Received: 5 November 2021 | Revised: 30 April 2022 | Accepted: 2 May 2022

DOI: 10.1111/hel.12900

Helicobacter WILEY

ORIGINAL ARTICLE

Efficacy and safety of low-dose rifabutin-based 7-day triple therapy as a third- or later-line *Helicobacter pylori* eradication regimen

Kazumi Inokuchi[1] | Hideki Mori[1,2] | Juntaro Matsuzaki[1,3] | Kenro Hirata[1] | Yosuke Harada[1] | Yoshimasa Saito[1,3,4] | Hidekazu Suzuki[1,5] | Takanori Kanai[1] | Tatsuhiro Masaoka[1,6]

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

[2]Translational Research Center for Gastrointestinal Disorders (TARGID), University of Leuven, Leuven, Belgium

[3]Division of Pharmacotherapeutics, Keio University Faculty of Pharmacy, Tokyo, Japan

[4]Department of Gastroenterology and Hepatology, Kitasato University Kitasato Institute Hospital, Tokyo, Japan

[5]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Tokai University School of Medicine, Isehara, Japan

[6]Department of Gastroenterology and Hepatology, International University of Health and Welfare, Mita Hospital Tokyo, Japan

Correspondence
Tatsuhiro Masaoka, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
Email: masaoka@keio.jp

Funding information
Keio University, School of Medicine

Abstract

Background: Rifabutin-based regimens are used as rescue therapy for refractory *Helicobacter pylori* infection; however, the duration for which treatment is required and side effects are concerning. This study assessed the efficacy and safety of 7-day rifabutin, amoxicillin, and vonoprazan triple therapy as third- or later-line treatment for *H. pylori* infection.

Materials and Methods: Patients who did not respond to second-line therapy were enrolled. After *H. pylori* infection was confirmed with the culture method, the patients received rifabutin-containing triple therapy (20 mg vonoprazan b.i.d., 500 mg amoxicillin q.i.d., and 150 mg rifabutin q.d.) for 7 days. Twelve weeks after the eradication therapy, successful eradication was confirmed using a ^{13}C urea breath test or the *H. pylori* stool antigen test. The results obtained from our previous study that reported a 10-day or 14-day esomeprazole based rifabutin-containing triple therapy as a third- or fourth-line rescue therapy treated patients were used as historical control. We determined the minimum inhibitory concentrations of amoxicillin and rifabutin. We also evaluated whether the patients were positive for the mutation of the *rpoB* gene.

Results: Intention-to-treat and per-protocol analyses showed that our regimen resulted in a high eradication rate (91.2%, 95% CI: 84%–99% and 92.7%, 95% CI: 86%–100%, respectively). Adverse events occurred in 31.6% of the patients, and two patients discontinued the therapy.

Conclusions: This is the first study to evaluate the efficacy and safety of a 7-day low-dose rifabutin-based triple therapy with vonoprazan and amoxicillin. Our results suggest that our regimen was effective and safe as a third- or later-line *H. pylori* eradication regimen. To clarify what component in this regimen are critical, subsequent studies using a factorial design (comparing vonoprazan-amoxicillin dual therapy vs. vonoprazan-rifabutin triple therapy) will be needed.

KEYWORDS
amoxicillin, potassium-competitive acid blocker, rescue therapy, rifabutin, *rpoB*, vonoprazan

1 | INTRODUCTION

Helicobacter pylori infection causes gastric cancer, duodenal or gastric ulcers, and gastric mucosa-associated lymphoid tissue lymphoma,[1] and its eradication has been shown to reduce the risk of upper gastrointestinal tract diseases.[1,2] Although the prevalence of *H. pylori* infection has decreased in some countries in recent years,[3] the *H. pylori* infection has been reported to develop in more than half of the global population.[4] Moreover, the increased antimicrobial resistance has become a major challenge for eradicating *H. pylori* worldwide.[5–8]

Rifabutin (RFB)-based treatment has been reported to be efficacious as a rescue therapy,[9–12] and recently, its potential as an effective first-line therapy was reported.[12] Regarding RFB-based regimens, most previous studies used RFB for 7–14 days.[13] We also previously reported the efficacy of 10- or 14-day eradication therapy with esomeprazole (EPZ), amoxicillin (AMX), and RFB as a third- and fourth-line regimen (hereinafter, called 10- and 14-day EAR, respectively). The eradication rate in the 14-day EAR group was higher than that in the 10-day EAR group (94.1% vs. 83.3%).[15] However, there were safety problems, in that 75.0% and 94.1% of the patients in the 10- and 14-day groups, respectively, showed adverse events, and the corresponding percentages of patients who withdrew treatment due to adverse events were 8.3% and 29.4%.[14]

Since 2015, vonoprazan (VPZ), a novel potassium-competitive acid blocker (P-CAB), has been available in Japan, and it can produce rapid, strong, and long-lasting gastric acid inhibition after its administration.[15,16] The safety of using VPZ compared with proton pump inhibitors (PPIs) has also been reported.[17] It was recently reported that VPZ-containing regimens achieved a higher eradication rate than the PPI-containing regimens as first-, second-, and third-line treatments.[15,16,18–20] Moreover, dual VPZ/ AMX therapy has been shown to be sufficient to eradicate *H. pylori*.[21]

Considering the evidence obtained thus far, in this study, we adjusted the dosage and duration of RFB and assessed the efficacy and safety of 7-day triple therapy with VPZ, AMX, and low-dose RFB as third- to seventh-line regimens for *H. pylori* eradication. Furthermore, we analyzed the correlation between the status of *rpoB* mutations associated with RFB resistance and the successful eradication of *H. pylori*.

2 | MATERIALS AND METHODS

2.1 | Study population

This prospective open-label study was conducted at Keio University Hospital (Tokyo, Japan) between June 2016 and February 2021. It was approved by the Institutional Review Board of the Keio University School of Medicine (jRCTs031180362) and registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000021928). Patients aged 20 years or higher and in whom eradication regimens had failed at least twice were included. The previous eradication regimens used were as follows. The first-line regimen consisted of PPI/P-CAB, AMX, and clarithromycin (CLR); the second-line regimen consisted of PPI/P-CAB, AMX, and metronidazole (MTZ). The third- to sixth-line eradication regimens were different in each case; however, RFB was not included in any previous regimens. Patients who were allergic to rifamycin, PPIs, or penicillin; had a history of tuberculosis or *Mycobacterium avium* complex infection, uveitis, or severe hepatic or renal impairment; pregnant; or taking voriconazole were excluded.

2.2 | Study design

H. pylori isolates were obtained from gastric biopsy specimens before starting the treatment. The minimum inhibitory concentrations (MICs) of AMX and RFB against *H. pylori* isolates and the *rpoB* mutation status of the *H. pylori* strains were determined using previously described methods.[14,19,20] Patients who did not undergo esophagogastroduodenoscopy after giving informed consent and whose *H. pylori* culture was negative were excluded from the analysis. After *H. pylori* infection was confirmed with the culture method, RFB-containing triple therapy (20 mg VPZ, b.i.d.; 500 mg AMX q.i.d.; 150 mg RFB q.d.) was administered to the patients for 7 days (7-day VAR). Twelve weeks after the end of the eradication therapy, successful eradication was confirmed using the ^{13}C urea breath test (UBT) or *H. pylori* stool antigen test.[22] The cut-off value for a negative UBT was less than 2.5‰.[23] The results obtained from our previous study that reported a 10-day or 14-day EAR therapy as a third- or fourth-line rescue therapy were used as historical control.[14] In the study, the enrolled patients were randomly assigned to receive a 10-day or 14-day eradication therapy with EPZ (20 mg, 4 times a day [q.i.d.]), AMX (500 mg, q.i.d.), and RFB (300 mg, once a day [q.d.]). Patients who failed the first- and second-line eradication therapy were enrolled between October 2013 and February 2015. This study was registered with the University Hospital Medical Information (UMIN) Clinical Trials Registry (UMIN000011963).

2.3 | Susceptibility of *H. pylori* to antimicrobial agents

The cut-off points for the MICs defining the antimicrobial resistance of *H. pylori* were 0.06 and 0.25 μg/ml for AMX and RFB, respectively, based on our previous report.[24]

2.4 | DNA preparation, PCR assay for sequencing to detect *rpoB* mutation

To determine the *rpoB* mutation in the *H. pylori* strains, we isolated *H. pylori* DNA using the method described previously.[23] Briefly, for DNA extraction, the Cica geneusR DNA extraction reagent AN (Kanto Chemical Co., Inc.) was incubated with *H. pylori* culture solution at 72°C for 6 min and at 94°C for 3 min. The resistance-determining region of the *rpoB* (codon 511 to 612) gene was amplified by polymerase chain reaction (PCR) using the following primers: *rpoB* (forward), 5′-AAATGATCACAAGCACCATC-3′; *rpoB* (reverse), 5′-ACCTTGCCATCCACAACC-3′. PCR was performed with 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min using a Gene Amp PCR system 9700 thermal cycler (Applied Biosystems) with KAPA SYBR Fast Master Mix (Nippon Genetics Co. Ltd.). PCR products for sequencing were qualified using a QIAquick Gel Extraction Kit (Qiagen). PCR templates of all strains were sequenced directly on both strands using a 3730xl DNA Analyzer (Thermo Fisher Scientific) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The sequences obtained were compared with published sequences of the *H. pylori rpoB* gene.[25]

2.5 | Outcomes

The main outcome measure was the overall eradication rate. The secondary outcome measures included the eradication rates of *rpoB* mutation-positive or mutation-negative strains, AMX and RFB-susceptible or -resistant *H. pylori* strains, other factors associated with eradication success, and frequency of adverse events. The results obtained were compared with those obtained for our 10- or 14-day EAR previously.[14]

2.6 | Treatment compliance and adverse events

Patients were checked for any adverse events that occurred during the treatment and 1 week after the eradication therapy. The patients were verbally checked whether they had any adverse events or not. Treatment adherence was evaluated by counting the leftover tablets at the end of the regimen. Poor compliance was defined as an intake of <80% of the study drugs.[14]

2.7 | Statistical analyses

The demographic characteristics, eradication rates, frequency of adverse events, the correlation between *rpoB* mutation status, and the MICs of RFB in the patients were compared using Fisher's exact test or Student's t-test, as appropriate. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corp.). Data are expressed as the mean ± standard deviation. Required sample size was estimated as 55 patients taking the following parameters into consideration: the expected eradication rate = 90%, the required minimum threshold eradication rate = 80%, two-sided alpha = 5%, and power = 80%, by referring to previous literature on RFB-based therapy as first- to fourth-line treatment for *H. pylori* eradication.[14,26]

3 | RESULTS

3.1 | Patient characteristics

Overall, 66 patients were enrolled in this study (Figure 1). Of these, three were excluded before treatment because they refused endoscopy ($n = 3$). Patients whose *H. pylori* culture was negative were also excluded ($n = 6$). Finally, 57 patients received the 7-day VAR. Two patients discontinued the regimen because one got a stomachache, and the other developed headache and conjunctival hyperemia on the first day.

As shown in Table 1, the mean patient age was 52.5 ± 9.5 years, and 47.4% (27/57) were male. Of the 57 patients, 3, 43, 10, and 1 patient received the third-, fourth-, fifth-line, and seventh-line eradication therapies.

Comparison with the historical control data revealed that the average patient age was higher in the 7-day VAR group than in the 14-day EAR group (52.5 ± 9.5 years vs 46.5 ± 8.8 years, p = 0.02). Furthermore, the percentage of alcohol drinkers was significantly higher in the 7-day VAR group than in the 10- and 14-day EAR groups in the intention-to-treat (ITT) population (66.7% vs. 33.3%, 47.1%, $p = 0.01$, 0.04, respectively). No intergroup differences were observed in sex, smoking habit, and body mass index (BMI). As shown in Table S1, 48 patients had a previous history of VPZ-AMX-containing or high dose AMX-containing treatments—27 patients had a history of VPZ-AMX (1500 mg)-containing treatment (eradicated/non-eradicated:25/2), 13 patients had a history of VPZ-AMX (≥2000 mg)-containing treatment (eradicated/non-eradicated:11/2), and 8 patients had a history of EPZ-AMX (2000 mg)-containing treatment (eradicated/non-eradicated:8/0).

3.2 | MICs of RFB and *rpoB* mutation status

The mean MIC of RFB was 0.00 ± 0.01 μg/ml, and all strains were RFB-sensitive. Mutations in *rpoB* were detected in three patients, all in V538. The MICs of *rpoB* for all three mutant strains were 0.00 μg/ml, of which two were successfully eradicated (Table 2).

3.3 | Eradication rates

The ITT and per-protocol (PP) analyses showed the eradication rates to be 91.2% (52/57, 95% Confidence Interval [95% CI]: 84%–99%) and 92.7% (51/55, 95% CI: 86%–100%), respectively

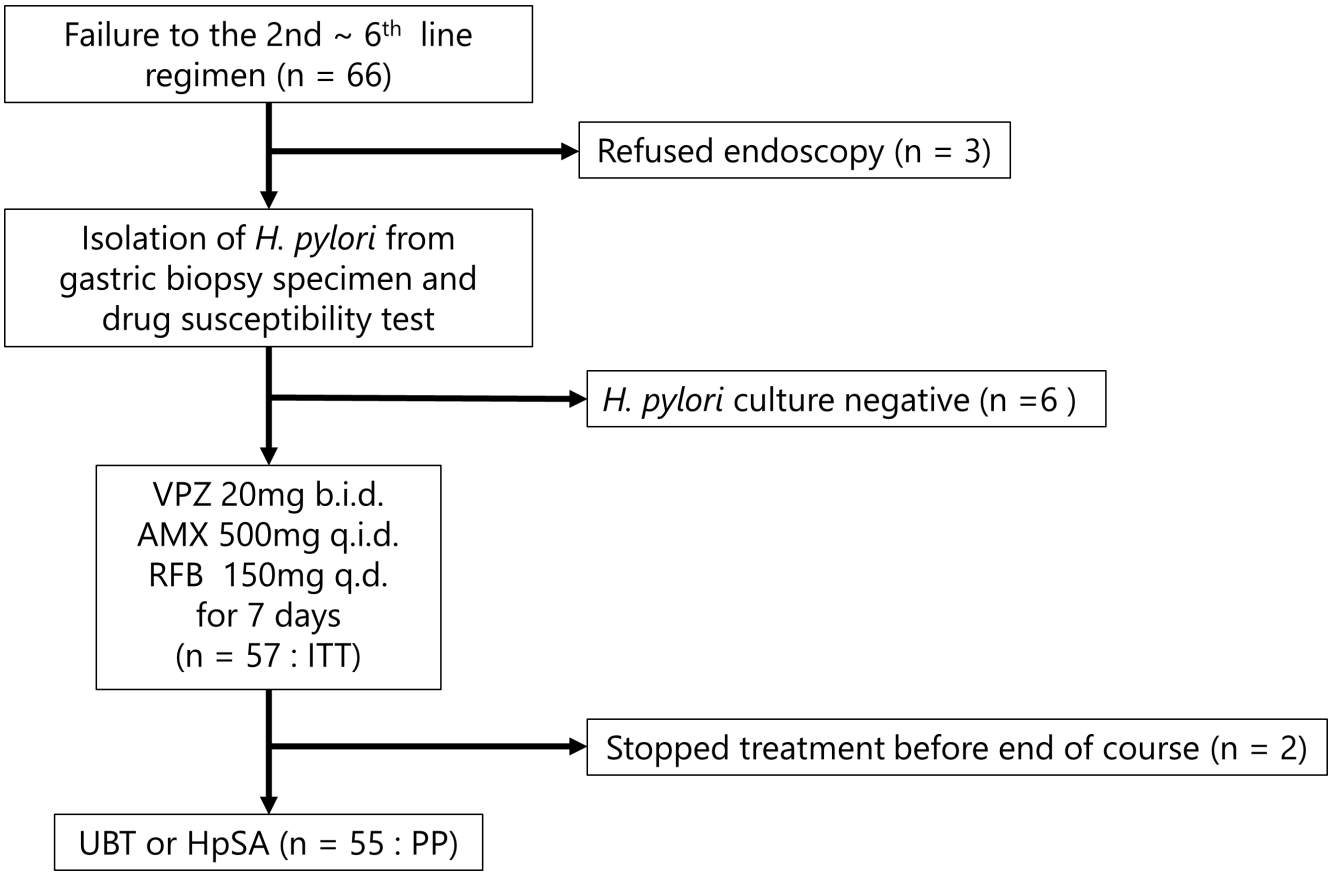

FIGURE 1 Flow diagram of the study. Abbreviations: AMX, amoxicillin; *H. pylori*, *Helicobacter pylori*, HpSA, H. pylori stool antigen test; ITT, intention-to-treat, PP, per-protocol; RFB, rifabutin; UBT, ^{13}C urea breath test; VPZ, vonoprazan

(Table 3). One patient discontinued treatment due to the development of adverse events on the first day and refused to be analyzed for successful eradication, while another patient, who discontinued medication on Day 4, was confirmed to have been successfully eradicated. Compared with the historical controls, the total eradication rate achieved with 7-day VAR did not significantly differ from those achieved with the 10-day EAR (ITT analysis: 83.3%, $p = 0.60$; PP analysis: 81.8%; $p = 0.26$) and 14-day EAR (ITT analysis: 94.1%, $p = 1.00$; PP analysis: 91.7%, $p = 1.00$) (Table 3). As shown in Table 4, all patients in whom the eradication therapies failed were treated with fourth- or fifth-line treatment. The number of smokers was significantly higher in the non-eradication group. However, there was no difference in the other patient characteristics and MICs of AMX and RFB between the patients in whom eradication therapy was successful and those in whom it was unsuccessful.

3.4 | Safety assessment

Adverse events occurred in 31.6% (18/57) of the patients in the 7-day VAR group, and two patients discontinued treatment (Table 5). One stopped medication because of stomachache on the fourth day, and the other discontinued the treatment because of headache and conjunctival hyperemia. In the ITT population, the occurrence of adverse events was lower in the 7-day VAR therapy group than those in the 14-day EAR therapy group (31.6% vs. 88.2%, $p < 0.01$)and the 10-day EAR therapy group (31.6% vs. 66.7%, $p = 0.046$). There were no severe adverse events, and all adverse events improved after the completion or discontinuation of the medication.

4 | DISCUSSION

In the present study, we assessed the efficacy and safety of 7-day VAR as a third- or later-line regimen to eradicate *H. pylori* infection. To the best of our knowledge, this is the first report of the use of 150 mg/day RFB in a 7-day regimen to eradicate *H. pylori* infection. The strength of this study is that all patients were assessed by *H. pylori* culture and antibiotic susceptibility testing. This means that all false-positive cases from the other tests were excluded, and all patients were assessed for AMX and RFB susceptibility and *rpoB* mutations.

The regimen used in the current study (7-day VAR) differs from our previously reported 10- and 14-day RFB-based regimens (10- and 14-day EAR) in three aspects. First, we shortened the duration of the regimen from 10 or 14 days to 7 days. Second, the dosage of RFB was reduced from 300 mg q.d. to 150 mg q.d. Third, VPZ was used to suppress gastric acid secretion instead of EPZ. Interestingly,

TABLE 1 Participant demographics

	7-day VAR therapy (*N* = 57)	10-day EAR therapy[a] (*N* = 12)	*p*-value[b]	14-day EAR therapy[a] (*N* = 17)	*p*-value[c]
Age (years, mean ± SD)	52.5 ± 9.5	50.3 ± 13.9	0.61[d]	46.5 ± 8.8	**0.02**[d]
Sex (male/female)	27/30	3/9	0.21[e]	9/8	078[e]
Smokers, *n* (%)	4 (7.0)	1 (8.3)	1.00[e]	3 (17.6)	034[e]
Alcohol drinkers, *n* (%)	38 (66.7)	4 (33.3)	**0.01**[e]	8 (47.1)	**0.04**[e]
BMI, mean ± SD, kg/m^2	22.6 ± 4.0	21.6 ± 2.5	0.39[d]	22.7 ± 4.7	0.96[d]
Presence of *rpoB* mutation, *n* (%)	3 (5.3)	1 (7.1)	0.54[e]	2 (11.8)	0.32[e]
Resistance to RFB (MIC: ≥0.25 μg/ml), *n* (%)	0	0		1 (5.9)	0.23[e]
RFB MIC (mean ± SD, μg/ml)	0 ± 0.01	0 ± 0.01	0.74[d]	0.06 ± 0.24	0.35[d]
Resistance to AMX (MIC: ≥0.6 μg/ml), *n* (%)	36 (63.2)	8 (66.7)	1.00[e]	8 (47.1)	0.27[e]
AMX MIC (mean ± SD, μg/ml)	0.12 ± 0.16	0.24 ± 0.56	0.47[d]	0.07 ± 0.08	0.24[d]
Third-line eradication therapy, *n* (%)	3 (5.3)	3 (25.0)	0.06[e]	6 (35.3)	**0.01**[e]
Fourth-line eradication therapy, *n* (%)	43 (75.4)	9 (75.0)	1.00[e]	11 (64.7)	0.53[e]
Fifth-line eradication therapy, *n* (%)	10 (16.1)	0		0	
Seventh-line eradication therapy, *n* (%)	1 (1.8)	0		0	

Note: Alcohol drinkers were defined as people who consumed at least one drink of alcohol per month. Bold values denote statistical significance at the $p < 0.05$ level.

Abbreviations: AMX, amoxicillin; BMI, body mass index; MIC, minimum inhibitory concentration; RFB, rifabutin; SD, standard deviation.

[a]Data of the 10- and 14-day EAR therapies have been cited from Mori et al[14] as historical control.

[b]7-day VAR vs 10-day EAR.

[c]7-day VAR vs 14-day EAR.

[d]Student's *t*-test.

[e]Fisher's exact test.

TABLE 2 Outcomes of patients with the *rpoB* mutation in the 7-day VAR and 10- or 14-day EAR groups

	MIC of RFB (μg/ml)	Amino acid change	Eradication result
VAR Patient 1	0	Val 538 Ile	success
VAR Patient 2	0	Val 538 Ile	failure
VAR Patient 3	0	Val 538 Ile	success
EAR 1[a]	0	Val 538 Ile	success
EAR 2[a]	0.03	Val 538 Ile	success
EAR 3[a]	1	Leu 525 Ile	success

Abbreviation: MIC, minimum inhibitory concentration; RFB, rifabutin.

[a]Data are cited from Mori et al.[14]

ITT and PP analyses showed that this 7-day VAR resulted in a sufficient eradication rate of 91.2% (52/57) and 92.7% (51/55), respectively, as a third- or later-line rescue treatment.

Regarding the duration, dosage, and frequency of RFB therapy, Gisbert et al reviewed and summarized the efficacy of RFB for *H. pylori* eradication by performing a meta-analysis and recommended treatment administration for 10 days considering the balance of eradication rate and frequency of adverse events. As for the dosage, RFB 300 mg/day is generally used for treating *H. pylori* infection.[13] All previously reported *H. pylori* eradication regimens consisting of 150 mg RFB daily were administered for more than 10 days.[13] However, the eradication rates were in the range of 40%–70%.[13,27,28] Our 7-day VAR resulted in a high eradication rate of >90% despite the shortening of the treatment duration and reducing the dosage of RFB.

An undoubtedly important factor influencing the results of this study is the use of VPZ. Since the availability of VPZ in 2015, many reports have shown the higher effectiveness of VPZ-containing regimens for *H. pylori* eradication compared with that of PPI-containing regimens.[29,30] Regarding the RFB regimen, Lim et al compared 7-day therapy with RFB (150 mg b.i.d.), AMX (1g t.i.d.), and lansoprazole (30 mg b.i.d. or 60 mg b.i.d.). In their study, the 60 mg lansoprazole group achieved a higher eradication rate than the 30 mg group (96.3% vs. 78.1%, $p = 0.047$).[31] This study suggested that strong acid suppression by high-dose PPIs is more effective in RFB-containing

TABLE 3 The eradication rate of 7-day VAR and historical 10- and 14-day EAR

	7-day VAR therapy	10-day EAR therapy[a]	*p*-value	14-day EAR therapy[a]	*p*-value
ITT analysis	91.2% (52/57)	83.3% (10/12)	0.60[b]	94.1% (16/17)	1.00 [b]
PP analysis	92.7% (51/55)	81.8% (9/11)	0.26[b]	91.7% (11/12)	1.00 [b]

Abbreviations: ITT, intention to treat; PP, per-protocol.

[a]Data of EAR therapy is cited from Mori et al[14] as historical control.

[b]Fisher's exact test.

TABLE 4 Factors influencing the eradication rate achieved with 7-day VAR

	7-day VAR, *N* = 57	Successful eradication (*N* = 52)	Eradication failure (*N* = 5)	*p*-value
Age (years, mean ± SD)	52.5 ± 9.5	51.7 ± 9.4	60.4 ± 8.3	0.51[a]
Male/Female	27/30	23/29	4/1	0.18[b]
Smokers, *n* (%)	4 (7.0)	2 (3.8)	2 (40.0)	**0.04**[b]
Alcohol drinkers, *n* (%)	52.5 ± 9.5	34 (65.4)	4 (80.0)	1.00 [b]
BMI, mean ± SD, kg/m^2	22.6 ± 4.0	22.7 ± 4.1	22.1 ± 2.3	0.75[a]
rpoB mutation, *n* (%)	3 (5.3)	2 (3.8)	1 (20.0)	0.25[b]
Resistance to RFB (MIC: ≥0.25 μg/ml), *n* (%)	0	0	0	N.A.
RFB MIC (mean ± SD, μg/ml)	0 ± 0.01	0 ± 0.01	0.00 ± 0.00	0.68[a]
Resistance to AMX (MIC: ≥0.6 μg/ml), *n* (%)	36 (63.2)	32 (61.5)	4 (80.0)	0.64[b]
AMX MIC (mean ± SD, μg/ml)	0.12 ± 0.16	0.12 ± 0.17	0.11 ± 0.09	0.88[a]
Fourth- or later-line eradication therapy, *n* (%)	54 (94.7)	49 (94.2)	5 (100)	1.00[b]

Note: Alcohol drinkers were defined as those who consumed at least one drink of alcohol per month. Bold values denote statistical significance at the $p < 0.05$ level.

Abbreviations: AMX, amoxicillin; BMI, body mass index; MIC, minimum inhibitory concentration; RFB, rifabutin; SD, standard deviation.

[a]Student's *t*-test.

[b]Fisher's exact test.

regimens. However, to our best knowledge, no studies have yet directly compared PPIs with VPZ in regimens containing RFB. In 2020, Hirata et al conducted a small trial of 10-day triple therapy with VPZ (20 mg b.i.d.), AMX (750 mg b.i.d.), and RFB (150 mg b.i.d.) and achieved a high eradication rate (100%, 19/19, 95% CI: 83%–100%).[32] Furthermore, our 7-day VAR therapy achieved a sufficient eradication rate compared with those achieved with the 10- and 14-day EAR, although there was no significant difference (Table 3). These results suggest that an additional effect of VPZ in the RFB-containing regimen can be expected.

We investigated the susceptibility of the isolates to antimicrobial and *rpoB* mutation agents responsible for RFB resistance,[33] as we did previously.[14] In the present study, the mean MIC of RFB was 0.00 μg/ml, and all strains were sensitive to RFB. Of these strains, three strains with *rpoB* mutation in V538 were identified, and the MICs of RFB for these strains were lower than the detection sensitivity (Table 2). Regarding our 10- and 14-day EAR study reported previously, the strains isolated from three patients showed the *rpoB* mutation, and mutations were detected at L525 and V538. Of these, the patients with the L525 mutation showed resistance to RFB; however, all were successfully eradicated. Concordant with these findings, the findings of the present study also detected the mutations at V538 and L525, and the patients harboring L525 mutation were resistant to RFB. It has been shown that mutations at L525 induce strong resistance to rifampicin.[34] Taken together, these studies suggest that the V538 mutation is not related to resistance to RFB in *H. pylori*. Furthermore, a new mutation, L547F, responsible for rifamycin resistance, has been reported recently.[35] However, in this study, L547F mutation was not detected.

The findings demonstrated no significant difference in eradication rate between the AMX-resistant and AMX-sensitive groups. Further, the study also indicated that the susceptibility of the strains

TABLE 5 Adverse events

	7-day VAR	10-day EAR[a]	*p*-value[b]	14-day EAR[a]	*p*-value[c]
ITT population, *n*	57	12		17	
Pateints who had adverse events, *n* (%)	18 (31.6)	8 (66.7)	**0.046**[d]	15 (88.2)	**<0.01**[d]
Treatment discontinuation, *n* (%)	1 (1.8)	1 (8.3)		5 (29.4)	
Soft stool, *n* (%)	5 (8.8)	2 (16.6)		2 (11.8)	
Fatigue, *n* (%)	4 (7.0)	0		1 (5.9)	
Stomachache, *n* (%)	3 (5.3)	0		0	
Discomfort in the eyes, *n* (%)	3 (5.3)	0		0	
Headache, *n* (%)	2 (3.5)	3 (25.0)		3 (17.7)	
Rash, *n* (%)	1 (1.8)	1 (8.3)		2 (11.8)	
Dysgeusia, *n* (%)	1 (1.8)	1 (8.3)		0	
Conjunctival hyperemia, *n* (%)	1 (1.8)	0		0	
Itching, *n* (%)	1 (1.8)	0		0	
Vertigo, *n* (%)	0	0		1 (5.9)	
Photophobia, *n* (%)	0	0		1 (5.9)	
Stomatitis, *n* (%)	0	1 (8.3)		0	
Diarrhea, *n* (%)	0	0		5 (29.4)	
Fever, *n* (%)	0	2 (16.6)		6 (35.3)	

Abbreviation: ITT, intention to treat. Bold values denote statistical significance at the $p < 0.05$ level.
[a]Data of the 10- and 14-day EAR therapies are cited from Mori et al[14] as historical control.
[b]7-day VAR vs 10-day EAR.
[c]7-day VAR vs 14-day EAR.
[d]Fisher's exact test.

to AMX and RFB was not a predictor of successful eradication. AMX resistance has been shown to be associated with decreased efficacy of AMX-containing regimens for *H. pylori* eradication.[36] Concordantly, in this study, a higher rate of AMX resistance was observed in the eradication failure group than that in the successful eradication group (Table 4). However, the sample size in this study cannot detect statistical significance, suggesting the requirement of a larger sample size to detect the precise effects of AMX-resistance on the treatment failure of AMX-containing regimens.

The percentage of adverse events associated with the 7-day VAR was lower than those related to the 10- and 14-day EAR therapies (7-day VAR vs. 10- and 14-day EAR: 47.4% vs. 75%, 94.1%).[14] The frequency of adverse events of a previous study that reported a 10-day triple therapy with VPZ (20 mg b.i. d.), AMX (750 mg b.i.d.), and RFB (150 mg b.i.d.) were comparable with our study (8/19, 42.1% vs, 27/56, 47.4%).[32] The incidence of adverse events has typically been reported to be associated with the RFB dose.[11,37] Patients treated with more than 450 mg RFB daily experienced more adverse events than those treated with 300 mg RFB daily.[13] On the contrary, Phillips et al reported that a duration of no more than one week should minimize adverse events.[38] The mean rate of RFB-associated adverse events for *H. pylori* eradication was reported to be 15% (95% CI: 14%–17%), mostly in Europe.[13] When limited to studies in Asia, the incidence of adverse events was relatively high, ranging from 11% to 94% (mean 44%, 95% CI: 20%–67%).[31,32,39–41] The incidence of adverse events for the 7-day VAR used in this study was 49.1%, which is high compared with the overall reported cases, but not as high as reported in Asia. As racial differences may affect adverse events, appropriate dosing may be necessary, particularly when RFB is used in Asian populations.

The eradication rate of tailored therapy was higher than that of conventional therapy.[42] However, drug susceptibility tests for *H. pylori* before *H. pylori* eradication and third- or fourth-line eradication are not covered by the Japanese health insurance system. In our study, most patients had a previous history of treatment with sitafloxacin-containing regimen (third to sixth-line) as non-health insurance covered treatment or clinical trial (data not shown).

Furthermore, our data revealed eradication failure in patients despite the lack of *rpoB* mutation or RFB/AMX resistance, which could be attributed to the smoking habits of the patients. The number of smokers was significantly higher in the eradication failure group than that in the successful eradication group (Table 1). Reportedly, smoking inhibits the activities of CYP3A4, which metabolizes both RFB and VPZ. Therefore, it has been speculated that smoking may lead to eradication failure, as it may slow down the clearance of drugs metabolized by CYP3A4.[43] These findings have also been supported

by the findings of a meta-analysis, which demonstrated that smoking increases the treatment failure rate for *H. pylori* eradication.[44] However, to understand the precise effect of smoking and its relation to treatment failure in patients lacking *rpoB* mutation, further studies exploring the activity or genotyping of CYP3A4 are required.

Efficacy of VPZ and low-dose AMX (1500 mg) dual therapy as first-line therapy has been recently reported in Japan.[45,46] In this study, we showed that in the 48 patients with a previous history of VPZ-AMX-containing or high dose (≥2000 mg) AMX-containing treatment, *H. pylori* infection was successfully eradicated in 90.0% of patients treated with a VPZ-AMX-containing regimen and 90.5% of patients treated with a high dose AMX-containing regimen (Table S1). These findings demonstrated that the VAR therapy in this study efficiently eradicated *H. pylori* in these patients, which could be attributed to the contribution of RFB. Therefore, we suggest that RFB in combination with VPZ and AMX as salvage therapy after treatments with VPZ and AMX or high dose AMX can efficiently eradicate *H. pylori*.

VPZ is metabolized mainly by CYP3A4 and partially by CYP2C19. Therefore, the influence of the CYP2C19 genotype status on gastric acid suppression by VPZ could be small.[47] In Asia, where CYP2C19 rapid metabolizers are uncommon and gastric acid secretion is generally low due to corpus gastritis, have shown that the combination high dose PPI–amoxicillin can produce higher cure rates than it in western populations.[48] Subsequent studies using a factorial design (i.e., with and without RFB) will be needed to address the question whether or what proportion of the observed outcome was related to the presence of RFB.

To elucidate whether VPZ is actually effective in RFB-containing treatments, direct comparison with 7 days regimen which contains PPI, AMX 2000 mg, and RFB 150 mg will be needed. However, this comparison has not been done yet, as the second-line treatment, the eradication rate by ITT analysis of 7 days omeprazole 40 mg, AMX 2000 mg, and RFB 300 mg containing regimen was 44.4%.[49] Moreover, the eradication rate by ITT analysis of 10 days low dose (150 mg) RFB-containing regimen was significantly lower than that of 10 days RFB 300 mg containing regimen (66.6% v.s. 86.6%).[27] As a rescue therapy, such eradication rate of over 90% by 7 days low dose RFB-containing regimen with PPIs has not been achieved yet. Therefore, these previous studies support the possibility that VPZ improved the eradication rate of 7 days low dose RFB-containing regimen. However, to clarify this point, subsequent studies using a factorial design (i.e., with and without VPZ) will be needed to address the question whether or what proportion of the observed outcome was related to the presence of VPZ.

This study had some limitations. First, this was an open-label, single-arm study. Second, the first- and second-line eradication regimens were AMX-CLR-PPI/P-CAB, AMX-MTZ-PPI/P-CAB, which differed from those used in other countries in some instances. For example, bismuth-containing quadruple therapy is used in many countries,[50] but not in Japan. Further, due to differences in available regimens or antimicrobial resistance in each region, comparing *H. pylori* eradication regimens used globally under exactly the same conditions is difficult.[51–53] This study lacks a control arm and subsequently lacks randomization and blinding, which may introduce bias. Further, the sample size is also small. However, the required sample size was obtained, to overcome these limitations, future studies involving more patients and control arms are essential.

5 | CONCLUSION

Seven-day low-dose RFB-based VAR therapy is effective and safe as a third- or later-line *H. pylori* eradication regimen. To clarify what component in this regimen are critical, subsequent studies using a factorial design (comparing VPZ-AMX dual therapy vs. VPZ-RFB triple therapy) will be needed.

AUTHOR CONTRIBUTIONS

TM and HM designed the research. KI, HM, JM, KH, HS, YS, and TM contributed to participant acquisition. KI, HM, JM, KH, YH, YS, and TM were involved in data analysis. KI and HM drafted the manuscript. All authors contributed to the interpretation of data, critically revised the manuscript, and approved the final manuscript version.

ACKNOWLEDGMENTS

The authors thank all the doctors who referred eligible patients for this clinical trial. We would also like to acknowledge the contributions of Hisako Kameyama, Yuta Yamamoto, and Tatsuto Mizuno, who assisted with the collection of data, and Editage (www.editage.jp) for English language editing. This study was supported by Keio Gijuku Academic Development Funds (to TM).

CONFLICT OF INTEREST

During the last two years, TM received service honoraria from Takeda Pharmaceutical Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Astra-Zeneca K.K., and Daiichi Sankyo Co., Ltd. HS received scholarship funds for research from Astellas Pharm Inc., Astra-Zeneca K.K., Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd., and received service honoraria from Astellas Pharma Inc., Astra-Zeneca K.K., Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd. TK received scholarship funds for research from Astellas Pharm Inc., Astra-Zeneca K.K., Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd. and received service honoraria from Astellas Pharma Inc., Otsuka Pharmaceutical Co., Ltd., and Takeda Pharmaceutical Co., Ltd.

ORCID

Hideki Mori https://orcid.org/0000-0001-8830-4671
Hidekazu Suzuki https://orcid.org/0000-0002-8994-6163
Tatsuhiro Masaoka https://orcid.org/0000-0002-4038-0322

REFERENCES

1. McColl KE. Clinical practice. *Helicobacter pylori* infection. *N Engl J Med*. 2010;362(17):1597-1604.
2. Lee YC, Chiang TH, Chou CK, et al. Association between *Helicobacter pylori* eradication and gastric cancer incidence: a systematic review

and meta-analysis. *Gastroenterology*. 2016;150(5):1113-1124.e1115.
3. Leja M, Grinberga-Derica I, Bilgilier C, Steininger C. Review: epidemiology of *Helicobacter pylori* infection. *Helicobacter*. 2019;24(suppl 1):e12635.
4. Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. *Gastroenterology*. 2017;153(2):420-429.
5. Suzuki S, Esaki M, Kusano C, Ikehara H, Gotoda T. Development of *Helicobacter pylori* treatment: how do we manage antimicrobial resistance? *World J Gastroenterol*. 2019;25(16):1907-1912.
6. Domanovich-Asor T, Craddock HA, Motro Y, Khalfin B, Peretz A, Moran-Gilad J. Unraveling antimicrobial resistance in *Helicobacter pylori*: global resistome meets global phylogeny. *Helicobacter*. 2021;26(2):e12782.
7. Mori H, Suzuki H, Matsuzaki J, Masaoka T, Kanai T. Acquisition of double mutation in gyrA caused high resistance to sitafloxacin in *Helicobacter pylori* after unsuccessful eradication with sitafloxacin-containing regimens. *United European Gastroenterol J*. 2018;6(3):391-397.
8. Nishizawa T, Suzuki H, Tsugawa H, et al. Enhancement of amoxicillin resistance after unsuccessful *Helicobacter pylori* eradication. *Antimicrob Agents Chemother*. 2011;55(6):3012-3014.
9. D'Elios MM, Silvestri E, Emmi G, Barnini T, Prisco D. *Helicobacter pylori*: usefulness of an empirical fourth-line rifabutin-based regimen. *Expert Rev Gastroenterol Hepatol*. 2012;6(4):437-439.
10. van Zanten SV, Desai S, Best L, et al. Rescue therapy using a rifabutin-based regimen is effective for cure of *Helicobacter pylori* infection. *Can J Gastroenterol*. 2010;24(5):303-306.
11. Gisbert JP, Calvet X. Review article: rifabutin in the treatment of refractory *Helicobacter pylori* infection. *Aliment Pharmacol Ther*. 2012;35(2):209-221.
12. Graham DY, Canaan Y, Maher J, Wiener G, Hulten KG, Kalfus IN. Rifabutin-based triple therapy (RHB-105) for *Helicobacter pylori* eradication: a double-blind, randomized controlled trial. *Ann Intern Med*. 2020;172(12):795-802.
13. Gisbert JP. Rifabutin for the treatment of *Helicobacter pylori* infection: a review. *Pathogens*. 2020;10(1):15.
14. Mori H, Suzuki H, Matsuzaki J, et al. Rifabutin-based 10-day and 14-day triple therapy as a third-line and fourth-line regimen for *Helicobacter pylori* eradication: a pilot study. *United European Gastroenterol J*. 2016;4(3):380-387.
15. Sakurai Y, Mori Y, Okamoto H, et al. Acid-inhibitory effects of vonoprazan 20 mg compared with esomeprazole 20 mg or rabeprazole 10 mg in healthy adult male subjects - a randomised open-label cross-over study. *Aliment Pharmacol Ther*. 2015;42(6):719-730.
16. Sugano K. Vonoprazan fumarate, a novel potassium-competitive acid blocker, in the management of gastroesophageal reflux disease: safety and clinical evidence to date. *Therap Adv Gastroenterol*. 2018;11:1756283X1774577.
17. Mizokami Y, Oda K, Funao N, et al. Vonoprazan prevents ulcer recurrence during long-term NSAID therapy: randomised, lansoprazole-controlled non-inferiority and single-blind extension study. *Gut*. 2018;67(6):1042-1051.
18. Mori H, Suzuki H, Omata F, et al. Current status of first- and second-line *Helicobacter pylori* eradication therapy in the metropolitan area: a multicenter study with a large number of patients. *Therap Adv Gastroenterol*. 2019;12:1756284819858511.
19. Sugimoto M, Yamaoka Y. Role of vonoprazan in *Helicobacter pylori* eradication therapy in Japan. *Front Pharmacol*. 2018;9:1560.
20. Murakami K, Sakurai Y, Shiino M, Funao N, Nishimura A, Asaka M. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for *Helicobacter pylori* eradication: a phase III, randomised, double-blind study. *Gut*. 2016;65(9):1439-1446.
21. Graham DY, Lu H, Shiotani A. Vonoprazan-containing *Helicobacter pylori* triple therapies contribution to global antimicrobial resistance. *J Gastroenterol Hepatol*. 2021;36(5):1159-1163.
22. Vaira D, Malfertheiner P, Mégraud F, et al. Diagnosis of *Helicobacter pylori* infection with a new non-invasive antigen-based assay. HpSA European study group. *Lancet*. 1999;354(9172):30-33.
23. Kato M, Asaka M, Ohara S, Toyota T. Clinical studies of 13C-urea breath test in Japan. *J Gastroenterol*. 1998;33(suppl 10):36-39.
24. Nishizawa T, Suzuki H, Matsuzaki J, et al. *Helicobacter pylori* resistance to rifabutin in the last 7 years. *Antimicrob Agents Chemother*. 2011;55(11):5374-5375.
25. Suzuki S, Suzuki H, Nishizawa T, et al. Past rifampicin dosing determines rifabutin resistance of *Helicobacter pylori*. *Digestion*. 2009;79(1):1-4.
26. Gisbert JP, Castro-Fernandez M, Perez-Aisa A, et al. Fourth-line rescue therapy with rifabutin in patients with three *Helicobacter pylori* eradication failures. *Aliment Pharmacol Ther*. 2012;35(8):941-947.
27. Perri F, Festa V, Clemente R, et al. Randomized study of two "rescue" therapies for *Helicobacter pylori*-infected patients after failure of standard triple therapies. *Am J Gastroenterol*. 2001;96(1):58-62.
28. Saracino IM, Pavoni M, Zullo A, et al. Rifabutin-based triple therapy or bismuth-based quadruple regimen as rescue therapies for *Helicobacter pylori* infection. *Eur J Intern Med*. 2020;81:50-53.
29. Sue S, Ogushi M, Arima I, et al. Vonoprazan- vs proton-pump inhibitor-based first-line 7-day triple therapy for clarithromycin-susceptible *Helicobacter pylori*: a multicenter, prospective, randomized trial. *Helicobacter*. 2018;23(2):e12456.
30. Yamada S, Kawakami T, Nakatsugawa Y, et al. Usefulness of vonoprazan, a potassium ion-competitive acid blocker, for primary eradication of *Helicobacter pylori*. *World J Gastrointest Pharmacol Ther*. 2016;7(4):550-555.
31. Lim HC, Lee YJ, An B, Lee SW, Lee YC, Moon BS. Rifabutin-based high-dose proton-pump inhibitor and amoxicillin triple regimen as the rescue treatment for *Helicobacter pylori*. *Helicobacter*. 2014;19(6):455-461.
32. Hirata Y, Yamada A, Niikura R, Shichijo S, Hayakawa Y, Koike K. Efficacy and safety of a new rifabutin-based triple therapy with vonoprazan for refractory *Helicobacter pylori* infection: a prospective single-arm study. *Helicobacter*. 2020;25(5):e12719.
33. Heep M, Beck D, Bayerdörffer E, Lehn N. Rifampin and rifabutin resistance mechanism in *Helicobacter pylori*. *Antimicrob Agents Chemother*. 1999;43(6):1497-1499.
34. Wang G, Wilson TJ, Jiang Q, Taylor DE. Spontaneous mutations that confer antibiotic resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother*. 2001;45(3):727-733.
35. Hays C, Burucoa C, Lehours P, Tran CT, Leleu A, Raymond J. Molecular characterization of *Helicobacter pylori* resistance to rifamycins. *Helicobacter*. 2018;23(1):e12451.
36. Chen MJ, Wu MS, Chen CC, et al. Impact of amoxicillin resistance on the efficacy of amoxicillin-containing regimens for *Helicobacter pylori* eradication: analysis of five randomized trials. *J Antimicrob Chemother*. 2017;72(12):3481-3489.
37. Brogden RN, Fitton A. Rifabutin. A review of its antimicrobial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs*. 1994;47(6):983-1009.
38. Phillips MC, Wald-Dickler N, Loomis K, Luna BM, Spellberg B. Pharmacology, dosing, and side effects of rifabutin as a possible therapy for antibiotic-resistant acinetobacter infections *Open Forum Infect Dis* 2020;7(11):ofaa460.
39. Wong WM, Gu Q, Lam SK, et al. Randomized controlled study of rabeprazole, levofloxacin and rifabutin triple therapy vs. quadruple therapy as second-line treatment for *Helicobacter pylori* infection. *Aliment Pharmacol Ther*. 2003;17(4):553-560.
40. Jeong MH, Chung JW, Lee SJ, et al. Comparison of rifabutin- and levofloxacin-based third-line rescue therapies for *Helicobacter pylori*. *Korean J Gastroenterol*. 2012;59(6):401-406.

41. Sung J, Kim N, Park YH, et al. Rifabutin-based fourth and fifth-line rescue therapy in patients with for *Helicobacter pylori* eradication failure. *Korean J Gastroenterol*. 2017;69(2):109-118.
42. Shinmura T, Adachi K, Yamaguchi Y, et al. Vonoprazan-based triple-therapy could improve efficacy of the tailored therapy of *Helicobacter pylori* infection. *J Gastrointestin Liver Dis*. 2019;28(4):389-395.
43. Rahmioglu R, Heaton J, Clement G, et al. Genetic epidemiology of induced CYP3A4 activity. *Pharmacogenet Genomics*. 2011;21(10):642-651.
44. Suzuki T, Matsuo K, Ito H, et al. Smoking increases the treatment failure for *Helicobacter pylori* eradication. *Am J Med*. 2006;119(3):217-224.
45. Suzuki S, Gotoda T, Kusano C, et al. Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line *Helicobacter pylori* treatment: a multicentre randomised trial in Japan. *Gut*. 2020;69(6):1019-1026.
46. Furuta T, Yamade M, Kagami T, et al. Dual therapy with vonoprazan and amoxicillin is as effective as triple therapy with vonoprazan, amoxicillin and clarithromycin for eradication of *Helicobacter pylori*. *Digestion*. 2020;101(6):743-751.
47. Yamasaki H, Kawaguchi N, Nonaka M, et al. In vitro metabolism of TAK-438, vonoprazan fumarate, a novel potassium-competitive acid blocker. *Xenobiotica*. 2017;47(12):1027-1034.
48. Shiotani A, Roy P, Lu H, Graham DY. Helicobacter pyloridiagnosis and therapy in the era of antimicrobial stewardship. *Therap Adv Gastroenterol*. 2021;21(14):17562848211064080.
49. Navarro-Jarabo JM, Fernández N, Sousa FL, et al. Efficacy of rifabutin-based triple therapy as second-line treatment to eradicate helicobacter pylori infection. *BMC Gastroenterol*. 2007;25(7):31. doi:10.1186/1471-230X-7-31
50. Tursi A, Franceschi M, Allegretta L, et al. Effectiveness and safety of Pylera® in patients infected by *Helicobacter pylori*: a multicenter, retrospective real life study. *Dig Dis*. 2018;36(4):264-268.
51. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of *Helicobacter pylori* infection. *Am J Gastroenterol*. 2017;112(2):212-239.
52. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence consensus report. *Gut*. 2017;66(1):6-30.
53. Kato M, Ota H, Okuda M, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2016 Revised Edition. *Helicobacter*. 2019;24(4):e12597.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Inokuchi K, Mori H, Matsuzaki J, et al. Efficacy and safety of low-dose rifabutin-based 7-day triple therapy as a third- or later-line *Helicobacter pylori* eradication regimen. *Helicobacter*. 2022;27:e12900. doi:10.1111/hel.12900