Protocol Number RHB-102-02

Randomized, Double-blind, Placebo-controlled, Phase 2 Trial of RHB-102 (Ondansetron 12 mg Bimodal Release Tablets) for Diarrhea Predominant Irritable Bowel Syndrome (IBS-D)

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Protocol Date:

Original:	10 December 2015
Amendment 1:	28 February 2016
Amendment 2:	13 April 2016
Amendment 3:	15 July 2016
Amendment 4:	16 February 2017

Amendment 4: List of Changes

Page/ section	Amendment 3	Amendment 4	Rationale
9, Synopsis and 18, Sec 3.1 Inclusion criterion 1	Male and female patients age≥18 years (with a minimum of 35% males in the study)	Male and female patients age≥18 years (with a minimum of 25% males in the study)	The initial intent of the study was to accrue a substantial number of male patients so that at least suggestive evidence of efficacy in men might be obtained. Due to the difficulty of accruing males, however, the Sponsor has decided to decrease the proportion of males required, understanding that this may make it impossible to assess the effect of gender on response to treatment. The study will still be stratified by gender.
10, Synopsis Second sentence in paragraph	Patients will then be randomized 60:40 to RHB-102 12 mg or placebo.	Patients will then be stratified by gender and randomized 60:40 to RHB-102 12 mg or placebo.	Added the phrase “stratified by gender” to clarify the procedure which is in effect. This phrase is already in the relevant section, 4.3, in the body of the protocol.
19, Sec 3.2 Exclusion Criterion 1.	Relevant abnormalities seen on colonoscopy if previously performed or if required per this protocol. If previously performed or if required per this protocol.. These include…	Relevant abnormalities seen on colonoscopy if previously performed or if required per this protocol.. These include….	Removed redundancy in protocol.
20, Sec 4.3 Randomization and Blinding	At least 35% and not more than 65% of patients entered are to be males.	At least 25% and not more than 65% of patients entered are to be males.	The minimum proportion of male patients is being reduced in order to speed accrual. The Sponsor understands that this may reduce the possibility of seeing a strong efficacy trend in the male subgroup.
24,Sec 5.2.2 On Study Procedures	-	Where medically necessary, Investigators may request additional (optional) lab tests from the central lab; however sites will have to seek approval from the medical monitor prior to requesting these tests.	
25, Sec 5.2.2 On Study Procedures	-	If patient is on every other day dosing: Predose: 44 - 52 hours after prior dose	Clarification for patients who are on every other day dose due to constipation

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Approvals

The investigator agrees to conduct the trial as described in the protocol in accordance with all FDA regulations, and according to current ICH GCP.

By agreement to this protocol, the investigator agrees to allow direct access to all essential documents, including source documents to authorized individuals representing the sponsor (including monitors, auditors and other personnel), to institutional review boards (IRBs) and to regulatory authorities.

[redacted]

Principal Investigator:

_____ _____
Date

Name:_____

Protocol Synopsis

Title	Randomized, Double-blind, Placebo-controlled, Phase 2 Trial of RHB-102 (Ondansetron 12 mg Bimodal Release Tablets) for Diarrhea Predominant Irritable Bowel Syndrome (IBS-D)
Study location	USA
Study drug	RHB-102, ondansetron 12 mg bimodal tablets
Comparator	Placebo
Rationale	Irritable bowel syndrome (IBS) is a functional bowel disorder in which abdominal pain or discomfort is associated with disordered defecation (Longstreth et al, 2006). One of the major types of IBS is diarrhea predominant, IBS-D. 5-HT3 antagonists have been shown to slow intestinal transit time in animals (Clayton et al, 1999) and humans (Garsed et al, 2013). In preliminary studies, ondansetron has demonstrated activity in IBS-D (Steadman et al, 1992; Clayton et al, 1999; Garsed et al, 2013). Unlike alosetron, ondansetron has not been noted to cause ischemic colitis (FDA labeling for Lotronex [alosetron], 2010; FDA labeling for Zofran [ondansetron], 2014). RHB-102 is a bimodal release formulation of ondansetron. It provides an initial release similar to immediate release ondansetron and then extended release over 24 hours. Because of its extended release properties, and once daily dosing it would appear to be an excellent candidate for treatment of IBS-D.
Objectives Primary:	Proportion of patients in each treatment group with response in stool consistency on study as compared to baseline. A weekly stool consistency responder is defined as a patient who experiences ≥50% reduction in the number of days per week with at least one stool with a Bristol Stool Scale score of 6 or 7 compared with baseline, and abdominal pain is unchanged or improved in comparison with baseline. A patient will be considered a stool consistency responder for the study if he or she is a stool consistency responder for ≥50% of the planned weeks of study.
Secondary:	Proportion of patients in each treatment group who are pain responders, per FDA guidance definition Proportion of patients in each treatment group who are overall responders, per FDA guidance definition Differences between treatment groups in Abdominal pain Abdominal discomfort Frequency of defecation Incidence and severity of adverse events
Exploratory:	Decrease in urgency
Population	Patients who meet Rome III criteria for IBS-D and do not have evidence of other gastrointestinal diseases which may be responsible for their symptomatology

Inclusion criteria:	1. Male and female patients age≥18 years (with a minimum of 25% males in the study) 2. Patient meets FDA guidance and Rome III criteria for IBS-D: a. Recurrent abdominal pain or discomfort over ≥6 months, with frequency ≥3 days/month in the last 3 months associated with ≥2 of the following: i. Improvement with defecation ii. Onset associated with a change in frequency of stool iii. Onset associated with a change in the form of stool b. Loose or watery stools (Bristol stool form scale 6 or 7) ≥2 days per week 3. Average worst daily pain intensity ≥3.0 for each of the two baseline weeks 4. Major laboratory parameters within the following limits (no worse than grade 1 abnormalities per NCI-CTCAE v4): a. Adequate hematologic function, as demonstrated by i. Hemoglobin $\geq$10 g/dL ii. ANC 1.5-10 x 10^9/L iii. Platelets $\geq$100 x 10^9/L b. Adequate liver and renal function as demonstrated by i. AST and ALT each $\leq$3.0 x ULN ii. Total bilirubin $\leq$1.5 x ULN iii. Creatinine $\leq$1.5 X ULN c. Euthyroid based on TSH and free T4 levels 5. Patients on thyroid hormone replacement must be on a stable dose for at least one month prior to study entry. 6. C-reactive protein ≤2 x upper limit of normal for lab 7. Patients of childbearing potential and male patients with partners of childbearing potential must utilize effective contraceptive measures Women of childbearing potential are women who have menstruated in the past 12 months, with the exception of women who have undergone surgical sterilization 8. All patients must sign informed consent.
Exclusion criteria:	1. Evidence of other cause for bowel disease: a. Relevant abnormalities seen on colonoscopy if previously performed or if required per this protocol These include but are not limited to Crohn's disease, ulcerative colitis, diverticulitis, ischemic colitis, microscopic colitis. b. History of and/or positive serologic test for celiac disease c. Known or suspected lactose intolerance. 2. History of abdominal surgery other than appendectomy or cholecystectomy at any time 3. Any elective major surgery (of any organ) planned for the period of the study, including follow-up 4. History of organic abnormalities of the GI tract including but not limited to intestinal obstruction, stricture, toxic megacolon, GI perforation, fecal impaction, gastric banding, adhesions or impaired intestinal circulation (e.g., aortoiliac disease) 5. Current or previous diagnosis of neoplasia (except non-GI neoplasia in complete remission ≥5 years, squamous and basal cell

<table>
<tr><td></td><td>carcinomas). With approval of the medical monitor, patients with curatively treated neoplasms in complete remission <5 years may be entered in the study.
6. Patients with a history of positive tests for ova or parasites or Clostridium difficile must be retested during the screening period and tests for the relevant agents must be negative
7. Use of any 5-HT_3 antagonist within 4 weeks of the start of baseline data collection
8. Use of rifaximin within 4 months of the start of baseline data collection
9. Use of any other agent specific for IBS (such as alosetron or eluxadoline) or for symptomatic treatment of IBS (such as antispasmotics and antidiarrheals other than loperamide) within 2 weeks of the start of baseline data collection
10. Uses of any investigational agent for any indication within 4 weeks of the start of baseline data collection
11. Congestive heart failure, bradyarrhythmia (baseline pulse<55/min), known long QT syndrome
12. Patients who have QTc prolongation>450 msec noted on screening ECG, or who are taking medication known to cause QT prolongation
Note: For current list of medications known to cause QT prolongation see:
https://www.crediblemeds.org/healthcare-providers/drug-list/
There are several risk categories. Use the list showing those drugs known to cause torsade de pointes (TdP).
13. Hypersensitivity or other known intolerance to ondansetron or other 5-HT_3 antagonists
14. Patient has taken apomorphine within 24 hours of screening
15. Pregnant or lactating
16. Patients with other major illnesses, either physical or psychiatric, or social situations which may interfere with participation in the study or interpretation of results
17. Patients with severe hepatic impairment, defined as Child-Pugh score ≥10 at baseline.</td></tr>
<tr><td>Design</td><td>This is a randomized double-blind, 2-arm parallel group study. After qualifying for the study and signing informed consent, patients will undergo a two-week observation period during which stool consistency and frequency data and symptom data will be collected. Patients will then be stratified by gender and randomized 60:40 to RHB-102 12 mg or placebo. Patients will continue on treatment for 8 weeks. Each medication will be given once daily.
<table><tr><td>Group</td><td>Treatment</td></tr><tr><td>A</td><td>RHB-102 12 mg</td></tr><tr><td>B</td><td>Placebo</td></tr></table></td></tr>
<tr><td>Methodology</td><td>All patients will undergo baseline evaluation including full history and physical, with particular attention to gastrointestinal symptomatology and findings, a standard set of safety laboratory examinations (CBC and platelet count, biochemical profile, urinalysis, serum TSH and free T4, INR), and 12-lead ECG. In addition, the following studies will be</td></tr>
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	performed to exclude other causes of gastrointestinal symptoms: • Serum testing for C-reactive protein and gluten sensitivity • Colonoscopy if required per protocol • Patients with a history of positive tests for ova, parasites or Clostridium difficile must undergo repeat testing, which must be negative, during the screening period. Starting during the baseline observation phase, all patients will keep diaries of symptomatology and stool frequency and consistency. Stool consistency will be assessed according to the Bristol Stool Form scale (Lewis and Heaton, 1997). Patients will keep diaries of stool frequency and consistency, symptoms, study medication compliance, and use of all medications, including rescue medications, throughout the study. Serum electrolyte assays (bicarbonate, calcium, chloride, magnesium, potassium, and sodium) will be performed at week 3 on study. Safety laboratory examinations will be performed during and after the treatment period in accordance with the study procedures schedule below. Patients will be questioned periodically regarding concomitant medication use and the occurrence of adverse events. **Patients must complete at least 12 days of all baseline diary entries within the 14 day screening period to be eligible to participate in the study. Patients completing fewer than 12 days of diary entries may, at the investigator's discretion, repeat the screening period diary. As long as the patient can complete and enter the study within 6 weeks, baseline laboratory studies need not be repeated. If repeating the 2 weeks' baseline diary will result in a period longer than 6 weeks from consent to start of treatment, the medical monitor must be consulted prior to randomization.**
Endpoints	
Efficacy:	Stool consistency each stool, per Bristol Stool Form scale Worst abdominal pain intensity per 24-hour period, on an 11-point Likert scale Frequency of bowel movements Worst discomfort per 24-hour period, on an 11-point Likert scale Interference of IBS with general functioning, on a 5-point Likert scale
Safety:	Occurrence of adverse events, both clinical and laboratory. Adverse events will be tabulated by system organ class (SOC) and preferred term (PT) using MedDRA version 13.1. Adverse events will be graded 1-4 according to NCI-CTCAE v4 criteria.
Statistics	Sample size will be calculated based on expected difference in primary endpoint between groups using 80% power, and a significance level of α=0.05. Based on Garsed et al, 2014, the expected response rates are 80% in the active group and 40% in the placebo group. To allow for a somewhat smaller difference, i.e., 70% response rate in active group and 40% in placebo group, and 60:40 rather than 1:1 randomization, a total of 120 patients will be randomized, 72 to RHB-102 and 48 to placebo. Safety assessments will be based on results from all patients who receive any study medication, either active or placebo.
Sample size calculation	
Study populations:	

Efficacy:	Efficacy data will be calculated, for the primary analysis, intent to treat, based on all patients randomized and who receive at least one dose of any study medication, either active or placebo. Per protocol analysis will include all patients who receive ≥75% of the planned study medication and have at least 12 days' baseline data and at least one full week (minimum 7 days of data) on study, or discontinue before that due to documented lack of efficacy.
Primary endpoint	The primary endpoint in the study is stool consistency response in the absence of increase in pain (<10% increase in weekly average of worst daily pain). A weekly stool consistency responder is defined as a patient who experiences during a week ≥50% reduction in the number of days per week with at least one stool that has a consistency of Type 6 or 7 compared with baseline, and abdominal pain is unchanged or improved in comparison with baseline. A patient will be characterized as an overall stool consistency responder if the patient was a weekly responder for ≥50% of the weeks of treatment.
Secondary endpoints:	Pain responder: Abdominal pain intensity weekly responder: patient who experiences a decrease in the weekly average of worst abdominal pain in the past 24 hours score ≥30% compared with baseline. A patient is characterized as an overall weekly responder if the patient meets both the stool consistency and pain response definitions for a given week. A patient is characterized as an overall study responder if the patient meets the criteria for overall weekly response for at least half the planned study weeks. Changes from baseline to each week on study for these parameters, as well as for each of the secondary efficacy parameters will be calculated and compared between active treatment group and placebo. Use of rescue medication (any and quantitatively) each week on study and overall across the study will be compared between each active treatment group and placebo.

Table 1. Study Procedures

Procedure / Week	-4 to -2	-2 and -1[a]	1	2[b]	3	5	7	9	13
Screening assessments[c]	X								
Physical examination inc VS[d]	X		X		X	X	X	X	X
Pregnancy test[e]	X		X						
Stool, pain and discomfort assessments[f]		X	X	X	X	X	X	X	X
CBC, biochemical profile[g]	X		X			X		X	X
Urinalysis	X					X		X	X
Electrolytes[h]					X				
Pharmacokinetic sampling[i]			X		X	X			
Concomitant medications	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X
Study medication dispensing			X			X			
Review of study medication use				X	X	X	X	X	

Notes:

[a]During weeks -2 and -1, patients will collect baseline data, including the daily assessments, use of concomitant medications including those for symptoms of IBS, and occurrence of adverse events. These data will be provided to and reviewed by the investigator and/or staff prior to the week 1 visit, at which time the patient will be randomized and start treatment.

[b]Week 2 visit may be a phone contact

[c]Informed consent, history, serum TSH and free T_4, urinalysis, INR, C-reactive protein, colonoscopy when required per protocol (see section 5.2.1, page 29, below), serology for celiac disease, stool for ova, parasites and C diff, respectively, if positive history for any of these, ECG.

[d]Physical examination including vital signs: full physical examination at baseline; interim examinations of pertinent systems with attention to areas of change based on symptoms at subsequent examinations. At each visit: temperature, blood pressure, pulse, respiratory rate, weight. Height to be recorded at initial screening visit only.

[e]For women of childbearing potential.

[f]Stool and symptom assessments: daily Bristol chart; diaries for urgency, worst abdominal pain and worst abdominal discomfort during each day, using 11-point Likert scale (0-10) for each, with 0= no pain or no discomfort and 10=as bad as can be; daily assessment of interference by IBS with activities of daily living on a 5-point Likert scale; daily count of number of bowel movements; adverse events also to be recorded in diary. Diary may be given to patient at initial screening visit or any time thereafter.

[g]Safety labs: CBC, platelet count, biochemical profile to include at a minimum albumin, alkaline phosphatase, ALT, AST, bicarbonate, total bilirubin, BUN, calcium, chloride, creatinine, glucose, magnesium, potassium, total protein, sodium.

[h]Serum Ca, Cl, HCO_3, K, Mg, Na. Need not be fasting or predose.

[i]Pharmacokinetic sampling will be performed on all patients on day 1 prior to initial dosing and on day 1 of weeks 3 and 5. See Table 2, page 24, for details of pharmacokinetic sampling procedure.

List of Abbreviations

°C	degrees Celsius
β-HCG	beta-human chorionic gonadotropin
5-HT3	5-hydroxytryptamine$_3$
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations
CO_2	Bicarbonate
CRF	case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GI	Gastrointestinal
HIPAA	Health Information Portability and Accountability Act
IBS	irritable bowel syndrome
IBS-D	irritable bowel syndrome – diarrhea predominant
ICH	International Committee on Harmonisation
IRB	institutional review board
Kg	kilogram
MedDRA	Medical dictionary for regulatory activities
Mg	milligram
mITT	Modified intent-to-treat
NCI-CTCAE v 4	National Cancer Institute common terminology criteria for adverse events, version 4
PP	Per protocol
SAE	serious adverse event
SAP	statistical analysis plan
SOC	System organ class
VS	vital signs
vs	Versus
WBC	white blood count
WOCBP	women of childbearing potential

1. Background and Rationale

1.1 Irritable Bowel Syndrome-Diarrhea Predominant (IBS-D)

Irritable bowel syndrome (IBS) is a functional bowel disorder in which abdominal pain or discomfort is associated with disordered defecation (Longstreth et al, 2006). Unlike inflammatory bowel diseases, no anatomic or biochemical abnormalities have been correlated with IBS. The disease may cause substantial discomfort and morbidity, and often results in considerable reduction in quality of life (Canavan et al, 2014). Over the past several decades, criteria for diagnosis and classification have been developed. The Rome criteria, most recently Rome III promulgated in 2006, are the most commonly used for diagnosis of IBS and definition of subgroups.

Over 10% of adults worldwide have symptoms of IBS (Canavan et al, 2014). In most studies, prevalence is much greater in females than males, with ratios of 1.5-3.0 to 1 reported in various studies.

Several subtypes of IBS have been defined, based on predominant stool pattern (Longstreth et al, 2006):

IBS-C: IBS with constipation

IBS-D: IBS with diarrhea

IBS-M: mixed IBS, sometimes constipation, sometimes diarrhea

Unsubtyped IBS: not classifiable into one of the above patterns

IBS-D is IBS with at least 25% of stools loose or watery and not more than 25% of stools hard or lumpy. Many different therapies, including pharmacologic, behavioral, yoga and probiotics, have been advocated for treatment of IBS. A recent article reviews pharmacological interventions for IBS (Chang et al, 2014). Their conclusion was that several pharmacologic interventions, including a 5-HT3 antagonist, alosetron, had significant activity in treatment of IBS.

1.2 Ondansetron for IBS-D

5-HT3 antagonists have been shown to slow intestinal transit time in animals (Clayton et al, 1999) and humans (Garsed et al, 2013). One 5-HT3 antagonist, alosetron (Lotronex) has been approved for treatment of IBS in women with severe, chronic IBS-D which has not responded adequately to conventional therapy and in whom anatomic or biochemical abnormalities of the gastrointestinal tract had been excluded. The drug was originally approved and then withdrawn due to the occurrence of ischemic colitis and other severe complications. It was eventually reintroduced with severe restrictions on who can prescribe it and the patients to whom it can be administered.

In preliminary studies, ondansetron has demonstrated activity in IBS-D (Steadman et al, 1992; Clayton et al, 1999; Garsed et al, 2013). Unlike alosetron, ondansetron has not been noted to cause ischemic colitis (FDA labeling for Lotronex [alosetron], 2010; FDA labeling for Zofran [ondansetron], 2014). Thus, due to its safety profile and the preliminary evidence demonstrating efficacy, ondansetron may be preferable to alosetron for treatment of IBS-D.

1.3 RHB-102

RHB-102 is a bimodal release oral formulation of ondansetron. It contains 12 mg of ondansetron, 3 mg immediate release and 9 mg in an extended release matrix. Pharmacokinetic studies in healthy volunteers have demonstrated rapid early release, with appearance of drug in the plasma within half

an hour of dosing, similarly to immediate release Zofran, but with sustained drug availability over 24 hours. Thus, RHB-102 should provide rapid relief, yet require only once daily dosing for a sustained effect. The safety profile of RHB-102 is similar to that of Zofran 8 mg, as demonstrated in volunteer studies.

1.4 Study Rationale

As noted above, there is preliminary evidence that ondansetron is effective in treatment of IBS-D and that it may be a safer alternative to alosetron. Ondansetron has never been developed through rigorous phase 3 studies for this indication.

The goal of this pilot study is to assess the efficacy of RHB-102 for treatment of IBS-D versus placebo. Information from this study will be used to determine whether RHB-102 is sufficiently effective to warrant further assessment in a phase 3 study, and to determine the treatment and assessment parameters to be used in such a study.

2. Objectives

2.1 Primary

Proportion of patients in each treatment group with response in stool consistency on study as compared to baseline.

A weekly stool consistency responder is defined as a patient who experiences ≥50% reduction in the number of days per week with at least one with a Bristol Stool Scale score of 6 or 7 compared with baseline, and abdominal pain is unchanged or improved in comparison with baseline. Baseline is the 2-week period prior to the start of dosing during which stool and other symptom data are collected. A patient will be considered a stool consistency responder for the study if he or she is a stool consistency responder for ≥50% of the planned weeks of study.

2.2 Secondary

Efficacy: Changes in the following assessed for each treatment group:

- Decrease in abdominal pain
- Decrease in abdominal discomfort
- Decrease in frequency of defecation
- Decrease in interference by IBS with activities of daily living

Results of each of the secondary efficacy endpoints will be compared between each active treatment group and the placebo group. In addition, a composite efficacy endpoint of stool consistency and abdominal pain, per FDA guidance, will be calculated for each patient. A patient is a composite responder (FDA endpoint) during a given week if he or she meets criteria for the primary endpoint, above, and meets the criteria for abdominal pain intensity response, a decrease in the weekly average of worst abdominal pain in the past 24 hours of ≥30% compared with baseline.

Safety: Incidence and severity of adverse events by treatment group

2.3 Exploratory

Decrease in urgency

This will be evaluated similarly to other secondary endpoints, above.

3. Study Population

Approximately 120 patients who meet FDA guidance and Rome III criteria (http://www.romecriteria.org/assets/pdf/19_RomeIII_apA_885-898.pdf) for IBS-D and do not have evidence of other gastrointestinal diseases which may be responsible for their symptomatology will be randomized to one of the two treatment groups described below.

3.1 Inclusion Criteria

1. Male and female patients age≥18 years (with a minimum of 25% males in the study)
2. Patient meets FDA guidance and Rome III criteria for IBS-D:
    a. Recurrent abdominal pain or discomfort over ≥6 months, with frequency ≥3 days/month in the last 3 months associated with ≥2 of the following:
        i. Improvement with defecation
        ii. Onset associated with a change in frequency of stool
        iii. Onset associated with a change in the form of stool
    b. Loose or watery stools (Bristol stool form scale 6 or 7) ≥2 days per week
3. Average worst daily pain intensity ≥3.0 for each of the two baseline weeks
4. Major laboratory parameters within the following limits (no worse than grade 1 abnormalities per NCI-CTCAE v4):
    a. Adequate hematologic function, as demonstrated by
        i. Hemoglobin ≥10 g/dL
        ii. ANC 1.5-10 x 10^9/L
        iii. Platelets ≥100 x 10^9/L
    b. Adequate liver and renal function as demonstrated by
        i. AST and ALT each ≤3.0 x ULN
        ii. Total bilirubin ≤1.5 x ULN
        iii. Creatinine ≤1.5 X ULN
    c. Euthyroid based on TSH and free T4 levels
5. Patients on thyroid hormone replacement must be on a stable dose for at least one month prior to study entry.
6. C-reactive protein ≤2 x upper limit of normal for lab
7. Patients of childbearing potential and male partners of females of childbearing potential must utilize acceptable contraceptive measures

    Women of childbearing potential are women who have menstruated in the past 12 months, with the exception of women who have undergone surgical sterilization
8. All patients must sign informed consent.

3.2 Exclusion Criteria

1. Evidence of other cause for bowel disease:
    a. Relevant abnormalities seen on colonoscopy if previously performed or if required per this protocol. These include but are not limited to Crohn's disease, ulcerative colitis, diverticulitis, ischemic colitis, microscopic colitis.
    b. History of and/or positive serologic test for celiac disease
    c. Known or suspected lactose intolerance.
2. History of abdominal surgery other than appendectomy or cholecystectomy at any time
3. Any elective major surgery (of any organ) planned for the period of the study, including follow-up
4. History of organic abnormalities of the GI tract including but not limited to intestinal obstruction, stricture, toxic megacolon, GI perforation, fecal impaction, gastric banding, adhesions or impaired intestinal circulation (e.g., aortoiliac disease)
5. Current or previous diagnosis of neoplasia (except non-GI neoplasia in complete remission ≥5 years, squamous and basal cell carcinomas). With approval of the medical monitor, patients with curatively treated neoplasms in complete remission <5 years may, be entered in the study.
6. Patients with a history of positive tests for ova or parasites or Clostridium difficile must be retested during the screening period and tests for the relevant agents must be negative
7. Use of any 5-HT_3 antagonist within 4 weeks of the start of baseline data collection
8. Use of rifaximin within 4 months of the start of baseline data collection
9. Use of any other agent specific for IBS (such as alosetron or eluxadoline) or for symptomatic treatment of IBS (such as antispasmotics and antidiarrheals other than loperamide) within 2 weeks of the start of baseline data collection
10. Uses of any investigational agent for any indication within 4 weeks of the start of baseline data collection
11. Congestive heart failure, bradyarrhythmia (baseline pulse<55/min), known long QT syndrome
12. Patients who have known QTc prolongation>450 msec, noted on prior ECG, or who are taking medication known to cause QT prolongation

    Note: For current list of medications known to cause QT prolongation see:

    https://www.crediblemeds.org/healthcare-providers/drug-list/

    There are several risk categories. Use the list showing those drugs known to cause torsade de pointes (TdP).
13. Hypersensitivity or other known intolerance to ondansetron or other 5-HT_3 antagonists
14. Patient has taken apomorphine within 24 hours of screening
15. Pregnant or lactating
16. Patients with other major illnesses, either physical or psychiatric, or social situations which may interfere with participation in the study or interpretation of results
17. Patients with severe hepatic impairment, defined as Child-Pugh score ≥10 at baseline

4. Study Design

4.1 Overview

This is a randomized double-blind, 2-arm parallel group study. After qualifying for the study, patients will undergo a two-week observation period during which stool consistency and frequency data and symptom data (listed below and in detail in Appendix I) will be collected. Patients will then be randomized 60:40 to once daily treatment with either RHB-102 12 mg or placebo. Patients will continue on treatment for 8 weeks. All medications will be given orally.

Group	Treatment
A	RHB-102 12 mg
B	Placebo

4.2 Study Drug Administration

Once patients have consented, completed all required pretreatment tests, and found to be eligible for the study, they will be randomized to one of the two treatment groups. Patients will be given a four week supply of drug and instructed to take it once daily, before breakfast with approximately 240 mL (8 oz) of water. If a patient forgets to take the medication before breakfast, he or she can take it later in the day; the time of administration should be recorded.

Patients will also be instructed to complete a diary, including information about stool consistency (Bristol chart), urgency worst abdominal pain, worst abdominal discomfort, interference with daily activities, concomitant medications (including rescue medications) and adverse events.

4.3 Randomization and Blinding

Patients will be stratified by gender (male/female) and randomized 60:40 to treatment with one of two matching products:

- RHB-102 12 mg tablets
- Placebo

At least 25% and not more than 65% of patients entered are to be males. If the planned enrollment for either gender is reached before the end of the study, no more patients of that gender will be enrolled in the study.

4.4 Concomitant Therapy

Patients can continue all concomitant medications, except those specifically excluded in this study, listed below:

- 5-HT3 antagonists (other than study medication)
- Other medications indicated specifically for treatment of IBS-D, e.g., eluxadoline
- Symptomatic treatments for IBS-D such as antispasmodics and antidiarrheals (other than loperamide)
- Apomorphine
- Medications known to cause QT prolongation. Note: For current list of medications known to cause QT prolongation see: https://www.crediblemeds.org/healthcare-providers/drug-list/

  There are several risk categories. Use the list showing those drugs known to cause torsade de pointes (TdP).

5-HT_3 antagonists, including ondansetron, have been associated with serotonin syndrome. When given as a single agent, ondansetron overdose has been associated with serotonin syndrome. However, most reports of serotonin syndrome have been associated with concomitant use of serotonergic drugs (e.g., selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors, mirtazapine, fentanyl, lithium, tramadol, and intravenous methylene blue). Some of the reported cases were fatal.

Symptoms associated with serotonin syndrome may include the following combination of signs and symptoms: mental status changes (e.g., agitation, hallucinations, delirium, and coma), autonomic instability (e.g., tachycardia, labile blood pressure, dizziness, diaphoresis, flushing, hyperthermia), neuromuscular symptoms (e.g., tremor, rigidity, myoclonus, hyperreflexia, incoordination), seizures, with or without gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea). Patients should be warned about and monitored for the emergence of serotonin syndrome, especially with concomitant use of other serotonergic drugs. If symptoms of serotonin syndrome occur, discontinue RHB-102 and initiate supportive treatment.

The dose of ondansetron used in this study is low. Therefore, the risk of serotonin syndrome is considered extremely low, and the potential benefit of control of IBS-D considered to outweigh the risk of serotonin syndrome. However, while the following classes of medications are allowed, use of these during the study is discouraged for patients who are not receiving these at baseline:

- Selective serotonin reuptake inhibitors (SSRIs)
- Serotonin and norepinephrine reuptake inhibitors (SNRIs)
- tricyclic antidepressants

Concomitant use of apomorphine with ondansetron is contraindicated based on reports of profound hypotension and loss of consciousness when apomorphine was administered with ondansetron.

Additional prohibited concomitant medications include systemic antibiotics (other than ≤14 days' treatment for acute illnesses), drugs enhancing gastrointestinal motility, laxatives, and antidiarrheal agents other than loperamide.

All medications which the patient took within the 30 days prior to the start of study therapy and those taken during the study and for 30 days after the last dose are to be recorded in the case report form. If the patient has required thyroid replacement therapy, this must be at a stable dose for at least one month prior to study entry.

The only rescue medication allowed is loperamide, which may not be taken during the baseline assessment period. During the treatment phase, loperamide may not be taken more often than a maximum daily dose of two 2 mg pills (total of 4 mg) and not more than 3 days in any given week.All rescue medication taken and the doses/number of pills used are to be recorded in the CRF.

4.5 Duration of Treatment

Patients will be treated daily for 8 weeks.

4.6 Dose Modification

Patients who develop constipation are to reduce dosing to once every other day. When constipation resolves, if symptoms recur or worsen, the patient may, in consultation with the investigator, increase the dose back to once daily. If constipation recurs, the patient is to again decrease the dose to once every other day.

If constipation persists despite decrease in dose to once every other day, study medication is to be discontinued.

Constipation is defined as no bowel movement for 48 hours or more.

4.7 Discontinuation of Treatment

Patients must discontinue therapy if they require any medication prohibited in this study. Additional reasons for discontinuation, at the investigator's discretion:

- Clinically significant adverse event, regardless of relation to study medication
- Lack of efficacy: worsening symptoms persisting for at least two weeks during the study

In addition, patients may elect to discontinue study therapy at any time.

The investigator shall attempt to obtain follow-up assessments per protocol regardless of when the patient discontinues therapy. The procedures and tests required for the week 9 visit per Table 1 should be performed at the time of early discontinuation of therapy. The follow-up visit (week 13 in Table 1) should be conducted 4 weeks after the early termination visit.

4.8 Replacement of Patients

Patients who are randomized but receive no study medication shall be replaced.

Patients who receive any study medication shall not be replaced.

5. Study Evaluations

5.1 Schedule of Evaluations

A flow chart of study evaluations is shown above, in Table 1. Informed consent must be obtained before any study-specific procedures are performed. However, data from procedures and assessments carried out prior to consent in the context of routine clinical care (e.g., endoscopies) may be used if they have been performed within study-required windows.

5.2 Description of Study Procedures

5.2.1 Baseline procedures

- Informed consent
- History and physical, including vital signs. History should include detailed description of IBS symptomatology and therapy.
- Physical examination should include all pertinent body systems.
- Vital signs should include height, weight, temperature, pulse, blood pressure, respiratory rate. All units should be metric.
- Baseline laboratory studies: CBC, biochemical profile, urinalysis, INR, serology for celiac disease and, for women of childbearing potential, serum or urine pregnancy test.
  - CBC to include: total RBC, total WBC count, absolute values for individual white blood cell types and platelet count.
  - Biochemical profile to include: albumin, alkaline phosphatase, ALT, AST, (total) bilirubin, BUN, calcium, chloride, CO_2, creatinine, glucose, magnesium, potassium, sodium, total protein.
  - Urinalysis to include at a minimum: routine: dipstick tests for protein, glucose, ketones, blood, pH, specific gravity; microscopic: erythrocytes, leukocytes, bacteria, crystals, casts.
  - Celiac serology cascade
  - Urine or serum pregnancy test (β-HCG) for women of childbearing potential
- Each patient's Child-Pugh score will be calculated based on physical examination and baseline laboratory data. Patients with Child-Pugh score≥10 will be disqualified and not randomized. See Appendix II for calculation of Child-Pugh score.
- 12-lead ECG
- Colonoscopy is required for patients at least age 50 at the time of consent or if they meet any of the following criteria:
  a. Documented weight loss within the past 6 months,
  b. Nocturnal IBS symptoms,
  c. Familial history of colon carcinoma, or
  d. Blood mixed with stool (excluding bleeding from hemorrhoids)
- Patients who have had a colonoscopy within 5 years of signing consent need not have a repeat colonoscopy unless they meet one or more of criteria a, b or d. If colonoscopy is required per protocol, biopsies are required. Biopsies are not mandatory for nonstudy colonoscopies.
- Stool and symptom assessments daily for 14 days, completing not more than two weeks prior to planned start of treatment (see Appendix I):
  - Bristol chart for stool consistency
  - Likert scales for urgency, worst pain and worst abdominal discomfort: 11-point scales
  - Likert scale for interference by IBS with activities of daily living: 5-point scale
  - Adverse events

- Concomitant medications (regardless of relationship to IBS) over 30 days prior to planned start of treatment

In general, colonoscopy if needed should be performed after collection of baseline stool and symptom data. If performed prior to collection of baseline stool and symptom data, sufficient time should elapse after colonoscopy so that patient returns to his or her basal bowel movement status prior to collection of the stool and symptom data.

Patients must complete at least 12 days of all baseline diary entries within the 14 day screening period to be eligible to participate in the study. Patients completing fewer than 12 days of diary entries may, at the investigator's discretion, repeat the screening period diary. As long as the patient can complete and enter the study within 6 weeks, baseline laboratory studies need not be repeated. If repeating the 2 weeks' baseline diary will result in a period longer than 6 weeks from consent to start of treatment, the medical monitor must be consulted prior to randomization.

5.2.2 On-study procedures

Patients will be seen on day 1 of treatment, day 1 of weeks 2 and 3, every 2 weeks thereafter while on treatment (weeks 5, 7, 9) and 4 weeks after the end of treatment. Assessments are to be performed at the times indicated in the chart of evaluations, with the following windows:

week 1 visit: within 6 weeks of consent;

weeks 2, 3, 5, 7 and 9 visits: ±2 days;

week 13 (follow-up) visit: ±4 days.

All the windows are based on day 1 of treatment, rather than cumulative changes. Thus, patients who adhere to the visit windows will not run out of study medication.

At the visits indicated in Table 1:

- Vital signs will be recorded,
- Patients will undergo a brief physical examination of pertinent systems,
- Study medication administration and concomitant medication use will be reviewed,
- Adverse events recorded.
- Completion of Bristol charts and Likert scales will be reviewed.
- At week 3 of the study (i.e., after 2 weeks of treatment), serum electrolytes will be assayed on all patients.
- CBC and biochemical profile will be repeated at weeks 5, 9 and at the end of study visit. Urinalysis and pregnancy test will be repeated only if clinically indicated. Where medically necessary, Investigators may request additional (optional) lab tests from the central lab; however sites will have to seek approval from the medical monitor prior to requesting these tests.
- Limited pharmacokinetic sampling will be performed, as shown in the following table:

Table 2. Pharmacokinetic sampling

	Time, relative to dose	
Week/day	Group A	Group B
1/1	Predose	Predose

3/1	Predose, 1 h postdose	6 h postdose
5/1	6 h postdose	Predose, 1 h postdose

All patients will have a predose (blank) sample drawn prior to their first dose of study medication. Patients will be randomized to give two additional samples for PK analysis either just prior to and one hour after the first dose of week 3 (i.e., after 2 weeks of treatment) or week 5 (i.e., after 4 weeks of treatment) and a fourth sample 6 h after the first dose of week 5 or week 3. On-treatment PK sampling windows are as follows:

- Predose: 20-28 hours after prior dose and before the dose on the day of sampling
- One hour post-dose: ±10 minutes
- Six hours post-dose: ±30 minutes

If patient is on every other day dosing:

- Predose: 44 -52 hours after prior dose

6. Safety Reporting

All patients will be assessed regularly for potential adverse events (AEs) occurring at any time after the patient signs the informed consent until 28 days after the last dose of study medication. AEs ongoing at the end of treatment will be followed until resolution or 28 days after the last dose of study medication, whichever comes first. All patients dosed will return to clinic approximately 4 weeks after the last dose of study medication and queried regarding the occurrence of AEs.

6.1 Terminology

An *Adverse Event (AE)* is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (may also be referred to as an adverse experience) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational or marketed) product, whether or not considered related to the medicinal (investigational or marketed) product and from any route of administration, formulation, or dose, including an overdose.

A *Serious Adverse Event (SAE)* is any untoward medical occurrence that at <u>any dose results in any of the following outcomes</u>:

death;

is a life-threatening adverse event (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe);

requires in-patient hospitalization or causes prolongation of existing hospitalization;

a persistent or significant disability/incapacity incapacity or substantial disruption of the ability to conduct normal life functions;

a congenital anomaly/birth defect;

is an important medical event. This is defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the other serious outcomes listed in the definition above. Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

If either the sponsor or investigator believes that the event is serious, the event must be considered serious and be evaluated by the Sponsor for expedited reporting.

A *suspected adverse reaction* means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

6.2 Assessment of Causal Relationship

The following categories and definitions for assessing the causal relationship of an event to the investigational product(s) are provided as a guide to be used for evaluating adverse events reported in this study to determine "suspected adverse reactions" that require expedited reported to regulatory agencies if they are unexpected. In addition to the assessment below, the aggregate number of

occurrences will be considered to decide whether the event is a reportable event and requires an IND safety report.

Table 3. Relationship of Study Medication to Adverse Events

Unrelated	The study drug almost certainly (or certainly) did not cause the event. Guidelines: There is no reasonable temporal relationship of the event to the administration of drug; The pattern is inconsistent with that known for the drug; and/or There is another obvious etiology.
Probably not related	It is more likely that the event is due to another etiology than due to the study drug. Guidelines: There is no reasonable temporal relationship of the event to the administration of drug; The pattern is inconsistent with that known for the drug; and/or There is another more likely etiology.
Possibly related	It is approximately equally likely that the event is due to the study drug as it is due to another etiology. Guidelines: There is a reasonable temporal relationship of the event to the study drug; The drug seems as likely as other etiologies to have caused the effect
Probably related	It is more likely that the event is due to the study drug than due to another etiology. Guidelines: There is a reasonable temporal relationship of the event to the study drug; The event may be consistent with a known pattern of drug (or drug class) effects; The drug seems more likely than other etiologies to cause the effect; The adverse event diminished upon cessation of study drug exposure or reduction in dose; and/or The adverse event worsened or recurred upon unintentional re-exposure to the study drug (Intentional rechallenge for the purpose of assigning causality should not be performed.)
Definitely related	The evidence is compelling that the study drug caused the adverse event. Guidelines: There is a reasonable temporal relationship of the event to the study drug; The event is consistent with a known pattern of drug (or drug class) effects; The drug is far more likely than other etiologies to have caused the effect; The adverse event diminished upon cessation of study drug exposure or reduction in dose; The adverse event worsened or recurred upon unintentional re-exposure to the study drug (Intentional rechallenge for the purpose of assigning causality should not be performed.)
Unknown	The data are inadequate to assign any of the above causal relationship categories to the study drug.

6.3 Adverse Event Grading and Coding

Adverse events will be graded according to the following criteria:

Grade	**Description**
1	Mild
2	Moderate
3	Severe
4	Life-threatening or disabling
5	Fatal

AEs will be coded, grouped and tabulated by MedDRA preferred terms by body system organ class. MedDRA version 13.1, used previously for RHB102 studies will be used for this study as well.

6.4 Handling of Serious Adverse Events

Adverse events classified as serious must be recorded on the AE page of the CRF and require expeditious handling and reporting to the sponsor, RedHill Biopharma, to comply with regulatory requirements. These SAEs will include deaths, regardless of their causal relationship to investigational product. All SAEs must be reported using the Serious Adverse Event Report form. To the extent possible, the descriptive terminologies and other SAE attributes entered on the SAE report form should approximate similar information in the CRF. **The completed SAE report form with supporting documentation must be provided to the sponsor within 24 hours of the study site personnel's initial notification/awareness of the event.** All telephone communication regarding SAE must be followed by a written report. Duly authorized study site personnel may sign completed SAE report forms; however, it is recommended that the investigator sign each final SAE report.

Collection of complete information concerning SAEs is extremely important. Thus, follow-up information that becomes available as the SAE evolves, as well as supporting documentation (e.g. hospital discharge summaries, additional lab and test results, autopsy reports, etc.), should be collected subsequently, if not available at the time of the initial report, and immediately sent to sponsor using the same procedure as the initial SAE report. Information on the SAE must be in sufficient detail to allow for a complete medical assessment of the case and independent determination of causality.

For ease of analysis, worldwide standardization, and regulatory reporting, the sponsor or its designee will code each reported adverse event or symptom to its corresponding preferred term and body system/organ class in the MedDRA dictionary version adopted for the study. The principal investigator will be responsible for assessing severity based on the intensity of the event as it presented using the criteria listed in section 6.3, above.

[redacted]

In addition, the study medical monitor may be contacted directly:

[redacted]

As required, all investigators will be notified of all AE reports that are determined to be serious, unexpected, and related (by the reporting investigator or sponsor) to the investigational product. The notification will be in the form of a Safety Update (Dear Doctor Letter).

The notification is considered an addendum to the current Investigator's Brochure; therefore, upon receiving such notices, the investigator must review and immediately submit a copy to the IRB

according to local regulations. The notification must be retained within the Investigator's Brochure. The investigator and IRB will determine if the informed consent requires revision.

Pregnancies will not be considered SAEs but will be handled and reported in a manner similar to SAEs. [redacted].

6.5 Laboratory Test Abnormalities

All new abnormal laboratory findings and those abnormal at baseline which change significantly (i.e., by at least one toxicity grade as defined in the National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] v4.03) are considered AEs. Laboratory AEs for which there is no clinical intervention will be extracted from the laboratory data. Laboratory AEs not listed in the NCI CTCAE v4.03 will be considered as grade 1 (mild) if there is no clinical effect or intervention. Laboratory values outside the normal range for certain parameters will not be considered AEs if they are generally not considered as indicating an abnormality; this includes such parameters as liver enzymes which are below the normal range. If there is a clinical sequela or intervention, the laboratory abnormality is to be graded according to the criteria used for clinical AEs, described above.

The NCI CTCAE v4.03 can be downloaded in pdf, Excel or OWL format at:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

6.6 Other Safety Considerations

At the follow-up visit, all patients will be questioned regarding the occurrence of adverse events, including serious and nonserious adverse events.

All AEs must be recorded and followed until resolution or for at least 28 days after discontinuation of study medication, whichever comes first.

Any clinically significant changes noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded on the AE page of the CRF.

7. Data Analysis and Statistical Considerations

7.1 Sample Size Considerations

Sample size is calculated based on expected results for achievement of the FDA-specified endpoint for stool consistency:

> A stool consistency weekly responder is defined as a patient who experiences ≥50% reduction in the number of days per week with at least one stool that has a consistency of type 6 or 7 compared with baseline.
>
> A patient will be characterized as an overall responder if the patient was a weekly responder for ≥50% of the weeks of treatment.

In addition, to be considered a responder for the week, the patient cannot have an increase in average abdominal pain >10% over baseline during that week.

Based on Garsed et al, 2013, it is expected that approximately 80% of patients in the active treatment group and 40% of patients in the placebo group will be responder. Using a continuity-corrected Chi-Square test, to achieve a statistically significant difference between the active group and the placebo group with 80% power and significance level of $\alpha = 0.05$ using 1:1 randomization, 28 patients per group must be treated. To allow for a smaller difference, i.e., 70% response rate in the active group and 40% in placebo group, and for a 3:2 randomization ratio, a total of 104 patients would be necessary to achieve this power. In order to allow for some further decrease in the actual difference in response rates between active and placebo, e.g., early drop-outs or major protocol violations, a total of 120 patients will be randomized, 72 to RHB-102 and 48 to placebo. If no substantial violations impact the results of the study, this sample size will achieve a power of 87.5%.

7.2 Statistical Methods

A detailed description of all statistical analyses to be performed for this study will be given in a statistical analysis plan (SAP). Any deviations from the analysis detailed in the protocol will be described in the SAP.

7.2.1 General Analysis and Coding

The statistical analysis will be performed using the software package SAS version 9.3 or higher (SAS Institute Inc., Cary, NC 27513, USA). All individual data as well as results of statistical analyses, whether explicitly discussed in the previous sections or not, will be presented in individual patient data listings and statistical summary tables.

In general, continuous variables will be summarized using the following standard descriptive summary statistics: number of observations, arithmetic mean, standard deviation, minimum, median, maximum. Categorical data will be described using absolute and relative frequencies. Shift tables will be provided, where appropriate. Confidence Intervals (CI) will be two-sided 95% CIs if not otherwise specified. If not specified otherwise, statistical tests will be two-sided with a significance level of $\alpha = 0.05$.

In general, if baseline values for (semi-) continuous variables are derived from the daily assessments during the two-week observation period preceding randomization, these will be defined as the average of the respective measurements over this two-week period.

All analyses and summary tables will be displayed by treatment group.

Any changes in the planned statistical methods described in the SAP will be documented in the clinical study report.

The following international dictionaries will be used for medical coding:

Diagnoses: Medical Dictionary for Regulatory Activities (MedDRA), version 13.1

Medications: World Health Organization (WHO) Drug Dictionary including ATC classification

AEs: MedDRA, version 13.1

7.2.2 Analysis Populations

The following analysis populations will be defined for the statistical analyses:

- The Safety population will include all patients who received at least 1 dose of study drug (even if the patient vomits immediately after drug administration). In the safety population patients will be analyzed by the actual treatment they received.
- The modified Intent-to-Treat (mITT) population will include all randomized patients who received at least 1 dose of study drug. In the mITT population, patients will be analyzed by the treatment they were randomized to.
- The Per-protocol (PP) population will include all patients from the mITT population who do not have a major protocol deviation that would affect treatment evaluation. Patients who discontinue the study due to documented lack of treatment effect will be included in the PP.
- The Pharmacokinetic Population (PKP) will include all patients with quantifiable concentrations of ondansetron in plasma and sufficient and reliable dosing and sampling information to permit estimation of elapsed time since the prior dose.

Major protocol deviations include but are not limited to:

- Lack of compliance defined as having administered <75% of the planned study medication until discontinuation.
- Fewer than 12 days (of planned 14 days) of baseline data.
- Fewer than 7 days of data if not discontinued before due to lack of efficacy.

Additional major protocol deviations will be defined in the SAP.

7.3 Baseline Characteristics and Demographics

Demographic and pre-treatment patient characteristics, including disease parameters and comorbidities, will be summarized with descriptive statistics.

7.4 Efficacy Analyses

All efficacy analyses will be conducted in the mITT and PP populations.

7.4.1 Primary Endpoint Analysis

The primary endpoint in the study is stool consistency response in the absence of increase in pain ≥10%.

A weekly stool consistency responder is defined in the FDA guidance on IBS-D as a patient who experiences during a week a ≥50% reduction in the number of days with at least one stool that has a consistency of Type 6 or 7 on the Bristol stool scale compared with baseline. In addition, to be considered a responder for the week, the patient cannot have an increase in average abdominal pain ≥10% over baseline during that week. A patient will be characterized as an overall stool consistency responder if the patient was a weekly responder for at least 50% of the weeks of treatment. For the handling of missing weekly data see section 7.4.3.

Let p_O denote the response rate under RHB-102 and p_P denote the response rate under placebo then the primary two-sided hypothesis to be tested is

$$H_0: p_O - p_P = 0$$

vs the alternative

$$H_1: p_O - p_P \neq 0.$$

A Chi-Square test with continuity correction at the significance level $\alpha = 0.05$ will be employed for the primary (confirmatory) analysis of the primary endpoint.

In addition, for each week the weekly response rates will also be compared between the treatment groups with a Chi-Square test with continuity correction.

To assess the effect of gender on the primary endpoint, a logistic regression model with treatment group and gender as covariates will be applied.

7.4.2 Secondary Endpoint Analyses

Secondary efficacy endpoints include the following:

- Proportion of patients in each treatment group who are pain responders, per FDA guidance definition
- Proportion of patients in each treatment group who are overall responders, per FDA guidance definition
- Differences between treatment groups in
  - Abdominal pain
  - Abdominal discomfort
  - Frequency of defecation
  - Interference by IBS with activities of daily living
  - Use of rescue medication

All secondary analyses are considered as exploratory, no adjustments for multiplicity will be made.

Pain response

A weekly pain responder is defined as a patient who experiences a decrease in the weekly average of worst abdominal pain in the past 24 hours score ≥30% compared with baseline and the number of days per week with at least one stool with consistency of type 6 or 7 that is the same as baseline or decreased and the number of stools of Type 6 or 7 on those days remains unchanged or decreased.

An overall pain responder is defined as patient who was a weekly pain responder for at least 50% of the weeks of treatment.

A Chi-Square test with continuity correction at the significance level $\alpha = 0.05$ will be employed for the comparison of the treatment groups regarding overall and weekly pain response.

To assess the effect of gender on the overall pain response, a logistic regression model with treatment group and gender as covariates will be applied.

Overall response

A patient is characterized as a weekly responder if the patient meets both the stool consistency and pain response definitions for a given week.

A patient is characterized as an overall study responder if the patient meets the criteria for both weekly stool consistency and pain response for at least for at least 50% of the weeks of treatment.

A Chi-Square test with continuity correction at the significance level $\alpha = 0.05$ will be employed for the comparison of the treatment groups regarding overall and weekly study response.

To assess the effect of gender on the overall study response, a logistic regression model with treatment group and gender as covariates will be applied.

Abdominal pain

Abdominal pain is measured as the worst abdominal pain during a 24-hour period, on an 11-point Likert scale ranging from 0 (no pain) to 10 (as bad as can be imagined). For each treatment week the change in the weekly average of worst abdominal pain from baseline will be compared between the treatment groups with an Analysis of Covariance (ANCOVA) model including treatment group, study site and gender as independent variables and baseline abdominal pain (see section 7.2.1) as covariate. To assess the impact of time a Repeated Measurement Model will be applied with the weekly changes from baseline as dependent variables, treatment group, study site, gender, week and treatment x week interaction as independent variables and baseline abdominal pain as covariate.

Abdominal discomfort

Abdominal discomfort is measured on an 11-point Likert scale ranging from 0 (no discomfort) to 10 (as bad as can be imagined). For each treatment week the change in the weekly average of abdominal discomfort from baseline will be compared between the treatment groups with an Analysis of Covariance (ANCOVA) model including treatment group, study site and gender as independent variables and baseline abdominal discomfort (see section 7.2.1) as covariate. To assess the impact of time a Repeated Measurement Model will be applied with the weekly changes from baseline as dependent variables, treatment group, study site, gender, week and treatment x week interaction as independent variables and baseline abdominal discomfort as covariate.

Interference by IBS with activities of daily living

Interference with daily activities is measured on a 5-point Likert scale ranging from 0 (no interference) to 4 (unable to carry out activities of daily living). For each treatment week the change in the weekly average of interference from baseline will be compared between the treatment groups with an Analysis of Covariance (ANCOVA) model including treatment group, study site and gender as independent variables and baseline interference score (see section 7.2.1) as covariate. To assess the impact of time a Repeated Measurement Model will be applied with the weekly changes from baseline as dependent variables, treatment group, study site, gender, week and treatment x week interaction as independent variables and baseline interference score as covariate

Frequency of defecation

For each treatment week the change in average daily frequency of defecation from baseline will be will be compared between the treatment groups with an Analysis of Covariance (ANCOVA) model including treatment group, study site and gender as independent variables and baseline daily

frequency of defecation (see section 7.2.1) as covariate. To assess the impact of time a Repeated Measurement Model will be applied with the weekly changes from baseline as dependent variable, treatment group, study site, gender, week and treatment x week interaction as independent variables and baseline daily frequency of defecation as covariate.

Use of rescue medication

Use of rescue medication will be analyzed qualitatively and quantitatively. For each treatment week and overall across the treatment phase the number of patients using any rescue medication will be compared between the treatment groups using a continuity corrected Chi-Square test. The number of days rescue medication is used in each week of treatment and overall will be compared between the treatment groups using the Wilcoxon Rank-Sum test.

7.4.3 Exploratory Endpoint Analysis

Urgency

Urgency is the feeling of a need to defecate and inability to control or incomplete control over defecation. Urgency over the course of the day, is measured on an 11-point Likert scale ranging from 0 (no urgency) to 10 (as bad as can be imagined). For each treatment week the change in the weekly average of urgency from baseline will be compared between the treatment groups with an Analysis of Covariance (ANCOVA) model including treatment group, study site and gender as independent variables and baseline urgency (see section 7.2.1) as covariate. To assess the impact of time a Repeated Measurement Model will be applied with the weekly changes from baseline as dependent variables, treatment group, study site, gender, week and treatment x week interaction as independent variables and baseline urgency as covariate.

7.4.4 Pharmacokinetic analyses

Nonlinear mixed-effects methods will be used to develop a population-based pharmacokinetic (PopPK) model of ondansetron concentrations in plasma after administration of RHB-102. Individual post hoc Bayesian estimates of pharmacokinetic (PK) model parameters will be used to estimate the steady-state peak (Cmax) and trough ($C\tau$) concentrations and the area under the curve of concentration versus time over the dosing interval ($AUC\tau$). Cmax, $C\tau$, and $AUC\tau$ will serve as exposure metrics in exploratory plots of exposure versus relevant efficacy and safety endpoints. Exposure-response (ER) models may be developed to describe emerging trends. PopPK and ER modeling may be described in a separate modeling and simulation analysis plan and reported separately from the clinical study report. The date and time will be recorded for the dose of RHB-102 (or placebo) taken prior to blood sampling to determine ondansetron concentration in plasma. This is the dose of the prior day for pre-dose PK samples.

7.4.5 Subgroup Analyses

The primary endpoint, improvement in stool consistency, and the secondary endpoints pain response and overall response will be analyzed for all patients and by gender. To evaluate the differences in effect by gender, additional efficacy endpoints may be analyzed by gender if trends towards differences (p-value from logistic regression ≤ 0.1) are seen in the endpoints initially analyzed by gender.

Further sub-group analyses may be performed as ad-hoc analyses after the protocol-defined analyses if this is suggested by the data.

7.4.6 Missing Data

If a patient fails to provide any assessments after baseline (e.g. if the diary is missing completely) he/she will be counted as a non-responder in any of the overall responder analyses (overall stool consistency response, overall pain response, overall study response). To be included in one of the weekly analyses for the primary or any of the secondary endpoints a patient has to provide data for this endpoint at least on 4 days of the respective week, otherwise the patient will be excluded from the analysis. If a patient fails to be included in more than two of the weekly responder analyses, he/she will also be counted as non-responder in any of the overall responder analyses. Otherwise missing values will not be imputed.

7.5 Safety Analyses

The safety and tolerability of RHB-102 will be determined by reported AEs, physical examinations, vital signs, and laboratory tests. All analyses of safety will be performed in the Safety population.

7.5.1 Adverse Events

The incidence of treatment-emergent AEs will be summarized for each treatment group by MedDRA system organ class (SOC) and preferred term. An AE is defined as treatment-emergent if its onset date/time is on or after the date/time of the first intake of study drug or if a pre-existing condition becomes worse after the date/time of first intake of study drug. An AE is defined as pre-treatment if its onset date/time is before the date/time of the first intake of study drug. AEs will be summarized according to the following:

- An overview of the absolute and relative frequencies of patients with at least one AE will be given for the categories pre-treatment and treatment-emergent by seriousness, severity, and causality.
- Further tables will present the absolute and relative frequencies of patients with at least one treatment-emergent AE classified by SOC and preferred term and, in addition, by severity and causality.
- The absolute and relative frequencies of patients with at least one SAE will be summarized by SOC and preferred term and, in addition, by severity and causality.
- Individual patient data listings will be provided for all deaths and discontinuation of study medication due to AEs.

Regarding the summaries by severity and causality, the following rule will be applied: if a patient experiences more than one AE within the same SOC or with the same preferred term, the AE with the highest severity or closest relationship to study drug will be used for the analysis.

7.5.2 Clinical Safety Laboratory Tests

Key laboratory data will be subjected to both a quantitative analysis (descriptive summary statistics) and qualitative analysis where frequencies of normal, abnormal low, and abnormal high values will be computed. Specific analytes to be evaluated will be listed in the SAP.

The following analyses will be performed:

- Standard descriptive summary statistics will be calculated at each scheduled measuring time point and the last individual measuring time point.

- Standard descriptive summary statistics will be calculated for the absolute change from baseline to each scheduled measuring time point after baseline and the last individual measuring time point.
- Shift tables displaying changes with respect to the normal range between baseline and each scheduled measuring time point after baseline and the last individual measuring time point will be provided. Shift tables will show changes by grade for analytes graded in the NCI CTCAE v 4.03 criteria.
- A listing of all patients with abnormal values at any time point will be given.

No inferential testing will be performed to compare the differences between the treatment groups; a paired-sample t-test may be provided for each group for change from baseline if data warrant.

7.5.3 Other Parameters

The results from physical examination, including vital signs, will be presented in the patient data listings. Findings on physical examination, including vital signs, which constitute clinically significant changes will be listed as adverse events.

8. Study Medications

Patients will receive a bottle containing 30 tablets of RHB102 12 mg or matching placebo. Thirty tablets will give the patient enough medication for 4 weeks treatment plus 2 days, to allow for minor delays in return to clinic on the assigned date.

Patients will be instructed to take one tablet daily, at the same time, preferably in the morning on arising. The tablets should be taken with water. Patients are to keep a diary of medication administration, noting the time of administration each day. At each visit, patients should bring their bottle of pills for a count. At week 5, remaining medication will be collected and the patient given a new bottle of study medication.

Medication should be stored at room temperature, 15-25°C.

9. Investigator Responsibilities

9.1 Compliance with Declaration of Helsinki and Good Clinical Practices

The study will be performed in accordance with the Declaration of Helsinki (1964) as revised, most recently in Fortaleza, Brazil (2013), US FDA regulations and the ICH Guideline for Good Clinical Practice, E6(R1). The investigator will ensure that all those concerned with conducting the study (such as pharmacists, research nurses and co-investigators) are provided with copies of the protocol and all safety information prior to the start of the study.

9.2 Institutional Review Board (IRB) Review and Approval

The investigator is responsible for obtaining IRB approval to conduct this study (including IRB approval of the Informed Consent form) and for ensuring continuing review as required by the IRB. Written confirmation of this approval and periodic review must be provided to the sponsor prior to the start of the study and at appropriate intervals.

9.3 Informed Consent

The investigator will inform patients as to the nature, expected duration and purpose of the study, the administration of the study medication, and the hazards involved, as well as the potential benefits that may come from treatment with this investigational drug. Informed consent must be obtained in accordance with US Code of Federal Regulations (21 CFR Part 50).

The patient will be informed that his/her medical records will be patient to review by the sponsor and possibly by a representative of the Food and Drug Administration. Patients will be informed that they are free to refuse participation in this clinical investigation, and if they should participate, it will be made clear to them that they may withdraw from this study at any time without prejudicing further care. Signed written informed consent must be obtained from every patient prior to study entry. The original will be kept by the investigator and will be patient to review by the sponsor; a copy will be given to the patient.

9.4 Confidentiality

All information provided to the investigator relevant to the study medication, as well as information obtained during the course of the study, will be regarded as confidential. The investigator and members of his/her research team agree not to disclose or publish such information in any way to any third-party without prior written permission from the sponsor, except as required by law.

9.5 Source Documentation

The investigator will allow inspections of the study site and documentation by clinical research and audit personnel from the sponsor, external auditors or representatives of regulatory authorities. The purpose of these inspections is to verify and corroborate the data collected on the case report forms. In order to do this direct access to the patients' medical or clinic records is necessary. The investigator will ensure that certain information is contained in the medical or clinic records of the patient and that the entries are signed and dated, as follows:

- sufficient data to allow verification of the entry criteria in terms of past and present medical and medication histories;
- a note on the day the patient entered the study describing the study number, the drug being evaluated, the study number assigned to that patient and a statement that consent was obtained;

- a note of each subsequent study visit including any concerns about adverse events or abnormal laboratory data and their resolution;
- notes of all concomitant medication taken by the patient including start and stop dates;
- a note of when the patient terminated from the study, the reason for termination and the patient's general condition at termination;
- a copy of the signed informed consent form should be kept in the medical records of each patient during the clinical phase of the study (thereafter it will be archived with the study file).

9.6 Drug Accountability

The investigator agrees to supervise the maintenance of records of the receipt, dispensing and return or destruction of study material supplied by the sponsor. Destruction of any material must be witnessed and documented in writing. The dispensing record must make it clear which patient received which material.

9.7 Data Monitoring and Collection

Suitably qualified and trained clinical research personnel of the sponsor will visit the study center at regular intervals during the study for monitoring purposes and to assist the research staff with any queries they may have.

9.8 Case Report Forms, Investigator's Study File and Record Retention

All case report forms and supporting source documentation must be available to the sponsor during monitoring visits.

Prior to review of the case report forms by the sponsor's representative and forwarding of the case report forms to the sponsor, they should be reviewed for completeness and legibility by the investigator or a member of the research team.

The investigator will maintain all records relating to the study (including copies of case report forms) for at least 2 years after written notification by the sponsor that the investigational drug program has been either completed or terminated, or that a New Drug Application (NDA) has been approved by the FDA. Should the investigator retire, relocate, or for other reasons withdraw from the responsibility of keeping the study records, custody must be transferred to a person who will accept that responsibility, and the sponsor must be notified in writing of the name and address of said person.

9.9 Non-Protocol Research

No investigational procedures other than those outlined in this protocol may be undertaken on the patients in this study without the prior written permission of the patient, the sponsor and the IRB.

10. Sponsor Responsibilities

10.1 General

The sponsor agrees to adhere to US FDA Guidelines on Good Clinical (Research) Practices and with the ICH Guideline for Good Clinical Practice, E6(R1). The sponsor has a legal responsibility to report fully to regulatory authorities the results of this study. It is the sponsor's responsibility to obtain appropriate regulatory approval to perform the study.

10.2 Case Report Forms

Case report forms will be provided by the sponsor or, upon agreement with the sponsor, forms generated by the investigative site may be used. If an electronic data collection system is used, the system will be compliant with applicable aspects of 21 CRF Part 11, ICH guidelines, GCP and HIPAA.

10.3 Data Monitoring and Collection

Suitably qualified and trained clinical research personnel of the sponsor will visit the study center at regular intervals during the study for monitoring purposes and to assist the research staff with any queries they may have. Case report forms and source documentation will be available for review during monitoring visits to the center. The function of this monitoring is to ensure compliance with the protocol, adherence to regulatory and good clinical (research) practice obligations, proper maintenance of records including drug accountability records, correct administration of study medications including storage conditions and accurate reporting of adverse events.

10.4 Audit

The sponsor has an obligation to audit a proportion of studies; this is usually undertaken by a department other than the clinical research department. Therefore the sponsor, an independent auditor or a regulatory authority may wish to audit the study site and documentation and these audits may take place as the study is running or up to several years later.

10.5 Confidentiality

The sponsor will not keep any material on file bearing any patient's name, and the patient's confidentiality will be maintained at all times.

11. Protocol Modifications

If necessary during the course of the study, the protocol may be modified by the sponsor in consultation with the investigator. Except in the case of modifications to resolve an imminent safety issue, any protocol modification or revision must be reviewed and approved by the investigator's IRB prior to implementation.

12. Publication

The sponsor reserves the right of decision regarding publication or presentation. If deemed necessary by the sponsor for protection of proprietary information prior to patent filing, the investigator agrees to delay for 60 days before any presentation or publication is submitted.

13. References

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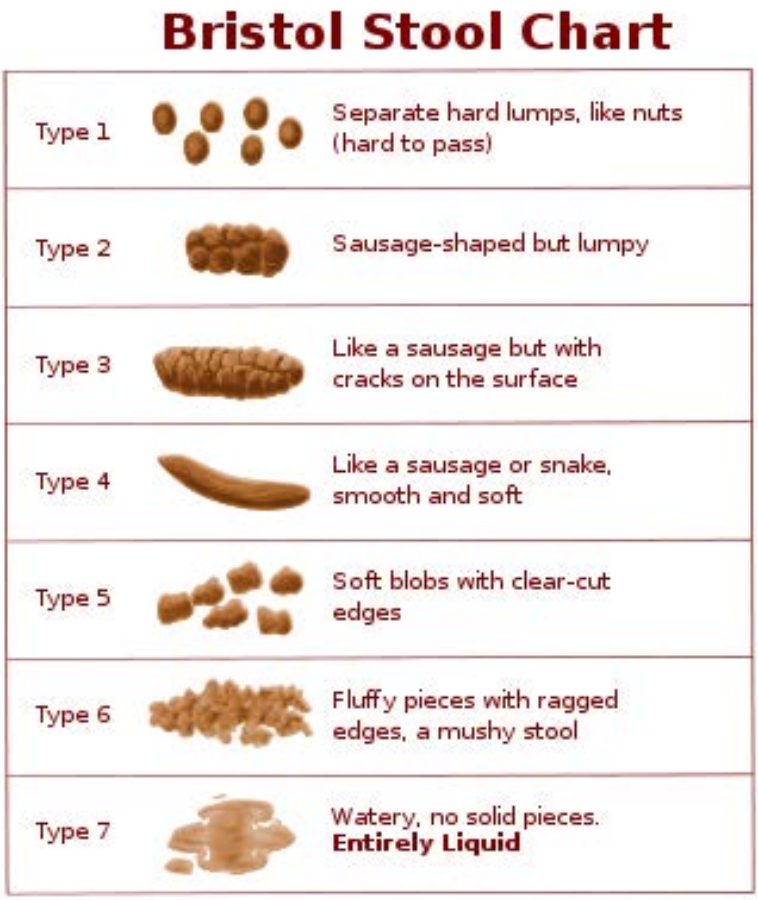
Appendices

Appendix I. Daily assessments

A. Stool consistency: Bristol scale



B. Likert scale for abdominal pain

How bad was your worst abdominal pain over the past 24 hours?

0	No pain
1	Barely felt pain
2	
3	Mild
4	
5	Moderate
6	
7	Severe
8	
9	Excruciating
10	As bad as can be imagined

C. Likert scale for urgency

How bad was your urgency over the past 24 hours?

0 No urgency
1 Barely noticed urgency over the course of the day
2
3 Mild
4
5 Moderate
6
7 Severe
8
9 Excruciating
10 As bad as can be imagined

D. Likert scale for abdominal discomfort

How much abdominal discomfort did you have over the past 24 hours?

0. No discomfort
1. Barely felt discomfort
2.
3. Mild
4.
5. Moderate
6.
7. Severe
8.
9. Excruciating
10. As bad as can be imagined

E. Likert scale for interference in daily activities

How much did your IBS interfere with your daily activities over the past 24 hours?

0 No interference
1 Mild interference
2 Moderate interference
3 Severe interference
4 Unable to carry out activities of daily living

Appendix II. Child-Pugh score

Measure	1 point	2 points	3 points
Total bilirubin, mg/dl	<2	2-3	>3
Serum albumin, g/dl	>3.5	2.8-3.5	<2.8
INR	<1.7	1.7-2.2	>2.2
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Score: 5-6: C-P A, mild

7-9: C-P B, moderate

10-15: C-P C, severe