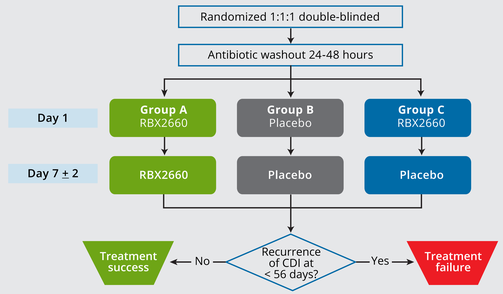
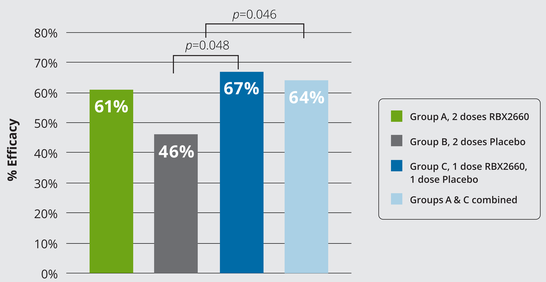


Figure 2: PUNCH™ CD2 Trial Results

- Groups A and C combined were superior to placebo (p=0.046)
- A second dose of RBX2660 one week later (Group A) did not provide additional clinical benefit



Results Summary

- RBX2660 successes have different microbiota composition at 7 and 30 days relative to baseline (Figure 3)
- RBX2660 failures have similar microbiota composition at 7 and 30 days relative to baseline (Figure 3)
- RBX2660 treatment increased Shannon and Simpson diversity at 7 days post-treatment in successes but not in failures (p<0.05; Figure 4)
- Taxonomic classes that diverged the most at baseline showed the largest longitudinal post-treatment change (Figure 5)

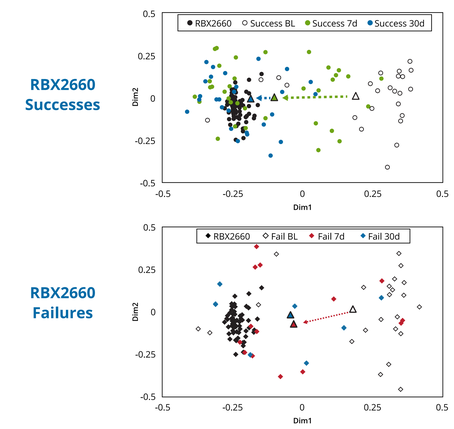
Results

140 stool samples collected from 58 patients who received at least one dose of RBX2660 (Groups A and C) were analyzed (Table 1)

Table 1

	Patients	# Patient samples			Product samples
		Baseline	7 days	30 days	
RBX2660 Successes	35	24	35	27	136
RBX2660 Failures	23	23	14	17	

Figure 3: Microbiomes from patients with successful response to RBX2660 have microbiomes that are different from baseline. Microbiomes from non-responders do not shift from baseline.



Comparisons were made at the sample level using non-metric multidimensional scaling based on the Bray Curtis measure. In the plot above, mean microbiomes for individual patients (dots) and group mean microbiomes (triangles) are shown. Arrows highlight the shift of group means from baseline toward cluster of RBX2660 product profile.

Figure 4: Patient microbiomes trend toward higher diversity for both Successes and Failures who received RBX2660 treatment

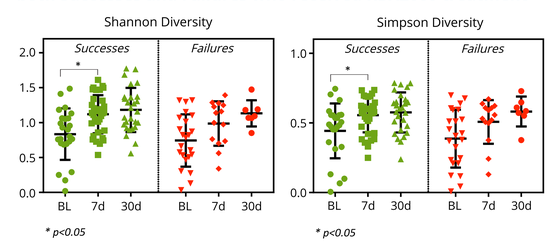
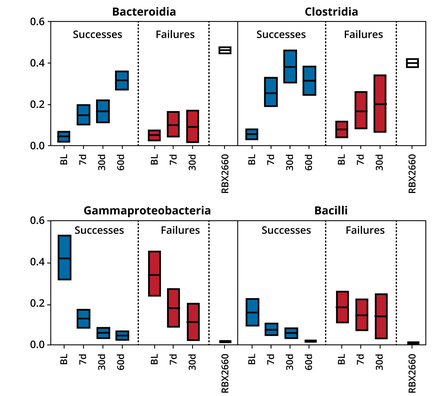
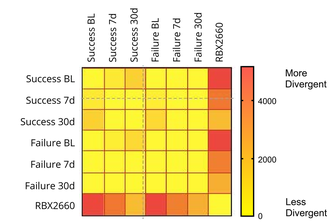


Figure 5: After RBX2660 treatment, Successes trend more toward RBX2660 product profile than Failures, particularly among Bacteroidia, Gammaproteobacteria, and Bacilli.



Dirichlet Comparisons were made at the taxa level using Dirichlet Multinomial parameter (pi), representing the group's mean relative taxa abundance, at each time point.

Figure 6: RBX2660 Successes diverge more from their respective baseline than Failures, consistent with taxonomic data.



Kullback-Leibler (KL) divergence analysis assesses the difference between microbial diversity at different time points or between different samples. KL divergence increases as color ranges from yellow to red. Similarity between two microbiomes decreases as KL divergence increases.

Conclusion

- We observe a general trend of the microbiomes among RBX2660 Successes toward a healthier composition, with higher Bacteroidia and Clostridia and lower Gammaproteobacteria and Bacilli
- RBX2660 treatment appears to increase microbiome diversity
- For this trial, there do not appear to be differences at baseline that predict response or failure

Disclaimer: This analysis was funded by Rebiotix Inc., Roseville, MN