PLACEBO RESPONDERS IN A RANDOMIZED CONTROLLED TRIAL OF RBX2660 FOR RECURRENT C. DIFFICILE INFECTION: PREDICTIVE VALUE OF 16s rRNA MICROBIOME ANALYSIS

Courtney Jones, BS[a] and Bill Shannon, PhD[b]

[a] Rebiotix Inc., Roseville, MN, USA; BioRankings LLC, St. Louis, MO, USA

OBJECTIVE: To determine whether 16s rRNA analysis could be used as a predictive biomarker in the assessment of the placebo response to a microbiome-based drug targeted at recurrent *C. difficile* infection.

BACKGROUND: Disruption of the gut microbiota has been demonstrated as a risk factor for *C. difficile* infection (CDI). Novel therapies aim to restore the gut microbiota to its pre-disease state as a protective factor against recurrence. Findings from recent controlled studies of fecal microbiota transplant have raised questions about a placebo response. We explored the potential of 16s rRNA analysis as a predictive biomarker.

METHODS: PUNCH CD 2 was a randomized placebo controlled study of RBX2660, a microbiota-based drug manufactured from live human-derived microbes and targeted at the prevention of recurrent CDI. Of the 133 patients in the study, a total of 44 patients were randomized to receive 2 doses of placebo. Longitudinal 16s rRNA analysis was performed on patient stool after the second dose using the Illumina MiSeq platform. The variable region V4 was targeted to identify the operational taxonomic units (OTUs) in each sample. We report the results of the first 20 consecutive patients in the placebo arm.

RESULTS: Of the 20 sequenced patients (55% female; mean age: 59.2 years), 8 experienced recurrent CDI symptoms prior to 56 days; 12 patients did not. At baseline, the microbiome profiles of the 20 patients were similar. At 7 and 30 days, OTU analysis showed increased divergence between patients who went on to further recurrence and those who did not. *Clostridiales* and *Enterobacteriales* predominated at all time points. Overall OTU analysis demonstrated a slight but non-significant variation between the two groups.

CONCLUSIONS: In this randomized controlled study of RBX2660 for recurrent CDI, 16s rRNA analysis was not predictive of which patients receiving placebo had a further recurrence of CDI symptoms and which did not. Further analysis of the entire cohort is needed to determine the possibility of a predictive analytical method.