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Ridinilazole Reduces Recurrence of *Clostridium difficile* Infection with Minimal Impact on the Gut Microbiota

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Introduction

- *C. difficile* infection (CDI) is one of 3 urgent antibiotic-resistant bacterial threats in the U. S., according to the CDC, and is the leading cause of hospital-acquired diarrhoea.
- A major clinical challenge is the high risk of recurrence (up to 35% following initial infection), with dysbiosis of the gut microbiome by mainstay CDI antibacterial therapy options a contributing factor.
- Ridinilazole (RDZ) is a novel, oral, antibacterial that is highly selective for *C. difficile* that has shown superior efficacy on Sustained Clinical Response (SCR), through a reduction of recurrent CDI and limited damage to the gut microbiome, compared to vancomycin (VAN), in a Phase 2 clinical trial.

Methods

- ***In vitro* Susceptibility:** Minimum inhibitory concentrations (MICs) against *C. difficile* were determined by agar dilution on Wilkins-Chalgren plates after 48h incubation at 37ºC, or, on Brucella plates following the CLSI guidelines M11-A7/A8. For the panel of bacteria, MICs were determined by agar dilution according to CLSI guidelines M11-A8 for the anaerobes and M7-A9 for the facultative and aerobic organisms.
- **Multi-center, Double-blind Phase 2 Clinical Trial:** One hundred subjects were randomized 1:1 to RDZ (200 mg BID) or VAN (125 mg QID) treatment for 10 days. Primary endpoint was SCR, defined as clinical response at end of therapy (EOT) with an absence of recurrence for the next 30 days. Recurrence was defined as signs and symptoms of CDI, a positive diagnostic test and the need for antimicrobial therapy. Primary analysis population was the modified intent-to-treat (MITT), which included all randomized subjects with a diagnosis of CDI by presence of free toxin in faeces
- **Microbiome Analysis:** Stool samples from 22 patients in each treatment arm were collected at study entry and at EOT, and, control stools were obtained from healthy volunteers. Bacterial DNA was extracted and amplicons of the V4 region of the 16SrRNA gene were generated by PCR, pooled in equimolar amount and sequenced. Taxonomic identification, OTU tables and beta diversity calculation were done in the software QIIME. The LEfSe algorithm was used to identify differences in microbiota composition between baseline and EOT. For the beta diversity, principal coordinate analysis was derived from weighted Unifrac distances between samples at baseline and EOT from subjects receiving ridinilazole or vancomycin, as well as healthy controls.

Results: In vitro *susceptibility*

- Ridinilazole demonstrated potent activity against 439 *C. difficile* clinical isolates with an overall MIC_{90} markedly lower than metronidazole and vancomycin, and, similar to fidaxomicin.
- There was no variation in activity by geographic region, antibiotic resistance profile or ribotype.

Table 1: Susceptibility of C. difficile Clinical Isolates to Ridinilazole and Comparator Antibiotics

		MIC range (mg/ml)	MIC_{90} (mg/ml)			
Study (Source of isolates)	N	RDZ	RDZ	VAN	MTZ	FDX
Corbett et al. 2015 (UK)[1]	82	0.06-0.25	0.125	2	8	0.06
Freeman et al. 2015 (EU)[2]	107	0.015-0.5	0.125	2	2	0.125
Goldstein et al. 2013 (US)[3]	50	0.125-0.5	0.25	4	2	0.5
Snydman et al. 2017 (US)[4]	200	0.125-0.5	0.25	2	1	0.5

RDZ: ridinilazole; VAN: vancomycin; MTZ: metronidazole; FDX: fidaxomicin

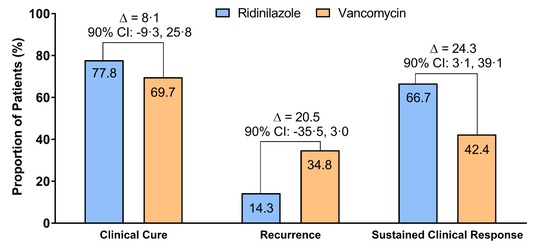
- Ridinilazole and fidaxomicin showed limited activity against Gram-negative anaerobes, especially against the Bacteroidetes, a dominant phylum of the human gut microbiome.
- Ridinilazole also exhibited limited activity against Gram-positive anaerobes compared to other agents.

Table 2: Spectrum of Activity

Bacteria (number of isolates)	RDZ MIC range (µg/ml)	MIC_{90} (µg/mL)			
		RDZ	FDX	VAN	MTZ
Bifidobacterium spp. (20)	16->512	>512	0.125	1	128
Lactobacillus spp. (20)	0.06->512	>512	>512	>512	>512
Eggerthella lenta (20)	>512	>512	≤0.03	4	0.5
Finegoldia magna (20)	0.03-512	64	2	0.5	1
Peptostreptococcus anaerobius (20)	0.125–128	64	≤0.03	0.5	1
Staphylococcus aureus (10)	>512	>512	16	1	>512
Enterococcus faecalis (10)	128->512	>512	8	4	>512
Enterococcus faecium (10)	64->512	128	8	256	>512
Streptococcus spp. (10)	0.5->512	>512	128	1	>512
Bacteroides spp. (50)	128->512	>512	>512	128	2
Parabacteroides spp. (10)	512->512	>512	>512	128	2
Fusobacterium spp. (20)	4->512	>512	>512	>512	0.5
Prevotella spp. (23)	32->512	>512	>512	512	1

Results: Clinical efficacy

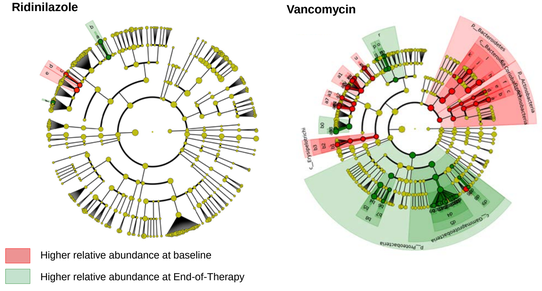
Figure 1: Clinical Cure and Sustained Clinical Response (SCR) in the Primary Analysis Population



- Ridinilazole demonstrated superiority (pre-specified 10% 2-sided) for SCR compared with vancomycin.
- The improved SCR rate was driven by a marked reduction in the rate of recurrent CDI.

Results: Gut Microbiome

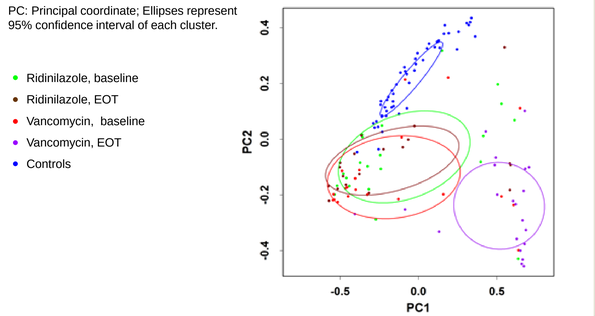
Figure 2: Effects of Vancomycin and Ridinilazole on Relative Abundance of Taxa at Baseline vs EOT



- Vancomycin treatment resulted in dramatic losses in Bacteroidetes (>3log), the most abundant phylum at baseline, in 4 families of the Firmicutes, including the Lachnospiraceae and Ruminococcaceae (>2 log), and in Actinobacteria (70% reduction).
- Simultaneously, an increase in Proteobacteria was observed, primarily in Enterobacteriaceae (> 2 log).
- In contrast, ridinilazole's effect was confined to the Firmicutes, with loss of *C. difficile* as well as modest decreases in the Clostridiaceae and Ruminococcaceae families.
- Beta diversity analyses confirmed that ridinilazole's effect on community structure was minimal, while vancomycin's was profound.

Figure 3: Beta diversity between Subjects Receiving Ridinilazole or Vancomycin, and Controls.

PC: Principal coordinate; Ellipses represent 95% confidence interval of each cluster.



Conclusions

- **Ridinilazole demonstrated potent activity against *C. difficile* clinical isolates with no major variation by geographic region, ribotype or antibiotic resistance phenotype.**
- **Ridinilazole effectively targets *C. difficile* in CDI patients with minimal impact on the gut microbiota.**
- **Preservation of the microbiome likely contributed to the low rate of recurrence, and superior efficacy on SCR, compared to vancomycin.**
- **These data provide evidence of ridinilazole being a microbiome sparing agent to both treat CDI and reduce the recurrence of CDI. Further clinical development is warranted.**

References: 1. Corbett, D., et al. (2015) J. Antimicrob. Chemother.; 2. Goldtein E.J. et al. (2013) Antimicrob Agents Chemother.; 3. Freeman J. et al. (2015) Antimicrob Agents Chemother.; 4. Snydman D.R. et al. (2017) ASM poster # 235. 5; Vickers, R. J., et al. (2017). Lancet Infect Dis.; 6. Thorpe C. M. et al. (2017) ECCMID poster # 1177.

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