Alterations in Microbial Diversity are Associated with Treatment Success with RBX2660, a Microbiota-Based Drug for the Prevention of Recurrent Clostridium difficile Infection: Results from PUNCH CD 2: a Randomized Double-Blind Placebo-Controlled Trial

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Disclosures

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  • Rebiotix, Inc
  • Merck

• Consulting
  • Assembly biosciences
  • Rebiotix, Inc (paid to institution)
Recurrent *C. difficile* is a Difficult Problem

- Approximately 83,000 cases of recurrent *C. difficile* infection (CDI) in the US every year
  - Difficult to treat with antibiotics for CDI
  - ~60% risk after 3 or more infections
- Disruption of the intestinal microbiome by antibiotics (both CDI and non-CDI)
  - Low colonization resistance
  - Increased spore survival

Microbiome based Therapies are Emerging

• Conventional fecal microbiota transplant
• Highly effective in preventing recurrent CDI
• Restore a disrupted gut microbiome
• Standard therapies such as RBX2660 are in clinical trials
• Lack of substantial data on microbiome changes with microbiota based therapies from placebo-controlled trials
Specific Aims

• Efficacy of a standard microbiome based therapy (RBX2660) for recurrent CDI

• Correlate efficacy of RBX2660 or placebo with longitudinal patient microbiome data
  • Do microbiome changes after therapy predict treatment response?
Investigational Product: RBX2660

- 50 g stool in 150 ml liquid suspension
  - $\geq 10^7$ live organisms/mL
  - Single-dose ready-to-use enema bag
  - Stored and shipped frozen at $\leq -80^\circ$C
- Thawed at 4°C for 24 hrs & delivered within 48 hrs
- Blinding: Investigational product or placebo shrouded in an opaque brown sleeve
  - Enema administrator (not involved in study follow-up) shrouds tubing and delivers enema
PUNCH CD 2 Clinical Trial Design

- Multi-center trial in the US & Canada between Dec 2014 & Nov 2015
- Participants randomized: 1:1:1 to the following
  - Group A: 2 doses of RBX2660
  - Group B: 2 doses of placebo
  - Group C: 1 dose each of RBX2660 & placebo
- Enemas administered on-site 7 days apart, no bowel preparation
- Failures in the blinded phase could receive up to 2 doses of open-label treatment
Patient Population

• Adults with ≥ 2 CDI recurrences or at least 2 CDI episodes resulting in hospitalization
• Positive *C. difficile* stool test with active symptoms within 60 days
• CDI symptoms controlled with antibiotics
• 24-48 hour antibiotic free period prior to dosing
• Common exclusions
  • Immunocompromized, IBS, IBD, Chronic diarrhea, celiac disease, chemotherapy, pregnancy, breast feeding
Endpoint Definitions: 8 Weeks

• **Success:**
  - Absence of diarrhea
  - No retreatment for CDI

• **Failure (all 4 criteria required):**
  - Presence of diarrhea, with or without other CDI symptoms
  - A positive stool test
  - Need for CDI retreatment
  - No other cause for diarrhea identified
## Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2 Doses of RBX2660 (N=41)</th>
<th>2 Doses of Placebo (N=44)</th>
<th>1 Dose of RBX2660 and 1 Dose of Placebo (N=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age – years</td>
<td>62.8 ±19</td>
<td>58.8 ± 19</td>
<td>61.5 ± 20</td>
</tr>
<tr>
<td>Female sex- no. (%)</td>
<td>25 (61)</td>
<td>30 (68)</td>
<td>24 (57)</td>
</tr>
<tr>
<td>Race-White – no. (%)</td>
<td>39 (95)</td>
<td>42 (96)</td>
<td>40 (95)</td>
</tr>
<tr>
<td>Antibiotic screening – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>38 (93)</td>
<td>39 (89)</td>
<td>36 (86)</td>
</tr>
<tr>
<td>Fidaxomicin</td>
<td>1 (2)</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (5)</td>
<td>3 (6)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Median number of CDI episodes – no., (IQR)</td>
<td>4.0 (2)</td>
<td>3.0 (1)</td>
<td>4.0 (1)</td>
</tr>
<tr>
<td>Mean duration of CDI episode, days</td>
<td>18.8 ± 14</td>
<td>19.5 ± 17</td>
<td>16.4 ± 11</td>
</tr>
<tr>
<td>Healthcare-acquired CDI infection – no. (%)</td>
<td>10 (5.7)</td>
<td>10 (6.0)</td>
<td>13 (7.8)</td>
</tr>
</tbody>
</table>
RBX2660 is More Effective than Placebo

2 doses of RBX2660 (A) - p=0.58
2 doses of placebo (B) - 46%
1 dose of RBX2660 & placebo (C) - 64%

p=0.048
Overall Success for At least 1 dose of RBX2660 (blinded and open-label): 88.6%

- 2 doses of RBX2660 (A): 89%
- Placebo failures: 88%
- 1 dose of RBX2660 & placebo (C): 90%
Sample Collection – Microbiome Analysis

• Stool samples were collected at baseline, 7 days, and 30 days after the 2nd blinded dose
  • Group A (n=23)
  • Group B (n=28)
  • Group C (n=22)

• Active group includes subjects who received at least one dose of RBX2660 (Group A + Group C)

<table>
<thead>
<tr>
<th></th>
<th>Active (RBX2660)</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fail</td>
<td>18</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>Success</td>
<td>27</td>
<td>13</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>28</td>
<td>73</td>
</tr>
</tbody>
</table>
Microbiome Analysis

• Longitudinal 16s rRNA analysis using the Illumina MiSeq platform

• The variable region V4 was targeted to identify the operational taxonomic units (OTUs) in each sample

• OTUs at each time point were compared to baseline and stratified by patient outcome
Microbiome Analysis

- Differentiation between sample community composition were visualized using a Kullback-Leibler (KL) divergence analysis model (BioRankings, St. Louis, MO)

Less Divergence
Microbiomes are more similar

More Divergence
Microbiomes are less similar
Kullback-Leibler Divergence Analysis

More Divergence

Less Divergence
No Differences in Baseline Microbiota Composition in Placebo and RBX2660 Treated Patients

Kullback-Leibler Divergence Analysis
Placebo Responders have Similar Microbiota Composition at 7 and 30 days to Baseline

Kullback-Leibler Divergence Analysis
RBX2660 Non-responders have Similar Microbiota Composition at 7 and 30 days to Baseline

More Divergence

Less Divergence

Kullback-Leibler Divergence Analysis

Fail Active Baseline
Fail Active 7 Days
Fail Active 30 Days
RBX2660 Responders have Different Microbiota Composition at 7 and 30 days from Baseline

Kullback-Leibler Divergence Analysis
RBX2660 Responders Trend towards Different Microbiota Composition at 7 days from Failures

More Divergence

Less Divergence

Kullback-Leibler Divergence Analysis
RBX2660 Responders have Different Microbiota Composition at 30 days from Failures and Placebo

Kullback-Leibler Divergence Analysis
Summary

• One dose of RBX2660 administered via enema is more effective than placebo for recurrent CDI

• Responders to RBX2660
  • Distinct microbiome changes from baseline in contrast to those who fail active treatment

• Responders to RBX2660
  • Different microbiota composition than responders to placebo

• Placebo responders demonstrate no changes in microbiota composition after treatment
Future Directions

• Larger phase III trials are needed to demonstrate safety and efficacy of standardized microbiome based therapies

• In depth microbiome analyses to determine specific taxa at baseline and follow-up that may predict outcomes
Conclusions

• RBX2660 administered via enema is an effective treatment for recurrent CDI

• Responders to active therapy have distinct microbiome profiles compared to failures
  • Importance of bacterial engraftment

• Response to active therapy but not to placebo correlates with changes from baseline microbiome

• Changes in microbial profiles after microbiota-based therapies may predict treatment response
Questions?
Overall Success for At least 1 dose of RBX2660 (blinded and open-label): 88.6%
RBX2660 is More Effective than Placebo

- 2 doses of RBX2660 (A): 61%
- 2 doses of placebo (B): 46%
- 1 dose of RBX2660 & placebo (C): 67%
- Group A and C: 64%

P-values:
- p=0.58
- p=0.048
- p=0.046