RBX Abstract for ACG2017

Developing Microbiome Rehabilitation Biomarkers for *Clostridium difficile* Infections: Evaluation and Plan of a Prototype Microbiome Restoration Index™

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Introduction
A healthy intestinal microbiome is important to human health, and microbiome disruption (dybiosis) is linked to many diseases, most notably recurrent *Clostridium difficile* infection (rCDI). This has spurred efforts to develop FDA-approved microbiome-based drugs to treat dysbiosis. Clinical efficacy for preventing rCDI has been demonstrated for some of these drugs, including RBX2660, a standardized microbiome-based therapeutic. However, quantitative biomarkers for microbiome restoration have not been established. Herein, we outline the challenges, preliminary data, and plan for developing a prototype Microbiome Rehabilitation Index™ (MRI™) as a qualifiable biomarker.

Methods
Gap analysis was conducted to map whether existing measures of microbiome data could meet FDA biomarker qualification criteria. As part of this analysis, reported microbiome data from rCDI clinical trials were assessed, including data from a double-blind, randomized, placebo-controlled Phase 2B trial of RBX2660, to determine if a clear relationship exists between microbiome changes and clinical outcome. Based on tentatively identified commonalities, a prototype MRI™ was formulated and evaluated for capability to predict rCDI trial outcomes.

Results
A key gap is standardization of data collection, reporting, and analysis tools. Sample extraction and 16S sequencing methods have progressed to the point that rapid and inexpensive protocols can be designed, but consensus tools for multidimensional data comparison are not established and would not be simple or rapid. A unidimensional parameter that captures key elements of multidimensional data could be an alternative. Toward that aim, rCDI patients who responded to RBX2660 treatment had increased *Bacteroidia* and *Clostridia* and decreased *Bacilli* and *Gammaproteobacteria* compared to baseline, changes that are consistent with reports for other rCDI therapies. Based on these data, a prototype MRI™ was constructed that could differentiate untreated rCDI patients from healthy populations (Fig 1A) and quantify a significant change as early as 7 days after treatment (Fig 1B).

Discussion
This prototype MRI™ is a promising model for a unidimensional measure of microbiome restoration, but significant refinement will be needed and may include other taxa, differential weighting of taxa, and/or diversity metrics. This framework enables prospective hypothesis testing in future trials toward qualification of biomarkers for microbiome rehabilitation.

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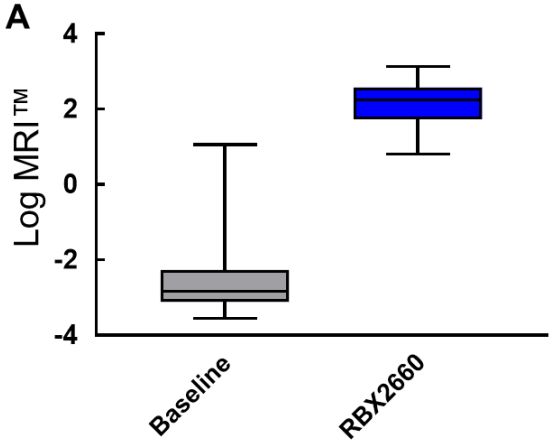
Figure 1. A) MRI™ from enrolled rCDI patients before treatment (baseline, grey) in a Phase 2B trial of RBX2660 and MRI™ of RBX2660 product consortium (RBX2660, blue). B) MRI™ values for a healthy population as defined by the Human Microbiome Project (HMP) and for 7, 30, and 60 days after RBX2660 treatment. The asterisk (*) indicates values that are significantly different from the baseline MRI™ (*P*<0.001), based on a Wilcoxon test.

A



B

Population	n	MRI™, median (IQR)
Baseline	24	0.001 (0.001, 0.003)
RBX2660 product	84	178 (56, 367)*
HMP	215	705 (322, 1356)*
7d	35	9 (0.4, 73)*
30d	27	16 (5, 89)*
60d	27	61 (7, 177)*