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

Poster Tu1879

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## 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 rCDI 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 CD 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.

| | Phase 2B (PUNCH CD2™) Randomized, blinded, placebo-controlled | | Phase 2 OL (PUNCH Open Label™) Open label with historical control |
|---|---|---|---|
| **Treatment** | **RBX2660** | **Placebo** | **RBX2660** |
| Total participants | 83 | 44 | 136 |
| Responders (Rate) | 53 (64%) | 20 (45%) | 107 (79%) |

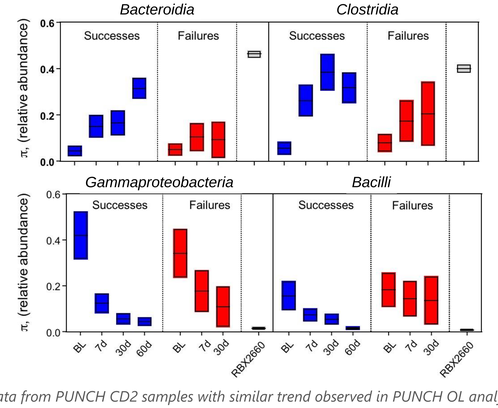
**Samples included in this analysis**

| | PUNCH CD2 | | | | PUNCH Open Label | |
|---|---|---|---|---|---|---|
| **Treatment** | **RBX2660** | | **Placebo** | | **RBX2660** | |
| # Baseline samples | 47 | | 23 | | 106 | |
| **7-day post treatment samples** | | | | | | |
| | Success | Failure | Success | Failure | Success | Failure |
| # Samples | 35 | 14 | 10 | 10 | 60 | 8 |

## 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 (CoreBiome, 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 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




*Data from PUNCH CD2 samples with similar trend observed in PUNCH OL analysis.*

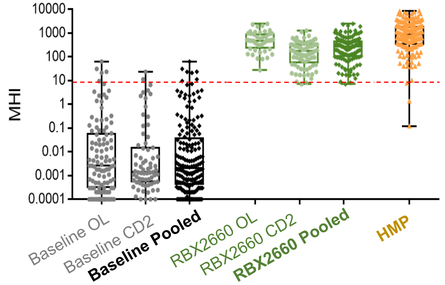
Because *Bacteroidia* and *Clostridia* are associated with colonization resistance, whereas high levels of *Gammaproteobacteria* or *Bacilli* are associated with microbiome disruption or dysbiosis, we combined the relative abundances of these four taxonomic classes into a unidimensional mathematical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

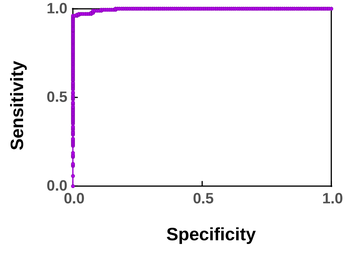
### Microbiome Health Index (MHI)

a unidimensional algorithm which captures changes in the relative abundance of taxonomic classes known to have relevance to microbiome health and colonization resistance

## MICROBIOME HEALTH INDEX (MHI) ANALYSIS

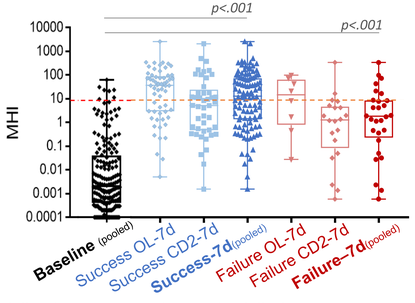
### MHI of "unhealthy" and "healthier" microbiota to define a putative efficacy cutpoint

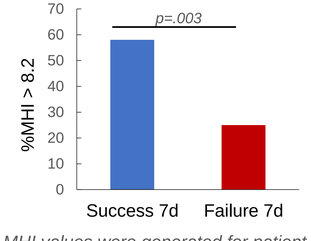



*MHI values for baseline and RBX2660 product did not differ between the Phase 2 studies, confirming pooling of values at each time point. Receiver Operating Characteristic (ROC) analysis of baseline (BL) vs RBX2660 yielded an area under the curve (AUC) of 0.996 and an optimal cut-off point of MHI=8.2 (red dotted line; sensitivity=0.96, specificity=0.99, likelihood ratio=0.8)*

### MHI determined at 7 days post-RBX2660 differs between responses and failures determined at 8 weeks



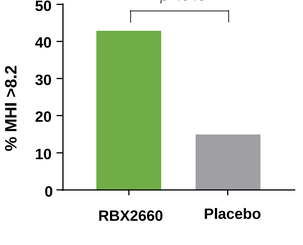




| | n | Median MHI | MHI >8.2 fraction | *p* vs BL |
|---|---|---|---|---|
| BL | 176 | 0.0023 | 0.04 | NA |
| 7d success | 106 | 11.1 | 0.58 | <.001 |
| 7d failure | 32 | 1.8 | 0.25 | <.001 |
| RBX2660 | 127 | 297 | 0.99 | <.001 |

*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, MHIs were also significantly higher 7±4 days after treatment (p<.001), but only 25% 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)*

### MHI during early treatment may provide a Microbiome Efficacy Endpoint that predicts clinical outcome

*Using MHI>8.2 as an exploratory endpoint (retrospective analysis), RBX2660 was superior to placebo, which concurs with clinical outcome*




## CONCLUSIONS

- MHI is agnostic from sequencing method and can effectively distinguish patients with dysbiosis from healthier patients.
- Retrospective MHI evaluation at 7 days post-treatment could establish a putative Microbiome Efficacy Endpoint that may predict 8-week clinical response.
- Future efforts will determine whether more specific taxonomic characterization below the class level and/or inclusion of diversity metrics provide a more precise index. Also, we will eva MHI prospectively in ongoing clinical trials as an explora endpoint.