Optimal dose of ramosetron in female patients with irritable bowel syndrome with diarrhea: A randomized, placebo-controlled phase II study

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Abstract

Background: Previous studies showed that 5 μg of ramosetron, a serotonin (5-hydroxytryptamine: 5-HT)-3 receptor antagonist, is only effective in male patients with irritable bowel syndrome (IBS) with diarrhea (IBS-D). We hypothesized that either 1.25, 2.5, or 5 μg of ramosetron would be effective in female patients with IBS-D.

Methods: This randomized, double-blind, placebo-controlled, phase II dose-finding exploratory trial included 409 female outpatients with IBS-D treated in Japan. They were administered oral placebo (n=102), or 1.25 μg (n=104), 2.5 μg (n=104), or 5 μg (n=99) of ramosetron once daily for 12 weeks after a 1-week baseline period. The primary endpoint was monthly responder rates of global improvement of IBS symptoms in the first month. Secondary endpoints included global improvement in the other months, abdominal pain/discomfort, weekly mean changes in the Bristol Stool Form Scale (BSFS), and IBS-QOL.

Key Results: Middle dose (2.5 μg) of ramosetron significantly improved abdominal pain/discomfort at second month (62.5%, P=.002), third month (60.6%, P=.005), and the last evaluation point (63.5%, P=.002) and weekly BSFS (P<.05) except at Week 8, 11, and 12 than placebo. IBS-QOL did not change. Ramosetron induced more constipation than placebo.

Conclusions & Inferences: The trial suggested that 2.5 μg of ramosetron is the most effective and least harmful option for treating female patients with IBS-D (Clinicaltrials.gov ID: NCT01274000).

KEYWORDS
5-hydroxytryptamine, abdominal discomfort, abdominal pain, global improvement, stool consistency

1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that is characterized by chronic or recurrent abdominal pain and/or abdominal discomfort associated with abnormal bowel...
movements. Treatment of IBS is important because IBS adversely affects the quality of life (QOL) of patients, leading to significant medical expenditure, and results in great losses to society. Categorizing IBS into the four major subtypes is one of the comprehensive approaches used to develop effective treatment. Based on the Rome III criteria, these types are IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), mixed-type IBS (IBS-M), and unsubtype IBS (IBS-U). Although these subtypes can transition from one to another, IBS-D persists in 30.8% of patients. Identification of treatment for IBS-D will not only help to establish IBS treatment but will also provide substantial insights into the pathophysiology of IBS.

Agents used to treat IBS-D-like symptoms include 5-hydroxytryptamine (5-HT)-3 antagonists, probiotics, polycarbophil, anticholinergics, trimebutine, loperamide, bile acid binders including cholestyramine, antiallergic agents, 5-aminosalicylic acid, and serotonergic psychoactive agents. Drugs in development that are likely to affect the management of IBS-D in the future are also noteworthy: rifaximin, absorbent drugs, tryptophan hydroxylase 1 inhibitors, mast cell stabilizers, and centrally acting benzodiazepines. Among these agents, 5-HT3 antagonists including alosetron, cilansetron, and ondansetron are the most distinct drugs that inhibit diarrhea. However, two unsatisfactory points have been widely recognized regarding the use of 5-HT3 antagonists for IBS-D. One is a gender-specific drug effect and the second is a serious adverse drug reaction, ischemic colitis. The risk of ischemic colitis is likely related to the compound per se and there is no clear evidence at present that ramosetron or ondansetron poses an increased risk. A great puzzle is the gender-specific drug effect of 5-HT3 antagonists on IBS-D as alosetron has been approved for women alone and ramosetron for men alone.

If we can demonstrate the efficacy of ramosetron in women with IBS-D, we will be able to use this 5-HT3 antagonist regardless of gender restriction. We hypothesized that the optimal dose of ramosetron for women with IBS-D is between 1.25 and 5 μg once daily. The rationale for this assumption is as follows: (i) The optimal dose of ramosetron in men with IBS-D is 5 μg once daily. (ii) The monthly responder rate for ramosetron 5 μg in women with IBS-D was similar to that in men at the last evaluation point of a 12-week study to that for men with IBS-D but differences from placebo were more apparent during the earlier weeks. (iii) The incidence of adverse events related to the mechanism of action of ramosetron was higher in women than in men. The aim of this study was to find a suitable dose for ramosetron in women with IBS-D.

### 2 | MATERIALS AND METHODS

#### 2.1 | Patient population

This study was conducted from November 2010 to November 2011 at 61 Japanese centers that have departments of gastroenterology. Female outpatients aged 20–64 years were diagnosed with IBS-D based on the Rome III criteria. The study protocol was designed in accordance with the Declaration of Helsinki and was approved by the institutional review board at each site. All patients provided written informed consent prior to participating in study-related procedures.

In Rome III criteria, irritable bowel syndrome with diarrhea (IBS-D) is defined by recurrent abdominal pain/discomfort for at least 3 days per month in the preceding 3 months, in association with two or more of the following: improved defecation, onset associated with a change in the frequency of stools, and/or onset associated with a change in the form (appearance) of stools. Furthermore, patients had loose ( mushy ) or watery stools at least 25% of the time and hard or lumpy stools for less than 25% of bowel movements.

Patients were eligible if they fulfilled the criteria for the last 3 months, with symptom onset at least 6 months prior to diagnosis. Organic diseases were excluded by colonoscopy or double-contrast barium enema if these examinations had not been performed within 5 years. Patients satisfying the inclusion and exclusion criteria for typical IBS-D symptoms during a 1-week baseline period were enrolled. Severity of abdominal pain/discomfort had to exceed mean scores of 0.7 or more assessed daily on a 5-point ordinate (numerical rating) scale ( 0, none; 1, mild; 2, moderate; 3, severe; 4, intolerable). The number of bowel movements had to exceed three times or more per week. Stool consistency was judged with the Bristol Stool Form Scale ( BSFS ) as follows: type 1, separate hard lumps, like nuts ( hard to pass ); type 2, sausage shaped but lumpy; type 3, like a sausage but with cracks on its surface; type 4, like a sausage or snake, smooth and soft; type 5, soft blobs with clear-cut edges ( passed easily ); type 6, fluffy pieces with ragged edges ( mushy stool ); or type 7, watery, no solid pieces, and entirely liquid. Following classification of stool consistency using BSFS, patients who had either type 1 or type 2 stools were excluded to enroll patients who were showing typical symptoms of IBS-D.

Patients who had not used drugs or undergone examinations that could affect the evaluation of study drug efficacy within 10 days prior to randomization; who recorded all items in the patient diary for 5 days or more during the baseline period; and who were not judged ineligible for the study according to the clinical laboratory test results obtained...
before the baseline period were randomized and then administered treatment. Based on a medical interview conducted by the attending physician before provisional registration, patients were excluded if any of the following were evident: a history of resection of the stomach, small intestine, or large intestine (excluding appendicitis or resection of benign polyps); history or current evidence of inflammatory bowel disease; history or current evidence of ischemic colitis, concurrent infectious enteritis, hyperthyroidism, hypothyroidism, or other diseases that may affect gastrointestinal transit or colonic function; history or current evidence of abuse of drugs or alcohol within the previous year; malignant tumors; current evidence of severe depression or a severe anxiety disorder that could potentially affect the evaluation of study drug efficacy; concurrent serious cardiovascular, respiratory, renal, hepatic, gastrointestinal (excluding IBS), hematological, or neurological/psychiatric diseases; or a history of drug allergies, concurrent endometriosis or uterine adenomyosis.

In addition, patients were excluded if they were pregnant or possibly pregnant, lactating or wished to become pregnant during the study period; if they were using drugs or undergoing examinations that could affect the evaluation of study drug efficacy; if they had been enrolled in previous clinical studies of ramosetron or had taken ramosetron; and if they were participating or had participated in other clinical studies within 12 weeks prior to study initiation. This study was registered at Clinicaltrials.gov ID: NCT01274000.

2.2 | Study design

This randomized, double-blinded, placebo-controlled, dose-finding clinical trial comprised a provisional registration period, a 1-week baseline period, and a 12-week treatment period, similar to previous studies.11,15,16 Following the baseline period, eligible patients were randomly assigned to 12-week oral treatment with placebo or ramosetron hydrochloride (1.25, 2.5, or 5 μg once daily) before breakfast. Visits were scheduled at Weeks 2, 4, 8, and 12 (or at discontinuation) to assess treatment efficacy, drug compliance, and occurrence of adverse events. Randomization was performed in a 1:1:1:1 ratio using a block size of four with a web-based randomization system. All patients, investigators, and sponsors were blinded until all observations and evaluations were completed. Statistical analysis plans were finalized, and all data had been locked. All authors had access to the study data and reviewed and approved the final manuscript.

2.3 | Data collection

During the baseline and treatment periods, patients recorded their IBS symptoms daily on paper diary cards at bedtime and electronically entered data into a database daily using an interactive voice response system to support daily completion of data recorded on the paper diary cards. This system of evaluating IBS symptoms has been previously reported to be reliable and valid.11,15,16 In the diary, patients recorded BSFS17 and stool frequencies and scored the severity of their abdominal pain/discomfort on a 5-point ordinate (numerical rating) scale. Every 7 days during the treatment period, patients also graded summarized IBS symptoms compared with the baseline period on a 5-point ordinate scale as follows; relief from overall IBS symptoms and abdominal pain/discomfort (0, completely relieved; 1, considerably relieved; 2, somewhat relieved; 3, unchanged; and 4, worsened) and improvement in abnormal bowel habits (0, nearly normalized; 1, considerably relieved; 2, somewhat relieved; 3, unchanged; and 4, worsened). Patients were assessed for disease-specific health-related QOL18 every 4 weeks using the Japanese version of the IBS-QOL measurement instrument.19

2.4 | Efficacy and safety endpoints

The primary endpoint was monthly responder rates of global assessment of relief of overall IBS symptoms at the first month. The Pharmaceuticals and Medical Devices Agency (PMDA) of Japan had approved the use of this global assessment as a primary endpoint for IBS studies since 2002.15,16 Patients with scores of 0 or 1 at each weekly evaluation point were defined as weekly responders, and patients who were weekly responders for at least two of the 4 weeks were defined as monthly responders. Drug efficacy at the first month is critical for IBS-D patients because a lack of efficacy during this period motivates patients to seek alternative therapies.11

The secondary endpoints were relief of abdominal pain/discomfort, improvement in abnormal bowel habits, stool consistency, stool frequency, urgency, feeling of incomplete evacuation, and IBS-QOL. Weekly responders of abdominal pain/discomfort or improvement in abnormal bowel habits were defined as patients with scores of 0 or 1 at each weekly evaluation point. Weekly responders of improvement in stool consistency were defined as patients with weekly mean BSFS scores of ≥3 to ≤5 during 1 week of the treatment period and a decrease of ≥1 point in mean BSFS scores from the baseline period. If more than two daily scores were missing during any week of the study period, the mean score for that week was defined as missing. Patients with missing mean BSFS scores were regarded as weekly non-responders. Patients who were weekly responders for at least two of the 4 weeks in a month were defined as monthly responders. All adverse events were recorded during the intervention period.

2.5 | Statistical analysis

Statistical analysis was performed using SAS Drug Development (ver. 3.4) and PC-SAS (ver. 9.1.3) (SAS Institute Inc., Cary, NC, USA). Sample sizes for a total of 360 patients (90 patients/group) were calculated to provide more than 80% power to detect a difference in monthly responder rates of global assessment of relief of overall IBS symptoms during the first month among the placebo group and the ramosetron 5-μg group using the Shirley-Williams test with a one-sided significance level of 0.025 based on assumptions regarding response rates in the placebo group (23.8%), and ramosetron 1.25-μg (23.8%), 2.5-μg (42.6%), and 5-μg (42.6%) groups. This assumption was based on gender difference trial (unpublished), previous phase II15 and phase III.16 Multiplicity was controlled by procedure of the test. We estimated that a total of 360 patients (90 patients/group) would be necessary.
for randomization. Efficacy analyses included the full analysis set (FAS), which was as complete as possible and as close as possible to the intention-to-treat ideal of including all randomized subjects. This analysis was authorized according to the International Conference on Harmonisation E9 generated by the regulatory authorities (European Medicines Agency, Food and Drug Administration [FDA]; and PMDA) and pharmaceutical industries of the European Union, US, and Japan based on each party’s agreement.  

The FAS included all patients who received at least one dose of the study drug during the treatment period and in whom more than one endpoint could be evaluated. To determine the robustness of the results, primary analyses were performed according to the per-protocol set. Safety analyses were performed for all patients who received at least one dose of the study drug during the treatment period. Significant level except for primary analysis was set at $P<0.05$.

Monthly responder rates for global assessment of relief of overall IBS symptoms at the first month were expressed as a percentage of responders, and 95% confidence intervals (95% CIs) were presented. The treatment groups were compared using the Shirley-Williams test with a one-sided significance level of 0.025. Monthly responder rates of abdominal pain/discomfort and improvement in abnormal bowel habits were similarly analyzed, but the chi-squared test was used, with a two-sided significance level of 0.05. The monthly responder rates for improvement of stool consistency were compared using the chi-squared test with a two-sided significance level of 0.05 in patients with weekly mean BSFS scores of >5 during the baseline period. Weekly changes in the severity of abdominal pain/discomfort and stool frequency, percentage of days without urgency, and percentage of days without a sense of incomplete evacuation were evaluated using a $t$ test. To compare the ramosetron groups with the placebo group, analysis of covariance was performed with the treatment groups as a factor and baseline scores as covariates to measure changes in the overall IBS-QOL and IBS-QOL subscale scores at each evaluation point from the baseline.

3 | RESULTS

3.1 | Overall study population

Written informed consent was provided by 603 patients. Of these, 194 patients dropped out and 409 patients were randomly allocated to the placebo group ($n=102$), or the ramosetron 1.25-μg ($n=104$), 2.5-μg ($n=104$), or 5-μg ($n=99$) group (Figure 1). Ultimately, 90 patients in the placebo group and 97 (1.25 μg), 98 (2.5 μg), and 82 (5 μg) patients in the ramosetron groups completed the study. The reasons for discontinuation are shown in Figure 1.

All the demographics and baseline characteristics shown in Table 1, except the duration of disease, were similar among patients
allocated to each group. Because no statistically significant difference was observed in the baseline characteristics related to the efficacy endpoint, data were not adjusted. The medication adherence rates were 97.8%, 97.9%, 95.9%, and 93.2% in the placebo, and the ramosetron 1.25-, 2.5-, and 5-μg groups, respectively.

### 3.2 The primary endpoint

The monthly responder rate for global assessment of relief of overall irritable bowel syndrome (IBS) symptoms at the first month was 28.4% (29/102) in the placebo group, 39.4% (41/104) in the ramosetron 1.25-μg group, 38.5% (40/104) in the ramosetron 2.5-μg group, and 40.4% (40/99) in the ramosetron 5-μg group. Comparisons between the placebo group and the ramosetron 5-μg group using the Shirley-Williams test showed no statistically significant difference ($P=.048$) and the lower dose groups were not tested. However, the differences in monthly responder rates in the ramosetron 1.25-μg group (11.0%; 95% CI, −1.8 to −23.8), ramosetron 2.5-μg group (10%; −2.8 to 22.8), and ramosetron 5-μg group (12%; −1.1 to 25.0) were 10% or greater than that in the placebo group. Moreover, at every other time point, ie, at the second month (17.6%; 4.2-30.9), the third month (13.7%; 0.3-27.1), and the last evaluation point (14.6%; 1.2-28.1), the difference in the monthly responder rate in the ramosetron 2.5-μg group was apparently greater than that in the placebo group, by ≥13% (Figure 2).

### 3.3 The secondary endpoints

In the ramosetron 2.5-μg group, the monthly responder rate for abdominal pain/discomfort at the second month ($P=.002$), the third month ($P=.005$), and the last evaluation point ($P=.002$) was significantly higher than that in the placebo group (Figure 3A). In the ramosetron 2.5-μg group as well, the monthly responder rate for improvement in abnormal bowel habits at the second month was significantly higher than that in the placebo group ($P=.016$; Figure 3B). Weekly BSFS scores in each ramosetron group were significantly lower than those in the placebo group with several exceptional weeks and groups (Figure 4A). In patients with weekly mean BSFS scores of >5 during the baseline period, the monthly responder rate for improvement of stool consistency was statistically significantly higher in the ramosetron groups (at the first month, the second month, and the last evaluation point in the ramosetron 1.25-μg group; at the first month, the third month, and the last evaluation point in the ramosetron

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**TABLE 1** Demographics and baseline characteristics

| Patient background | Placebo ($n=102$) | Ramosetron 1.25 μg ($n=104$) | Ramosetron 2.5 μg ($n=104$) | Ramosetron 5 μg ($n=99$) | $P$-value |
|--------------------|------------------|-----------------------------|-----------------------------|--------------------------|-----------|
| Age (years)        | 42.8±12.1        | 40.8±12.1                   | 40.3±12.0                   | 41.1±11.0                | .452      |
| Duration of disease (months) | 193.8±145.1     | 171.0±141.3                 | 131.3±113.8                 | 148.7±124.4              | .005      |
| Severity of abdominal pain/discomfort (0-4) | 1.59±0.6         | 1.71±0.6                    | 1.69±0.6                    | 1.77±0.7                 | .178      |
| Bristol Stool Form Scale (1-7)$^a$ | 5.3±0.6          | 5.3±0.6                     | 5.3±0.5                     | 5.4±0.5                  | .422      |
| Stool frequency (times/day) | 2.1±0.9          | 2.2±1.2                     | 2.1±1.0                     | 2.1±1.1                  | .916      |

Data are expressed as mean±SD.

$^a$Some data were missing; so, actual patient numbers were 98 in the placebo group, and 103 (ramosetron 1.25-μg group), 98 (ramosetron 2.5-μg group), and 97 (ramosetron 5-μg group). $P$-values were calculated using analysis of variance.

**FIGURE 2** Primary endpoints expressing efficacy of ramosetron. Monthly responder rates for relief of overall irritable bowel syndrome (IBS) symptoms. Height: responder rate (%). Error bar: 95% CI

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allocated to each group. Because no statistically significant difference was observed in the baseline characteristics related to the efficacy endpoint, data were not adjusted. The medication adherence rates were 97.8%, 97.9%, 95.9%, and 93.2% in the placebo, and the ramosetron 1.25-, 2.5-, and 5-μg groups, respectively.
2.5-μg group; and at the first month, the second month, and the last evaluation point in the ramosetron 5-μg group) than that in the placebo group (Figure 4B). Weekly changes in stool frequency in each ramosetron group were significantly greater than those in the placebo group with several exceptional weeks and groups (Figure 4C). Ramosetron did not significantly improve IBS-QOL compared with placebo.

### 3.4 Safety

Safety was evaluated in all 409 patients. Adverse events were observed in 44 patients (43.1%) in the placebo group, 57 patients (54.8%) in the ramosetron 1.25-μg group, 57 patients (54.8%) in the ramosetron 2.5-μg group, and 70 patients (70.7%) in the ramosetron 5-μg group. The incidences of constipation and hard stool were higher in the ramosetron groups than those in the placebo group, which was considered to be caused by the pharmacological action of ramosetron (highest in the ramosetron 5-μg group; Table 2). All the events involving constipation and hard stool observed in this study were mild and improved immediately. Serious adverse events included gastroenteritis (one patient) in the placebo group, granulocytopenia (one patient) in the ramosetron 1.25-μg group, and blood potassium increased (one patient) in the ramosetron 2.5-μg group. This increase in blood potassium was mild although an association with the study drug could not be ruled out. There was no occurrence of ischemic colitis.

### 4 Discussion

This study clearly suggests that for women with IBS-D, the optimal dose of ramosetron is 2.5 μg once daily. The optimal dose for women is half the dose (5 μg) for men with IBS-D. These findings support the study hypothesis. Moreover, we succeeded in answering the question why ramosetron failed to be effective in female patients with IBS-D in previous studies. In these studies, the number of female patients per arm was 20–30 in phase II and 42–54 in phase III studies. The sample sizes of female patients were thus too small to permit detection of gender-related differences in the optimal dose of ramosetron.
FIGURE 4  The secondary endpoints supporting the efficacy of ramosetron: stool consistency and frequency. *P<.05, **P<.01, ***P<.001 vs placebo, chi-squared test. (A) Weekly average score for Bristol Stool Form Scale (BSFS) scores (means±95% CI). (B) Monthly responder rates for improvement in stool consistency in patients who had bowel movements for ≥5 days, with a mean score of >5 on the BSFS during the baseline period. (C) Weekly changes in stool frequency (means±95% CI)
In contrast, the sample size of nearly 100 per arm in this study was enough to reveal the clinically useful dose of ramosetron in women. The FDA recommends that the drug industry follow the guidelines for clinical studies that investigate IBS-D.21 With respect to abdominal pain intensity, a weekly or daily responder was defined as a patient with at least a 30% decrease in their weekly averaged score for the worst abdominal pain in the past 24 h, compared with baseline.21 A weekly responder for stool consistency was defined as a patient with at least a 50% decrease in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline, while a daily responder was defined as a patient whose stool consistency is less than 5 for all bowel movements on that day or who has no bowel movement.21 A patient is categorized as a weekly or daily responder if the patient is a responder in terms of both pain intensity and stool consistency but the FDA also allows use of just one major IBS sign or symptom based on the mechanism of drug action. We could not apply these guidelines precisely because they were published after this study. However, the ramosetron 2.5-μg group nearly satisfied the criteria for a better responder rate regarding abdominal pain/discomfort at the second month, the third month, and the last evaluation point and better BSFS below 5 throughout the treatment period, when compared with the placebo group. Although FDA composite score could not be calculated, the efficacy of ramosetron in this study approximates the FDA requirement.

Many factors are thought to influence gender differences in terms of the effect of 5-HT3 antagonists in patients with IBS-D.13,22 Alosetron in women with IBS-D inhibits small bowel transit, colonic transit, and the drug metabolizing enzyme, cytochrome P450, to a greater extent than that in men.13 Brain activation patterns in response to visceral nociception differ in women and men, and women have

| TABLE 2 | Incidence of adverse events |
|----------|-----------------------------|
| Event                | Placebo (n=102)  | Ramosetron 1.25 μg (n=104) | Ramosetron 2.5 μg (n=104) | Ramosetron 5 μg (n=99) |
| All adverse events   | 44 (43.1%)  | 57 (54.8%)  | 57 (54.8%)  | 70 (70.7%)  |
| Gastrointestinal disorders | 22 (21.6%)  | 33 (31.7%)  | 35 (33.7%)  | 46 (46.5%)  |
| Abdominal distension | 5 (4.9%)  | 3 (2.9%)  | 9 (8.7%)  | 5 (5.1%)  |
| Constipation         | 6 (5.9%)  | 13 (12.5%)  | 12 (11.5%)  | 25 (25.3%)  |
| Hard stool           | 7 (6.9%)  | 20 (19.2%)  | 24 (23.1%)  | 30 (30.3%)  |
| Nausea               | 0 (0.0%)  | 1 (1.0%)  | 0 (0.0%)  | 3 (3.0%)  |
| Hepatobiliary disorders | 0 (0.0%)  | 1 (1.0%)  | 1 (1.0%)  | 4 (4.0%)  |
| Hepatic dysfunction  | 0 (0.0%)  | 1 (1.0%)  | 1 (1.0%)  | 4 (4.0%)  |
| Infections and infestations | 19 (18.6%)  | 16 (15.4%)  | 19 (18.3%)  | 22 (22.2%)  |
| Cystitis             | 1 (1.0%)  | 3 (2.9%)  | 2 (1.9%)  | 0 (0.0%)  |
| Nasopharyngitis      | 14 (13.7%)  | 8 (7.7%)  | 13 (12.5%)  | 16 (16.2%)  |
| Pharyngitis          | 0 (0.0%)  | 1 (1.0%)  | 1 (1.0%)  | 2 (2.0%)  |
| Enteritis infectious | 0 (0.0%)  | 0 (0.0%)  | 3 (2.9%)  | 1 (1.0%)  |
| Investigations       | 5 (4.9%)  | 15 (14.4%)  | 12 (11.5%)  | 8 (8.1%)  |
| Increased blood urea | 1 (1.0%)  | 1 (1.0%)  | 1 (1.0%)  | 2 (2.0%)  |
| Increased gamma-glutamyltransferase | 0 (0.0%)  | 0 (0.0%)  | 1 (1.0%)  | 2 (2.0%)  |
| Positive urinary protein | 0 (0.0%)  | 4 (3.8%)  | 0 (0.0%)  | 1 (1.0%)  |
| Musculoskeletal and connective tissue disorders | 1 (1.0%)  | 5 (4.8%)  | 2 (1.9%)  | 4 (4.0%)  |
| Back pain            | 1 (1.0%)  | 2 (1.9%)  | 0 (0.0%)  | 2 (2.0%)  |
| Nervous system disorders | 0 (0.0%)  | 6 (5.8%)  | 2 (1.9%)  | 4 (4.0%)  |
| Headache             | 0 (0.0%)  | 5 (4.8%)  | 0 (0.0%)  | 2 (2.0%)  |
| Somnolence           | 0 (0.0%)  | 1 (1.0%)  | 0 (0.0%)  | 2 (2.0%)  |
| Reproductive system and breast disorders | 1 (1.0%)  | 1 (1.0%)  | 2 (1.9%)  | 2 (2.0%)  |
| Genital hemorrhage   | 0 (0.0%)  | 0 (0.0%)  | 0 (0.0%)  | 2 (2.0%)  |
| Respiratory, thoracic and mediastinal disorders | 2 (2.0%)  | 0 (0.0%)  | 0 (0.0%)  | 5 (5.1%)  |
| Upper respiratory tract inflammation | 2 (2.0%)  | 0 (0.0%)  | 0 (0.0%)  | 3 (3.0%)  |
| Oropharyngeal pain   | 0 (0.0%)  | 0 (0.0%)  | 0 (0.0%)  | 2 (2.0%)  |

Data are expressed as number (%). Events with an incidence of ≥2% in any of the ramosetron groups are listed.
more activation of the amygdala, anterior cingulate cortex, and medial prefrontal cortex.\textsuperscript{22} Although ramosetron is believed to act mainly on the 5-HT3 receptors in the myenteric plexus and vagal afferent neurons,\textsuperscript{23–25} alosetron was proven to have a greater effect than placebo in women with IBS-D, alleviating activation of the amygdala, which is rich in 5-HT3 receptors.\textsuperscript{26} In animal experiments, estrogen sensitizes visceral nociception.\textsuperscript{27} Progesterone reduces the contraction amplitude of the circular and longitudinal muscle and contraction frequency and exerts its inhibitory effect on colonic smooth muscle via changes in the cytoplasmic calcium concentration.\textsuperscript{28} These factors likely relate to the finding that a lower dose of ramosetron is sufficient to improve symptoms of IBS-D but also causes more constipation in women than in men. In fact, 5 μg of ramosetron caused constipation in 3.4% of IBS-D men,\textsuperscript{11} while in this study, the same dose caused constipation in 25.3% of IBS-D women. Moreover, this is why women with IBS-D given 2.5 μg of ramosetron ranked the drug highly for relief from overall IBS symptoms.

There are some limitations to this study. First, efficacy of ramosetron was not proven for the primary endpoint at the first month. However, this is a dose-finding explanatory phase II study. Moreover, monthly responder rates for global assessment of relief of overall IBS symptoms at the second month, the third month, and the last evaluation point improved. In addition, results for abdominal pain/discomfort and stool consistency, which can follow the evaluation method of the FDA guidance\textsuperscript{21} were also meaningful. Second, IBS-QOL was not influenced in this study. This is not surprising because improvement of IBS-QOL may require a larger sample size\textsuperscript{11} or a longer assessment duration for detection.\textsuperscript{29} The feasibility of this assumption is clearly shown in the following phase III trial for IBS-D women with larger sample size ($n$=284 for placebo vs $n$=292 for 2.5 μg of ramosetron) after this study.\textsuperscript{30} Therefore, this study is positioned as an important clinical trial that disclosed how much dose of ramosetron is clinically optimal for IBS-D women.

In conclusion, this study suggests that 2.5 μg of ramosetron may be the most effective and least harmful option for treating female patients with IBS-D. There was no occurrence of ischemic colitis. The optimal dose of ramosetron for women may be lower than that for men. Further works on studies using 5-HT3 antagonist for IBS-D are warranted.

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CONFLICTS OF INTEREST

No competing interests declared. Shin Fukudo, Kei Matsueda, Ken Haruma, and Michio Hongo are contracted medical consultants to Astellas Pharma Inc. Motoko Ida, Hisatake Hayase, Hikaru Akiho, and Yoshiohiro Nakashima are employees of Astellas Pharma Inc.

AUTHOR CONTRIBUTION

SF, MI, HH, and HA designed the study, assessed the data, and wrote the manuscript; YN performed statistical analyses; KM, KH, and MH provided important scientific comments on study design, data analysis, and manuscript content. All authors have approved the final version of the article, including the authorship list.

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