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

Sahil Khanna MBBS, MS, Ken Blount PhD, Courtney Jones BS, Bill Shannon PhD MBA, Sharina Carter PhD
1Division of Gastroenterology and Hepatology Mayo Clinic, Rochester, MN, USA; 2Rebitox Inc, Roseville, MN, USA; 3BioRankings LLC, St. Louis, MO, USA

Background

• Recurrent C. difficile infections (CDI) 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 CDI patient microbiomes was evaluated by comparing pre- and post-treatment samples collected from PUNCH CD2—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 CDI 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 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.
• Differences 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.

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

<table>
<thead>
<tr>
<th>RBX2660 Successes</th>
<th>RBX2660 Failures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td># Patient samples</td>
</tr>
<tr>
<td>Baseline</td>
<td>7 days</td>
</tr>
<tr>
<td>RBX2660</td>
<td>44</td>
</tr>
</tbody>
</table>

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.

Figure 1: PUNCH CD2 Trial Design

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.

Figure 4: RBX2660 Successes diverge more from their respective baseline than Failures, consistent with taxonomic data.

Figure 5: After RBX2660 treatment, Successes trend more toward healthy microbiomes, particularly among Bacteroidia, Gammaproteobacteria, and Bacilli.

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

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 Rebitox Inc., Roseville, MN.