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 diversity & altered intestinal microbiome compared to healthy patients. RBX2660, a standardized microbiota-based drug, is designed to restore microbiome diversity & composition in patients'. The effect of RBX2660 on rCDI patient microbiomes was evaluated by comparing pre- & post-treatment samples from PUNCH CD 2—a randomized, double-blind, placebo-controlled study.

Methods
rCDI subjects were randomized to receive blinded treatments of 2 doses of RBX2660 (Group A), 2 doses of placebo (Group B), or 1 dose each of RBX2660 & placebo (Group C), by enema 7 days apart. Subjects submitted stool samples at baseline, day 7, 30, & 60 after treatment. Stool samples from responders to RBX2660 treatment per protocol defined as the absence of CDI for 8 weeks after treatment were compared to non-responders.

Relative taxonomic abundances at the class level were determined using 16s rRNA sequencing analysis for 94 stool samples from 45 patients in Groups A & C. Relative abundance data were grouped longitudinally using Bray-Curtis dissimilarity index. Analyses were performed based on the Dirichlet-Multinomial distribution to compare group mean relative taxonomic abundances; Simpson & Shannon diversity indices were compared among groups longitudinally.

Results
Baseline patient microbiomes were similar across response groups. RBX2660 treatment shifted the relative microbiome densities with taxa-specific increase in Bacteroidia, Clostridia, and decrease in Gamma-proteobacteria abundance. A larger shift from baseline microbiome was seen in responders to RBX2600 compared to non-responders (Figure 1). Microbiome changes in responders were durable to 60 days. RBX2660 treatment increased Shannon and Simpson diversity at 7 days post-treatment in responders but not in non-responders ($p < 0.05$).

Conclusion
RBX2660 treatment shifts patient intestinal microbiomes with greater alterations seen in those with a successful clinical outcome.

Figure 1. Responders to RBX2660 have a greater change in taxa abundance from baseline relative to non-responders at 30 days. Dirichlet-Multinomial parameter pi presented as mean (95%CI).