MICROBIOME AND BILE ACID RESTORATION WAS CONSISTENT ACROSS THREE CLINICAL TRIALS OF RBX2660 FOR RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION: A COMBINED ANALYSIS

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FERRING PHARMACEUTICALS

INTRODUCTION

- Recurrent *Clostridioides difficile* infections (rCDI) are an urgent public health threat associated with disruption of the microbiome
- RBX2660 is a microbiota-based investigational live biotherapeutic that has been evaluated in >600 clinical trial participants for reducing recurrence of rCDI
- Here we report the combined microbiome and metabolomic analysis of participants in three trials of RBX2660
- This analysis determined the correlations of clinical response with
  - microbiome composition and diversity
  - a recently published Microbiome Health Index (MHI-A) biomarker of post-antibiotic dysbiosis and restoration [1]
  - bile acid (BA) compositions, given BA directly impact the life cycle of *C. difficile (C. diff)* through multiple and complex mechanisms, including stimulation of *C. diff* germination by conjugated primary BA and inhibition of *C. diff* outgrowth by deconjugated secondary BA [2]

STUDY DESIGN

- This analysis included samples from RBX2660- or placebo-treated participants in the randomized, double-blind, placebo-controlled trials PUNCH CD2 (NCT02589847; n=153) [3], PUNCH CD3 (NCT03244644; n=887) [4] , and from RBX2660-treated participants in the PUNCH OLS (NCT03931941; n=653) [5]
- Clinical response was defined as the absence of CDI recurrence at eight weeks after treatment
- Participants who opted in submitted stool samples prior to blinded study treatment (BL), 1, 4 and 8 weeks and up to 24 months after study treatment
- Samples were sequenced using shallow shotgun methods. Operational taxonomic unit (OTU) data were used to calculate
  - relative taxonomic abundance
  - the Microbiome Health Index™ (MHI-A)—a microbiome biomarker of post-antibiotic dysbiosis and restoration

Microbiome Health Index (MHI-A) [1]

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

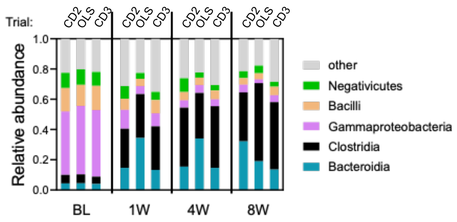
- A liquid chromatography mass spectrometry method [6] was used to quantify 33 bile acids for participant samples from PUNCH CD2 and CD3

CONCLUSIONS

1. **There is a totality of evidence that clinical response to microbiota-based investigational live biotherapeutic RBX2600 in rCDI patients is associated with restoration of microbiome and bile acid compositions**
2. **RBX2660-associated restorative changes are characteristic of shifts from an antibiotic-induced dysbiosis to a healthier state**

Figure 1

CLINICAL RESPONSE TO RBX2660 WAS ASSOCIATED WITH A SHIFT IN MICROBIOME COMPOSITION

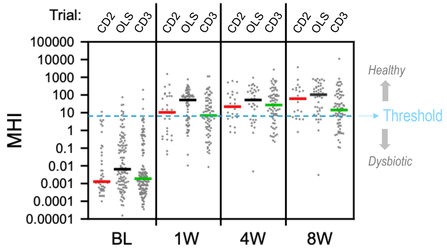


Relative to baseline, microbiome composition shifted significantly in treatment responders for all three trials. In all three trials, Bacteroidia and Clostridia were increased and Gammaproteobacteria and Bacilli decreased after treatment.

- *In all three trials, baseline was characterized by predominance of Gamma-proteobacteria and Bacilli*
- *Post-treatment shifted to predominance of Clostridia and Bacteroidia (healthy commensals) as early as 1W*
- *The shifts to Clostridia and Bacteroidia were maintained at the 8W efficacy endpoint as well as to the end of the follow-up sampling periods (6M for CD2 and CD3; 24M for OLS) (not shown)*

Figure 2

CLINICAL RESPONSE TO RBX2660 WAS ASSOCIATED WITH RESTORATION OF MHI-A

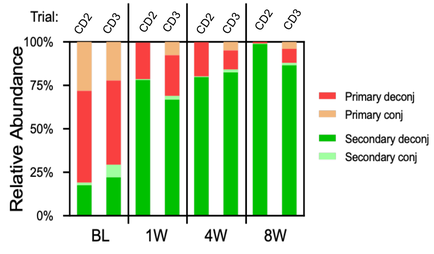


MHI-A is a biomarker of antibiotic-induced dysbiosis and restoration.[1] In all three trials, MHI-A was restored from dysbiotic (below threshold) to healthy (above threshold) levels.

- *The MHI threshold is determined by Receiver Operated Characteristic curves and indicates high diagnostic accuracy (AUROC = 0.99) [1]*
- *Participant samples are predicted to be healthy or dysbiotic if above or below the cutoff threshold, respectively*
- *Before treatment, majority and median of samples from all trials were below cutoff*
- *As early as 1W after treatment the majority and medians had shifted above the cutoff and maintained to the 8W efficacy endpoint as well as to the end of the follow-up sampling periods (6M for 2 and 3; 24M for OLS) (not shown)*

Figure 3

CLINICAL RESPONSE TO RBX2660 WAS ASSOCIATED WITH RESTORATION OF SECONDARY BILE ACIDS

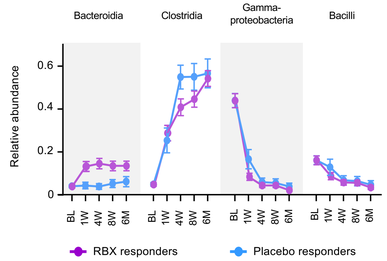


Primary bile acids (BA) produced by the liver are metabolized to secondary by healthy microbiota. In PUNCH CD2 and CD3, bile acid compositions were restored from primary BA predominance before to secondary BA predominance after treatment —a composition more resistant to *C. difficile* colonization and infection.

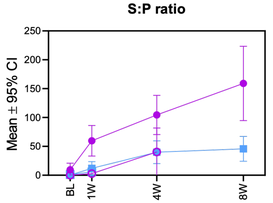
- *BA composition can influence C. diff colonization: several primary BA stimulate germination; several secondary BA repress C. diff growth*
- *Before treatment, primary BA were predominant, consistent with a dysbiotic state*
- *As early as 1W after, there was a shift to predominance of secondary BA, which continued and was maintained to the 8W efficacy endpoint*

Figure 4

GREATER RESTORATIVE SHIFTS IN RESPONDERS ARE ASSOCIATED WITH RBX2660 TREATMENT



RBX2660-treated responders demonstrated more extensive recovery of Bacteroidia and decreased Gammaproteobacteria relative to placebo-treated responders.



Predictors	p-value
Outcome (Responder and Non-responder)	0.00033
Treatment (RBX2660 and Placebo)	0.02575

RBX2660 leads to significant secondary: primary BA (S:P) ratio increase relative to non-responders and placebo-treated responders

- *S:P ratio was restored earlier and to a greater extent among RBX2660 responders than PBO responders*
- *S:P ratio restoration was maintained to the 8W efficacy endpoint*
- *This is consistent with the microbiome restoration and indicates a likely mechanism why RBX2660 showed greater clinical response*

REFERENCES

[1] Blount et al. (2022) Frontiers in Microbiology 2022 Jan 4;12:781275. doi: 10.3389/fmicb.2021.781275. [2] Mullish & Allegretti (2021) 14:17562848211017725 [3] Dubberke et al. (2018) Clinical Infectious Diseases 67(8):1198-204. [4] Lee et al. (2021) Digestive Disease Week, Oral Session 2155 [5] Kraft et al. (2021) Digestive Disease Week, Poster Sa611 [6] Ferdyan et al. (2020) *Open Forum Infect Dis.* 7(Suppl 1): S15–S16.