ORIGINAL ARTICLE

Determining an optimal dose of linaclotide for use in Japanese patients with irritable bowel syndrome with constipation: A phase II randomized, double-blind, placebo-controlled study

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Abstract
Background: Clinical testing to determine a suitable dose of linaclotide for Japanese patients with irritable bowel syndrome with constipation (IBS-C) was needed.

Methods: This was a randomized, double-blind, placebo-controlled, dose-finding trial. Japanese patients with IBS-C diagnosed using Rome III criteria (n = 559, men/women: 49/510) were randomly assigned to 1 of 4 linaclotide doses (0.0625, 0.125, 0.25, or 0.5 mg) or placebo for the 12-week treatment period. The primary endpoint was responder rate of global assessment of relief of IBS symptoms during 12 weeks. The secondary endpoints included responder rates of complete spontaneous bowel movement (CSBM), SBM and abdominal pain/discomfort relief and others.

Key Results: The primary endpoint was 23.2%, 36.2%, 38.7%, 34.8%, and 38.3% in placebo (n = 112), 0.0625 (n = 116), 0.125 (n = 111), 0.25 (n = 112), and 0.5 (n = 107) mg of linaclotide groups with the difference from the placebo group in each linaclotide group (13.0%, 15.5%, 11.6%, 15.1%, P > .05). Monthly responder rate of global assessment of relief of IBS symptoms at month 3 (48.6%), responder rate of CSBM during 12 weeks (45.8%), and responder rate of abdominal pain/discomfort relief during 12 weeks (32.7%) in the 0.5 mg were significantly higher than those in placebo group (29.5%, P < .01; 25.9%, P < .01; and 18.8%, P < .05 respectively). The most frequent adverse event in the linaclotide groups was diarrhea.

Conclusions & Inferences: This study suggests that a linaclotide dose of 0.5 mg may be appropriate in Japanese patients with IBS-C.

KEYWORDS
abdominal pain, constipation, guanylate cyclase C activator, linaclotide, stool consistency

Abbreviations: 95% CI, 95% confidence interval; BSFS, Bristol Stool Form Scale; cGMP, cyclic guanosine monophosphate; FDA, Food and Drug Administration; GC-C, guanylate cyclase C; IBS-C, irritable bowel syndrome with predominant constipation; IBS-D, irritable bowel syndrome with predominant diarrhea; IBS-M, irritable bowel syndrome with mixed bowel habits; IBS-QOL, irritable bowel syndrome-quality of life; IBS-U, irritable bowel syndrome unclassified; QOL, quality of life.

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1 | INTRODUCTION

Irritable bowel syndrome (IBS) is one of the functional gastrointestinal (GI) disorders mediated by brain-gut interactions.\(^1\) Irritable bowel syndrome is defined as chronic or recurrent abdominal pain associated with abnormal bowel movements.\(^2\) Irritable bowel syndrome is very common and despite heterogeneity of the 83 qualified studies, pooled prevalence of IBS calculated from 288 103 participants of the general population in 41 global countries is 8.8% (95% confidence interval [CI]: 8.7%-8.9%).\(^3\) Irritable bowel syndrome greatly disturbs quality of life (QOL) of patients.\(^6\) Individuals with IBS incur direct and indirect costs up to approximately 1.4 times that of healthy control individuals.\(^5\) Irritable bowel syndrome is also associated with substantial costs to healthcare systems and society.\(^6\) Developing effective treatment for IBS is beneficial not only to individuals but also to the society.

Recent therapeutic strategies for IBS greatly focuses on differential treatments among subtypes of IBS.\(^7\) Based on the Rome IV criteria,\(^2\) these subtypes are IBS with predominant diarrhea (IBS-D), IBS with predominant constipation (IBS-C), IBS with mixed bowel habits (IBS-M), and IBS unclassified (IBS-U). Among these subtypes, IBS-C is relatively prevalent among geographic regions, ranging from 12.7% for Asia, 13.6% for North America/Western Europe/Australia/New Zealand, 39.4% for Latin America, to 43.3% for the Middle East.\(^3\) Japanese epidemiologic survey showed that constipation increases risk of mortality due to cardiovascular disease.\(^8\) Although these findings demonstrate only an association, if there is a mechanistic link related to prolonged exposure to environmental toxins as can be hypothesized, development of a novel effective and well-tolerated treatment for IBS-C effective across geographic regions is indispensable.

Linacotide is a 14-amino acid peptide that activates the guanylate cyclase C (GC-C) receptor located in the apical cell membrane of the gut epithelium.\(^9\)\(^,\)\(^10\) Activation of GC-C results in the production and release of in cyclic guanosine monophosphate (cGMP) in enterocytes. Intracellular cGMP results in increased secretion of chloride ion and bicarbonate ion into the intestinal lumen and concomitant fluid secretion.\(^11\)\(^\text{-13}\) Distinct benefits of linacotide were shown in patients with IBS-C.\(^14\)\(^,\)\(^15\) Extracellular cGMP has been shown in animal models to act on basolateral afferent neurons to calm abdominal pain-sensing nerves.\(^16\) Phase 3 studies\(^17\)\(^\text{-19}\) conducted in North America evaluated by the Food and Drug Administration (FDA) of the United States (US) and European Medicines Agency demonstrated that linacotide 0.29 mg once per day is a safe and effective dose for patients with IBS-C. Therefore, superficially it seems to be quite natural to assume that linacotide 0.29 mg should also be effective for patients with IBS-C in Japan. However, concerning the pharmacological properties of linacotide, different diet\(^16\) or microbiota\(^12\) may modify the effect of the agent. Clinical testing was needed to find a suitable dose of linacotide for patients with IBS-C in Japan. We tested the hypothesis that the optimal dose of linacotide for patients with IBS-C in Japan is similar to that in the USA or Europe.

2 | METHODS

2.1 | Patient population

This study was conducted from August 2012 to December 2013 at 66 centers with departments of gastroenterology in Japan. Male and female outpatients aged 20-64 years with IBS-C based on the Rome III diagnostic criteria\(^21\) were eligible (Rome IV criteria were not available at the time this trial was started). In brief, patients had to have recurrent abdominal pain or discomfort at least 3 days/month in the last 3 months associated with two or more of the following: improvement with defecation, onset associated with a change in frequency of stool, and/or onset associated with a change in form (appearance) of stool. In addition, patients were required to have IBS symptom onset at least 6 months prior to enrollment. Patients also had to have hard or lumpy stools with at least 25% of bowel movements and loose (mushy) or watery stools with less than 25% of bowel movements.\(^21\) Organic diseases were excluded by colonoscopy or double-contrast barium enema if these examinations had not been performed within 5 years. The study protocol was designed in accordance with the Declaration of Helsinki and was approved by institutional review boards at all sites. All patients provided written informed consent prior to participating in study.

Patients demonstrating the inclusion and exclusion criteria of typical IBS-C symptoms during a 2-week baseline period were enrolled. Weekly mean abdominal pain/discomfort severity score had to be at least 2.0 assessed daily on a 5-point ordinate rating scale (1, none; 2, mild; 3, moderate; 4, severe; and 5, very severe).\(^15\) The concept spontaneous bowel movement (SBM) was defined as defe- cation without laxatives, enema, or manual maneuvers.\(^22\) Complete SBM (CSBM) was defined as an SBM associated with a sensation of complete bowel emptying.\(^14\) Patients were required to demonstrate less than 3 of mean CSBM/week and less than or equal to 5 of mean SBM/week. Stool consistency was classified using with the Bristol Stool Form Scale (BSFS) as follows: type 1, separate hard lumps like nuts (difficult to pass); type 2, sausage shaped but lumpy; type 3, like sausage but with cracks on its surface; type 4, like 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.²² Patients who had either one or more SBMs of type 7 of or two or more SBMs of type 6 were excluded. In addition, 25% or more of SBMs had to be type 1 or 2 of SBM. Patients who had not used drugs or undergone examinations that could affect the evaluation of study drug efficacy within 17 days prior to randomization; who recorded all items in the patient diary for 5 days or more during each week of the baseline period, and who were not judged ineligible for the study according to the clinical laboratory test results received before the baseline period were randomized and subsequently administered treatment.

### 2.2 | Study design

This randomized, double-blinded, placebo-controlled, parallel-group, comparative phase II-dose finding study was comprised of a screening period, a 2-week pretreatment period and a 12-week treatment period (ClinicalTrial.gov number NCT01714843). Following the pretreatment period, eligible patients were randomly assigned to 12-week oral treatments with placebo or 0.0625, 0.125, 0.25, or 0.5 mg of linaclotide (code number ASP0456) once daily before breakfast. Visits were scheduled at week 2, 4, 8, and 12 (or at discontinuation) to assess treatment efficacy, drug compliance, and occurrence of adverse events. Randomization was performed in a 1:1:1:1:1 ratio using a block size of 5 based on randomization list developed by a third-party contract research organization (ADJUST Co., Ltd., Hokkaido, Japan). All patients, investigators, and sponsors were blinded until all observations and evaluations were completed, statistical analysis plans were finalized, all data had been entered, and the database was locked. All authors had access to the study data and had reviewed and approved the final manuscript.

### 2.3 | Data collection

During the pretreatment and treatment periods, patients recorded their IBS symptoms every day in a paper diary at bedtime and with each bowel movement, and subsequently electronically entered these data into a database daily using an interactive voice response system. This system of evaluating IBS symptoms has been previously reported as reliable and valid.²³,²⁴ In the diary, patients recorded stool frequencies and BSFS types and scored the severity of their abdominal pain/discomfort on the 5-point ordinate scale. Straining was assessed on 5-point ordinate scale (1, not at all; 2, a little bit; 3, a moderate amount; 4, a great deal; and 5, an extreme amount). Sensation of incomplete evacuation was assessed as a binary scale (0, absent or 1, present). Every 7 days during the treatment period, patients also graded their global assessment of relief of IBS symptoms, abdominal pain/discomfort relief, and improvement in abdominal bowel habits compared with the baseline period on a 7-point ordinate scale as follows; 1, completely relieved; 2, considerably relieved; 3, somewhat relieved; 4, unchanged; 5, somewhat worse; 6, considerably worse; and 7, as bad as I can imagine. Patients also assessed their disease-specific QOL²⁵ every month using the Japanese version of the IBS-QOL²⁶.

### 2.4 | Efficacy and safety endpoints

The primary endpoint was responder rate of the global assessment of relief of IBS symptoms during 12 weeks. Patients with assessment of 1 or 2 for a weekly evaluation point were defined as weekly responders, and patients who were weekly responders for at least 6 of the 12 weeks were considered to be overall responders. The Pharmaceuticals and Medical Devices Agency approved use of global assessment of relief of IBS symptoms as a primary endpoint for IBS studies.²³,²⁴ Secondary endpoints included relief of abdominal pain/discomfort, improvement in abnormal bowel habits, stool frequency, SBM responder, CSBM responder, stool consistency, severity of abdominal pain/discomfort, straining, and IBS-QOL. Weekly responders of abdominal pain/discomfort relief or abnormal bowel habits improvement were defined as patients with a score of 1 or 2 ranging from 1 to 7 at the weekly evaluation point and patients who were weekly responders for at least 6 of the 12 weeks were considered to be overall responders. Weekly responders of SBM or CSBM were defined as patients with 3 or more SBMs or CSBMs and with 1 or more increase of SBM or CSBM from baseline at the each weekly evaluation point, and patients who were weekly responders for at least 6 of the 12 weeks were considered to be overall responders. All adverse events were recorded during the treatment 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 of 525 patients (105 patients/group) were calculated to provide more than 80% power to detect a difference in global assessment of relief of IBS symptoms during 12 weeks (i.e., the primary endpoint) between placebo and linaclotide based on the US phase II clinical study and US phase III clinical studies.¹⁵ The responder rate of global assessment of relief of IBS symptoms during 12 weeks of placebo, 0.072, 0.145, 0.29 and 0.579 mg in US phase II clinical study were 21.18% (18/85), 35.44% (28/79), 36.59% (30/82), 52.38% (44/84) and 49.44% (44/89) respectively. These results were ad-hoc analysis to design this (0456-CL-0021) study. The responder rate of placebo and 0.29 mg in US phase III clinical studies were 16.63% (67/403) and 39.40% (158/401) in MCP-103-302 study, and 18.48% (73/395) and 37.04% (150/405) in LIN-MD-31 study, respectively. Because there are little different doses between US studies and our study, we assumed same efficacy between 0.072, 0.145, 0.29, and 0.579 mg, and 0.0625, 0.125, 0.25, and 0.5 mg, respectively, and 0.25 mg reaches plateau. Based on these results and assumptions, we set the responder rate of each group as follows: placebo 17.5%, 0.0625 mg 24.4%, 0.125 mg 31.3%, 0.25 mg 38.2% and 0.5 mg 38.2%.

Efficacy analyses were performed on the full analysis set, which was as complete as possible and as close as possible to the intention-to-treat (ITT) ideal of including all randomized subjects.²⁷ The full analysis set included all patients who received at least 1 dose of the study drug during the treatment period and in whom more than 1
endpoint could be evaluated. To determine the robustness of the results, primary analyses were also performed according to the per-protocol set defined as subset of the subjects in the full analysis set who completed the protocol. Safety analyses were performed for all patients who received at least 1 dose of the study drug during the treatment period.

As ad hoc additional efficacy analysis, weekly responders of CSBM+1 and abdominal pain/discomfort severity score were defined as patients with increase in ≥1 CSBM and a decrease in ≥30% in abdominal pain/discomfort score from baseline at each weekly evaluation point respectively. Weekly responders of these evaluation items for at least 6 of the 12 weeks were considered to be overall responders, referring to FDA guidance. Composite responder rate was the proportion of patients who had a composite response of both CSBM+1 and abdominal pain/discomfort severity score, which was referring to the composite responder defined by FDA guidance. Moreover, we added the more stringent SBM responder rate as SBM5+1 overall responder rate. Weekly responder of SBM5+1 was defined as patients with 5 or more SBMs and with 1 or more increase in SBM from baseline at each weekly evaluation point. Patients who were weekly responder for at least 6 of the 12 weeks were considered as overall responders.

Global assessment of relief of IBS symptoms during 12 weeks is expressed as a percentage of patients who are overall responders, and 95% CIs are presented. The linaclootide treatment groups were compared with placebo using Fisher’s exact test with a 2-sided significance level of 0.05 and multiplicity was adjusted by the Hochberg method. Other responder rate parameters were similarly analyzed but without multiplicity adjustment. For comparing change in baseline parameters in linaclootide groups vs placebo, analysis of covariance was performed with the treatment groups as a factor and baseline scores as covariates; these parameters were weekly mean CSBM frequency, weekly mean SBM frequency, weekly mean stool form score, abdominal pain/discomfort severity score, straining severity score, the overall IBS-QOL and IBS-QOL subscale score. Only the primary analysis was adjusted for multiplicity.

3 | RESULTS

3.1 | Overall study population

Among patients who provided written informed consent (n = 1276), 717 of them dropped out prematurely and 559 were randomly allocated. As was defined in the protocol, one patient in the placebo group with no data after the administration of study drug was excluded from

![Flowchart](image-url)  
**FIGURE 1** Flowchart of patient progress throughout the study. Note that 113 patients were allocated to the placebo group at first but 1 patient withdraw consent (*). This patient provided no data. From the rule of full analysis set, placebo group consisted of 112 patients as was shown in the Table 1.
full analysis set used in efficacy analyses. The full analysis set consisted of placebo group (n = 112) and 0.0625 mg (n = 116), 0.125 mg (n = 111), 0.25 mg (n = 112) and 0.5 mg (n = 107) of linaclotide groups (Figure 1). Finally, 102 patients in the placebo group and 95 (0.0625 mg), 97 (0.125 mg), 99 (0.25 mg) and 95 (0.5 mg) patients in linaclotide groups completed the study. The reasons for discontinuation were shown in Figure 1.

All the demographics and baseline characteristics, except the severity of abdominal pain/discomfort, were similar among all the patients allocated to each group (Table 1). No significant difference was observed in the baseline characteristics related to the efficacy endpoint. The medication compliance rate was 99.35%, 98.61%, 98.10%, 98.09%, and 98.41% in placebo, 0.0625, 0.125, 0.25, and 0.5 mg linaclotide groups, respectively.

### 3.2 Evaluation of the primary endpoint

Responder rate of the global assessment of relief of IBS symptoms during 12 weeks was 23.2%, 36.2%, 38.7%, 34.8%, and 38.3% in placebo, 0.0625, 0.125, 0.25, and 0.5 mg of linaclotide groups (Figure 2). Comparisons between the placebo group and each of the linaclotide groups by Fisher’s exact test adjusted by the Hochberg method showed no statistically significant difference. However, the differences in the responder rate of the global assessment of relief of IBS symptoms during 12 weeks between placebo group and each linaclotide group were ≥10% which is considered to be clinically meaningful (13.0%; 95% CI: -1.1 to 24.3, 15.1%; 95% CI: 2.1-28.1, for 0.0625, 0.125, 0.25 and 0.5 mg respectively).

### FIGURE 2 Primary endpoint expressing efficacy of linaclotide. Responder rate of global assessment of relief of IBS symptoms during 12 weeks (%). Column height: responder rate (%). Error bar: 95% CI. P-values derived by Fisher’s exact test compared to placebo were adjusted by Hochberg method to treat multiplicity.
3.3 Evaluation of secondary endpoints

At Month 1, the monthly responder rate of the global assessment of relief of IBS symptoms in every linaclotide group was significantly greater than that in the placebo group (Figure 3A). At Month 3, the responder rates of global assessment in 0.5 mg linaclotide group and 0.125 mg linaclotide group were significantly greater than that in the placebo group. At Weeks 1-12, with the exception of Week 4 and 7, the weekly responder rates of the global assessment of relief of IBS symptoms in 0.5 mg linaclotide group were significantly greater than
that in the placebo group (Figure 3B). At some weeks but not as many weeks as the 0.5 mg linaclotide group, the weekly responder rates of global assessment in the other linaclotide dose groups were significantly greater than that in the placebo group.

In 0.5 mg linaclotide group, the responder rate of CSBM during 12 weeks (45.8%, 95% CI: 36.1-55.7) was statistically significantly higher than that in the placebo group (25.9%, 95% CI: 18.1-35.0, \( P = .003 \)) (Figure 3C). The responder rates of CSBMs during 12 weeks in 0.0625 mg (34.5%, 25.9-43.9), 0.125 mg (32.4%, 23.9-42.0), and 0.25 mg (37.5%, 28.5-47.1) of linaclotide groups suggested a possible dose-dependent increase, but was not statistically significant. Also in the 0.5 mg linaclotide group, all other responder rates, including the responder rate of abnormal bowel habits improvement during 12 weeks, and the responder rate of abdominal pain/discomfort relief during...
FIGURE 3 (continued)
12 weeks, were statistically significantly higher than those in the placebo group (Figure 3D and E). At Week 1-2 and 8, the weekly responder rates of abdominal pain/discomfort relief in 0.5 mg linaclotide group were significantly greater than that in the placebo group (Figure 3F).

Change in weekly mean CSBM frequency during 12 weeks were significantly higher in linaclotide groups than that in the placebo group (Figure 3G). A significant improvement was observed at almost every week except for 0.0625 mg linaclotide group (Figure 3H).

Change in weekly mean stool form score during 12 weeks and change in weekly mean straining severity score during 12 weeks were significantly higher in linaclotide groups than that in the placebo group (Figure 3I and J). Linaclotide did not significantly improve IBS-QOL compared with placebo. The other outcomes were shown in the Figures S1-S4. Change in weekly mean abdominal pain/discomfort severity score during 12 weeks was not clearly different between placebo group and each group of linaclotide (Figure S5). In 0.25 and 0.5 mg groups of linaclotide, responder rate of CSBM+1 during 12 weeks was statistically significantly higher than that in the placebo group. Responder rate of abdominal pain/discomfort severity score during 12 weeks and composite responder rate of CSBM+1 and abdominal pain/discomfort severity score during 12 weeks referring to FDA guidance were not clearly different between placebo group and each group of linaclotide (Figure S6A-C).

3.4 | Safety

Safety was evaluated in 559 patients. Adverse events were observed in 41 patients (36.3%), 39 patients (33.6%), 56 patients (50.5%), 46 patients (41.1%), and 50 patients (46.7%) in placebo, 0.0625, 0.125, 0.25, and 0.5 mg of linaclotide groups. The incidences of diarrhea considered to be caused by the pharmacological action of linaclotide were higher in every linaclotide group than that in the placebo group (Table 2). All the events of diarrhea in this study were mild or moderate. No patient in any group had severe diarrhea. Diarrhea resolved in 54 cases by the end of the study and in 6 cases after the treatment period. Drug-related serious adverse events including hepatic dysfunction (2 patients) in 0.0625 mg linaclotide group and 0.25 mg linaclotide group and diverticulitis (1 patient) in 0.5 mg linaclotide group occurred. There was no dose-dependent increase in number of patients who withdrew from the study.

4 | DISCUSSION

The results of this trial on the treatment of IBS-C patients in Japan with linaclotide supports an optimal dose of 0.5 mg/day which is different from the dose approved for this indication in the US and EU (0.29 mg/day).15,17-19 We judged linaclotide 0.5 mg/day to be optimal because, in this trial of Japanese patients, this dose was associated with the best monthly responder rate of global assessment of relief of IBS symptoms at month 3, the best weekly responder rate of global assessment of relief of IBS symptoms at the last week, the best CSBM responder rate during 12 weeks, the best change in weekly mean CSBM frequency during 12 weeks, the best change in weekly mean stool form score during 12 weeks, and the best change in weekly mean straining severity score during 12 weeks compared to the other linaclotide groups. Moreover, 0.5 mg of linaclotide group showed better SBM responder rate during 12 weeks, responder rate

**TABLE 2** Incidence of adverse events

| Event                        | Placebo (n = 113) | 0.0625 mg (n = 116) | 0.125 mg (n = 111) | 0.25 mg (n = 112) | 0.5 mg (n = 107) |
|------------------------------|-------------------|---------------------|--------------------|------------------|-----------------|
| All adverse events           | 41 (36.3%)        | 39 (33.6%)          | 56 (50.5%)         | 46 (41.1%)       | 50 (46.7%)      |
| Gastrointestinal disorders   | 10 (8.8%)         | 13 (11.2%)          | 28 (25.2%)**       | 24 (21.4%)**     | 19 (17.8%)      |
| Diarrhea                     | 3 (2.7%)          | 8 (6.9%)            | 17 (15.3%)***      | 20 (17.9%)***    | 15 (14.0%)**    |
| Abdominal pain               | 1 (0.9%)          | 2 (1.7%)            | 4 (3.6%)           | 2 (1.8%)         | 2 (1.9%)        |
| Infections and infestations  | 24 (21.2%)        | 15 (12.9%)          | 20 (18.0%)         | 15 (13.4%)       | 26 (24.3%)      |
| Nasopharyngitis              | 18 (15.9%)        | 9 (7.8%)            | 13 (11.7%)         | 10 (8.9%)        | 17 (15.9%)      |
| Influenza                    | 1 (0.9%)          | 1 (0.9%)            | 1 (0.9%)           | 2 (1.8%)         | 4 (3.7%)        |
| Abnormal laboratory test     | 6 (5.3%)          | 9 (7.8%)            | 12 (10.8%)         | 7 (6.3%)         | 5 (4.7%)        |
| Increased plasma triglyceride| 1 (0.9%)          | 0                   | 4 (3.6%)           | 2 (1.8%)         | 1 (0.9%)        |
| Decreased leukocyte count    | 0                 | 3 (2.6%)            | 0                  | 0                | 0               |
| Nervous system disorders     | 4 (3.5%)          | 1 (0.9%)            | 1 (0.9%)           | 1 (0.9%)         | 0               |
| Headache                     | 3 (2.7%)          | 1 (0.9%)            | 0                  | 0                | 0               |

Data were expressed as numbers (%). Events with an incidence of ≥2% in any groups were listed. P-values were calculated by using Fisher exact test as ad-hoc analysis.

*P < .05, **P < .01, ***P < .001 vs placebo.
of abnormal bowel habits improvement during 12 weeks, responder rate of abdominal pain/discomfort relief during 12 weeks, and change in weekly mean SBM frequency during 12 weeks than placebo group. The responder rate for the primary endpoint, global assessment of relief of IBS symptoms during 12 weeks, in the 0.5 mg linaclotide group also tended to be better than that in placebo group (note that only this endpoint was adjusted for multiplicity in the analysis, as it was the primary endpoint; without adjustment, all linaclotide doses would have shown significantly better responder rates compared to placebo). In addition, diarrhea was the only adverse event to be reported significantly more frequently in linaclotide groups than in the placebo group, and the rate was actually lower in the 0.5 mg linaclotide group compared to the 0.125 and 0.25 mg linaclotide groups. Therefore, based on the efficacy and safety results of this study, linaclotide 0.5 mg appears to be the optimal dose for treatment of IBS-C in Japan.

We tried to provide an explanation for the difference in optimal dose in Japan compared to US and EU. Linaclotide activates the GC-C receptor located in the apical membrane of the epithelial cells of the gut. This activation causes accumulation of intracellular cGMP in the gut epithelium. Thus, chloride ions and bicarbonate ion are secreted into the intestinal lumen with concomitant secretion of fluid. The GC-C receptor has a genetic polymorphism associated with attention deficit/hyperactive disorder which is highly comorbid with pediatric constipation. To our knowledge, the distribution pattern and functional role of this common polymorphism of GC-C receptor gene has not been reported in healthy controls and patients with IBS-C in the USA, Europe, or Japan. However, a heterozygous missense mutation (c.2519G→T) with gain of function in the GC-C receptor gene GUCY2C was found to cause familial diarrhea syndrome. Therefore, some studies should be focused on difference in polymorphic distribution and function of GC-C receptor gene between people in Japan and other countries in the future.

The other possibility of the origin of difference in optimal dose of linaclotide between USA/Europe and Japan is a possible difference in natural ligand of the GC-C receptor. Guanylin-family hormones including guanylin and uroguanylin are endogenous ligands of the GC-C receptor that are synthesized in the intestine and released both luminally and into the circulation. Guanylin and uroguanylin are assumed to bind to GC-C receptor competitively with linaclotide. Interestingly, the distribution of the guanylin (GUCA2A) gene polymorphism rs2071499 is different between European and Japanese population (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2071499). This SNP is in the coding region and changes the serine (mRNA: TTC) at position 7 (within the cleaved signal peptide) to phenylalanine (mRNA: TAC), known as missense mutation. While it is not yet known how this polymorphism may impact the expression or activity of guanylin, there may be effects that influence the clinical response to linaclotide. Further study is necessary to clarify this relation between GUCA2A gene and intraluminal guanylin effect.

Diet is also very different between western countries and Japan. Americans consume more fat in their diet (https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweia-data-tables/) than Japanese (http://www.mhlw.go.jp/bunya/kenkou/kenkou_eiou_chousa.html). Diet-induced obesity causes a loss of guanylin expression in the colon with subsequent GUCY2C silencing. Therefore, Japanese diet may induce more expression of intraluminal guanylin than western diet. The gut microbiota is also very different between western countries and Japan. Not only guanylin-family hormones but also heat-stable enterotoxins which are produced by enterotoxigenic Escherichia coli bind to GC-C receptors. The gut metagenome analyses showed that porphyranases and agarases are frequent in the Japanese population and that they are absent in metagenome data from North American individuals. It is possible that the natural ligands for GC-C receptors produced by gut microbiota in Japanese patients act as competitors of linaclotide. Linaclotide is stable in the acidic environment of the stomach and is converted to the metabolite MM-419447 in the small intestine. The disulfide bonds of both peptides are reduced in the small intestine, where they are subsequently proteolyzed and degraded. Actually, Japanese have more abundant Bifidobacteria than other nations and many Bifidobacteria have exopeptidase. Therefore, a difference in endogenous or bacterial proteases might also help to explain 0.5 mg/day as the optimal dose for IBS-C patients in Japan. Moreover, there is 10- to 100-fold inter-individual variability in GC-C mRNA expression in normal intestinal epithelial cells across the population. This study suggests the importance of how the effect of linaclotide may be determined by endogenous and exogenous factors in each individual.

This study has some limitations. Firstly, the primary endpoint of this study was not significant when adjusted for multiplicity. Nonetheless, the responder rate of global assessment of relief of IBS symptoms during 12 weeks in 0.5 mg of linaclotide group tended to be better than that in placebo group but this was not statistically significant. The differences in the global assessment of relief of IBS symptoms for 12 weeks between placebo group and each linaclotide group were clinically meaningful. The CSBM responder rate during 12 weeks and the responder rate of abdominal pain/discomfort relief during 12 weeks in this study were key secondary endpoints as these parameters are recommended as primary endpoints by the FDA guidance for IBS treatment. A second limitation is that the IBS-QOL was not more improved by linaclotide than by placebo in this study. Improvement of QOL in IBS patients by pharmacotherapy may require a longer duration of therapy and/or a larger sample size. Further studies on linaclotide for IBS-C patients in Japan are needed.

In conclusion, this study clearly showed evidence that linaclotide can be clinically applicable outside the USA. However, unlike the US phase 2 study, our phase 2 study results suggest that a linaclotide dose of 0.5 mg may be appropriate in Japanese patients with IBS-C.

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CONFLICTS OF INTEREST
Shin Fukudo, Atsushi Nakajima, Yoshihide Fujiyama, and Hiroto Miwa are contracted medical consultants to Astellas Pharma Inc. Masanori Kosako, Ayako Nakagawa, Hiraku Akiho, and Yoshihiro Nakashima are employees of Astellas Pharma Inc. Jeffrey Johnston was an employee of Ironwood Pharmaceuticals at the time of the study and is a medical consultant to Ironwood now.

AUTHOR CONTRIBUTION
SF, MK, AN, HA designed the study, assessed the data, and wrote the manuscript; YN performed statistical analyses; AN, YF, JMJ, and HM 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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REFERENCES
1. Drossman DA, Hasler WL. Rome IV - functional GI disorders: disorders of gut-brain interaction. Gastroenterology. 2016;150:1257-1261.
2. Lacy BE, Mearin F, Chang L, et al. Bowel disorders. Gastroenterology. 2016;150:1393-1407.
3. Sperber AD, Dumitrascu D, Fukudo S, et al. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review. Gut. 2017;66:1075-1082. https://doi.org/10.1136/gutjnl-2015-311240.
4. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome (IBS). Nat Rev Dis Primers. 2016;2:16014.
5. Leong SA, Barghout V, Birnbaum HG, et al. The economic consequences of irritable bowel syndrome: a US employer perspective. Arch Intern Med. 2003;163:929-935.
6. Canavan C, West J, Card T. Review article: the economic impact of the irritable bowel syndrome. Aliment Pharmacol Ther. 2014;40:1023-1034.
7. Kux L. Department of Health and Human Services, Food and Drug Administration [docket no. FDA-2012-D-0146]: Guidance for industry on irritable bowel syndrome—clinical evaluation of drugs for treatment: availability. Fed Regist. 2012;77:32124-32125.
8. Honkura K, Tomata Y, Sugiyama K, et al. Defecation frequency and cardiovascular disease mortality in Japan: the Ohsaki cohort study. Atherosclerosis. 2016;246:251-256.
9. Currie MG, Fok FK, Kato J, et al. Guanylin: an endogenous activator of intestinal guanylate cyclase. Proc Natl Acad Sci U S A. 1992;89:947-951.
10. Kita T, Smith CE, Fok KF, et al. Characterization of human uroguanylin: a member of the guanylin peptide family. Am J Physiol. 1994;266:F342-F348.
11. Hamra FK, Forte LR, Eber SL, et al. Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. Proc Natl Acad Sci U S A. 1993;90:10464-10468.
12. Giannella RA. Escherichia coli heat-stable enterotoxins, guanylin, and their receptors: what are they and what do they do? J Lab Clin Med. 1995;125:173-181.
13. Forte LR. Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology. Regul Pept. 1999;81:25-39.
14. Andresen V, Camilleri M, Busciglio IA, et al. Effect of 5 days linaclotide on transit and bowel function in females with constipation-predominant irritable bowel syndrome. Gastroenterology. 2007;133:761-768.
15. Johnston JM, Kurtz CB, Macdougall JE, et al. Linaclotide improves abdominal pain and bowel habits in a phase IIb study of patients with irritable bowel syndrome with constipation. Gastroenterology. 2010;139:1877-1886.e2.
16. Castro J, Harrington AM, Hughes PA, et al. Linaclotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3’5’-monophosphate. Gastroenterology. 2013;145:1334-1346.e1-11.
17. Chey WD, Lembo AJ, Lavins BJ, et al. Linaclotide for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. Am J Gastroenterol. 2012;107:1702-1712.
18. Rao S, Lembo AJ, Shiff SJ, et al. A 12-week, randomized, controlled trial with a 4-week randomized withdrawal period to evaluate the efficacy and safety of linaclotide in irritable bowel syndrome with constipation. Am J Gastroenterol. 2012;107:1714-1724.
19. Quigley EM, Tack J, Chey WD, et al. Randomised clinical trials: linaclotide phase 3 studies in IBS-C - a prespecified further analysis based on European Medicines Agency-specified endpoints. Aliment Pharmacol Ther. 2013;37:49-61.
20. Franciscon CF, Sperber AD, Fang X, et al. Multicultural aspects in functional gastrointestinal disorders (FGIDs). Gastroenterology. 2016;150:1344-1354.
21. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology. 2006;130:1480-1491.
22. Fukudo S, Hongo M, Kaneko H, et al. Lubiprostone increases spontaneous bowel movement frequency and quality of life in patients with chronic idiopathic constipation. Clin Gastroenterol Hepatol. 2015;13:249-301.e5.
23. Fukudo S, Ida M, Akiho H, et al. Effect of ramosetron on stool consistency in male patients with irritable bowel syndrome with diarrhea. Clin Gastroenterol Hepatol. 2014;12:953-959.e4.
24. Fukudo S, Kinoshita Y, Okumura T, et al. Ramosetron reduces symptoms of irritable bowel syndrome with diarrhea and improves quality of life in women. Gastroenterology. 2016;150:358-366.e8.
25. Patrick DL, Drossman DA, Frederick IO, et al. Quality of life in persons with irritable bowel syndrome: development and validation of a new measure. Dig Dis Sci. 1998;43:400-411.

26. Kanazawa M, Drossman DA, Shinozaki M, et al. Translation and validation of a Japanese version of the irritable bowel syndrome-quality of life measure (IBS-QOL-J). BioPsychoSoc Med. 2007;1:6.

27. International Conference on Harmonisation: guidance on statistical principles for clinical trials—availability: FDA. Notice. Fed Regist. 1998;63:49583-49598.

28. Hochberg Y. A sharper Bonferroni procedure for multiple significance testing. Biometrika. 1988;75:800-803.

29. Gong R, Ding C, Hu J, et al. Role for the membrane receptor guanylyl cyclase-C in attention deficiency and hyperactive behavior. Science. 2011;333:1642-1646.

30. Liu L, Li H, Wang Y, et al. Association between GUC2C and ADHD: evidence from both categorical and quantitative traits. Psychiatry Res. 2014;220:708-710.

31. McKeown C, Hisle-Gorman E, Eide M, et al. Association of constipation and fecal incontinence with attention-deficit/hyperactivity disorder. Pediatrics. 2013;132:e1210-e1215.

32. Fiskerstrand T, Arshad N, Haukanes BI, et al. Familial diarrhea syndrome caused by an activating GUCY2C mutation. N Engl J Med. 2012;366:1586-1595.

33. Busby RW, Kessler MM, Bartolini WP, et al. Pharmacologic properties, metabolism, and disposition of linaclotide, a novel therapeutic peptide approved for the treatment of irritable bowel syndrome with constipation and chronic idiopathic constipation. J Pharmacol Exp Ther. 2013;344:196-206.

34. Lin JE, Colon-Gonzalez F, Blomain E, et al. Obesity-induced colorectal cancer is driven by caloric silencing of the guanylin-GUCY2C paracrine signaling axis. Cancer Res. 2016;76:339-346.

35. Nishijima S, Suda W, Oshima K, et al. The gut microbiome of healthy Japanese and its microbial and functional uniqueness. DNA Res. 2016;23:125-133.

36. Hehemann JH, Correc G, Barbeyron T, et al. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature. 2010;464:908-912.

37. Minagawa E, Kaminogawa S, Tsukasaki F, et al. Exopeptidase profiles of bifidobacteria. J Nutr Sci Vitaminol (Tokyo). 1985;31:599-606.

38. Bharucha AE, Waldman SA. Taking a lesson from microbial diarrheagenesis in the management of chronic constipation. Gastroenterology. 2010;138:813-817.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

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