Microbiome Profile is Distinct in Patients with Successful Response to Microbiota-Based Drug RBX2660 Relative to Placebo Responders

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Background

- Recurrent Clostridium difficile infections (rCDI) are associated with an altered microbiome composition and diversity compared to healthy patients.
- RBX2660, a standardized microbiota-based drug designed to rehabilitate patients’ microbiomes, was superior to placebo for preventing rCDI in a Phase 2B clinical trial.
- The Phase 2B placebo-treated patients demonstrated a response rate that is consistent with the results from placebo-treated groups of other randomized-controlled rCDI trials.
- This study sought to understand whether there was a significant difference in the extent of microbiome recovery between patients who responded in the RBX2660 treatment groups and the placebo treatment group.

Methods

- Patients with rCDI who 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 subjects with successful outcomes.
- 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).
- Differentiation between sample communities was visualized using a Kulback-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

- 157 stool samples collected from 57 patients classified as responders (A = 21, B = 15, C = 21). Microbiome data for all patients at study entry were combined as a treatment-naïve baseline. For MDS and relative abundance analyses, Groups A and C were pooled as “active” and compared to placebo treatments longitudinally (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Placebo 7D</th>
<th>Placebo 30D</th>
<th>Placebo 60D</th>
<th>RBX2660 7D</th>
<th>RBX2660 30D</th>
<th>RBX2660 60D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>136</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

- At 7, 30, and 60 days, microbiomes from RBX2660-treated patients had high KL divergence from baseline and significantly different means from baseline (p<0.001) (Figure 3).
- RBX2660 treatment increased the relative abundances of Bacteroides, and decreased Gammaproteobacteria and Bacilli more than placebo treatment (Figure 4).
- Recurrent CDI patient microbiomes were dissimilar to RBX2660 product profile at baseline. Active responders became more similar following RBX2660 treatment (Figure 5).

Conclusion

- Among patients who were recurrence-free at 8 weeks, those who received RBX2660 had significantly greater microbiome changes than those who received placebo. Most notably Bacteroides were significantly higher after RBX2660.
- These changes are consistent with the hypothesis that RBX2660 can restore a healthier microbiome in rCDI patients. Longer-term studies are needed to compare the durability of microbiome changes and recurrence-free rates.

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