Developing Microbiome Rehabilitation Biomarkers for *Clostridium difficile* Infections: Continued Evaluation of a Prototype Microbiome Health Index™

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Background
A healthy intestinal microbiome is important to human health, and microbiome disruption (dybiosis) is linked to many diseases, most notably recurrent *Clostridium difficile* infections (rCDI). This has spurred efforts to develop FDA-approved microbiota-based drugs to restore a healthier microbiome, including RBX2660, a standardized microbiota-based therapeutic with demonstrated clinical efficacy for preventing rCDI. Because clear biomarkers of microbiome restoration have not been established, we have undertaken evaluation of unidimensional Microbiome Health Indices (MHI). Herein we evaluate pooled MHI data from two Phase 2 clinical trials of RBX2660.

Methods
MHI is based on the association of Bacteroidia and Clostridia with colonization resistance and Gammaproteobacteria and Bacilli with dysbiosis. MHIs were calculated from sequencing data from patient fecal samples and RBX2660 product samples from a randomized, blinded, placebo-controlled Phase 2B trial and from an open-label Phase 2 trial of RBX2660 to prevent rCDI. Data from the Phase 2B trial were based on 16S sequencing and data from the open-label trial were based on shallow shotgun sequencing. MHI values from the two trials were compared and pooled. Receiver operator characteristic (ROC) analysis was used to define an MHI cut-point for distinguishing rCDI subjects prior to treatment (baseline) from the RBX2660 drug product. Post-treatment MHIs from patients were assessed longitudinally and by outcome.

Results
Baseline and RBX2660 MHI values were not significantly different between the two trials, despite data derivation from different sequencing methods ($p>.05$). ROC analysis indicated that the pooled baseline samples could be distinguished from the pooled RBX2660 profile with a maximum likelihood ratio of 121 (AUC=0.99, sensitivity=0.96, specificity=0.99, cutpoint=8.2). Among patients who responded to treatment, MHIs were significantly higher 7±4 days after treatment ($p<.001$) with 58% of responders having an MHI>8.2. Among patients who failed treatment, MHIs were also significantly higher 7±4 days after treatment ($p<.001$), but only 21% were above the MHI=8.2 cutpoint. More importantly, MHI of successes could be distinguished from failures at 7±4 days post-treatment ($p=.003$, Wilcoxon test).

Conclusion
MHI values pre- and post-RBX2660 treatment are consistent across two controlled Phase 2 clinical trials and using two different sequencing methods, suggesting potential for generalized utility. MHI can effectively distinguish patients with dysbiosis from healthier patients. Significant MHI increases can be measured post-treatment, and in this analysis MHI can differentiate successes from failures at 7±4 days post-treatment. These results generate prospectively evaluable hypotheses for future clinical trials and emphasize the value of a unidimensional MHI.

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Keywords: *Clostridium difficile* infection, RBX2660, microbiome rehabilitation

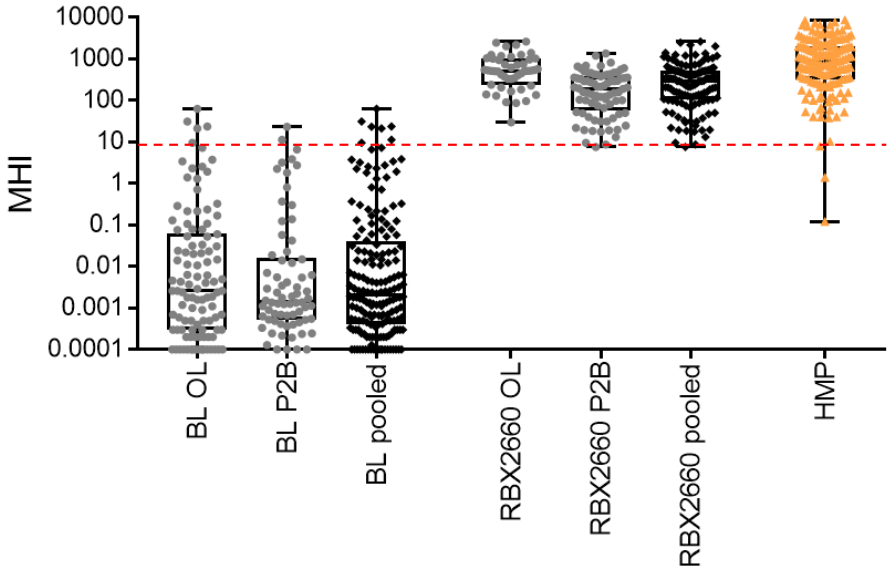

Figure 1. MHI values for pre- and post-treatment samples, RBX2660, and HMP. MHI cutpoint for distinguishing baseline from RBX2660 is shown as a hashed red line.