Consistent and Reproducible Production of a Microbiota-based Drug for Recurrent *C. difficile* Infection: Application of a Novel Diagnostic for Dysbiosis

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Disclosure

- Program Manager, Rebiotix Inc., Roseville, MN USA
Background

- Perturbation of the gut microbiota by antibiotics is a major risk factor for both primary and recurrent *Clostridium difficile* infection (CDI).
- Restoration of the gut microbiota protects against CDI recurrence.\(^1,2\)
- RBX2660 is manufactured from live human-derived microbes using standardised, quality controlled-processes.
- Tested in >300 patients in 3 robust, FDA-regulated clinical trials including a randomized, double-blind placebo controlled Phase 2 trial (PUNCH CD 2).

Objectives

• Use a novel platform (GA-map Dysbiosis Test, Genetic Analysis, Oslo, Norway) to assess if the RBX2660 manufacturing process alters the normal microbiome.

• Determine, using this unique platform, whether or not a normal microbiome is maintained by healthy donors over time.
Methods

• A total of 70 drug substance samples sourced from 17 unrelated donors from August 2014 to February 2016 were compared with 70 matched samples of finished drug product using the GA-map Dysbiosis Test.
  – Donor characteristics: median age: 23 years; range 18 to 57 years; 94% male
• Includes a small sub-set of 4 donors with longitudinal data
• Product tested was collected over 4-13 months.
  – Stored at -80°C to preserve samples
  – Drug substance and drug product were collected and frozen on the same day
Methods

• The GA-map Dysbiosis Test was developed as a diagnostic tool for IBS/IBD.
• Validated in accordance with EU requirements to identify and characterize dysbiosis.¹
• Uses 54 probes targeting V3 to V7 of the bacterial 16s rRNA gene to characterize and identify bacteria present.
• Covers approximately 300-400 bacteria at different taxonomic levels and provides an assessment of the “normal” or “dysbiotic” microbial community by using multiple variable regions.
• Enables serial assessment of fecal bacterial community abundance profile and potentially clinically relevant alterations in the microbiota over time.

Methods

**GA-map:** 1180 bp

**Illumina:** 459 bp

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In silico development of highly specific probe-set

Fecal sample processing

Model calibration and verification: development of a DI algorithm

Test validation in healthy IBS and IBD subjects

- gDNA from fecal samples of healthy volunteers applied to GA-map™ technology platform
- gDNA from fecal samples applied to GA-map™ technology platform
- Algorithm-based read-out
  - DI>2 = dysbiosis

Binding Labeling

Generation of bacterial profile
## Comparative Signal Strength of Bacteria: DP vs. DS

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Signal Strength in DP vs. DS</th>
<th>Mean Difference (95% CIM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteroidetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>Increased</td>
<td>0.07 (0.03, 0.11)</td>
</tr>
<tr>
<td><em>Parabacteroides</em></td>
<td>Increased</td>
<td>0.12 (0.07, 0.17)</td>
</tr>
<tr>
<td><em>Alistipes</em></td>
<td>Increased</td>
<td>0.17 (0.11, 0.23)</td>
</tr>
<tr>
<td><strong>Firmicutes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lachnospirae</em></td>
<td>Decreased</td>
<td>-0.13 (-0.15, -0.11)</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>Decreased</td>
<td>-0.16 (-0.20, -0.13)</td>
</tr>
<tr>
<td><em>Negativicutes</em></td>
<td>Increased</td>
<td>0.03 (0.01, 0.06)</td>
</tr>
<tr>
<td><em>Clostridia</em></td>
<td>Decreased</td>
<td>-0.18 (-0.20, -0.16)</td>
</tr>
<tr>
<td><strong>Actinobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>Decreased</td>
<td>-0.33 (-0.38, -0.28)</td>
</tr>
</tbody>
</table>

DP = drug product; DS = drug source, CIM = confidence interval of mean

- High accuracy: 83.4% at cross validation
- Drug substance and drug product profiles largely conserved.
Principal Component Analysis Score Plots

17 donors, N=140 samples

- Drug product
- Drug substance

log 10 transformed data of 54 probes in GA-map Dysbiosis Test
Principal Component Analysis Score Plots

17 donors, N=140 samples

log 10 transformed data of 54 probes in GA-map Dysbiosis Test
Drug substance shows slightly more variance than the drug product: SD=0.76 vs. SD=0.82
Summary

- The GA-map Dysbiosis Test confirms that bacterial community abundance in the drug substance is conserved in manufactured RBX2660. Dysbiosis is not instigated with the standardized manufacturing process.
- The GA-map Dysbiosis Test also demonstrated that donors maintain a consistent, normal microbiome over time, corresponding with previous findings.¹

Discussion

• Validated using healthy controls and patients with IBS and IBD from Norway, Sweden, Denmark and Spain.
  – Validity of test when used in different populations (North America, Asia, Middle East, etc.) for diagnosis or identification of a “normal” microbiome?
• Ability to monitor alterations in the microbiome of patient’s with CDI to potentially predict therapeutic outcome or relapse?
  – Longitudinal patient samples – how do they change compared to dysbiotic index?
• Further exploration of healthy donors over time – how long do donors maintain a stable microbiome?
Acknowledgements

• Genetic Analysis Team
  – Christina Casén
  – Hans Fagerun

• Rebiotix Technical Team
Thank you!