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Characterization of Stool Microbiota in Patients With Diarrhea-Predominant Irritable Bowel Syndrome (IBS-D) Receiving Repeat Treatments With Rifaximin in the TARGET 3 Study

Mark Pimentel, MD[1]; Anthony A. Fodor, PhD[2]; Pamela L. Golden, PhD[3]; Enoch Bortey, PhD[3]; William P. Forbes, PharmD[3]

[1]Cedars-Sinai Medical Center, Los Angeles, CA, USA; [2]University of North Carolina at Charlotte, Charlotte, NC, USA; [3]Salix, a Division of Valeant Pharmaceuticals North America LLC, Bridgewater, NJ, USA

INTRODUCTION

- Patients with irritable bowel syndrome (IBS) have qualitative and quantitative alterations in the gut microbiota compared with healthy individuals[1-3]; therefore, targeting the gut microbiota may be an effective treatment for diarrhea-predominant IBS (IBS-D)
- Rifaximin, an oral, minimally absorbed antimicrobial agent, significantly improved IBS-D symptoms versus placebo in three phase 3, randomized, placebo-controlled studies (TARGET 1, 2, and 3)[4,5]
- Rifaximin inhibits bacterial ribonucleic acid (RNA) synthesis by binding to the β-subunit of bacterial deoxyribonucleic acid (DNA)-dependent RNA polymerase[6]; rifaximin also has been shown to alter bacterial metabolism[7,8] and modulate gastrointestinal (GI) epithelial cell physiology, the latter impacting the ability of some pathogenic bacteria to adhere to the GI lining[9,10]
- However, the potential impact of repeat treatment with rifaximin on the stool microbiota of patients with IBS-D has not been characterized previously

OBJECTIVE

- To assess the effect of rifaximin repeat treatment on the stool microbiota by genomic characterization of the stool bacteria using next-generation sequencing techniques

METHODS

Patient Population

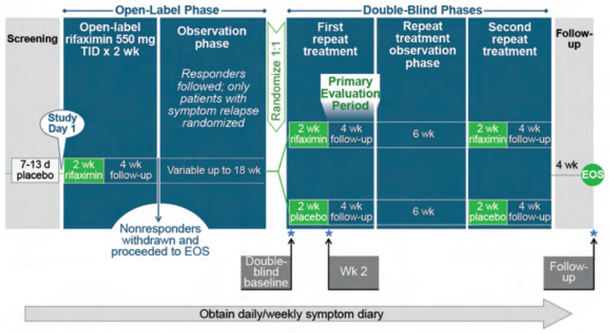
- Adults were eligible who were diagnosed with IBS-D (based on Rome III criteria) with average symptom severity scores during the screening phase of ≥3 for IBS-related abdominal pain and bloating, and stools for ≥2 days per week meeting criteria for Bristol Stool Scale (BSS) type 6 or 7 stool consistency
  - Exclusion criteria included a history of inflammatory bowel disease or having taken antidiarrheals, antispasmodics, narcotics, drugs indicated for IBS, probiotics, or antibiotics within 14 days of study entry
- For stool microbiota analyses, patients were selected randomly for inclusion, regardless of response to therapy

Study Design

- Randomized, double-blind, phase 3, placebo-controlled, multicenter, multinational study
- Patients received open-label rifaximin 550 mg 3 times daily (TID) for 2 weeks, followed by a 4-week treatment-free follow-up period to assess response (Figure 1)
  - A responder was defined as a patient meeting weekly response criteria for both abdominal pain (≥30% decrease from baseline in mean weekly pain score) and stool consistency (≥50% decrease from baseline in number of days/week with BSS type 6 or 7 stool consistency) for ≥2 of 4 weeks during follow-up
  - Nonresponders to open-label rifaximin were withdrawn from the study
- Responders were subsequently followed, treatment-free, until relapse or for up to 18 additional weeks (observation phase)
  - Patients who relapsed (loss of response for either abdominal pain or stool consistency for ≥3 out of a consecutive, rolling 4-week period during the 18-week observation phase) were randomized (1:1) to receive 2 double-blind repeat treatments (two 2-week courses) of rifaximin 550 mg TID or placebo, with repeat courses separated by 10 weeks
- For DNA sequencing, DNA was extracted using the QIAmp® DNA Stool Mini Kit (Qiagen, Germantown, MD); sequencing of the V4 hypervariable region of 16S ribosomal ribonucleic acid (rRNA) gene was performed using the HiSeq 2500 System (Illumina, Inc., San Diego, CA)
- The Ribosomal Database Project classifier (version 2.6) was used to assign forward and backward reads to bacterial families
- Analyses compared double-blind baseline (randomization) samples with those obtained following first repeat treatment and those obtained at the end of the study

METHODS

Figure 1. Study Design



*Stool sample collection for assessment of treatment effect versus placebo.
EOS = end of study.

- Univariate matched pairs *t*-test was used to compare changes from baseline; analysis of covariance was used to assess treatment differences
- The Shannon diversity index was calculated, which considers both richness (abundance) and evenness of the species present; richness and evenness were also individually evaluated
  - **Richness:** number of bacterial species present in a sample, adjusted for different numbers of sequences observed from each sample
  - **Evenness:** score of the relative abundance of the different species (range, 0–1; 1 = complete evenness)
  - **Diversity:** combination of richness and evenness that measures the overall complexity of a community

RESULTS

- 103 patients were randomly selected for inclusion in the stool microbiota analysis (Table); demographics were similar for patients who relapsed and were subsequently randomized to the double-blind repeat treatment phase with rifaximin (n = 37) or placebo (n = 36)
- For the paired end reads, ~89% of the pairs produced a matching Ribosomal Database Project call to family from both ends
- At baseline of the first repeat treatment, the Shannon diversity index score was 1.79 in the rifaximin group and 1.73 in the placebo group; after 2 weeks of repeat treatment, diversity index scores were essentially unchanged for rifaximin (1.70; $P = 0.3$) and placebo (1.74; $P = 0.8$), with no significant difference between groups ($P = 0.4$), and remained unchanged during follow-up (Figure 2)
- The stool microbiota richness significantly decreased from double-blind baseline to the end of the first repeat treatment in the rifaximin group (mean Δ -1.2; $P = 0.03$) and versus placebo ($P = 0.02$)—this change appeared to be short-lived and was not observed during follow-up (mean Δ from baseline, 0.1-0.2; $P \geq 0.8$; Figure 3)
  - Stool microbiota richness in the placebo group remained unchanged from baseline at both the end of first repeat treatment (Δ = 0.2; $P = 0.7$) and during follow-up (mean Δ, -0.2-1.1; $P \geq 0.1$)

RESULTS

- No significant changes in stool microbiota evenness were observed between the 2 groups ($P \geq 0.3$; Figure 4)
  - Change from baseline to end of first repeat treatment for rifaximin was 0.0 ($P = 0.2$) and for placebo was 0.0 ($P = 0.9$); during follow-up, changes from baseline were -0.1-0.0 ($P \geq 0.04$) and 0.0-0.0 ($P = 0.9$) for rifaximin and placebo, respectively

Table. Demographics

Characteristic	Double-Blind Population: Rifaximin 550 mg TID (n = 37)	Double-Blind Population: Placebo (n = 36)
Age, y, mean (SD)	46.9 (15.0)	47.7 (14.8)
Sex, male:female (%)	29.7:70.3	27.8:72.2
Race, n (%)		
White	28 (75.7)	32 (88.9)
Black	5 (13.5)	1 (2.8)
Other	4 (10.8)	3 (8.3)
Concomitant antibiotic use, n (%)	6 (16.2)	11 (30.6)

SD = standard deviation.

Figure 2. Change From Baseline in Shannon Diversity Index During Double-Blind Repeat Treatment With Rifaximin

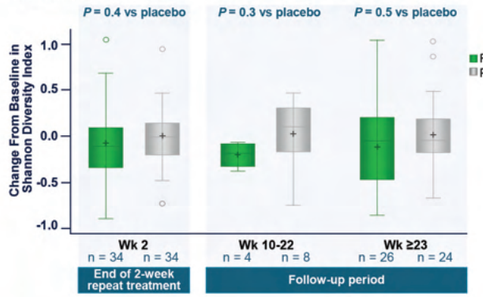


Figure 3. Change From Baseline in Richness of Stool Microbiota During Double-Blind Repeat Treatment With Rifaximin

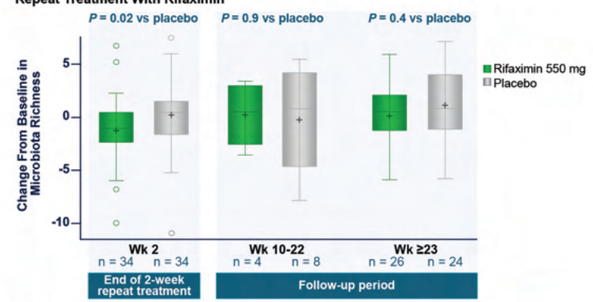
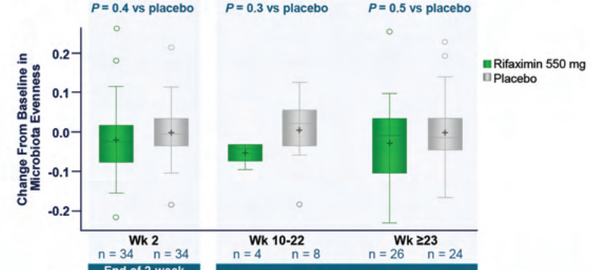


Figure 4. Change From Baseline in Evenness of Stool Microbiota During Double-Blind Repeat Treatment With Rifaximin



CONCLUSION

- **Overall, no sustained disturbance of the stool microbiota was observed in patients with IBS-D who received repeat treatment with rifaximin**

REFERENCES **1.** Kassinen A, et al. *Gastroenterology*. 2007;133(1):24-33. **2.** Carroll IM, et al. *Gut Pathog*. 2010;2(1):19. **3.** Carroll IM, et al. *Neurogastroenterol Motil*. 2012;24(6):521-530. **4.** Lembo A, et al. Presented at: American College of Gastroenterology 2014 Annual Scientific Meeting; October 17-22, 2014; Philadelphia, PA. **5.** Pimentel M, et al. *N Engl J Med*. 2011;364(1):22-32. **6.** Xifaxan® (rifaximin) tablets [package insert]. Raleigh, NC: Salix Pharmaceuticals, Ltd.; 2014. **7.** Bajaj JS, et al. *PLoS One*. 2013;8(4):e60042. **8.** Maccaferri S, et al. *J Antimicrob Chemother*. 2010;65(12):2556-2565. **9.** Brown EL, et al. *Antimicrob Agents Chemother*. 2010;54(1):388-396. **10.** Schrodt C, et al. *PLoS One*. 2013;8(7):e68550.

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