**DEVELOPING MICROBIOME REHABILITATION BIOMARKERS FOR CLOSTRIDIUM DIFFICILE INFECTIONS: CONTINUED EVALUATION OF A PROTOTYPE MICROBIOME HEALTH INDEX™ (MHI™)**

Ken Blount PhD1, Courtney Jones BS1, Elena Deych MS2, Bill Shannon PhD MBA2

1Rebiotix Inc., Roseville, MN, USA; 2BioRankings LLC, St. Louis, MO, USA

**BACKGROUND**

- Dysbiosis, or disruption of a healthy microbiome, is strongly associated with Clostridium difficile infections (CDI). RBX2660 is a standardized, stabilized microbiota restoration therapy in clinical development for preventing recurrence of CDI (rCDI).
- In two recent Phase 2 clinical trials, RBX2660 was more efficacious than controls for preventing rCDI, and associated studies indicated that RBX2660 restored a healthier microbiome among responding participants.
- Since quantitative biomarkers for microbiome dysbiosis and/or restoration have not been established, we are evaluating a prototype unidimensional Microbiome Health Index (MHI).
- Herein we report a pooled MHI analysis of two Phase 2 trials of RBX2660 for preventing CDI and evaluate the potential of MHI as a predictor of clinical efficacy.

**METHODS**

- Included in this analysis are 127 RBX2660 product samples and 339 stool samples collected at indicated time points from a total of 176 participants with recurrent CDI who received at least one dose of RBX2660 as part of the PUNCH CD2 2 Phase 2B trial (NCT02299570) or the PUNCH Open Label Phase 2 trial (NCT02589847).
- Success was defined as the absence of CDI at 8 weeks after the last treatment.

**MICROBIOME ANALYSIS**

- PUNCH CD2 participant samples underwent 16S sequencing. Data from the PUNCH OL participant samples were generated using an ultra-shallow shotgun sequencing that generates taxonomic profiles with species-level resolution (CoreGenome, Minneapolis, MN).
- Relative taxonomic abundances at the class level were calculated from OTU data for each time, treatment, outcome group, and the mean and upper/lower confidence limits defined by fitting to a Dirichlet-multinomial distribution using maximum likelihood estimation.
- Taxa levels from PUNCH CD2 participants with successful response to RBX2660 approach product profile levels over time.

**MICROBIOME HEALTH INDEX (MHI) ANALYSIS**

- MHI of “unhealthy” and “healthier” microbiota to define a putative efficacy endpoint.
- MHI during early treatment may provide a Microbiome Efficacy Endpoint that may predict clinical outcome.
- MHI values were generated for patient samples collected at 7d following RBX2660 treatment and categorized by the patient’s primary endpoint response in the clinical trial (success or failure). Among patients who failed treatment, MHI's were also significantly higher 7d post-treatment compared to MHI’s at baseline, and MHI of successes could be distinguished from failures at 7d 4 days post-treatment (p<.001 Wilcoxon test).

**CONCLUSIONS**

- Microbiome Health Index (MHI) is an unidimensional algorithm which captures changes in the relative abundance of taxonomic classes known to have importance to microbiome health and colonization resistance.
- MHI values for baseline and RBX2660 product did not differ between the Phase 2 studies confirming pooling of values at each time point. Receiver Operating Characteristic (ROC) analysis of baseline (BL) vs RBX2660 yielded an area under the curve (AUC) of 0.996 and an optimal cutoff point of MHI=8.2 (red dotted line; sensitivity=0.96, specificity=0.99, likelihood ratio=0.8).
- MHI of “unhealthy” and “healthier” microbiota to define a putative efficacy endpoint.