Developing Microbiome Rehabilitation Biomarkers for *Clostridium difficile* Infections: Evaluation and Plan of a Prototype Microbiome Health Index™ (MHI™)

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Rebiotix® Microbiota Restoration Therapy

Poster P87

BACKGROUND

- Because *Clostridium difficile* infections (CDI) are strongly associated with dysbiosis, or disruption of a healthy microbiome, there are several clinical development programs in progress to develop FDA-approved microbiome-based drugs. However, quantitative biomarkers for microbiome dysbiosis and/or restoration have not been established.
- In the PUNCH CD2 Phase 2B trial, a single dose of RBX2660, a standardized microbiota-based product, performed significantly better than placebo, and microbiome analysis indicated that RBX2660 restored a healthier microbiome, as defined by the RBX2660 product profile and the Human Microbiome Project (HMP).
- Herein, we outline and evaluate a unidimensional index, the Microbiome Health Index (MHI), that describes microbiome rehabilitation among patients from that trial.

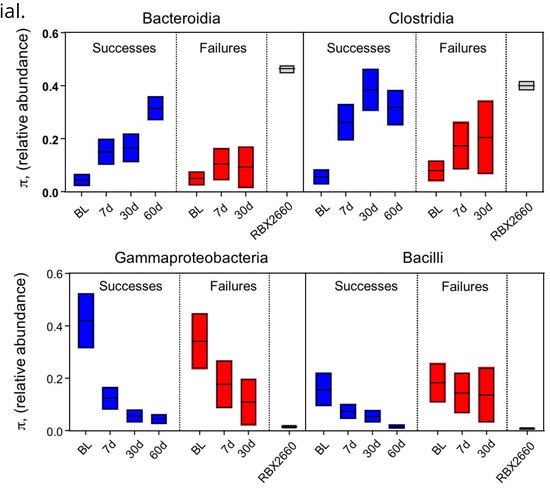
METHODS

- Included in this analysis are 84 RBX2660 product samples and 154 stool samples collected at the indicated time points from 58 patients with recurrent CDI who received at least one blinded dose of RBX2660 as part of the PUNCH™ CD2 Phase 2B trial. In that trial, success was defined as the absence of CDI at 8 weeks after the last blinded treatment.
- 16s rRNA analysis using the Illumina MiSeq platform was performed on stool samples, targeting the variable region V4 to identify the operational taxonomic units (OTUs) in each sample.

PUNCH™ CD2 CLINICAL TRIAL

In a separate analysis, we presented the following conclusions:

- Bacteroidia/Clostridia consistently increased after treatment, whereas Gammaproteobacteria/Bacilli consistently decrease. These changes were more pronounced among successes than failures. A similar trend was observed in our Phase 2 open-label trial.



Because Bacteroidia and Clostridia are associated with colonization resistance, whereas high levels of Gammaproteobacteria or Bacilli are associated with microbiome disruption or dysbiosis, we combined the relative abundances of these four taxonomic classes into a unidimensional mathematical expression:

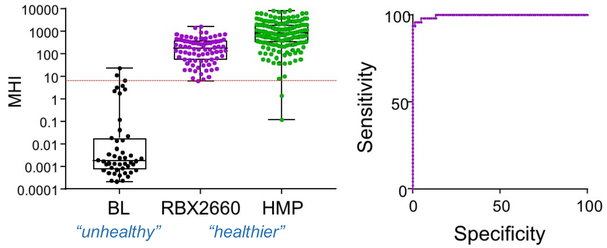
Microbiome Health Index (MHI)

a unidimensional expression of changes in four taxonomic classes known to have relevance to microbiome health and colonization resistance

$$\frac{(\text{RA, Bacteroidia} + \text{RA, Clostridia})}{(\text{RA, Gammaproteobacteria} + \text{RA, Bacilli})}$$

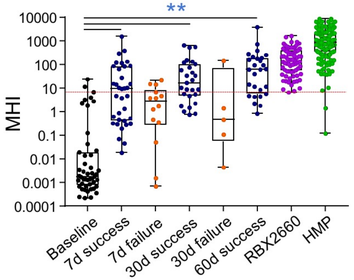
RA = relative abundance of each taxonomic class

MHI can distinguish between baseline "unhealthy" and "healthier" microbiota



Receiver Operating Characteristic (ROC) analysis of baseline vs RBX2660 yielded an area under the curve (AUC) of 0.996 and an optimal cut-off point of MHI = 7.1 (red dotted line; sensitivity = 0.96, specificity = 0.99, likelihood ratio = 0.8).

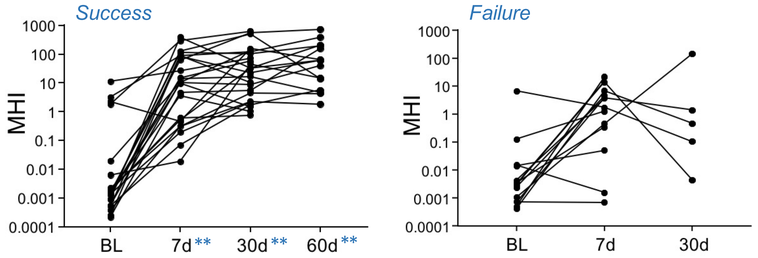
MHI differs among successful and failed response to RBX2660



	n	Median MHI	Fraction of patients >7.1	p vs BL
BL	47	0.0018	0.04	NA
7d success	35	9.4	0.51	<0.001
30d success	27	16	0.67	<0.001
60d success	27	61	0.74	<0.001
7d failure	13	1.7	0.23	0.057
30d failure #	5	0.47	0.20	NA
RBX2660	84	182	0.99	<0.001

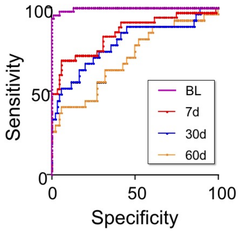
Treatment failures prior to 56d primary endpoint received open-label treatment with RBX2660; no further samples were included after open-label treatment.

Successful patients show continual MHI increase



*** p <0.001 by Sign pairwise test compared to baseline . MHI at 7d is not significantly different among successes and failures (p=0.24, Kolmagorov-Smirnov test).*

High MHI correlates to MHI of RBX2660-treated microbiota



Analysis of ROC curves at each time point confirms that patient microbiota converge toward and are less distinguishable from RBX2660 product over time.

Comparison to RBX2660 product	Sensitivity	Specificity	Odds Ratio	AUC
BL	96	99	80	0.996
7d success	49	99	41	0.850
30d success	33	99	28	0.79
60d success	26	99	22	0.700

This analysis highlights the potential for defining MHI as a continuous rather than binary metric

CONCLUSIONS

- MHI can effectively distinguish patients with dysbiosis from healthier patients, as defined by the RBX2660 product profile and the Human Microbiome Project.
- MHI significantly increased as early as 7d in responders compared to baseline, and continued to increase at 30d and 60d post-RBX2660 treatment.
- MHI did not significantly increase at 7d in failures compared to baseline.
- Future efforts will seek to determine whether more specific taxonomic characterization below the class level and/or inclusion of diversity metrics provide a more precise index.
- Most importantly, we will evaluate MHI prospectively in ongoing clinical trials as an exploratory endpoint.