A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

ClinicalTrials.gov Identifier: NCT03977155

Final Protocol, Version 3.0 (Amendment 2), dated 12-August-2019
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Final Protocol, Version 1.0, dated 25-January-2019

CLINICAL TRIAL PROTOCOL

Study Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)
Study Number:	BOS-589-201
Regulatory Identification Number(s):	IND 142421
Study Phase:	2a
Investigational Product:	BOS-589
Indication:	Diarrhea-predominant Irritable Bowel Syndrome
Sponsor:	Boston Pharmaceuticals, Inc. 55 Cambridge Parkway, Suite 400 Cambridge, Massachusetts 02142 USA

Version	**Date**
Final v.3.0	12 August 2019

Confidentiality Statement

SPONSOR SIGNATURE PAGE

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Protocol Number: BOS-589-201

Version Number and Date: v.3.0, 12 August 2019

I, the undersigned, have approved the final version of this Clinical Trial Protocol.

DocuSigned by:
PPD

Date

DocuSigned by:
PPD

Date

INVESTIGATOR AGREEMENT

Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

Protocol Number: BOS-589-201

Version Number and Date: v.3.0, 12 August 2019

I, the undersigned, have read the protocol and agree to conduct the trial in compliance with the International Council for Harmonization (ICH) guidelines and any other applicable regulatory requirements; as well as Good Clinical Practice (GCP) standards (CPMP/ICH/135/95).

I will provide copies of the protocol and all pertinent information to all individuals who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the investigational product and the conduct of the study.

I will use only the Informed Consent Form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this study.

I agree that the sponsor or its representatives will have access to any source documents from which case report form information may have been generated.

Investigator's Signature	Date

Name of Investigator *(typed or printed)*

Institution Name

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ABBREVIATIONS

AE	adverse event
AKT	protein kinase B
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration versus time curve
AUC_{0-4}	area under the concentration versus time curve from time zero to 4 hours postdose
AUC_{0-12}	area under the concentration versus time curve from time zero to 12 hours postdose
AUC_{0-t}	area under the concentration versus time curve from time zero to the last measurable concentration
BSFS	Bristol Stool Form Scale
BMI	body mass index
CFR	Code of Federal Regulations
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CNS	central nervous system
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CSR	clinical study report
CYP	cytochrome P450
ECG	electrocardiogram
EDC	electronic data capture
ENS	enteric nervous system
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
FDR	first-degree relative
GCP	Good Clinical Practice
GDNF	glial cell line-derived neurotrophic factor
GFR-α	glial cell line-derived neurotrophic factor family receptor-alpha
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
HBV	hepatitis B virus
HCV	hepatitis C virus

HDPE	high-density polyethylene
hERG	human ether-à-go-go related gene
HRT	hormone replacement therapy
IB	Investigator's Brochure
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome-constipation
IBS-GS	Irritable Bowel Syndrome Global Scale
IBS-D	irritable bowel syndrome-diarrhea
IBS-M	irritable bowel syndrome-mixed
IBS-SS	Irritable Bowel Syndrome Severity Score
IBS-U	irritable bowel syndrome-unclassified
IC_{50}	half-maximal inhibitory concentration
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
ILC3	group 3 innate lymphoid cells
IRB	Institutional Review Board
ITT	intention-to-treat
IV	intravenous
IWRS	interactive web response system
JAK	Janus kinase
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no observed adverse effect level
NRS	numeric rating scale
NTEAE	non-treatment-emergent adverse event
PBO	placebo
PD	pharmacodynamic(s)
PE	physical examination
P-gp	P-glycoprotein
PI3K	phosphoinositide 3-kinase
PK	pharmacokinetic(s)
PRO	patient-reported outcome
PYY	pancreatic peptide YY
QC	quality control
QTcB	QT interval corrected by Bazett's formula

QTcF	QT interval corrected by Fridericia's formula
RAS	rat sarcoma
RBC	red blood cell
RET	REarranged during Transfection
RIBA	Recombinant Immunoblot Assay
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SK-N-AS	a human neuroblastoma cell line
STAT	signal transducer and activator of transcription proteins
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
TT	a human thyroid medullary carcinoma cell line
ULN	upper limit of normal
WAP	worst abdominal pain
WOCBP	woman of childbearing potential

1 PROTOCOL SUMMARY

1.1 Synopsis

<table>
<tr><td>Sponsor:</td><td colspan="2">Boston Pharmaceuticals Inc.</td></tr>
<tr><td>Title:</td><td colspan="2">A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)</td></tr>
<tr><td>Protocol Number:</td><td colspan="2">BOS-589-201</td></tr>
<tr><td>Phase:</td><td colspan="2">2a</td></tr>
<tr><td rowspan="5">Objectives and Endpoints:</td><td>OBJECTIVES</td><td>ENDPOINTS</td></tr>
<tr><td colspan="2">Primary</td></tr>
<tr><td>• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.</td><td>• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
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<tr><td colspan="2">Secondary</td></tr>
<tr><td>• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.</td><td>• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• Change in stool frequency, measured by</td></tr>
</table>

		the total number of
		spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the steady-state pharmacokinetics (PK) of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).
Study Design:	A phase 2a, randomized, double-blind, PBO-controlled, multicenter trial to provide proof-of-principle efficacy of BOS-589 in IBS-D patients and to inform dose selection for subsequent development. The study will comprise 3 phases: A **Pre-treatment Phase** of up to 5 weeks, in which all patients will be assessed to determine eligibility. This phase will consist of initial screening assessments after which eligible patients will enter a Run-in Period of up to 3 weeks. During the Run-in period the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medication use,	

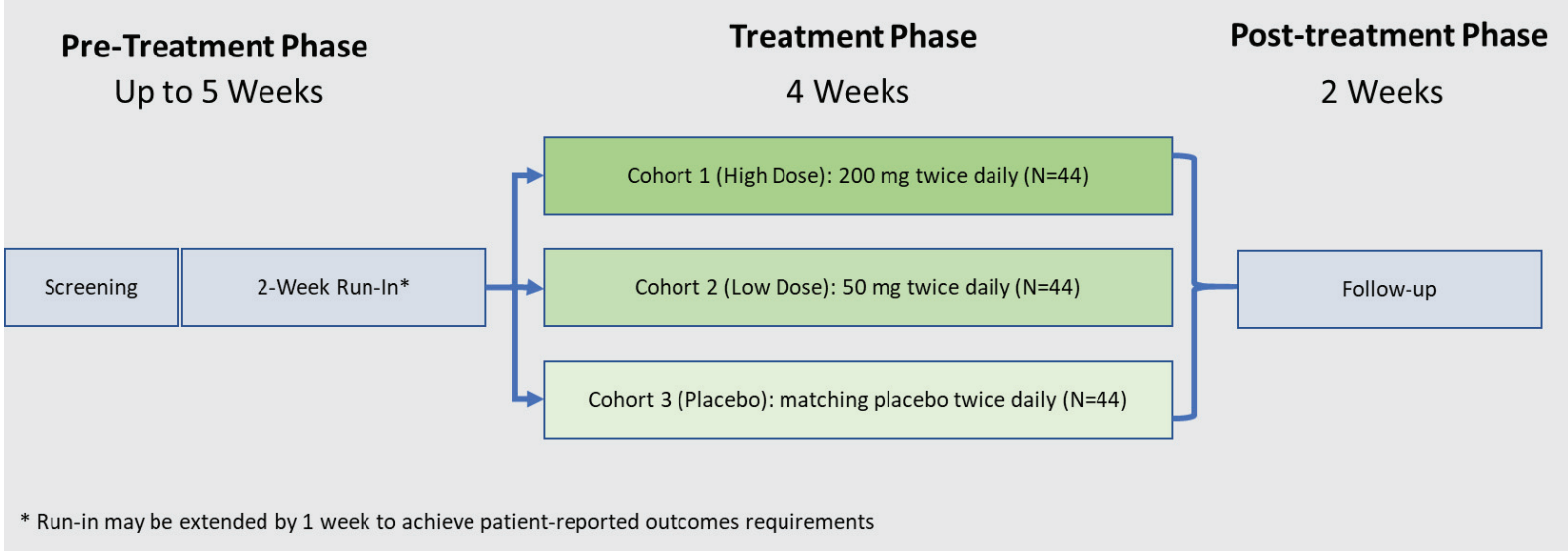
	to confirm disease activity, and diary compliance. Upon completion of the Run-in Period, patients will return to the study site to confirm eligibility for randomization into the double-blinded Treatment Phase. A **Treatment Phase**, in which eligible patients will be randomized (1:1:1) to receive BOS-589 or PBO twice daily (bid) for a total of 4 weeks. BOS-589 will be administered at 1 of 2 dose levels. Patients will be randomized to the following cohorts: • Cohort 1 (High Dose): 200 mg BOS-589 bid orally; • Cohort 2 (Low Dose): 50 mg BOS-589 bid orally; • Cohort 3 (PBO): matching PBO oral tablets bid. During the treatment phase, patients will continue to complete the electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine. A **Post-treatment Phase**, in which all patients who complete 4 weeks of treatment will return to the clinical for a 2-week follow-up visit. Patients who discontinue treatment early will be asked to return to the clinic for safety assessments.
Study Population:	Male and female patients 18 to 65 years of age, inclusive, with a diagnosis of IBS-D.
Number of Patients Planned:	Approximately 300 patients will be screened with the intent of randomizing 132 patients for the study.
Duration of Patient Participation and Study:	The duration of patient participation is anticipated to be up to 11 weeks. The duration of the study is anticipated to be approximately 12 months.
Study Sites:	Up to 66 sites in the United States.
Investigational Product:	BOS-589 (oral doses bid).
Reference	Matching PBO (oral doses bid).

Treatment:	
Concomitant Product:	Not applicable.
Statistical Methods:	**Efficacy:** This is a phase 2a proof-of-principle study. Descriptive and inferential statistical methods will be employed. A standalone Statistical Analysis Plan will be developed and approved prior to database lock. A hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change from baseline to Day 29 in the patient-reported outcome for WAP measured daily on an 11-point numeric rating scale. Mean change from baseline will be measured as a continuous variable and compared between treatment groups in the following testing order using a t-test of equal variances with a two-tailed alpha of 0.05: Hypothesis 1: Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3); If statistically significant at $P < 0.05$, then proceed to Hypothesis 2. Hypothesis 2: Cohort 1 (High Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$, then proceed to Hypothesis 3. Hypothesis 3: Cohort 2 (Low Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4. Hypothesis 4: Cohort 1 (High Dose) versus Cohort 2 (Low Dose).

	Safety: Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for Regulatory Activities Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All serious AEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced. All AEs will be listed for individual patients, showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs related to investigational product will be generated.

1.2 Study Schema

Figure 1. Study Schematic



1.3 Schedule of Activities

Table 1. Schedule of Activities

	Screening Days -36 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Informed consent	X							
Demographics	X							
Medical and surgical history	X							
Rome IV assessment	X							
Concomitant medication review	X	X	X	X	X	X	X	X
Adverse event review and evaluation		X- continuous - X						
Physical examination[c]	X		X				X	
Height, weight, and body mass index	X[d]		X		X		X	X
Vital signs	X		X	X	X	X	X	X
12-lead electrocardiogram	X						X	
Clinical safety laboratory tests[e]	X		X		X		X	X
HIV, HCV/HBV testing	X							
Urine pregnancy test	X		X				X	X
C-reactive protein and erythrocyte sedimentation rate tests	X		X		X		X	X
Biomarker sample collection			X		X		X	X
Pharmacokinetic sample collection[f]			X	X	X	X		
Worst abdominal pain	X	X[g]	X- [h] - X					X[h]
Bristol Stool Form Scale	X	X[g]	X- [h] - X					X[h]

	Screening Days -36 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Spontaneous bowel movements (total number of stools)		X[g]	X- -[h]- X					X[h]
IBS-GS		X[g]	X- -[h]- X					X[h]
Rescue medications		X[g]	X- -[h]- X					X[h]
IBS-SS			X				X	X
Eligibility review	X		X					
Randomization			X					
Administration of investigational product			Day 1 - Day 28					

HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IBS-GS = Irritable Bowel Syndrome Global Scale; IBS-SS = Irritable Bowel Syndrome Severity Score.

[a] Run-in may be extended by up to one week in accordance with the investigators judgement. The full 5 week (Days -36 to -1) may be used to randomize eligible patients. However, efforts should be made to randomize eligible patients within 1 to 3 days after completing run-in period.

[b] Procedures performed at Visit 7/End of Treatment or upon Early Termination. Early Termination procedures are to be performed in close proximity to discontinuation of dosing for patients who are withdrawn from the study. Other procedures may be performed at Investigator or Sponsor discretion.

[c] Full physical exam to be conducted at Screening. Symptom-directed physical examinations should be conducted at subsequent visits.

[d] Height and body mass index to be collected at Screening Visit only.

[e] Hematologic, complete serum chemistry, urinalysis panels and fecal calprotectin to be completed as noted in Appendix 1. Patients should refrain from food or drinks (other than water) for at least 8 hours prior to obtaining a fasting glucose level.

[f] Pharmacokinetic (PK) serial blood samples are to be collected on Day 1 and Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If PK serial blood samples cannot be collected on Day 15, PK serial blood samples should be collected on Day 22. A predose sample will be obtained on all other Treatment Study Visits. Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for 0.5 and 1 hour postdose, and ± 10 minutes for 2 and 4 hours postdose.

[g] Data will be collected daily for entire Run-in Period.

[h] Data will be collected daily until Early Termination or until End-of-Study Visit, whichever is later.

2 INTRODUCTION

2.1 Study Rationale

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) illness with a prevalence of approximately 5% to 20% globally and characterized by a constellation of clinical symptoms. Establishing a diagnosis and assessing response to treatment remains challenging because there are no biomarkers that reliably correlate with disease state.

BOS-589 is an oral agent with limited systemic absorption that inhibits RET (REarranged during Transfection), a receptor tyrosine kinase that is hypothesized to play a key role in the maintenance of a healthy enteric nervous system (ENS). Inhibition of RET may represent a novel therapeutic strategy for the treatment of IBS by attenuating visceral hypersensitivity and/or colonic motility.

BOS-589 has been evaluated in healthy human subjects at doses up to 200 mg twice daily (bid) for 2 weeks. Consistent with its low oral bioavailability, BOS-589 was safe and well tolerated with no significant safety signals identified. The purpose of this study is to evaluate the efficacy, safety, and tolerability of BOS-589 in the treatment of patients with diarrhea-predominant IBS (IBS-D).

2.2 Background

2.2.1 Disease Under Study

Irritable bowel syndrome is a relatively common GI illness characterized by a number of clinical symptoms, including abdominal pain and discomfort, abnormal bowel habits, and bloating (CCI). Subtypes of IBS, based on the predominant bowel habit(s) reported by IBS patients, include diarrhea (IBS-D), constipation (IBS-C), mixed (IBS-M), or unclassified (IBS-U). Currently approved medications for IBS address the restoration of patients' bowel habits and are minimally effective in addressing abdominal pain and discomfort. Because the etiology of the disease has not been clearly established, diagnosis is difficult and relies primarily on the presence of a specific symptom complex occurring in the absence of an alternative explanation. The development of criteria by expert panels, with recent iterations as recently as 2016, has improved the diagnosis and management of IBS patients (CCI).

It is generally believed that the sensory inputs/outputs in the ENS and central nervous system (CNS) are altered in patients with IBS and this contributes to the signs and symptoms they experience. For example, patients with IBS have a heightened and disproportionate sensory experience (visceral hypersensitivity) for a given stimulus (CCI). Visceral hypersensitivity and abnormal bowel habits may result from visceral afferent neurons or increased nerve fiber density and sprouting that have been observed in the intestinal mucosal tissues of IBS patients (CCI ; CCI). The sensitizing event causing visceral

hypersensitivity may be transient or chronic; however, its impact on the CNS and ENS may be long lasting (CCI). Supernatants from these intestinal tissues can stimulate nerve outgrowth and increase neuronal signaling *in vitro*, suggesting that neurotrophic factors and/or inflammatory cytokines might be mediators of neuronal plasticity in IBS (CCI ; CCI). Given the central role the ENS may play in mediating signals between the CNS and the intestine, ENS modulation may provide therapeutic benefit to IBS patients by impacting visceral hypersensitivity and intestinal motility.

The only drugs currently approved by the Food and Drug Administration (FDA) for the treatment of IBS are alosetron (for women only), rifaximin, and eluxadoline for refractory IBS-D, and plecanatide, linaclotide, and lubiprostone for IBS-C. The majority of these drugs, and those used off label to treat IBS, target patients' symptoms by altering GI motility and are minimally effective in addressing abdominal pain and discomfort. Of the FDA-approved agents, only alosetron is hypothesized to modulate the CNS and ENS (CCI). Therefore, an agent such as BOS-589, which may ameliorate visceral hypersensitivity and potentially impact intestinal motility, would address a significant unmet medical need for IBS-associated pain.

2.2.2 The "RET" ("REarranged During Transfection") Gene and the Enteric Nervous System

The RET gene, localized on human chromosome 10q11.2, encodes a receptor-type tyrosine kinase with an extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain (CCI). The ligands for RET have been identified as neurotrophic factors of the glial cell line-derived neurotrophic factor (GDNF) family, including GDNF, neurturin, artemin, and persephin. Ligand binding to its corresponding GDNF family receptor-alpha (GFRα) co-receptor triggers RET dimerization and subsequent transphosphorylation of intracellular tyrosines (CCI) and leads to the activation of different intracellular signaling cascades, including the Janus kinase/signal transducer and activator of transcription proteins (JAK/STAT), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), and rat sarcoma/extracellular signal-regulated kinase (RAS/ERK) pathways.

Mice deficient in the GDNF ligand, its coreceptor GFRα1, or the RET protein itself, exhibit severe defects in kidney and ENS development. This implicates RET signaling as critical to the development of normal kidneys and the ENS (CCI). The role of RET in the development of the ENS is also apparent in patients with Hirschsprung's disease, who frequently suffer from colonic obstruction because of a lack of normal colonic innervation. In Hirschprung's disease, different loss-of-function mutations that occur in the *RET* gene account for the highest proportion of both familial and sporadic cases of the disease (CCI).

While its role during the development of the ENS has been well established, recent reports also implicate a significant role for RET in the maintenance and plasticity of the adult ENS. Neurons within the submucosal and myenteric plexus of the adult human

colon have been shown to express RET and its coreceptors, GFRα1 and GFRα2, while the GDNF ligand is expressed in the muscularis mucosa and in circular and longitudinal muscle tissue (CCI). Systemic administration of GDNF in adult rodents results in significant increases in submucosal neuron density in both the small intestine and colon and altered function (CCI). Furthermore, a conditional knockout of the RET co-receptor, GFRα3, results in decreased colonic hypersensitivity implicating a role for RET signaling in visceral nociception (CCI). Therefore, by reducing RET signaling, inhibition of RET may modulate ENS activity.

2.2.3 Investigational Product

BOS-589, formerly GSK3352589, is a potent and selective inhibitor of RET that has been shown to reduce visceral hypersensitivity in an animal model of IBS and inhibit cholinergic-induced increases in colonic motility (details are provided in the BOS-589 Investigator's Brochure [IB]). The results from these preclinical studies suggest that inhibition of RET with a potent, selective, and gut-restricted small molecule may represent a novel therapeutic strategy for the treatment of abdominal pain and defecation abnormalities (i.e., diarrhea) in patients with IBS through the attenuation of visceral hypersensitivity and/or cholinergically mediated ion transport and colonic motility. The patient population likely to derive the greatest benefit would comprise individuals with IBS-D with increased GI motility.

2.2.3.1 Nonclinical Summary

A range of *in vitro* and *in vivo* studies have been conducted to investigate the primary, secondary, and safety pharmacology of BOS-589. Details are provided in the BOS-589 IB.

2.2.3.1.1 *In Vitro* Studies

CCI

CCI

CCI

2.2.3.1.2 *In Vivo* Studies

2.2.3.1.3 Pharmacokinetics and Product Metabolism in Animals

2.2.3.1.4 Toxicology and Nonclinical Assessment of Safety


CCI
CCI
CCI
CCI
CCI

2.2.3.2 Clinical Experience

Study CCI [redacted] evaluated the safety, tolerability, pharmacokinetics (PK), and exploratory pharmacodynamics (PD) of escalating single and multiple oral doses of BOS-589 ranging from 2 to 400 mg in the fasted state, and 25 mg in the fed state. All doses in the single ascending-dose portion of the study were generally safe and well tolerated, with a safety profile similar to that of placebo (PBO). Similarly, repeat-dose administration of BOS-589 for 14 days at doses ranging from 5 to 200 mg bid in the fed state was generally safe and well tolerated. There were no drug-related clinically significant changes in safety laboratory tests, vital signs, ECGs, or stool patterns as assessed by the Bristol Stool Form Scale (BSFS) in this study. There were no serious adverse events (SAEs) considered related to the administration of BOS-589. A review of all GI adverse events (AEs) demonstrated that the occurrence of GI AEs was similar in the PBO- and BOS-589-treatment groups. There was no pattern or trend with dose escalation suggestive of a treatment effect.

The PK profile of BOS-589 demonstrated limited oral bioavailability and systemic exposure, with plasma concentrations generally less than 2 ng/mL, when measured utilizing a sensitive analytical method to detect concentrations as low as 5 pg/mL. Exposures were dose dependent and less than dose proportional, although box plots of dose-normalized parameters suggested dose proportionality between 15 mg and 100 mg. Dosing was escalated to the highest possible dose because predicted mean systemic exposures did not exceed the defined plasma toxicokinetic limits ($AUC_{0\text{-}t}$ and C_{max} of 40.4 ng*h/mL and 26.7 ng/mL, respectively) and there was no evidence of dose-limiting toxicities. There was accumulation of BOS-589 with repeat dosing, but systemic exposures remained very low after 14 days of dosing, with respective geometric mean (CV%) $AUC_{0\text{-}t}$ and C_{max} values of 24.1 (45.9) h*ng/mL and 1.53 (43.6) ng/mL for the highest dose of 200 mg bid; BOS-589 was likely at steady state before that time. Accumulation was lower for doses below 100 mg bid (1.3 to 1.8 fold) and highest for the highest dose of 200 mg bid (2.0 to 2.7 fold).

The study included a pilot food-effect group to evaluate the magnitude of a food challenge on the bioavailability of single-dose BOS-589. Following administration of a single dose of BOS-589 25 mg in the fed state, there was a small decrease in exposure with food, with decreases in mean C_{max} and AUC values in the range of 20% to 35%. The time to C_{max} (T_{max}) remained the same with or without food. These food-effect results were deemed not clinically important; dosing BOS-589 with or without food is not anticipated to affect future evaluation of safety or efficacy.

Because RET is expressed in enteroendocrine cells lining the intestinal mucosa, an exploratory objective of the study was to explore the effect of BOS-589 on glucagon-like peptide-1 (GLP-1) and pancreatic peptide YY (PYY) excursions in plasma; however, no clear relationship or impact on BOS-589 administration and peptide secretion was identified.

2.3 Risk/Benefit Assessment

Summaries of findings from nonclinical and clinical studies conducted with BOS-589 can be found in the IB (refer to the IB for additional details). The following section outlines the risk assessment and mitigation strategy for this protocol.

The current study, BOS-589-201, represents the first administration of BOS-589 to patients with IBS-D. Considerations for safety monitoring are derived primarily from the literature regarding RET expression in the intestine, nonclinical data, and clinical experience dosing BOS-589 to normal healthy volunteers (Study CCI), in which no clinically relevant risks were identified that would preclude dosing a RET inhibitor for up to 4 weeks in patients with IBS-D.

2.3.1 Risk Assessment

CCI

CCI

CCI

CCI

2.3.2 Benefit Assessment

Patients randomized to the active treatment arms may potentially experience improvement in their IBS-D during the course of the study. Those randomized to the PBO arm are not expected to obtain any benefit beyond that of their background treatment.

2.3.3 Overall Risk/Benefit Conclusion

On the basis of nonclinical and clinical study results to date, limited effective alternative treatments, and the strength of the scientific hypothesis under evaluation, BOS-589 is considered to have a favorable benefit-risk profile for patients with IBS-D.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).	• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.	• Incidence of adverse events (AEs) serious adverse events (SAEs), discontinuations because of AEs, and any treatment-related severe AEs.
Secondary	
• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.	• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). • Change in stool frequency, measured by the total number of spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the steady-state pharmacokinetics of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).

4 STUDY DESIGN

4.1 Overall Design

This study is a phase 2a, randomized, double-blind, PBO-controlled, multicenter trial designed to provide the first proof-of-principle efficacy of BOS-589 in IBS-D patients, and to inform dose selection for subsequent development. The study will consist of a pre-treatment phase, a 4-week double-blind treatment phase, and a 2-week post-treatment follow-up period.

Pre-Treatment Phase: During the pre-treatment phase, patients will be evaluated for up to 5 weeks to assess eligibility. The pre-treatment phase will consist of initial screening assessments and a Run-in period.

After the initial screening assessments have been performed, eligible patients will enter a Run-in period of up to 3-weeks. During the Run-in period, the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine use, to confirm disease activity and diary compliance. Patients will also be requested to discontinue any prohibited medications during this phase of the study.

Upon completion of the Run-in period, patients will return to the study site to confirm eligibility for randomization into a 4-week double-blinded Treatment Phase.

Treatment Phase: Eligible patients will be randomized (1:1:1) into the following cohorts:

- Cohort 1 (High Dose): 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 50 mg BOS-589 bid orally
- Cohort 3 (PBO): matching PBO oral tablets bid orally

During the 4 weeks of double-blind treatment, patients will continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary, as described in Section 8.3.

Post-treatment Phase: A 2-week post-treatment follow-up visit will occur for patients who complete the Treatment Phase. During the 2 weeks of follow-up, patients should continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary.

Patients who prematurely discontinue treatment should return to the study center to complete the early termination assessments as soon as possible after stopping the treatment.

Data analyses will occur after all patients in the trial have completed the last visit or procedure shown in the Schedule of Activities, Section 1.3.

Post-study Access to Therapy: No post-treatment access to therapy will be provided to patients randomized in the study.

4.1.1 Study Duration for Patients

Unless shortened by intolerable AEs or rapid disease progression, each patient's participation in this study is anticipated to last up to 11 weeks.

The total duration of this study is anticipated to be approximately 12 months, including patient enrollment, treatment, and follow-up.

4.1.2 Number of Patients

Approximately 300 patients will be screened to randomize approximately 132 patients with IBS-D (44 patients per cohort).

4.1.3 Replacement of Patients

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who have received at least 1 dose of investigational product and are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

4.1.4 Number of Sites

Up to 66 sites in the United States may participate in this study.

4.2 Rationale for Study Design

Key aspects of the study (e.g., eligibility, cohort size, stopping criteria, safety data collection, efficacy assessments, use of PBO as control, and use of patient-reported outcome [PRO] tools) are based upon generally accepted clinical trial methodologies for phase 2a efficacy studies and prior studies conducted in patients with IBS.

The Rome IV (CCI [redacted]) diagnostic criteria for IBS is the best accepted tool for standardized IBS diagnosis.

A Run-in Phase is designed to account for fluctuations in symptoms and the potential for wide variations in bowel habits; patient diaries that record frequency and severity of daily symptoms will be used to ensure that symptom severity fluctuations are identified and taken into account for subject eligibility. Patients will also be assessed on their ability to record their disease-related information in the required manner.

The use of PBO as a control comparator in IBS clinical trials is acceptable given the lack of consistently effective treatments. Placebo is an important component of IBS clinical trials given the high PBO effect in this population.

The key primary and secondary endpoints in this study are based on PRO assessments. These assessments evaluate pain, defecation, and IBS signs and symptoms. Abdominal pain intensity is measured using an 11-point (i.e., 0 to 10) numeric rating scale (NRS) that asks patients daily to rate their *worst abdominal pain (WAP) over the past 24-hours* as recommended by the FDA guidance on IBS (FDA, May 2012). The use of the NRS for pain has been validated in IBS clinical studies (CCI). The IBS Severity Score (IBS-SS) (CCI) and IBS Global Scale (IBS-GS) (CCI) are validated and standard methods of assessing IBS symptoms in clinical trials.

Although treatment durations longer than 4 weeks will be required for true assessment of efficacy in IBS patients, in this initial phase 2a study, 4 weeks should be sufficient to identify any efficacy signal that warrants further clinical invention.

The use of multiple enrolling sites is aimed at maximizing external validity and to minimizing the potential influence of regional variations in diet, exercise habits, and ethnicity.

4.3 Justification for Dose

CCI

CCI

CCI

CCI

CCI [redacted]

4.4 End of Study Definition

The end of the study is defined as completion of the last visit or procedure shown in the Schedule of Activities in the trial globally. A patient will be considered to have completed the study if he or she has completed all phases of the study, including the last visit or the last scheduled procedure shown in the Schedule of Activities, Section 1.3.

5 STUDY POPULATION

Before any study-specific activities/procedures, the appropriate written informed consent must be obtained. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Age

1. Male and female patients must be 18 to 65 years of age, inclusive, at the time of signing informed consent.

Run-in eDiary Compliance

2. Patient has completed the daily electronic diary on at least 6 of the 7 days during the week prior to randomization AND at least 11 of the 14 days during the 2 weeks prior to randomization. Patients have up to 3 weeks to meet these criteria.

Type of Patient and Disease Characteristics

3. Patient meets the diagnosis of IBS based on the Rome IV diagnostic criteria (CCI):*

    - Recurrent abdominal pain occurring, on average, at least 1 day per week and associated with 2 or more of the following:
        - Related to defecation;
        - Associated with a change in frequency of bowel movements;
        - Associated with a change in form (appearance) of stool;

    * These criteria must be fulfilled for the last 3 months prior to randomization and onset must have occurred at least 6 months prior to randomization.

4. Patient meets the diagnosis of IBS-D subtype based on Rome IV diagnostic criteria (CCI). On days when the patient experiences IBS symptoms:

    - At least 25% of stools are loose or watery; AND
    - Fewer than 25% of stools are hard.

5. Over the final 7 days of the run-in period, the patient has an average of WAP scores in the prior 24 hours of 3.0 to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable).

6. Over the final 7 days of the run-in period, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools), and there must be at least 2 days with stool consistency of Type ≥ 6.

7. Patient is not planning to change his or her usual diet and lifestyle during the course of the study.

Diagnostic Assessments

8. Patient must undergo or previously have undergone (a) an appropriate evaluation for their IBS symptoms, including an evaluation for organic/structural etiologies (if in the presence of alarm symptoms); and (b) age-appropriate screening for colorectal cancer, if applicable.

    a) If at least one of the following alarm features are present, then the patient must have had a colonoscopy that should include biopsy since the onset of symptoms or within the past 5 years (whichever is less):

        - Documented and unexplained weight loss of ≥ 10% within the past 6 months;
        - Nocturnal diarrhea;
        - Blood mixed with stool (except hemorrhoidal bleeding, defined as occasional blood found on the toilet paper only or limited dripping of blood into the toilet bowl after defecation;
        - Unexplained iron-deficiency anemia.

    b) If no alarm features are present, then the patient must have had a colonoscopy or other appropriate exam, based on criteria as outlined below (Colorectal cancer screening tests other than colonoscopy are considered second-tier and can be discussed with the sponsor [CCI]):

        - Age ≥ 50 (≥ 45 if African-American): Colonoscopy within the past 10 years;
        - First-degree relative (FDR) diagnosed with colorectal cancer under age 60 OR 2 FDRs diagnosed with colorectal cancer at any age: Colonoscopy within past 5 years, *beginning* 10 years before age of youngest FDR (at time of diagnosis);
        - Age ≥ 40 AND FDR diagnosed with colorectal cancer (at any age): Colonoscopy within past 10 years;

9. Patient is negative for serum tissue transglutaminase IgA antibody (tTG-IgA) plus has evidence of detectable serum IgA within the normal reference range.

Weight

10. Body mass index (BMI) within the range 16 to 39 kg/m^2 (inclusive).

Gender

11. Male and female patients are eligible if:

    a. Male patients:

       A male patient must agree to use contraception as detailed in Appendix 2 of this protocol during the treatment period and for at least 14 weeks after the last dose of investigational product. Patients must also agree to refrain from donating sperm during this period.

    b. Female patients:

       A female patient is eligible to participate if she is not pregnant (see Appendix 2), not breastfeeding, and at least 1 of the following conditions applies:

       i. Not a woman of childbearing potential (WOCBP) as defined in Appendix 2; OR

       ii. A WOCBP who agrees to follow the contraceptive guidance in Appendix 2 during the treatment period and for at least 5 weeks after the last dose of investigational product.

Informed Consent

12. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the Informed Consent Form (ICF) and in this protocol.

13. Patient is willing to be compliant with study procedures, including completing the daily electronic diary during the Run-in Period and throughout the study.

5.2 Exclusion Criteria

An individual for whom any of the following criteria apply will be excluded from participation in this study:

Gastrointestinal-related Medical Conditions

1. At the time of screening, patient has a diagnosis of an IBS subtype other than IBS-D, based on Rome IV criteria (CCI). Based on stool patterns on days that the patient experiences symptoms, other IBS subtypes are defined as follows:

    a. IBS-C: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements;

    b. IBS-M: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≥ 25% of bowel movements;

    c. IBS-U: hard or lumpy stools ≤ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements.

2. Patient has a history of inflammatory or immune-mediated GI disorders including (but not limited to) inflammatory bowel disease (i.e., Crohn's disease, ulcerative colitis, microscopic colitis, and celiac disease).

3. Patient has had an episode of diverticulitis within 3 months prior to Screening.

4. Patient has a history of intestinal obstruction, stricture, toxic megacolon, GI perforation, fecal impaction, gastric banding, bariatric surgery, adhesions, ischemic colitis, or impaired intestinal circulation (e.g., aortoiliac occlusive disease).

5. Patient has any of the following surgical history:

    a. Cholecystectomy with ANY history of post-cholecystectomy biliary tract pain. A successful cholecystectomy with no postoperative biliary tract pain is not exclusionary;

    b. Any abdominal surgery within the 3 months prior to Screening;

    c. Major gastric, esophageal, hepatic, pancreatic, or intestinal surgery (appendectomy, hemorrhoidectomy, or polypectomy greater than 3 months post-surgery are allowed).

6. Patient has a history or current evidence of laxative abuse.

Other Medical Conditions

7. Patient has a history of a cardiovascular event, including stroke, myocardial infarction, congestive heart failure, or transient ischemic attack within 6 months prior to Screening.

8. Patient has a history of malignancy within 5 years prior to Screening (except squamous and basal cell carcinomas of the skin and cervical carcinoma *in situ*).

9. Patient has a history of alcohol abuse or binge drinking.

10. Patient has uncontrolled hypertension, defined as systolic blood pressure > 180 mmHg or a diastolic blood pressure > 100 mmHg at the time of Screening.

11. Patient has a history of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.

Laboratory Assessments

12. Fecal calprotectin > 150µg/g ([redacted]).

13. Hemoglobin A1c level ≥ 8.0% or a confirmed fasting plasma glucose level ≥ 180 mg/dL.

14. Confirmed alanine aminotransferase (ALT) > 2x upper limit of normal (ULN).

15. Confirmed total bilirubin > ULN, unless the patient has a documented history of Gilbert's syndrome.

16. Confirmed QT interval corrected by Fridericia's formula (QTcF) or QT interval corrected by Bazett's formula (QTcB) > 500 msec.

17. Evidence of active hepatitis B virus (HBV) infection, based on a positive hepatitis B surface antigen (HBsAg) screen.

18. Evidence of hepatitis C virus (HCV) infection based on a positive HCV antibody screen. Patients with a positive HCV antibody at screening may be eligible if confirmatory testing (i.e., Recombinant Immunoblot Assay [RIBA], or HCV RNA viral load) provided by the study site is negative. Patients who have been successfully treated for HCV are eligible if an undetectable HCV viral load at least 6 months after completion of treatment can be demonstrated.

19. Human immunodeficiency virus (HIV)-1 or HIV-2 antibody positive.

Prior/Concomitant Therapy

20. Within 14 days of randomization, patient has used either of the following:

    - 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron, ondansetron, or ramosetron);
    - Eluxadoline;
    - Any of the strong inhibitors of P-glycoprotein listed in Appendix 4.

21. Within 14 days of randomization, patient has used any of the following:

- Loperamide; (Note: loperamide can be used during the study as rescue medication based on protocol specified guidelines [see Section 6.7.1]);
- Bile acid sequestrants such as cholestyramine, colestipol, or colesevelam*
- Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
- Narcotic- or opioid-containing agents;
- Cannabis-containing products;
- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents).

22. Within 14 days of randomization, receipt of any prescribed or over-the-counter, systemic, herbal (including St. John's wort), or topical medication, or any expectation of requiring use of such medication while participating in the study that, in the opinion of the Investigator, would interfere with study procedures, compromise safety, or the scientific integrity of the data.

23. Patient has used, or is expected to use, the following antibiotics:

    - Rifaximin within 90 days prior to randomization;
    - Other oral antibiotics within 28 days of randomization (with the exception of topical antibiotics or a 1-day course with an antibiotic).

    However, a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomly assigned to study drug.

24. Within 3 months prior to randomization, patient has had significant changes to his or her antidepressant regimen (i.e., addition of a new agent, discontinuation of a prior agent, significant modifications to the dose of a current medication). Patients on

* Patients with diagnosis of or suspected to have bile acid malabsorption are not specifically excluded given the challenge of diagnosing these patients; however, use of bile acid sequestrants are prohibited

chronic stable doses of antidepressants will be allowed to participate in the study. As-needed use of buspirone and benzodiazepines for anxiety is permitted during the study;

25. Within 30 days prior to randomization (or 5 half-lives, if known), the patient has received an investigational drug, or is currently enrolled in an investigational study.

Other Exclusions

26. Patient is unable to swallow solid oral dosage forms whole with the aid of liquid (patients may not chew, divide, dissolve, or crush the study drug).

27. Patient has an elective surgery planned or expects to need elective surgery at any time during the study.

28. Patient is pregnant or breastfeeding.

29. Patient has any medical or psychological disorder or condition that, in the opinion of the Investigator, would compromise the wellbeing of the patient or the study or prevent the patient from meeting or performing study requirements.

30. Patient has poor peripheral venous access.

31. Patient is an employee of the Investigator or study center with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members of the employees or the Investigator.

5.3 Lifestyle Considerations

During this study, patients will be asked to abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests.

Only on days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities; Section 1.3), patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study. Investigational product should be taken with approximately 240 mL water.

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened with Medical Monitor approval.

6 INVESTIGATIONAL PRODUCT

6.1 Investigational Products

6.1.1 Investigational Product Description

Investigational Product Name:	BOS-589	Placebo
Dosage Formulation:	Tablet	Tablet
Unit Dose Strengths/Dosage Levels:	50 mg (2 x 25-mg tablets) and 200 mg (2 x 100-mg tablets)	Visually matching placebo tablets across both BOS-589 dose levels
Route of Administration:	Oral	Oral
Dosing Instructions:	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush
Packaging and Labeling:	Investigational product will be provided in white, opaque, high-density polyethylene (HDPE) bottles with a child-resistant closure. Each bottle will be labeled as per country requirement	Investigational product will be provided in white, opaque, HDPE bottles with a child-resistant closure. Each bottle will be labeled as per country requirement
Manufacturer:	WuXi STA, Shanghai, China	WuXi STA, Shanghai, China

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all investigational product received and any discrepancies are reported and resolved before use of the investigational product.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for investigational product accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

6.2.2 Product Storage and Stability

All investigational product must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

Further guidance and information for the final disposition of unused investigational products are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias

This is a randomized, double-blinded, PBO-controlled study. Patients should be randomized as close as possible to the time of the planned first dose of study treatment.

Randomization will be conducted via an interactive web response system (IWRS) in a 1:1:1 ratio for patients to receive BOS-589 200 mg, BOS-589 50 mg, or PBO. Patients will be assigned to the treatment arms using a permuted block randomization stratified by site (44 patients per group). The randomization schedule will be generated by the Study Biostatistician and will be transferred to the IWRS team for loading into the system. The randomization file will be held by the Study Biostatistician until the end of the trial and when the database has been locked.

The sponsor, site staff, and patients will be blinded to the treatment assignment. BOS-589 and PBO will be similar in appearance, and provided in white, opaque, high-density polyethylene (HDPE) bottles, each with a child-resistant closure. Each bottle will be labeled as per country requirement.

Only in a medical emergency will a patient's treatment assignment be unblinded, and this process will be performed and documented in the IWRS. Every effort should be made to contact the Medical Monitor to discuss unblinding prior to breaking the blind.

6.4 Dosing and Administration

Only patients enrolled in the study may receive investigational product and only authorized site staff may supply or administer investigational product.

Eligible patients will be randomized (1:1:1) to receive for a total of 4 weeks BOS-589 at 1 of 2 dose levels or PBO bid. Patients will be randomized to the following cohorts:

- Cohort 1 (High Dose): 2 x 100 mg tablets for the 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 2 x 25 mg tablets for the 50 mg BOS-589 bid orally
- Cohort 3 (PBO): 2 x visually matched PBO tablets bid orally

For each dose level and at each dosing timepoint, the 2 tablets are to be swallowed with approximately 240 mL room temperature water and are to be swallowed whole (i.e., not divided, crushed, dissolved, or chewed). On days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities, Section 1.3, patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study.

Patients will be instructed to take the investigational product approximately every 12 hours at approximately the same time each day. If a patient misses a dose at a given timepoint, and the time is within 4 hours of the regularly scheduled dosing time, the patient should be instructed to take the investigational product. If it has been longer than 4 hours, the patient should skip the missed dose at that timepoint and resume the regularly scheduled dosing schedule at the next scheduled timepoint.

In the event of vomiting following administration, BOS-589 should not be taken until the next scheduled dose.

6.5 Dose modification

Study treatment should be interrupted if the patient reports any treatment-related severe AE. Therapy should only be restarted after approval by the Medical Monitor.

If therapy is restarted, the same dose should be administered as that prior to occurrence of the AE. If the patient is still having tolerability issues and an AE reoccurs, the dose may be reduced from 2 tablets of study treatment bid to 1 tablet of study treatment bid, without breaking the study blind.

If another AE occurs after the study treatment dose reduction, study treatment will be permanently discontinued and the subject followed until the end of the study.

6.6 Investigational Product Compliance

All patients in this study will commence therapy on site on Study Day 1; oral investigational product will be administered under clinic staff supervision. After patients are discharged to continue therapy at home, compliance will be assessed on subsequent visits by returned tablet count. Administration of investigational product and any deviation(s) from the prescribed dosage regimen should be recorded in the case report form (CRF) and Drug Accountability Record.

6.7 Concomitant Therapy

Refer to Appendix 4. for more details on which concomitant medications are permitted and which are excluded while patients are on study.

Any prior or concomitant therapy (including over the counter or prescription medicines, vitamins and/or supplements) taken 28 days prior to the first dose of investigational product through the End-of-Study Visit must be recorded, along with:

- Reason for use;
- Dates of administration including start and end dates;
- Dosage information including dose and frequency.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications, vaccines, vitamins, and supplements.

6.7.1 Rescue Medicine

During the double-blind Treatment Phase of the study, patients will be allowed to take loperamide rescue medication for the acute treatment of uncontrolled diarrhea. It is recommended that patients refrain from loperamide unless a minimum of 2 or more uncontrolled diarrhea events that cause significant discomfort and prevent normal everyday activities are experienced in a given 24-hour period.

If loperamide must be used, patients must not exceed the 2 mg (unit dose), which may be taken once approximately every 6 hours and must not exceed the following dosing requirements:

- No more than 4 unit doses over a continuous 24-hour time period (8 mg/day);
- No more than 7 unit doses over a continuous 48-hour time period (14 mg over 2 days);
- No more than 11 unit doses over a continuous 7-day time period.

The use of loperamide rescue medication should be recorded electronically.

6.8 Intervention After the End of the Study

BOS-589 will not be provided to study patients after the end of the study.

7 DISCONTINUATION / WITHDRAWAL

7.1 Discontinuation of Investigational Product

A patient may discontinue from study treatment at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. Discontinuation from the investigational product does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to, changes from baseline) after enrollment, the Investigator or qualified designee will determine if any change in patient management is needed. Any new clinically relevant finding will be reported as an AE.

Upon investigational product discontinuation, patients should complete all procedures collected in the Day 29/End-of-Treatment visit as applicable per the Schedule of Activities (Section 1.3).

7.2 Discontinuation/Withdrawal from the Study

A patient may withdraw from the study at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for the following reasons:

- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the patient;
- Disease progression that requires discontinuation of the investigational product;
- Pregnancy;
- If the patient meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation after consultation with the Medical Monitor;
- Significant investigational product noncompliance;
- Termination of the study by the sponsor.

If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a patient withdraws from the study, he or she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

See Schedule of Activities, Section 1.3, for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The reason for patient discontinuation or withdrawal from the study will be recorded on the CRF.

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

Clinical and/or laboratory criteria that may warrant study drug discontinuation.

If a patient experiences any of the following clinical or laboratory events, study treatment should be immediately suspended:

- Any SAE considered study drug-related;
- No bowel movement for 4 days;
- Any clinical evidence suggestive severe constipation, obstipation or intestinal obstruction; gastrointestinal hemorrhage, significant gastrointestinal infection;
- Abnormal liver functions concerning for drug-induced as follows[1]:
    - Confirmed ALT > 5x ULN
    - Confirmed ALT > 3x ULN and any of the following signs or symptoms (fatigue, nausea, vomiting, right upper quadrant pain, fever, rash or eosinophilia)[2]
    - Confirmed ALT > 3x ULN and total bilirubin > 2x ULN or INR > 1.5 (**should be repeated in 48-72 hours**)

[1]Should any of these events occur, a full evaluation of other causes of liver enzyme elevation should be undertaken and patients would be followed at regular intervals until labs return to normal/stabilize, or an alternate diagnosis is confirmed.

[2]If patients develop asymptomatic elevations of the ALT of > 3x ULN and, if there are persistent elevations upon repeat testing, close observation should be implemented, and discontinuation of the drug should be considered.

Depending on the nature, severity, and outcome, the Sponsor will decide to (and document with supporting rationale) either: 1) discontinue study drug; 2) request additional safety data; or 3) continue dosing at current dose or reduced dose (see Section 6.5).

If 2 or more patients experience any of the above criteria (note: study drug-related SAEs must be in the same system organ class) the study enrollment will be halted. The Sponsor, an independent GI physician not involved with the study, and the lead

investigator will review available data to determine whether individual patient unblinding is warranted, amendments are required for the protocol, and whether patients currently on treatment should also discontinue study drug. A decision to continue (with or without modifications) or discontinue the study will be made. All supporting rationale will be documented.

7.3 Lost to Follow-up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site will attempt to contact the patient and reschedule the missed visit as soon as possible; counsel the patient on the importance of maintaining the assigned visit schedule; and ascertain if the patient wishes to and/or should continue in the study.

- Before a patient is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods. These contact attempts should be documented in the patient's medical record or study file.

- Should the patient continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

Planned timepoints for all assessments are provided in the Schedule of Activities (Section 1.3).

8.1 Study Periods

The study will comprise 3 phases:

1. **Pre-Treatment Phase** (up to 5 weeks), in which all patients will be assessed for eligibility. The pre-treatment phase will consist of initial screening assessments (Visit 1) and a Run-in period (Visit 2 through Visit 3).

2. A **Treatment Phase** (4 weeks), in which all patients will return to the clinical for randomization and start of treatment (Visit 3 – Day 1) followed by weekly visits (Visit 4 – Day 8 [± 1 day], Visit 5 – Day 15 [± 1 day], Visit 6 – Day 22 [± 1 day]) until Visit 7 – Day 29 (± 1 day), and

3. A **Follow-up Phase** (2 weeks), in which patients who complete Visit 7, will be asked to return for an End-of-Study Follow-up visit (Visit 8 – Day 43 [± 3 days]) for assessment of treatment outcome (e.g., safety, durability of effect).

8.2 Screening Assessments

After signing the ICF(s), the patient will undergo the initial screening assessments and procedures as described in the Schedule of Activities (Section 1.3) to determine eligibility.

Eligible patients will enter the Run-in period. At the beginning of the Run-in period, patients will receive instructions for completing an electronic diary to collect daily information related to their IBS-D symptoms, their bowel function, and rescue medication use.

Patients who meet the following requirements will be eligible for participation and immediate randomization into the double-blind treatment phase (i.e., all diary conditions listed must be met to qualify for randomization):

- have completed the daily electronic diary on at least 6 of the 7 days during the week prior to randomization AND at least 11 of the 14 days during the 2 weeks prior to randomization

- over the final 7 days of the run-in period, the patient has an average of WAP scores in the prior 24 hours of 3.0 to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable),

- over the final 7 days of the run-in period, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools), and there must be at least 2 days with stool consistency of Type ≥6, and

- have not used any loperamide rescue medication in the 2 weeks prior to randomization

Eligible patients will be instructed to return to the study site for the Baseline visit on Day 1.

If patients do not meet all of the 4 diary conditions required for entry during the 2 week run-in period, the run-in may be extended for an additional week in accordance with investigator judgment (for a total run-in period of up to 3 weeks). Eligibility will then be based on the most recent 14 days of the extended run-in period.

8.3 Efficacy Assessments

The efficacy objectives of the study are to evaluate the effect of BOS-589, relative to PBO after 4 weeks of therapy, on pain, defecation, and key IBS-D related signs and symptoms.

During the 4 weeks of the double-blind treatment phase, patients will be required to access their electronic diary every day, preferably at the same time each day, to record IBS-D symptom data and information related to their bowel function and rescue medication use.

8.3.1 Worst Abdominal Pain

Throughout the 4 weeks of the double-blind treatment phase, patients will be asked to rate their WAP in the past 24 hours. The patient-reported WAP in the past 24 hours will be recorded on a 0 to 10 scale, where 0 corresponds to no pain and 10 corresponds to worst imaginable pain.

8.3.2 Bristol Stool Form Scale

Patients will be asked to record daily stool consistency according to the BSFS most representative of the past 24 hours and worst stool consistency (defined as the loosest stool with the highest BSFS score) in the past 24 hours. The patient-reported BSFS consistency score is based on a 1 to 7 scale where 1 corresponds to a hard stool and 7 corresponds to watery diarrhea (CCI [redacted]). Please refer to Appendix 3.

8.3.3 IBS-D Global Symptom Score

Patients will be asked to record daily their overall IBS-D global symptoms in the prior 24 hours. The patient-reported daily IBS-GS is based on a 0 to 4 scale where:

- 0 corresponds to no symptoms;
- 1 corresponds to mild symptoms;
- 2 corresponds to moderate symptoms;
- 3 corresponds to severe symptoms; and
- 4 corresponds to very severe symptoms.

8.3.4 IBS Severity Score System

Patients will be asked to complete 5 questions regarding the severity of their IBS. Each of the 5 questions generate a maximum score of 100, leading to a total possible score of 500.

8.4 Safety and Other Assessments

8.4.1 Adverse Events and Serious Adverse Events

See Section 8.5.

8.4.2 Physical Examinations

A complete physical examination (PE) will be performed at Screening. At a minimum, a targeted PE will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) with additional symptom-directed examination performed as clinically indicated. Any clinically significant PE finding noted at Screening will be recorded as medical history in the source document. Any clinically significant PE finding noted at after enrollment will be reviewed for reporting as an AE (see Section 8.5).

A complete PE will include, at a minimum, assessment of the cardiovascular, respiratory, GI, and neurological systems. A targeted PE will include, at a minimum, assessments of the lungs, cardiovascular system, and abdomen (liver and spleen).

Height and weight will also be measured and BMI will be calculated and the data recorded at the timepoints specified in the Schedule of Activities (Section 1.3).

Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.4.3 Vital Signs

At the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection, vital signs will be measured in a supine position after 5 minutes rest and will include oral temperature, systolic and diastolic blood pressure, pulse, and

respiratory rate. Any clinically significant abnormal vital sign value will be recorded as an AE (see Section 8.5).

8.4.4 Electrocardiograms

Single 12-lead ECGs will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection. Electrocardiogram interval measurements and interpretation (normal, abnormal not clinically significant, abnormal clinically significant) will be recorded in the source document. Any clinically significant change in ECG interpretation will be recorded as an AE (see Section 8.5).

8.4.5 Clinical Safety Laboratory Assessments

See Appendix 1 for the list of clinical laboratory tests to be performed and to the Schedule of Activities (Section 1.3) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record in the AE section of the CRF any clinically relevant changes occurring during the study. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 1, must be conducted in accordance with the laboratory manual and the Schedule of Activities.

If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the Investigator (e.g., SAE, or AE, or dose modification), then the results must be recorded in the CRF.

8.5 Adverse Events and Serious Adverse Events

Adverse events will be reported by the patient (or, when appropriate, by a caregiver, surrogate, or the patient's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting and documenting events that meet the definition of an AE or SAE and are responsible for following all AEs.

8.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of investigational product.

8.5.2 Events of Special Interest

Not applicable.

8.5.3 Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death because of progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life threatening;

  The term 'life threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization;

  In general, hospitalization signifies that the subject or patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.

  Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE;

- Results in persistent disability/incapacity;

  The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea,

vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised when deciding if SAE reporting is appropriate in other situations such as important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient or may require medical or surgical intervention to prevent any of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.5.4 Classification of an Adverse Event

8.5.4.1 Assessment of Severity

The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities;

- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities;

- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as ‘serious’ when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

8.5.4.2 Assessment of Causality

All AEs must have their relationship to the investigational product and/or study participation assessed by the Investigator who examines and evaluates the patient based on temporal relationship and his or her clinical judgment. Alternative causes, such as underlying disease, concomitant therapy, and other risk factors, as well as the temporal relationship of the event to investigational product administration will be considered and investigated. The Investigator will also consult the IB in his or her assessment.

The degree of certainty about causality will be graded using the categories below:

- **Related** – The AE is known to occur with the investigational product, there is a reasonable possibility that the investigational product caused the AE, or there is a temporal relationship between the investigational product and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the investigational product and the AE.

- **Not Related** – There is not a reasonable possibility that the administration of the investigational product caused the event, there is no temporal relationship between the investigational product and event onset, or an alternate etiology has been established.

For each AE, the Investigator must document in the medical notes that he or she has reviewed the event and has provided an assessment of causality. The Investigator may change his or her opinion of causality in light of follow-up information and updated causality assessment reported.

8.5.4.3 Expectedness

The Investigator will be responsible for determining whether an SAE is expected or unexpected. An SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the investigational product.

8.5.5 Time Period and Frequency for Event Assessment and Follow-up

All AEs will be collected from the signing of the ICF until the End-of-Study Follow-up visit.

Whenever possible, all AEs should be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the patient is stable.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, whether considered investigational product related or not, and must include an assessment of if there is a reasonable possibility that the investigational product caused the event. The Investigator will also submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he or she considers the event to be reasonably related to the investigational product or study participation, the Investigator must promptly notify the sponsor.

8.5.6 Adverse Event Reporting

At each study visit, patients will be evaluated for new AEs and the status of existing AEs. Care will be taken not to introduce bias when evaluating for AEs. The Investigator should use open-ended questions when soliciting information from a patient regarding AEs, followed by appropriate questions that clarify the patient's verbatim description of AEs or change in concomitant medications.

When an AE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE.

The Investigator will then record all relevant AE information in the CRF. It is not acceptable for the Investigator to send photocopies of the patient's medical records in lieu of completion of the CRF page. New or updated information will be recorded in the originally completed CRF. However, there may be instances when copies of medical records for certain cases are requested. In this case, all patient identifiers, with the exception of the patient number, will be redacted on the copies of the medical records before submission.

If a patient dies during participation in the study or during a recognized follow-up period, the Investigator will provide a copy of any postmortem findings, including histopathology when available.

If a site receives report of a new SAE from a study patient or receives updated data on a previously reported SAE after database lock of the electronic data capture (EDC) system, then the site can report this information on a paper SAE form or to the sponsor by telephone. See the Study Manual for additional information on SAE reporting.

The study sponsor, or designee, will be responsible for notifying all applicable regulatory authorities of any required safety events in compliance with country-specific regulatory requirements. In addition, the sponsor must notify applicable regulatory authorities and all participating Investigators of suspected unexpected serious adverse reactions (SUSARs), from clinical trials or any other source.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the Institutional Review Board/ Independent Ethics Committee (IRB/IEC), if appropriate according to local requirements.

8.5.7 Reporting of Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. Pregnancy in a patient's partner is not considered an AE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs. Elective abortions without complications should not be handled as AEs; however, an induced therapeutic abortion to terminate a pregnancy because of complications or medical reasons must be reported as an SAE. The underlying medical diagnosis for this procedure should be reported as the SAE term. A spontaneous abortion in a study patient is always considered an SAE.

All pregnancies in female patients and, if indicated, pregnancies in female partners of male patients, will be collected after the start of investigational product and until 5 weeks after the last dose. Pregnancy outcomes, defined as the first well baby visit for live births, or spontaneous or induced abortion, will be documented even after the patient is withdrawn from or completes the study.

If a pregnancy is reported, the Investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 2.

8.6 Treatment of Overdose

There are no data on overdose because this is the first study of BOS-589 in patients and, in a prior study in healthy human volunteers, no evidence of overdose was reported. There is no definition of what constitutes an overdose and no known antidote. Any patient who receives a higher dose than that intended (i.e., more than 200 mg per dose; more than 400 mg in a given day) should be monitored closely, managed with appropriate supportive care, and followed up appropriately. If possible, a blood sample for PK should be collected as soon as is feasible from any patient who takes a higher dose than that intended.

If AEs or SAEs are reported and are considered related to a patient receiving a higher dose than intended, dosing should be interrupted if deemed necessary by the Investigator and the case discussed further with the Medical Monitor.

8.7 Pharmacokinetics

Plasma samples of approximately 4 mL will be collected for measurement of plasma concentrations of BOS-589 at the following times and as specified in the Schedule of Activities (Section 1.3).

- On Day 1 at predose and at 0.5, 1, 2, and 4 hours postdose.

- On Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If samples cannot be obtained on Day 15, then samples should be obtained on Day 22.

- A predose sample will be obtained on all other Treatment Study Visits.

Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for the 0.5 and 1 hour postdose timepoints, and ± 10 minutes for the 2 and 4 hours postdose timepoints.

Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual time of dosing and actual date and time (24-hour clock time) of each sample will be recorded.

Drug concentration information that may unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

Any changes in the timing or addition of timepoints for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

The following PK parameters will be estimated from the plasma concentrations of BOS-589 using either traditional noncompartmental methods or a population-PK model:

AUC_{0-t} Area under the plasma concentration versus time curve from time zero to the last quantifiable sample;

AUC_{0-4} Area under the plasma concentration versus time curve from time zero to 4 hours postdose;

C_{max} Maximum observed plasma concentration;

C_{min} Minimum observed plasma concentration.

T_{max} time to maximum concentration

In addition to parameters above, the AUC from time zero to 4 hours postdose (AUC_{0-12}) may be calculated using predose concentrations other than Day 1 predose to impute a 12-hour concentration. Details will be provided in the PK analysis plan.

8.8 Biomarkers/Pharmacodynamics

CCI [redacted]

9 STATISTICAL CONSIDERATIONS

9.1 Timing of Analyses

Formal hypothesis testing will occur at the end of the study and post database lock. There are no planned Data, Safety, or Adjudication Committees for this study and no planned statistical interim analyses.

9.2 Sample Size Determination

This is a phase 2a study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint. The primary endpoint is defined as a change in the 24-hour WAP score at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and compared between treatment groups in a hierarchical testing order using a t-test of equal variances and a two-tailed alpha of 0.05.

The sample size was based on the first hypothesis test in the hierarchical plan, with 80% power, two-sided alpha of 0.05, and a standard deviation (SD) of 3 points on the NRS to detect a 1.6 minimum change between treatment groups (N = 120 patients). To account for attrition and a potential higher PBO rate, a total of 132 patients will be randomized to one of 3 treatment groups in a 1:1:1 ratio (44 patients per treatment group).

9.3 Populations for Analyses

1. The Intention-to-Treat (ITT) analysis dataset will comprise all randomized patients;

2. The Per-protocol Analysis Dataset will comprise patients in the ITT Analysis Dataset who received at least 1 dose of investigational product and did not have any major protocol deviations;

3. The Safety Analysis Dataset will comprise all patients who received at least 1 dose of investigational product;

4. The Pharmacokinetic Analysis Dataset will comprise all randomized patients who received the active study drug and have available serum time concentration data.

9.4 Statistical Analyses

Detailed methodology for descriptive and inferential statistical analyses of the data collected in the study will be documented in the Statistical Analysis Plan (SAP). The SAP will be prepared by the Study Biostatistician and agreed upon by the Sponsor. The SAP will be finalized and approved prior to database lock. The SAP will take precedence over the protocol for details regarding the statistical analyses to be conducted for the study. In addition to the SAP, other graphical representations of the results may be produced after review of the data (*post hoc*). Any major modifications of the Primary Endpoint's definition and/or its analysis will be reflected in a protocol amendment.

In general, descriptive statistical methods will be used to summarize the data from this study. Unless stated otherwise, the term "descriptive statistics" refers to number of patients (n), mean, median, SD, minimum, and maximum for continuous data, and frequencies and percentages for categorical data.

All statistical analyses will be performed using Statistical Analysis System (SAS®) software Version 9.4 or higher.

9.4.1 Efficacy Analyses

9.4.1.1 Primary Endpoint

This is a proof-of-principle study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change in the 24-hour WAP score at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and compared between treatment groups in the following testing order using a t-test of equal variances and a two-tailed alpha of 0.05:

Hypothesis 1:

Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 2.

Hypothesis 2:

Cohort 1 (High Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 3.

Hypothesis 3:

Cohort 2 (Low Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4.

Hypothesis 4:

Cohort 1 (High Dose) versus Cohort 2 (Low Dose).

9.4.1.2 Secondary Endpoint

Mean daily BSFS score (stool type) and mean daily frequency of bowel movements will be tabulated and summarized by study day for each treatment for the ITT and Per-protocol Analysis Dataset populations. Mean daily BSFS score (stool type) will be analyzed separately based on the most representative stool consistency in the past 24 hours and the worst stool consistency (defined as loosest stool with the highest BSFS score) in the past 24 hours (each measure averaged over the week prior to each respective timepoint). Time profiles and box plots will also be presented by study day and by treatment group. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.3 Other Efficacy Endpoints

Scores from IBS-GS and IBS-SS questionnaires will be summarized for the ITT and Per-protocol Analysis Dataset populations. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.4 Missing Data

Study implementation procedures and training will assist in limiting the amount of missing data in the trial. However, sensitivity analyses may be performed when indicated for the study endpoint and the amount of missing data to be handled. Details of the statistical methods for handling missing data will be described in the SAP.

9.4.2 Safety Analyses

9.4.2.1 Adverse Events

Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All SAEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced.

All AEs will be listed for individual patients showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs (TEAEs) related to investigational product will be generated.

Any event reported on the CRF that occurs on at the time of or after the initiation of investigational product is defined as treatment-emergent. Additionally, an AE that is reported to have started on Day 1 without an associated onset time will be assumed to have occurred after the initiation of investigational product. Hence, AEs occurring on Day 1 with no associated onset time will be assumed to be treatment emergent.

Serious AEs associated with a protocol-specified procedure and occurring after the time of consent but before administration of first dose of investigational product will be defined as non-treatment-emergent AEs (NTEAEs).

All safety analyses will be performed in the Safety Analysis Dataset.

9.4.2.2 Clinical Laboratory Evaluations

Laboratory data will be listed for all patients. Laboratory data will be reviewed by the Investigator as they become available. All abnormal laboratory results will be evaluated by the Investigator as either clinically significant or not clinically significant.

Descriptive summaries of clinical laboratory results will be presented by date and time of collection. The number and percentage of patients experiencing treatment-emergent graded toxicities will be summarized by treatment arm and severity grade. Laboratory toxicity shifts from baseline to post-baseline assessments will be summarized by treatment arm. Changes from baseline in laboratory tests will be summarized for each treatment arm.

9.4.2.3 Twelve-lead Electrocardiograms, Vital Signs, and Physical Examinations

Twelve-lead ECG and vital signs data will be listed and summarized.

Physical examination findings will be captured and listed as part of a patient's medical history or as AEs as applicable.

9.4.3 Other Analyses

9.4.3.1 Pharmacokinetic Analysis

A full description of the methods for evaluating the PK of BOS-589 in this patient population will be provided in a PK data analysis plan. The PK data from this study may be combined with data from other studies to complete a population-PK analysis.

A full list of PK parameters to be calculated is included in Section 8.7.

9.4.4 Baseline Descriptive Statistics

Demographic and baseline characteristics will be summarized for all patients overall and by treatment arm. Summary statistics (e.g., number of patients, mean, median, SD, and range) will be generated for continuous variables (e.g., age and weight) and the number and percentage of patients within each category will be presented for categorical variables (e.g., gender, ethnicity, race).

A detailed description of patient disposition will be provided. It will include:

- A summary of overall patient enrollment status (consented, screened, screen failures, replacements, randomized);
- A summary of patients who discontinued the study;
- An account of identified protocol deviations.

All patients who are consented for the study will be accounted for in the summation. The number of patients who do not qualify for certain analysis populations will be summarized.

9.4.5 Subgroup Analyses

No subgroup analyses are powered for the study. Subgroup analyses may be performed for descriptive purposes as described in the SAP.

9.5 Planned Interim Analyses

No formal efficacy interim analysis is planned for the purposes of statistically comparing the data prior to the completion of the study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines;
- Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines;
- Applicable laws and regulations.

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted, and reviewed and approved as necessary, to the relevant Competent Authorities, IRBs and IECs before the study is initiated.

Any amendments to the protocol will require regulatory approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC;
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures;
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Informed Consent/Assent Process

An initial sample ICF is provided for the Investigator to prepare the informed consent document to be used at his or her site. Updates to the sample ICF are to be communicated formally in writing from the Sponsor or Sponsor’s designee to the Investigator. The written ICF is to be prepared in the language of the potential patient population.

The Investigator or his/her representative will explain the nature of the study to the patient or his/her legally authorized representative and answer all questions regarding the study.

Patients must be informed that their participation is voluntary. Patients or their legally authorized representative will be required to sign a statement of informed consent, and/or assent, that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Patients must be re-consented to the most current version of the ICF(s) during their participation in the study if one becomes available.

A copy of the ICF(s) must be provided to the patient or the patient's legally authorized representative.

The Investigator is also responsible for asking the patient if the patient has a primary care physician and if the patient agrees to have his/her primary care physician informed of the patient's participation in the clinical study if it is a local requirement. If the patient agrees to such notification, the Investigator is to inform the patient's primary care physician of the patient's participation in the clinical study. If the patient does not have a primary care physician and the Investigator will be acting in that capacity, the Investigator is to document such in the patient's medical record.

If a patient is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written ICF and any other written information to be provided to patients, is read and explained to the patient or the patient's legally acceptable representative, and after the patient or the patient's legally acceptable representative has orally consented to the patient's participation in the trial and, if capable of doing so, has signed and personally dated the ICF, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the patient or the patient's legally acceptable representative, and that informed consent was freely given by the patient or the patient's legally acceptable representative (Refer to ICH GCP guideline, Section 4.8.9).

10.1.3 Confidentiality and Privacy

Patient confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, and the sponsor and its designees. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to patients. Therefore, the study documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the study data will be released to any unauthorized third party without prior written approval of the sponsor.

Study patient research data will not include the patient's contact or identifying information. Rather, individual patients and their research data will be identified by a unique study identification number. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred. Research data will be used in accordance with local data protection laws.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/IEC or regulatory agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

10.1.4 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices, Good Manufacturing Practices).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.5 Data Handling and Record Keeping

10.1.5.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site, under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

The Investigator must maintain adequate source documentation. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into a 21 CFR Part 11-compliant EDC system or transmitted electronically (e.g., laboratory data). The data system(s) includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Data recorded in the CRF should be consistent with the data recorded on the source documents or the discrepancies must be explained.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data. The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.5.2 Study Records Retention

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations.

No records will be destroyed without the written consent of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

It is the responsibility of the sponsor to inform the Investigator when these documents no longer need to be retained.

10.1.6 Study and Site Discontinuation and Closure

The sponsor or its designee reserves the right to suspend or prematurely terminate a study site or study at any time for any reason and at the sole discretion of the sponsor.

An Investigator may initiate site closure at any time, provided there is reasonable cause and sufficient notice is given to the sponsor in advance of the intended termination.

Written notification, documenting the reason for site or study suspension or termination, will be provided by the suspending or terminating party to study participants,

Investigator, funding agency, the Investigational New Drug sponsor, and regulatory authorities.

If the study is prematurely terminated or suspended, the Investigator will promptly inform study participants, the IRB/IEC, and sponsor, and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants;
- Demonstration of efficacy that would warrant stopping;
- Insufficient compliance to the protocol, regulatory requirements, or GCP guidelines;
- Data that are not sufficiently complete and/or evaluable;
- Determination that the primary endpoint has been met;
- Determination of futility;
- Inadequate recruitment of patients.

10.1.7 Dissemination of Clinical Study Data

A Clinical Study Report (CSR) will be prepared in accordance with the ICH guideline on structure and contents of CSRs and any applicable regulatory and legal requirements.

10.1.8 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

11 REFERENCES

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U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (May 2012). Guidance for Industry, Irritable Bowel Syndrome – Clinical Evaluation of Drugs for Treatment. Retrieved from https://www.fda.gov/downloads/Drugs/Guidances/UCM205269.pdf.

CCI [redacted]

CCI [redacted]

12 APPENDICES

Appendix 1. Clinical Laboratory Tests

The tests detailed in Table 2 will be performed by the central laboratory with the exception of erythrocyte sedimentation rate and urine pregnancy tests. The central laboratory will provide kits and instructions to perform these assessments on site.

Local laboratory results are only required in the event that the central laboratory results are not available in time for either investigational product administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either an investigational product decision or response evaluation, the results must be entered into the CRF.

Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Pregnancy testing: Refer to Section *5.1* for Screening pregnancy criteria.

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count Red blood cell (RBC) count Hemoglobin Hematocrit		RBC Indices: Mean corpuscular volume Mean corpuscular Hemoglobin % Reticulocytes	White blood cell count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical Chemistry	Blood urea nitrogen	Potassium	Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase	Total and direct bilirubin
	Creatinine	Sodium	Alanine aminotransferase/Serum glutamic pyruvic transaminase	Total protein
	Glucose (fasted)[a]	Calcium	Alkaline phosphatase	
CCI [redacted]	CCI [redacted]	CCI [redacted]	CCI [redacted]	
Routine Urinalysis	Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Microscopic examination (if blood or protein is abnormal)			

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Other Screening Tests	Hemoglobin A1c Follicle-stimulating hormone and estradiol (as needed in women of nonchildbearing potential only) Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential) Serology (HIV antibody, hepatitis B surface antigen, and hepatitis C virus antibody) serum tissue transglutaminase IgA antibody (tTG-IgA) and IgA (if applicable)

[a] Patients should refrain from food or drinks (other than water) for at least 8 hours prior to obtaining a fasting glucose level.

Investigators must document their review of each laboratory safety report.

Laboratory results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

Appendix 2. Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories **are not considered WOCBP**

1. Premenarchal
2. Premenopausal female with 1 of the following:
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy
    c. Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's review of the patient's medical records, medical examination, or medical history interview.

3. Postmenopausal female
    a. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
    b. Females on HRT and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Male patients.

Male patients with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the treatment period and for at least 14 weeks after the last dose of investigational product:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent.
- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year, as described in Table 3, when having penile-vaginal intercourse with a WOCBP who is not currently pregnant, unless confirmed to be azoospermic (vasectomized or secondary to medical cause).

In addition, male patients must refrain from donating sperm for the duration of the study and for 14 weeks after the last dose of investigational product.

Male patients with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration for the duration of the study and for 14 weeks after the last dose of investigational product.

Female patients.

Female patients of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception, as described in Table 3, consistently and correctly.

Table 3. Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[a]
Failure rate of <1% per year when used consistently and correctly.

Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b]

- Oral
- Intravaginal
- Transdermal

Progestogen only hormonal contraception associated with inhibition of ovulation[b]

- Oral
- Injectable

Highly Effective Methods That Are User Independent

- Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b]
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion

Vasectomized Partner
A vasectomized partner is a highly effective contraception method, provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.

Sexual Abstinence
Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the investigational product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the patient.

NOTES:
[a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for patients participating in clinical studies. Should be initiated 2 or more menstrual cycles prior to screening.
[b] Hormonal contraception may be susceptible to interaction with the investigational product, which may reduce the efficacy of the contraceptive method. In the case of hormonal contraception as the primary method of contraception, a supplementary barrier method (preferably male condom) should be utilized during the treatment period and for at least 5 weeks after the last dose of investigational product.

Pregnancy Testing:

- Any WOCBP should only be included after a negative pregnancy test.
- Additional urine pregnancy testing should be performed at the Day 29 visit, and the Day 43 End-of-Study Follow-up visit.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected

Collection of Pregnancy Information:

- All occurrences of pregnancies must be reported on a Pregnancy Notification and Outcome Form (within 24 hours of awareness of any such pregnancy that occurs for either the study patient or for the female partner of a male patient); all pregnancies will be followed to outcome, which means until either the first well-baby visit for live births or until documented spontaneous or induced abortion.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

- Pregnancies that occur beyond 90 days after the last dose of study drug will only need to be reported if there is an associated SAE that the Investigator assessed as related to study drug.

- Safety Team will follow-up with the Investigator every 3 months regarding pregnancy outcome. The Investigator must follow the pregnancy to conclusion in order to determine the outcome. If the case is beyond 30 days from the expected due date and no information has been received regarding the outcome, the Safety Team will contact the Investigator to request outcome.

Male Patients With Partners Who Become Pregnant

- The Investigator will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to male patients who receive BOS-589.

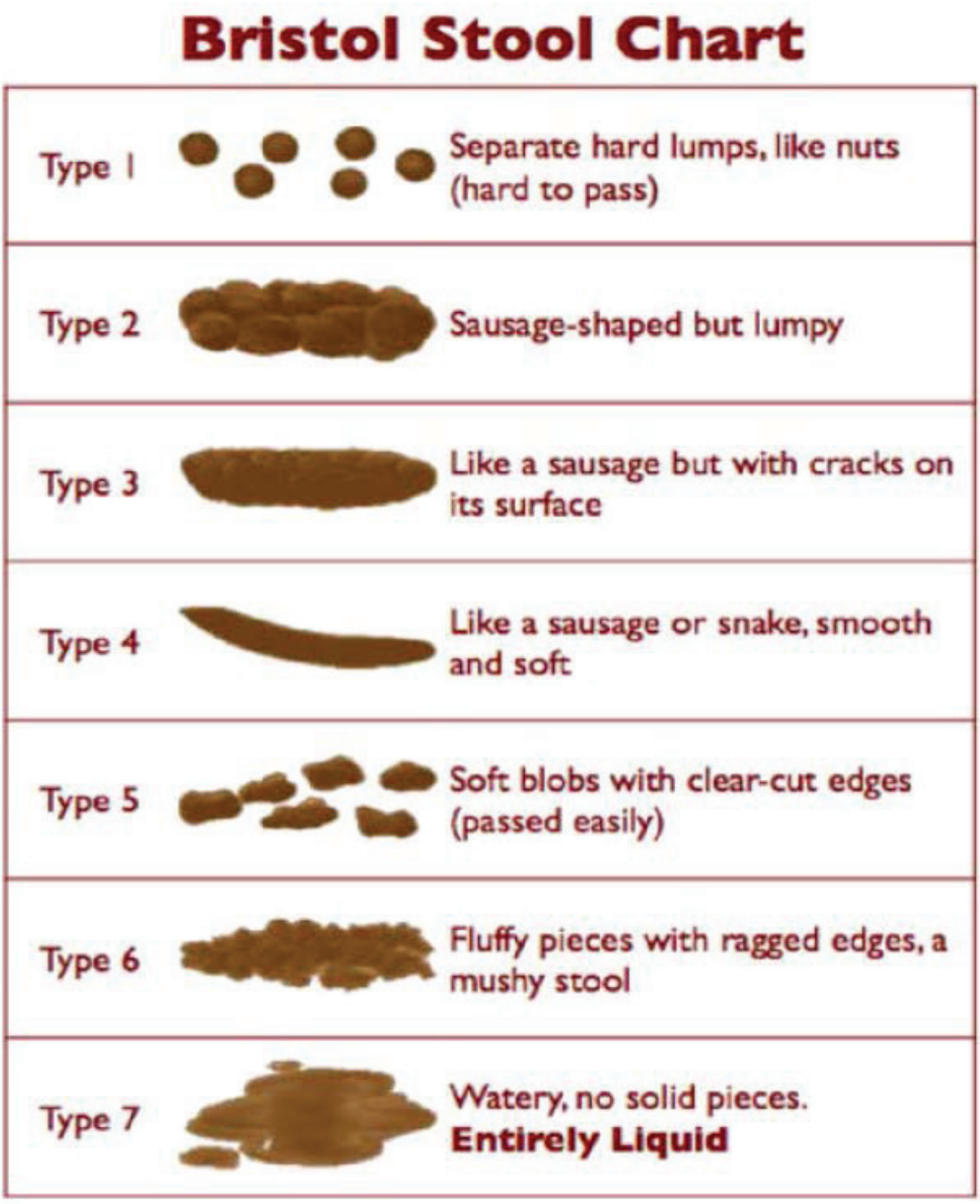
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the sponsor as outlined above.

Female Patients Who Become Pregnant

- The Investigator will collect pregnancy information on any female patient who becomes pregnant while participating in this study. Information will be recorded on the appropriate form as noted above.

- Any female patient who becomes pregnant while participating in the study will be withdrawn from the study.

Appendix 3. The Bristol Stool Form Scale



([redacted])

Appendix 4. Concomitant Medications

If the study site/investigator has any questions regarding permitted or excluded medications, the medical monitor should be contacted for further discussion.

Medications permitted during the course of the study:

- Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or supplements) that in the opinion of the Investigator, should not interfere with study procedures, compromise safety, or the scientific integrity of the data;
- Stable doses of medications for allergies, migraines (with the exception of opioids for acute treatment), anxiety (e.g., as-needed use of benzodiazepines), depression or other chronic conditions at the discretion of the Investigator;
- Stable doses of antidepressants. As-needed use of buspirone and benzodiazepines for anxiety;
- Loperamide can be used during study as rescue medication based on protocol-specified guidelines (see Section 6.7.1) for allowable on-study rescue medications);
- Acetaminophen, up to 2 g/day for up to 3 consecutive days;
- The Investigator should monitor closely for potential side effects related to any of the following medicines which are considered moderate to highly sensitive substrates of CYP3A4.

alprazolam	dronedarone	nisoldipine
aprepitant	ebastine	pimozide
atorvastatin	eletriptan	rivaroxaban
avanafil	felodipine	sildenafil
budesonide	lomitapide	simvastatin
buspirone	lovastatin	tadalafil
colchicine	midazolam	triazolam
darifenacin	naloxegol	vardenafil

Medications excluded during the course of the study:

- 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron, ondansetron, or ramosetron);
- Eluxadoline;
- Bile acid sequestrants such as cholestyramine, colestipol, or colesevelam;
- Strong inhibitors of P-glycoprotein:

amiodarone	dronedarone	quinidine
carvedilol	itraconazole	ranolazine
clarithromycin	propafenone	verapamil

- Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
- Narcotic- or opioid-containing agents;
- Cannabis-containing products;
- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents);
- Planned use of rifaximin or oral antibiotics, with the exception of topical antibiotics or a 1-day course with an antibiotic); a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomized;
- Any investigational drug.

Appendix 5. Protocol Amendment History

DOCUMENT HISTORY	
Document	**Date of Issue**
Amendment 2, v.3.0	12 August 2019
Amendment 1, v.2.0	09 May 2019
Original, v.1.0	05 January 2019

Appendix 6. Protocol Summary of Changes

Section	Description of Changes	Rationale
Global	Replace: Protocol Date and Version: ~~09 May 2019; v. 2.0~~ With: Protocol Date and Version: **12 August 2019**; v. **3.0**	Administrative
Global	Change: Changed Version number from Amendment 1 (v. 2.0) to **Amendment 2** (v. **3**.0) and Version date from 09 May 2019 to **12 August 2019**.	Administrative
Global	Made administrative and editorial (formatting, typographical, and grammatical) updates.	Administrative
PROTOCOL SUMMARY, Section 1.1, Synopsis Section 1.2, Study Schema Section 4.1, Overall Design Section 8.1, Study Periods	Replace: A Pre-treatment Phase of up to 4 weeks, in which all patients will be assessed to determine eligibility. With: A Pre-treatment Phase of up to **5** weeks, in which all patients will be assessed to determine eligibility.	Update to pre-treatment length of time.
PROTOCOL SUMMARY, Section 1.1, Synopsis	Replace: The duration of patient participation is anticipated to be up to ~~10~~ weeks. With: The duration of patient participation is anticipated to be up to **11** weeks.	Update to account for the extra week of screening
PROTOCOL SUMMARY, Section 1.3, Schedule of Assessments, Table 1	Replace:  With:	Update to screening window to align with update to pre-treatment length of time.

Section	Description of Changes	Rationale
		
PROTOCOL SUMMARY, Section 1.3, Schedule of Assessments, Table 1	Add: **The full 5 week (Days -36 to -1) may be used to randomize eligible patients. However, efforts should be made to randomize eligible patients within 1 to 3 days after completing run-in period.**	Protocol clarification on length of time for randomization
PROTOCOL SUMMARY, Section 1.3, Schedule of Assessments, Table 1 Appendix 1. Clinical Laboratory Tests	Add: **Patients should refrain from food or drinks (other than water) for at least 8 hours prior to obtaining a fasting glucose level.**	Protocol clarification added on patient fasting.
Section 4.1.1, Study Duration for Patients	Replace: Unless shortened by intolerable AEs or rapid disease progression, each patient's participation in this study is anticipated to last up to ~~10~~ weeks. With: Unless shortened by intolerable AEs or rapid disease progression, each patient's participation in this study is anticipated to last up to **11** weeks.	Protocol clarification on length of time for patient participation to align with update to pre-treatment length of time
Section 5.1, Inclusion Criteria	Replace: 5. Over ~~the week prior to randomization~~, the patient has an average of worst abdominal pain (WAP) scores in the prior 24 hours of ~~4.0~~ to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable). With: 5. Over **the final 7 days of the run-in period**, the patient has an average of WAP scores in the prior 24 hours of **3.0** to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable).	Protocol clarification
Section 5.1, Inclusion Criteria	Replace: 6. Over ~~the week prior to randomization~~, the	Protocol clarification

Section	Description of Changes	Rationale
	patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools) and ~~at least 5~~ days with ~~a BSFS score ≥ 5.0~~. With: 6. Over **the final 7 days of the run-in period**, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools), **and there must be at least 2** days with **stool consistency of Type ≥ 6**.	
Section 5.1, Inclusion Criteria	Delete: ~~7. Over the week prior to randomization, the patient has an average daily IBS-GS score of ≥ 2.0 (on a 0 to 4 scale, where 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe).~~	Protocol clarification
Section 5.1, Inclusion Criteria	Add: a) If at least one of the following alarm features are present, then the patient must have had a colonoscopy **that should include biopsy** since the onset of symptoms or within the past 5 years (whichever is less):	Protocol clarification
Section 5.1, Inclusion Criteria	Add: b) If no alarm features are present, then the patient must have had a colonoscopy or other appropriate exam, based on criteria as outlined below **(Colorectal cancer screening tests other than colonoscopy are considered second-tier and can be discussed with the sponsor [CCI]):**	Protocol clarification
Section 5.2, Exclusion Criteria	Replace: 12. Fecal calprotectin ≧ 50µg/g. With: 12. Fecal calprotectin **>** 150µg/g (CCI).	In careful literature review CCI), an exclusion cutoff of >150 µg/g along with colonoscopy when appropriate has adequate sensitivity and specificity to distinguish patients with IBS from other gastrointestinal disorders, especially in those patients with appropriate workup

Section	Description of Changes	Rationale
		(history, physical examination, and colonoscopy) as required in this study.
Section 5.2, Exclusion Criteria	Add: 18. Evidence of hepatitis C virus (HCV) infection based on a positive HCV antibody screen**. Patients with a positive HCV antibody at screening may be eligible if confirmatory testing (i.e., RIBA, or HCV RNA viral load) provided by the study site is negative.** Patients who have been successfully treated for HCV are eligible if an undetectable HCV viral load at least 6 months after completion of treatment can be demonstrated).	Protocol clarification
Section 5.2, Exclusion Criteria	Add: 20. Within 14 days of randomization, patient has used either of the following: • 5-hydroxytriptamine (5-HT)3 or 5 HT4 receptor antagonists (e.g., alosetron, **ondansetron, or ramosetron**); • **Eluxadoline;**	Protocol clarification
Section 5.2, Exclusion Criteria	Add: • **Bile acid sequestrants such as cholestyramine, colestipol, or colesevelam*** Add footnote at bottom of page: * **Patients with diagnosis of or suspected to have bile acid malabsorption are not specifically excluded given the challenge of diagnosing these patients; however, use of bile acid sequestrants are prohibited**	Protocol clarification
Section 6.7.1, Rescue Medicine	Replace: During the double-blind Treatment Phase of the study, patients will be allowed to take loperamide rescue medication for the acute treatment of uncontrolled diarrhea. ~~Loperamide at a unit dose of~~ 2 mg may be taken once approximately every 6 hours ~~with the following guidelines~~: With: During the double-blind Treatment Phase of the study, patients will be allowed to take loperamide rescue medication for the acute treatment of uncontrolled diarrhea. **It is recommended that patients refrian**	Protocol clarification

Section	Description of Changes	Rationale
	from loperamide unless a minimum of 2 or more uncontrolled diarrhea events that cause significant discomfort and prevent normal everyday activities are experienced in a given 24-hour period. **If loperamide must be used, patients must not exceed the** 2 mg **(unit dose), which** may be taken once approximately every 6 hours **and must not exceed the following dosing requirements:**	
Section 7.2, Discontinuation/ Withdrawal from the Study	Replace: • No bowel movement for ~~7~~ days; With: • No bowel movement for **4** days;	Protocol clarification
Section 8.2, Screening Assessments	Replace: Patients who ~~are:~~ • ~~Compliant in completing the screening diary on a daily basis on at least 6 of the 7 days during the week prior to randomization AND on at least 11 of the 14 days during the 2 weeks prior to randomization, and~~ • ~~Have an average WAP score in the past 24 hours of between 4.0 and 8.0 on a 0 to 10 scale over the week prior to randomization, and~~ • ~~Have an average daily BSFS of ≥ 5.0 and at least 5 days with a BSFS score ≥ 5.0 on a 1 to 7 scale over the week prior to randomization, and~~ • ~~Have an average daily IBS-GS score of ≥ 2.0 on a 0 to 4 scale over the week prior to randomization, and~~ • ~~Who~~ have not used any loperamide rescue medication in the 2 weeks prior to randomization ~~will be eligible for participation and immediate randomization into the double-blind treatment phase (i.e., all 5 diary conditions must be met to qualify for randomization).~~ With: Patients who **meet the following requirements will be eligible for participation and immediate randomization into the double-blind treatment phase (i.e., all diary conditions listed must be met to qualify for randomization):** • **have completed the daily electronic diary**	Protocol clarification

Section	Description of Changes	Rationale
	on at least 6 of the 7 days during the week prior to randomization AND at least 11 of the 14 days during the 2 weeks prior to randomization • **over the final 7 days of the run-in period, the patient has an average of WAP scores in the prior 24 hours of 3.0 to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable)** • **over the final 7 days of the run-in period, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools), and there must be at least 2 days with stool consistency of Type ≥6, and**	
Section 8.2, Screening Assessments	Replace: If ~~after 2 weeks,~~ patients do not meet all of the ~~5~~ diary conditions required for entry, the run-in may be extended ~~by up to one~~ week in accordance with investigator judgement (for a total run-in period of up to 3 weeks). With: If patients do not meet all of the **4** diary conditions required for entry **during the 2 week run-in period**, the run-in may be extended **for an additional** week in accordance with investigator judgement (for a total run-in period of up to 3 weeks). **Eligibility will then be based on the most recent 14 days of the extended run-in period.**	Protocol clarification
Section 8.3.2, Bristol Stool Form Scale	Add: Patients will be asked to record daily stool consistency according to the BSFS most representative of the past 24 hours **and worst stool consistency (defined as loosest stool with the highest BSFS score) in the past 24 hours**. The patient-reported BSFS consistency score is based on a 1 to 7 scale where 1 corresponds to a hard stool and 7 corresponds to watery diarrhea ([redacted]). Please refer to Appendix 3.	Collection of additional data during the study
Section 8.4.2, Physical	Replace: A complete physical examination (PE) will be	Protocol clarification

Section	Description of Changes	Rationale
Examinations	performed at Screening. A ~~symptom-directed~~ PE will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) ~~and~~ as clinically indicated~~, and results recorded in the source document~~. Any clinically significant PE finding noted at Screening will be recorded as medical history. Any clinically significant PE finding noted at after enrollment will be reviewed for reporting as an AE (see Section 8.5). With: A complete physical examination (PE) will be performed at Screening. A**t a minimum, a targeted** PE will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) **with additional symptom-directed examination performed** as clinically indicated. Any clinically significant PE finding noted at Screening will be recorded as medical history **in the source document**. Any clinically significant PE finding noted at after enrollment will be reviewed for reporting as an AE (see Section 8.5).	
Section 8.4.2, Physical Examinations	Delete: A targeted PE will include, at a minimum, assessments of the ~~skin,~~ lungs, cardiovascular system, and abdomen (liver and spleen).	Protocol clarification
Section 8.5.4.3, Expectedness	Add: The Investigator will be responsible for determining whether an **S**AE is expected or unexpected. An **S**AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the investigational product.	Protocol clarification
Section 8.7, Pharmacokinetics	Add: T_{max} time to maximum concentration	Protocol clarification
Section 9.4.1.2, Secondary Endpoint	Add: Mean daily BSFS score (stool type) and mean daily frequency of bowel movements will be tabulated and summarized by study day for each treatment for the ITT and Per-protocol Analysis Dataset populations. **Mean daily BSFS score (stool type) will be analyzed separately based on the most representative stool consistency in the past 24 hours and the worst stool consistency (defined as loosest stool with the highest BSFS score) in**	Collection of additional data during the study

Section	Description of Changes	Rationale
	the past 24 hours (each measure averaged over the week prior to each respective timepoint). Time profiles and box plots will also be presented by study day and by treatment group. Pooled active data may be compared to PBO for the analyses, and also by cohort.	
Section 11, REFERENCES	Add: CCI Add: CCI	Administrative change
Appendix 2, Contraceptive Guidance and Collection of Pregnancy Information	Add: • Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year, as described in Table 3, when having penile-vaginal intercourse with a WOCBP who is not currently pregnant**, unless confirmed to be azoospermic (vasectomized or secondary to medical cause).**	Protocol clarification
Appendix 2, Contraceptive Guidance and Collection of Pregnancy Information	Add: Progestogen only hormonal contraception associated with inhibition of ovulation[b]	Administrative correction to indicate correct footnote
Appendix 2, Contraceptive Guidance and Collection of Pregnancy Information	Delete: Highly Effective Methods That Are User Independent[a]	Administrative correction to indicate correct footnote
Appendix 2, Contraceptive Guidance and Collection of Pregnancy Information	Add: Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for patients participating in clinical studies. **Should be initiated 2 or more menstrual cycles prior to screening.**	Protocol clarification

Section	Description of Changes	Rationale
Appendix 2, Contraceptive Guidance and Collection of Pregnancy Information	Replace: Hormonal contraception may be susceptible to interaction with the investigational product, which may reduce the efficacy of the contraceptive method. In ~~this~~ case~~, 2 highly effective methods of contraception~~ should be utilized during the treatment period and for at least 5 weeks after the last dose of investigational product. With: Hormonal contraception may be susceptible to interaction with the investigational product, which may reduce the efficacy of the contraceptive method. In **the** case **of hormonal contraception as the primary method of contraception, a supplementary barrier method (preferably male condom)** should be utilized during the treatment period and for at least 5 weeks after the last dose of investigational product.	Protocol clarification
Appendix 4, Concomitant Medication	Add: Medications excluded during the course of the study: • 5-hydroxytriptamine (5-HT)3 or 5-HT4 receptor antagonists (e.g., alosetron**, ondansetron, or ramosetron);** • **Eluxadoline;** • **Bile acid sequestrants such as cholestyramine, colestipol, or colesevelam);**	Protocol clarification

CLINICAL TRIAL PROTOCOL

Study Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)
Study Number:	BOS-589-201
Regulatory Identification Number(s):	IND 142421
Study Phase:	2a
Investigational Product:	BOS-589
Indication:	Diarrhea-predominant Irritable Bowel Syndrome
Sponsor:	Boston Pharmaceuticals, Inc. 55 Cambridge Parkway, Suite 400 Cambridge, Massachusetts 02142 USA

Version	**Date**
Final v.2.0	09 May 2019

Confidentiality Statement

SPONSOR SIGNATURE PAGE

Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

Protocol Number: BOS-589-201

Version Number and Date: v.2.0, 09 May 2019

I, the undersigned, have approved the final version of this Clinical Trial Protocol.

PPD

5/10/2019
Date

PPD

5/10/2019
Date

INVESTIGATOR AGREEMENT

Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

Protocol Number: BOS-589-201

Version Number and Date: v.2.0, 09 May 2019

I, the undersigned, have read the protocol and agree to conduct the trial in compliance with the International Conference on/Council for Harmonization (ICH) guidelines and any other applicable regulatory requirements; as well as Good Clinical Practice (GCP) standards (CPMP/ICH/135/95).

I will provide copies of the protocol and all pertinent information to all individuals who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the investigational product and the conduct of the study.

I will use only the Informed Consent Form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this study.

I agree that the sponsor or its representatives will have access to any source documents from which case report form information may have been generated.

Investigator's Signature	Date

Name of Investigator *(typed or printed)*

Institution Name

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ABBREVIATIONS

AE	adverse event
AKT	protein kinase B
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration versus time curve
AUC_{0-4}	area under the concentration versus time curve from time zero to 4 hours postdose
AUC_{0-12}	area under the concentration versus time curve from time zero to 12 hours postdose
AUC_{0-t}	area under the concentration versus time curve from time zero to the last measurable concentration
bid	twice daily
BSFS	Bristol Stool Form Scale
BMI	body mass index
CFR	Code of Federal Regulations
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CNS	central nervous system
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CSR	clinical study report
CYP	cytochrome P450
ECG	electrocardiogram
EDC	electronic data capture
ENS	enteric nervous system
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
FDR	first-degree relative
GCP	Good Clinical Practice
GDNF	glial cell line-derived neurotrophic factor
GFR-α	glial cell line-derived neurotrophic factor family receptor-alpha
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
HBV	hepatitis B virus
HCV	hepatitis C virus

HDPE	high-density polyethylene
hERG	human ether-à-go-go related gene
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
IB	Investigator's Brochure
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome-constipation
IBS-GS	Irritable Bowel Syndrome Global Scale
IBS-D	irritable bowel syndrome-diarrhea
IBS-M	irritable bowel syndrome-mixed
IBS-SS	Irritable Bowel Syndrome Severity Score
IBS-U	irritable bowel syndrome-unclassified
IC_{50}	half-maximal inhibitory concentration
ICF	Informed Consent Form
ICH	International Conference on/Council for Harmonisation
IEC	Independent Ethics Committee
ILC3	group 3 innate lymphoid cells
IRB	Institutional Review Board
ITT	intention-to-treat
IV	intravenous
IWRS	interactive web response system
JAK	Janus kinase
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no observed adverse effect level
NRS	numeric rating scale
NTEAE	non-treatment-emergent adverse event
PBO	placebo
PD	pharmacodynamic(s)
PE	physical examination
P-gp	P-glycoprotein
PI3K	phosphoinositide 3-kinase
PK	pharmacokinetic(s)
PRO	patient-reported outcome
PYY	pancreatic peptide YY
QC	quality control
QTcB	QT interval corrected by Bazett's formula

QTcF	QT interval corrected by Fridericia's formula
RAS	rat sarcoma
RBC	red blood cell
RET	REarranged during Transfection
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SD	standard deviation
SK-N-AS	a human neuroblastoma cell line
STAT	signal transducer and activator of transcription proteins
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
TT	a human thyroid medullary carcinoma cell line
ULN	upper limit of normal
WAP	worst abdominal pain
WOCBP	woman of childbearing potential

1 PROTOCOL SUMMARY

1.1 Synopsis

<table>
<tr><td>Sponsor:</td><td colspan="2">Boston Pharmaceuticals Inc.</td></tr>
<tr><td>Title:</td><td colspan="2">A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)</td></tr>
<tr><td>Protocol Number:</td><td colspan="2">BOS-589-201</td></tr>
<tr><td>Phase:</td><td colspan="2">2a</td></tr>
<tr><td rowspan="5">Objectives and Endpoints:</td><td>OBJECTIVES</td><td>ENDPOINTS</td></tr>
<tr><td colspan="2">Primary</td></tr>
<tr><td>• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.</td><td>• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• Incidence of adverse events (AEs), serious adverse events (SAEs), discontinuations because of AEs, and any treatment-related severe AEs.</td></tr>
<tr><td colspan="2">Secondary</td></tr>
<tr><td>• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.</td><td>• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• Change in stool frequency, measured by the total number of</td></tr>
</table>

		spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the steady-state pharmacokinetics (PK) of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).
Study Design:	A phase 2a, randomized, double-blind, PBO-controlled, multicenter trial to provide proof-of-principle efficacy of BOS-589 in IBS-D patients and to inform dose selection for subsequent development. The study will comprise 3 phases: A **Pre-treatment Phase** of up to 4 weeks, in which all patients will be assessed to determine eligibility. This phase will consist of initial screening assessments after which eligible patients will enter a Run-in Period of up to 3 weeks. During the Run-in period the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medication use, to confirm disease activity, and diary compliance.	

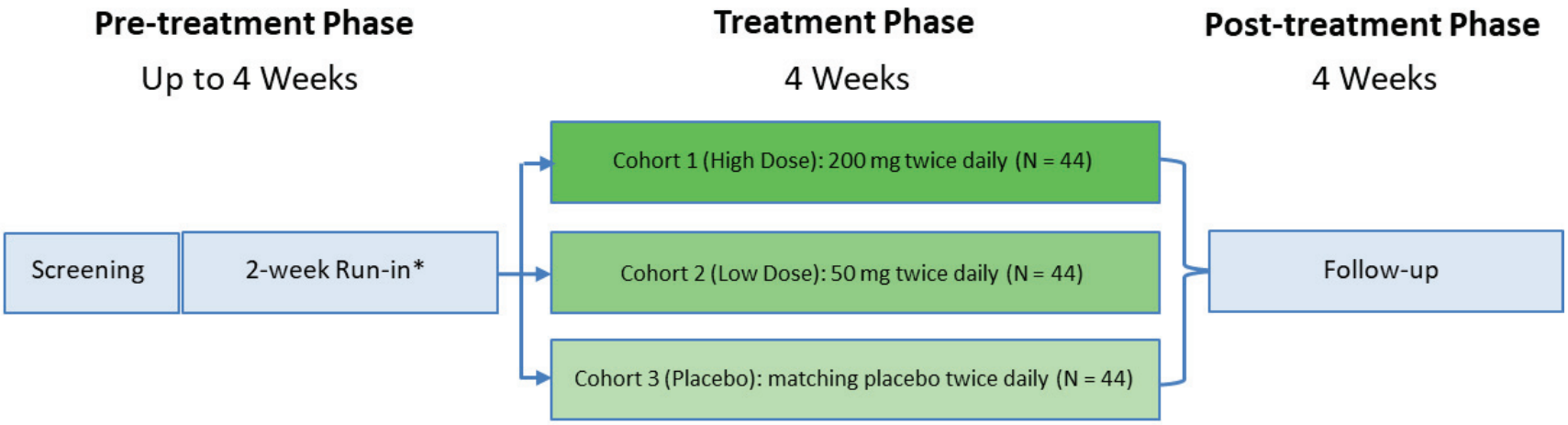
	Upon completion of the Run-in Period, patients will return to the study site to confirm eligibility for randomization into the double-blinded Treatment Phase. A **Treatment Phase**, in which eligible patients will be randomized (1:1:1) to receive BOS-589 or PBO twice daily (bid) for a total of 4 weeks. BOS-589 will be administered at 1 of 2 dose levels. Patients will be randomized to the following cohorts: • Cohort 1 (High Dose): 200 mg BOS-589 bid orally; • Cohort 2 (Low Dose): 50 mg BOS-589 bid orally; • Cohort 3 (PBO): matching PBO oral tablets bid. During the treatment phase, patients will continue to complete the electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine. A **Post-treatment Phase**, in which all patients who complete 4 weeks of treatment will return to the clinical for a 2-week follow-up visit. Patients who discontinue treatment early will be asked to return to the clinic for safety assessments.
Study Population:	Male and female patients 18 to 65 years of age, inclusive, with a diagnosis of IBS-D.
Number of Patients Planned:	Approximately 300 patients will be screened with the intent of randomizing 132 patients for the study.
Duration of Patient Participation and Study:	The duration of patient participation is anticipated to be up to 10 weeks. The duration of the study is anticipated to be approximately 12 months.
Study Sites:	Up to 66 sites in the United States.
Investigational Product:	BOS-589 (oral doses bid).
Reference Treatment:	Matching PBO (oral doses bid).
Concomitant Product:	Not applicable.

Statistical Methods:	**Efficacy:** This is a phase 2a proof-of-principle study. Descriptive and inferential statistical methods will be employed. A standalone Statistical Analysis Plan will be developed and approved prior to database lock. A hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change from baseline to Day 29 in the patient-reported outcome for WAP measured daily on an 11-point numeric rating scale. Mean change from baseline will be measured as a continuous variable and compared between treatment groups in the following testing order using a t-test of equal variances with a two-tailed alpha of 0.05: Hypothesis 1: Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3); If statistically significant at $P < 0.05$, then proceed to Hypothesis 2. Hypothesis 2: Cohort 1 (High Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$, then proceed to Hypothesis 3. Hypothesis 3: Cohort 2 (Low Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4. Hypothesis 4: Cohort 1 (High Dose) versus Cohort 2 (Low Dose). **Safety:** Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for

	Regulatory Activities Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All serious AEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced. All AEs will be listed for individual patients, showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs related to investigational product will be generated.

1.2 Study Schema

Figure 1. Study Schematic



* Run-in may be extended by 1 week to achieve for patient-reported outcomes requirements

1.3 Schedule of Activities

Table 1. Schedule of Activities

	Screening Days -29 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Informed consent	X							
Demographics	X							
Medical and surgical history	X							
Rome IV assessment	X							
Concomitant medication review	X	X	X	X	X	X	X	X
Adverse event review and evaluation		X- continuous - X						
Physical examination[c]	X		X				X	
Height, weight, and body mass index	X[d]		X		X		X	X
Vital signs	X		X	X	X	X	X	X
12-lead electrocardiogram	X						X	
Clinical safety laboratory tests[e]	X		X		X		X	X
HIV, HCV/HBV testing	X							
Urine pregnancy test	X		X				X	X
C-reactive protein and erythrocyte sedimentation rate tests	X		X		X		X	X
Biomarker sample collection			X		X		X	X
Pharmacokinetic sample collection[f]			X	X	X	X		
Worst abdominal pain	X	X[g]	X- [h] - X					X[h]
Bristol Stool Form Scale	X	X[g]	X- [h] - X					X[h]

	Screening Days -29 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Spontaneous bowel movements (total number of stools)		X[g]	X- -[h]- X					X[h]
IBS-GS		X[g]	X- -[h]- X					X[h]
Rescue medications		X[g]	X- -[h]- X					X[h]
IBS-SS			X				X	X
Eligibility review	X		X					
Randomization			X					
Administration of investigational product			Day 1 - Day 28					

HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IBS-GS = Irritable Bowel Syndrome Global Scale; IBS-SS = Irritable Bowel Syndrome Severity Score.

[a] Run-in may be extended by up to one week in accordance with the investigators judgement.

[b] Procedures performed at Visit 7/End of Treatment or upon Early Termination. Early Termination procedures are to be performed in close proximity to discontinuation of dosing for patients who are withdrawn from the study. Other procedures may be performed at Investigator or Sponsor discretion.

[c] Full physical exam to be conducted at Screening. Symptom-directed physical examinations should be conducted at subsequent visits.

[d] Height and body mass index to be collected at Screening Visit only.

[e] Hematologic, complete serum chemistry, urinalysis panels and fecal calprotectin to be completed as noted in Appendix 1.

[f] Pharmacokinetic (PK) serial blood samples are to be collected on Day 1 and Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If PK serial blood samples cannot be collected on Day 15, PK serial blood samples should be collected on Day 22. A predose sample will be obtained on all other Treatment Study Visits. Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for 0.5 and 1 hour postdose, and ± 10 minutes for 2 and 4 hours postdose.

[g] Data will be collected daily for entire Run-in Period.

[h] Data will be collected daily until Early Termination or until End-of-Study Visit, whichever is later.

2 INTRODUCTION

2.1 Study Rationale

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) illness with a prevalence of approximately 5% to 20% globally and characterized by a constellation of clinical symptoms. Establishing a diagnosis and assessing response to treatment remains challenging because there are no biomarkers that reliably correlate with disease state.

BOS-589 is an oral agent with limited systemic absorption that inhibits RET (REarranged during Transfection), a receptor tyrosine kinase that is hypothesized to play a key role in the maintenance of a healthy enteric nervous system (ENS). Inhibition of RET may represent a novel therapeutic strategy for the treatment of IBS by attenuating visceral hypersensitivity and/or colonic motility.

BOS-589 has been evaluated in healthy human subjects at doses up to 200 mg twice daily (bid) for 2 weeks. Consistent with its low oral bioavailability, BOS-589 was safe and well tolerated with no significant safety signals identified. The purpose of this study is to evaluate the efficacy, safety, and tolerability of BOS-589 in the treatment of patients with diarrhea-predominant IBS (IBS-D).

2.2 Background

2.2.1 Disease Under Study

Irritable bowel syndrome is a relatively common GI illness characterized by a number of clinical symptoms, including abdominal pain and discomfort, abnormal bowel habits, and bloating (CCI [redacted]). Subtypes of IBS, based on the predominant bowel habit(s) reported by IBS patients, include diarrhea (IBS-D), constipation (IBS-C), mixed (IBS-M), or unclassified (IBS-U). Currently approved medications for IBS address the restoration of patients' bowel habits and are minimally effective in addressing abdominal pain and discomfort. Because the etiology of the disease has not been clearly established, diagnosis is difficult and relies primarily on the presence of a specific symptom complex occurring in the absence of an alternative explanation. The development of criteria by expert panels, with recent iterations as recently as 2016, has improved the diagnosis and management of IBS patients (CCI [redacted]).

It is generally believed that the sensory inputs/outputs in the ENS and central nervous system (CNS) are altered in patients with IBS and this contributes to the signs and symptoms they experience. For example, patients with IBS have a heightened and disproportionate sensory experience (visceral hypersensitivity) for a given stimulus (CCI [redacted]). Visceral hypersensitivity and abnormal bowel habits may result from visceral afferent neurons or increased nerve fiber density and sprouting that have been observed in the intestinal mucosal tissues of IBS patients (CCI [redacted]; CCI [redacted]). The sensitizing event causing visceral hypersensitivity may be transient or chronic; however, its impact on the CNS and ENS

may be long lasting (CCI). Supernatants from these intestinal tissues can stimulate nerve outgrowth and increase neuronal signaling *in vitro*, suggesting that neurotrophic factors and/or inflammatory cytokines might be mediators of neuronal plasticity in IBS (CCI ; CCI). Given the central role the ENS may play in mediating signals between the CNS and the intestine, ENS modulation may provide therapeutic benefit to IBS patients by impacting visceral hypersensitivity and intestinal motility.

The only drugs currently approved by the Food and Drug Administration (FDA) for the treatment of IBS are alosetron (for women only), rifaximin, and eluxadoline for refractory IBS-D, and plecanatide, linaclotide, and lubiprostone for IBS-C. The majority of these drugs, and those used off label to treat IBS, target patients' symptoms by altering GI motility and are minimally effective in addressing abdominal pain and discomfort. Of the FDA-approved agents, only alosetron is hypothesized to modulate the CNS and ENS (CCI). Therefore, an agent such as BOS-589, which may ameliorate visceral hypersensitivity and potentially impact intestinal motility, would address a significant unmet medical need for IBS-associated pain.

2.2.2 The "RET" ("REarranged During Transfection") Gene and the Enteric Nervous System

The RET gene, localized on human chromosome 10q11.2, encodes a receptor-type tyrosine kinase with an extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain (CCI). The ligands for RET have been identified as neurotrophic factors of the glial cell line-derived neurotrophic factor (GDNF) family, including GDNF, neurturin, artemin, and persephin. Ligand binding to its corresponding GDNF family receptor-alpha (GFRα) co-receptor triggers RET dimerization and subsequent transphosphorylation of intracellular tyrosines (CCI) and leads to the activation of different intracellular signaling cascades, including the Janus kinase/signal transducer and activator of transcription proteins (JAK/STAT), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), and rat sarcoma/extracellular signal-regulated kinase (RAS/ERK) pathways.

Mice deficient in the GDNF ligand, its coreceptor GFRα1, or the RET protein itself, exhibit severe defects in kidney and ENS development. This implicates RET signaling as critical to the development of normal kidneys and the ENS (CCI). The role of RET in the development of the ENS is also apparent in patients with Hirschsprung's disease, who frequently suffer from colonic obstruction because of a lack of normal colonic innervation. In Hirschprung's disease, different loss-of-function mutations that occur in the *RET* gene account for the highest proportion of both familial and sporadic cases of the disease (CCI).

While its role during the development of the ENS has been well established, recent reports also implicate a significant role for RET in the maintenance and plasticity of the adult ENS. Neurons within the submucosal and myenteric plexus of the adult human colon have been shown to express RET and its coreceptors, GFRα1 and GFRα2, while the GDNF ligand is expressed in the muscularis mucosa and in circular and longitudinal

muscle tissue (CCI [redacted]). Systemic administration of GDNF in adult rodents results in significant increases in submucosal neuron density in both the small intestine and colon and altered function (CCI [redacted]). Furthermore, a conditional knockout of the RET co-receptor, GFRα3, results in decreased colonic hypersensitivity implicating a role for RET signaling in visceral nociception (CCI [redacted]). Therefore, by reducing RET signaling, inhibition of RET may modulate ENS activity.

2.2.3 Investigational Product

BOS-589, formerly GSK3352589, is a potent and selective inhibitor of RET that has been shown to reduce visceral hypersensitivity in an animal model of IBS and inhibit cholinergic-induced increases in colonic motility (details are provided in the BOS-589 Investigator's Brochure [IB]). The results from these preclinical studies suggest that inhibition of RET with a potent, selective, and gut-restricted small molecule may represent a novel therapeutic strategy for the treatment of abdominal pain and defecation abnormalities (i.e., diarrhea) in patients with IBS through the attenuation of visceral hypersensitivity and/or cholinergically mediated ion transport and colonic motility. The patient population likely to derive the greatest benefit would comprise individuals with IBS-D with increased GI motility.

2.2.3.1 Nonclinical Summary

A range of *in vitro* and *in vivo* studies have been conducted to investigate the primary, secondary, and safety pharmacology of BOS-589. Details are provided in the BOS-589 IB.

2.2.3.1.1 *In Vitro* Studies

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI

2.2.3.1.2 *In Vivo* Studies

CCI

2.2.3.1.3 Pharmacokinetics and Product Metabolism in Animals

CCI

CCI

CCI

CCI

CCI [redacted]

2.2.3.1.4 Toxicology and Nonclinical Assessment of Safety

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI

CCI

CCI

CCI

CCI

2.2.3.2 Clinical Experience

Study CCI evaluated the safety, tolerability, pharmacokinetics (PK), and exploratory pharmacodynamics (PD) of escalating single and multiple oral doses of BOS-589 ranging from 2 to 400 mg in the fasted state, and 25 mg in the fed state. All doses in the single ascending-dose portion of the study were generally safe and well tolerated, with a safety profile similar to that of placebo (PBO). Similarly, repeat-dose administration of BOS-589 for 14 days at doses ranging from 5 to 200 mg bid in the fed

state was generally safe and well tolerated. There were no drug-related clinically significant changes in safety laboratory tests, vital signs, ECGs, or stool patterns as assessed by the Bristol Stool Form Scale (BSFS) in this study. There were no serious adverse events (SAEs) considered related to the administration of BOS-589. A review of all GI adverse events (AEs) demonstrated that the occurrence of GI AEs was similar in the PBO- and BOS-589-treatment groups. There was no pattern or trend with dose escalation suggestive of a treatment effect.

The PK profile of BOS-589 demonstrated limited oral bioavailability and systemic exposure, with plasma concentrations generally less than 2 ng/mL, when measured utilizing a sensitive analytical method to detect concentrations as low as 5 pg/mL. Exposures were dose dependent and less than dose proportional, although box plots of dose-normalized parameters suggested dose proportionality between 15 mg and 100 mg. Dosing was escalated to the highest possible dose because predicted mean systemic exposures did not exceed the defined plasma toxicokinetic limits (AUC_{0-t} and C_{max} of 40.4 ng*h/mL and 26.7 ng/mL, respectively) and there was no evidence of dose-limiting toxicities. There was accumulation of BOS-589 with repeat dosing, but systemic exposures remained very low after 14 days of dosing, with respective geometric mean (CV%) AUC_{0-t} and C_{max} values of 24.1 (45.9) h*ng/mL and 1.53 (43.6) ng/mL for the highest dose of 200 mg bid; BOS-589 was likely at steady state before that time. Accumulation was lower for doses below 100 mg bid (1.3 to 1.8 fold) and highest for the highest dose of 200 mg bid (2.0 to 2.7 fold).

The study included a pilot food-effect group to evaluate the magnitude of a food challenge on the bioavailability of single-dose BOS-589. Following administration of a single dose of BOS-589 25 mg in the fed state, there was a small decrease in exposure with food, with decreases in mean C_{max} and AUC values in the range of 20% to 35%. The time to C_{max} (T_{max}) remained the same with or without food. These food-effect results were deemed not clinically important; dosing BOS-589 with or without food is not anticipated to affect future evaluation of safety or efficacy.

Because RET is expressed in enteroendocrine cells lining the intestinal mucosa, an exploratory objective of the study was to explore the effect of BOS-589 on glucagon-like peptide-1 (GLP-1) and pancreatic peptide YY (PYY) excursions in plasma; however, no clear relationship or impact on BOS-589 administration and peptide secretion was identified.

2.3 Risk/Benefit Assessment

Summaries of findings from nonclinical and clinical studies conducted with BOS-589 can be found in the IB (refer to the IB for additional details). The following section outlines the risk assessment and mitigation strategy for this protocol.

The current study, BOS-589-201, represents the first administration of BOS-589 to patients with IBS-D. Considerations for safety monitoring are derived primarily from the literature regarding RET expression in the intestine, nonclinical data, and clinical experience dosing BOS-589 to normal healthy volunteers (Study CCI [redacted]), in

which no clinically relevant risks were identified that would preclude dosing a RET inhibitor for up to 4 weeks in patients with IBS-D.

2.3.1 Risk Assessment

CCI

CCI

CCI

CCI

2.3.2 Benefit Assessment

Patients randomized to the active treatment arms may potentially experience improvement in their IBS-D during the course of the study. Those randomized to the PBO arm are not expected to obtain any benefit beyond that of their background treatment.

2.3.3 Overall Risk/Benefit Conclusion

On the basis of nonclinical and clinical study results to date, limited effective alternative treatments, and the strength of the scientific hypothesis under evaluation, BOS-589 is considered to have a favorable benefit-risk profile for patients with IBS-D.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).	• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.	• Incidence of adverse events (AEs) serious adverse events (SAEs), discontinuations because of AEs, and any treatment-related severe AEs.
Secondary	
• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.	• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). • Change in stool frequency, measured by the total number of spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the steady-state pharmacokinetics of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).

4 STUDY DESIGN

4.1 Overall Design

This study is a phase 2a, randomized, double-blind, PBO-controlled, multicenter trial designed to provide the first proof-of-principle efficacy of BOS-589 in IBS-D patients, and to inform dose selection for subsequent development. The study will consist of a pre-treatment phase, a 4-week double-blind treatment phase, and a 2-week post-treatment follow-up period.

Pre-Treatment Phase: During the pre-treatment phase, patients will be evaluated for up to 4 weeks to assess eligibility. The pre-treatment phase will consist of initial screening assessments and a Run-in period.

After the initial screening assessments have been performed, eligible patients will enter a Run-in period of up to 3-weeks. During the Run-in period, the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine use, to confirm disease activity and diary compliance. Patients will also be requested to discontinue any prohibited medications during this phase of the study.

Upon completion of the Run-in period, patients will return to the study site to confirm eligibility for randomization into a 4-week double-blinded Treatment Phase.

Treatment Phase: Eligible patients will be randomized (1:1:1) into the following cohorts:

- Cohort 1 (High Dose): 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 50 mg BOS-589 bid orally
- Cohort 3 (PBO): matching PBO oral tablets bid orally

During the 4 weeks of double-blind treatment, patients will continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary, as described in Section 8.3.

Post-treatment Phase: A 2-week post-treatment follow-up visit will occur for patients who complete the Treatment Phase. During the 2 weeks of follow-up, patients should continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary.

Patients who prematurely discontinue treatment should return to the study center to complete the early termination assessments as soon as possible after stopping the treatment.

Data analyses will occur after all patients in the trial have completed the last visit or procedure shown in the Schedule of Activities, Section 1.3.

Post-study Access to Therapy: No post-treatment access to therapy will be provided to patients randomized in the study.

4.1.1 Study Duration for Patients

Unless shortened by intolerable AEs or rapid disease progression, each patient's participation in this study is anticipated to last up to 10 weeks.

The total duration of this study is anticipated to be approximately 12 months, including patient enrollment, treatment, and follow-up.

4.1.2 Number of Patients

Approximately 300 patients will be screened to randomize approximately 132 patients with IBS-D (44 patients per cohort).

4.1.3 Replacement of Patients

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who have received at least 1 dose of investigational product and are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

4.1.4 Number of Sites

Up to 66 sites in the United States may participate in this study.

4.2 Rationale for Study Design

Key aspects of the study (e.g., eligibility, cohort size, stopping criteria, safety data collection, efficacy assessments, use of PBO as control, and use of patient-reported outcome [PRO] tools) are based upon generally accepted clinical trial methodologies for phase 2a efficacy studies and prior studies conducted in patients with IBS.

The Rome IV (CCI [redacted]) diagnostic criteria for IBS is the best accepted tool for standardized IBS diagnosis.

A Run-in Phase is designed to account for fluctuations in symptoms and the potential for wide variations in bowel habits; patient diaries that record frequency and severity of daily symptoms will be used to ensure that symptom severity fluctuations are identified and taken into account for subject eligibility. Patients will also be assessed on their ability to record their disease-related information in the required manner.

The use of PBO as a control comparator in IBS clinical trials is acceptable given the lack of consistently effective treatments. Placebo is an important component of IBS clinical trials given the high PBO effect in this population.

The key primary and secondary endpoints in this study are based on PRO assessments. These assessments evaluate pain, defecation, and IBS signs and symptoms. Abdominal pain intensity is measured using an 11-point (i.e., 0 to 10) numeric rating scale (NRS) that asks patients daily to rate their *worst abdominal pain over the past 24-hours* as recommended by the FDA guidance on IBS (FDA, May 2012). The use of the NRS for pain has been validated in IBS clinical studies (CCI). The IBS Severity Score (IBS-SS) (CCI) and IBS Global Scale (IBS-GS) (CCI) are validated and standard methods of assessing IBS symptoms in clinical trials.

Although treatment durations longer than 4 weeks will be required for true assessment of efficacy in IBS patients, in this initial phase 2a study, 4 weeks should be sufficient to identify any efficacy signal that warrants further clinical invention.

The use of multiple enrolling sites is aimed at maximizing external validity and to minimizing the potential influence of regional variations in diet, exercise habits, and ethnicity.

4.3 Justification for Dose

CCI

CCI

CCI

CCI

CCI [redacted]

4.4 End of Study Definition

The end of the study is defined as completion of the last visit or procedure shown in the Schedule of Activities in the trial globally. A patient will be considered to have completed the study if he or she has completed all phases of the study, including the last visit or the last scheduled procedure shown in the Schedule of Activities, Section 1.3.

5 STUDY POPULATION

Before any study-specific activities/procedures, the appropriate written informed consent must be obtained. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Age

1. Male and female patients must be 18 to 65 years of age, inclusive, at the time of signing informed consent.

Run-in eDiary Compliance

2. Patient has completed the daily electronic diary on at least 6 of the 7 days during the week prior to randomization AND at least 11 of the 14 days during the 2 weeks prior to randomization. Patients have up to 3 weeks to meet these criteria.

Type of Patient and Disease Characteristics

3. Patient meets the diagnosis of IBS based on the Rome IV diagnostic criteria (CCI [redacted]):*

   - Recurrent abdominal pain occurring, on average, at least 1 day per week and associated with 2 or more of the following:
     - Related to defecation;
     - Associated with a change in frequency of bowel movements;
     - Associated with a change in form (appearance) of stool;

   * These criteria must be fulfilled for the last 3 months prior to randomization and onset must have occurred at least 6 months prior to randomization.

4. Patient meets the diagnosis of IBS-D subtype based on Rome IV diagnostic criteria (CCI [redacted]). On days when the patient experiences IBS symptoms:

   - At least 25% of stools are loose or watery; AND
   - Fewer than 25% of stools are hard.

5. Over the week prior to randomization, the patient has an average of worst abdominal pain (WAP) scores in the prior 24 hours of 4.0 to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable).

6. Over the week prior to randomization, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools) and at least 5 days with a BSFS score ≥ 5.0.

7. Over the week prior to randomization, the patient has an average daily IBS-GS score of ≥ 2.0 (on a 0 to 4 scale, where 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe).

8. Patient is not planning to change his or her usual diet and lifestyle during the course of the study.

Diagnostic Assessments

9. Patient must undergo or previously have undergone (a) an appropriate evaluation for their IBS symptoms, including an evaluation for organic/structural etiologies (if in the presence of alarm symptoms); and (b) age-appropriate screening for colorectal cancer, if applicable.

    a) If at least one of the following alarm features are present, then the patient must have had a colonoscopy since the onset of symptoms or within the past 5 years (whichever is less):

        - Documented and unexplained weight loss of ≥ 10% within the past 6 months;
        - Nocturnal diarrhea;
        - Blood mixed with stool (except hemorrhoidal bleeding, defined as occasional blood found on the toilet paper only or limited dripping of blood into the toilet bowl after defecation;
        - Unexplained iron-deficiency anemia.

    b) If no alarm features are present, then the patient must have had a colonoscopy or other appropriate exam, based on criteria as outlined below:

        - Age ≥ 50 (≥ 45 if African-American): Colonoscopy within the past 10 years;
        - First-degree relative (FDR) diagnosed with colorectal cancer under age 60 OR 2 FDRs diagnosed with colorectal cancer at any age: Colonoscopy within past 5 years, *beginning* 10 years before age of youngest FDR (at time of diagnosis);

- Age ≥ 40 AND FDR diagnosed with colorectal cancer (at any age): Colonoscopy within past 10 years;

10. Patient is negative for serum tissue transglutaminase IgA antibody (tTG-IgA) plus has evidence of detectable serum IgA within the normal reference range.

Weight

11. Body mass index (BMI) within the range 16 to 39 kg/m^2 (inclusive).

Gender

12. Male and female patients are eligible if:

a. Male patients:

A male patient must agree to use contraception as detailed in Appendix 2 of this protocol during the treatment period and for at least 14 weeks after the last dose of investigational product. Patients must also agree to refrain from donating sperm during this period.

b. Female patients:

A female patient is eligible to participate if she is not pregnant (see Appendix 2), not breastfeeding, and at least 1 of the following conditions applies:

i. Not a woman of childbearing potential (WOCBP) as defined in Appendix 2; OR

ii. A WOCBP who agrees to follow the contraceptive guidance in Appendix 2 during the treatment period and for at least 5 weeks after the last dose of investigational product.

Informed Consent

13. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the Informed Consent Form (ICF) and in this protocol.

14. Patient is willing to be compliant with study procedures, including completing the daily electronic diary during the Run-in Period and throughout the study.

5.2 Exclusion Criteria

An individual for whom any of the following criteria apply will be excluded from participation in this study:

Gastrointestinal-related Medical Conditions

1. At the time of screening, patient has a diagnosis of an IBS subtype other than IBS-D, based on Rome IV criteria (CCI [redacted]). Based on stool patterns on days that the patient experiences symptoms, other IBS subtypes are defined as follows:

    a. IBS-C: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements;

    b. IBS-M: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≥ 25% of bowel movements;

    c. IBS-U: hard or lumpy stools ≤ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements.

2. Patient has a history of inflammatory or immune-mediated GI disorders including (but not limited to) inflammatory bowel disease (i.e., Crohn's disease, ulcerative colitis, microscopic colitis, and celiac disease).

3. Patient has had an episode of diverticulitis within 3 months prior to Screening.

4. Patient has a history of intestinal obstruction, stricture, toxic megacolon, GI perforation, fecal impaction, gastric banding, bariatric surgery, adhesions, ischemic colitis, or impaired intestinal circulation (e.g., aortoiliac occlusive disease).

5. Patient has any of the following surgical history:

    a. Cholecystectomy with ANY history of post-cholecystectomy biliary tract pain. A successful cholecystectomy with no postoperative biliary tract pain is not exclusionary;

    b. Any abdominal surgery within the 3 months prior to Screening;

    c. Major gastric, esophageal, hepatic, pancreatic, or intestinal surgery (appendectomy, hemorrhoidectomy, or polypectomy greater than 3 months post-surgery are allowed).

6. Patient has a history or current evidence of laxative abuse.

Other Medical Conditions

7. Patient has a history of a cardiovascular event, including stroke, myocardial infarction, congestive heart failure, or transient ischemic attack within 6 months prior to Screening.

8. Patient has a history of malignancy within 5 years prior to Screening (except squamous and basal cell carcinomas of the skin and cervical carcinoma *in situ*).

9. Patient has a history of alcohol abuse or binge drinking.

10. Patient has uncontrolled hypertension, defined as systolic blood pressure > 180 mmHg or a diastolic blood pressure > 100 mmHg at the time of Screening.

11. Patient has a history of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.

Laboratory Assessments

12. Fecal calprotectin ≥ 50µg/g.

13. Hemoglobin A1c level ≥ 8.0% or a confirmed fasting plasma glucose level ≥ 180 mg/dL.

14. Confirmed alanine aminotransferase (ALT) > 2x upper limit of normal (ULN).

15. Confirmed total bilirubin > ULN, unless the patient has a documented history of Gilbert's syndrome.

16. Confirmed QT interval corrected by Fridericia's formula (QTcF) or QT interval corrected by Bazett's formula (QTcB) > 500 msec.

17. Evidence of active hepatitis B virus (HBV) infection, based on a positive hepatitis B surface antigen (HBsAg) screen.

18. Evidence of hepatitis C virus (HCV) infection based on a positive HCV antibody screen (patients who have been successfully treated for HCV are eligible if an undetectable HCV viral load at least 6 months after completion of treatment can be demonstrated).

19. Human immunodeficiency virus (HIV)-1 or HIV-2 antibody positive.

Prior/Concomitant Therapy

20. Within 14 days of randomization, patient has used either of the following:

    - 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron);
    - Any of the strong inhibitors of P-glycoprotein listed in Appendix 4.

21. Within 14 days of randomization, patient has used any of the following:

    - Loperamide; (Note: loperamide can be used during the study as rescue medication based on protocol specified guidelines [see Section 6.7.1]);
    - Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
    - Narcotic- or opioid-containing agents;

- Cannabis-containing products;
- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents).

22. Within 14 days of randomization, receipt of any prescribed or over-the-counter, systemic, herbal (including St. John's wort), or topical medication, or any expectation of requiring use of such medication while participating in the study that, in the opinion of the Investigator, would interfere with study procedures, compromise safety, or the scientific integrity of the data.

23. Patient has used, or is expected to use, the following antibiotics:

    - Rifaximin within 90 days prior to randomization;
    - Other oral antibiotics within 28 days of randomization (with the exception of topical antibiotics or a 1-day course with an antibiotic).

    However, a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomly assigned to study drug.

24. Within 3 months prior to randomization, patient has had significant changes to his or her antidepressant regimen (i.e., addition of a new agent, discontinuation of a prior agent, significant modifications to the dose of a current medication). Patients on chronic stable doses of antidepressants will be allowed to participate in the study. As-needed use of buspirone and benzodiazepines for anxiety is permitted during the study;

25. Within 30 days prior to randomization (or 5 half-lives, if known), the patient has received an investigational drug, or is currently enrolled in an investigational study.

Other Exclusions

26. Patient is unable to swallow solid oral dosage forms whole with the aid of liquid (patients may not chew, divide, dissolve, or crush the study drug).

27. Patient has an elective surgery planned or expects to need elective surgery at any time during the study.

28. Patient is pregnant or breastfeeding.

29. Patient has any medical or psychological disorder or condition that, in the opinion of the Investigator, would compromise the wellbeing of the patient or the study or prevent the patient from meeting or performing study requirements.

30. Patient has poor peripheral venous access.

31. Patient is an employee of the Investigator or study center with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members of the employees or the Investigator.

5.3 Lifestyle Considerations

During this study, patients will be asked to abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests.

Only on days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities; Section 1.3), patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study. Investigational product should be taken with approximately 240 mL water.

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened with Medical Monitor approval.

6 INVESTIGATIONAL PRODUCT

6.1 Investigational Products

6.1.1 Investigational Product Description

Investigational Product Name:	BOS-589	Placebo
Dosage Formulation:	Tablet	Tablet
Unit Dose Strengths/Dosage Levels:	50 mg (2 x 25-mg tablets) and 200 mg (2 x 100-mg tablets)	Visually matching placebo tablets across both BOS-589 dose levels
Route of Administration:	Oral	Oral
Dosing Instructions:	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush
Packaging and Labeling:	Investigational product will be provided in white, opaque, high-density polyethylene (HDPE) bottles with a child-resistant closure. Each bottle will be labeled as per country requirement	Investigational product will be provided in white, opaque, HDPE bottles with a child-resistant closure. Each bottle will be labeled as per country requirement
Manufacturer:	WuXi STA, Shanghai, China	WuXi STA, Shanghai, China

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all investigational product received and any discrepancies are reported and resolved before use of the investigational product.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for investigational product accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

6.2.2 Product Storage and Stability

All investigational product must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

Further guidance and information for the final disposition of unused investigational products are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias

This is a randomized, double-blinded, PBO-controlled study. Patients should be randomized as close as possible to the time of the planned first dose of study treatment.

Randomization will be conducted via an interactive web response system (IWRS) in a 1:1:1 ratio for patients to receive BOS-589 200 mg, BOS-589 50 mg, or PBO. Patients will be assigned to the treatment arms using a permuted block randomization stratified by site (44 patients per group). The randomization schedule will be generated by the Study Biostatistician and will be transferred to the IWRS team for loading into the system. The randomization file will be held by the Study Biostatistician until the end of the trial and when the database has been locked.

The sponsor, site staff, and patients will be blinded to the treatment assignment. BOS-589 and PBO will be similar in appearance, and provided in white, opaque, high-density polyethylene (HDPE) bottles, each with a child-resistant closure. Each bottle will be labeled as per country requirement.

Only in a medical emergency will a patient's treatment assignment be unblinded, and this process will be performed and documented in the IWRS. Every effort should be made to contact the Medical Monitor to discuss unblinding prior to breaking the blind.

6.4 Dosing and Administration

Only patients enrolled in the study may receive investigational product and only authorized site staff may supply or administer investigational product.

Eligible patients will be randomized (1:1:1) to receive for a total of 4 weeks BOS-589 at 1 of 2 dose levels or PBO bid. Patients will be randomized to the following cohorts:

- Cohort 1 (High Dose): 2 x 100 mg tablets for the 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 2 x 25 mg tablets for the 50 mg BOS-589 bid orally
- Cohort 3 (PBO): 2 x visually matched PBO tablets bid orally

For each dose level and at each dosing timepoint, the 2 tablets are to be swallowed with approximately 240 mL room temperature water and are to be swallowed whole (i.e., not divided, crushed, dissolved, or chewed). On days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities, Section 1.3, patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study.

Patients will be instructed to take the investigational product approximately every 12 hours at approximately the same time each day. If a patient misses a dose at a given timepoint, and the time is within 4 hours of the regularly scheduled dosing time, the patient should be instructed to take the investigational product. If it has been longer than

4 hours, the patient should skip the missed dose at that timepoint and resume the regularly scheduled dosing schedule at the next scheduled timepoint.

In the event of vomiting following administration, BOS-589 should not be taken until the next scheduled dose.

6.5 Dose modification

Study treatment should be interrupted if the patient reports any treatment-related severe AE. Therapy should only be restarted after approval by the Medical Monitor.

If therapy is restarted, the same dose should be administered as that prior to occurrence of the AE. If the patient is still having tolerability issues and an AE reoccurs, the dose may be reduced from 2 tablets of study treatment bid to 1 tablet of study treatment bid, without breaking the study blind.

If another AE occurs after the study treatment dose reduction, study treatment will be permanently discontinued and the subject followed until the end of the study.

6.6 Investigational Product Compliance

All patients in this study will commence therapy on site on Study Day 1; oral investigational product will be administered under clinic staff supervision. After patients are discharged to continue therapy at home, compliance will be assessed on subsequent visits by returned tablet count. Administration of investigational product and any deviation(s) from the prescribed dosage regimen should be recorded in the case report form (CRF) and Drug Accountability Record.

6.7 Concomitant Therapy

Refer to Appendix 4. for more details on which concomitant medications are permitted and which are excluded while patients are on study.

Any prior or concomitant therapy (including over the counter or prescription medicines, vitamins and/or supplements) taken 28 days prior to the first dose of investigational product through the End-of-Study Visit must be recorded, along with:

- Reason for use;
- Dates of administration including start and end dates;
- Dosage information including dose and frequency.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported

in the CRF are concomitant prescription medications, over-the-counter medications, vaccines, vitamins, and supplements.

6.7.1 Rescue Medicine

During the double-blind Treatment Phase of the study, patients will be allowed to take loperamide rescue medication for the acute treatment of uncontrolled diarrhea. Loperamide at a unit dose of 2 mg may be taken once approximately every 6 hours with the following guidelines:

- No more than 4 unit doses over a continuous 24-hour time period (8 mg/day);
- No more than 7 unit doses over a continuous 48-hour time period (14 mg over 2 days);
- No more than 11 unit doses over a continuous 7-day time period.

The use of loperamide rescue medication should be recorded electronically.

6.8 Intervention After the End of the Study

BOS-589 will not be provided to study patients after the end of the study.

7 DISCONTINUATION / WITHDRAWAL

7.1 Discontinuation of Investigational Product

A patient may discontinue from study treatment at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. Discontinuation from the investigational product does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to, changes from baseline) after enrollment, the Investigator or qualified designee will determine if any change in patient management is needed. Any new clinically relevant finding will be reported as an AE.

Upon investigational product discontinuation, patients should complete all procedures collected in the Day 29/End-of-Treatment visit as applicable per the Schedule of Activities (Section 1.3).

7.2 Discontinuation/Withdrawal from the Study

A patient may withdraw from the study at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for the following reasons:

- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the patient;
- Disease progression that requires discontinuation of the investigational product;
- Pregnancy;
- If the patient meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation after consultation with the Medical Monitor;
- Significant investigational product noncompliance;
- Termination of the study by the sponsor.

If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a patient withdraws from the study, he or she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

See Schedule of Activities, Section 1.3, for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The reason for patient discontinuation or withdrawal from the study will be recorded on the CRF.

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

Clinical and/or laboratory criteria that may warrant study drug discontinuation.

If a patient experiences any of the following clinical or laboratory events, study treatment should be immediately suspended:

- Any SAE considered study drug-related;
- No bowel movement for 7 days;
- Any clinical evidence suggestive severe constipation, obstipation or intestinal obstruction; gastrointestinal hemorrhage, significant gastrointestinal infection;
- Abnormal liver functions concerning for drug-induced as follows[1]:
    - Confirmed ALT > 5x ULN
    - Confirmed ALT > 3x ULN and any of the following signs or symptoms (fatigue, nausea, vomiting, right upper quadrant pain, fever, rash or eosinophilia)[2]
    - Confirmed ALT > 3x ULN and total bilirubin > 2x ULN or INR > 1.5 (**should be repeated in 48-72 hours**)

[1]Should any of these events occur, a full evaluation of other causes of liver enzyme elevation should be undertaken and patients would be followed at regular intervals until labs return to normal/stabilize, or an alternate diagnosis is confirmed.

[2]If patients develop asymptomatic elevations of the ALT of > 3x ULN and, if there are persistent elevations upon repeat testing, close observation should be implemented, and discontinuation of the drug should be considered.

Depending on the nature, severity, and outcome, the Sponsor will decide to (and document with supporting rationale) either: 1) discontinue study drug; 2) request additional safety data; or 3) continue dosing at current dose or reduced dose (see Section 6.5).

If 2 or more patients experience any of the above criteria (note: study drug-related SAEs must be in the same system organ class) the study enrollment will be halted. The Sponsor, an independent GI physician not involved with the study, and the lead investigator will review available data to determine whether individual patient unblinding

is warranted, amendments are required for the protocol, and whether patients currently on treatment should also discontinue study drug. A decision to continue (with or without modifications) or discontinue the study will be made. All supporting rationale will be documented.

7.3 Lost to Follow-up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site will attempt to contact the patient and reschedule the missed visit as soon as possible; counsel the patient on the importance of maintaining the assigned visit schedule; and ascertain if the patient wishes to and/or should continue in the study.

- Before a patient is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods. These contact attempts should be documented in the patient's medical record or study file.

- Should the patient continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

Planned timepoints for all assessments are provided in the Schedule of Activities (Section 1.3).

8.1 Study Periods

The study will comprise 3 phases:

1. **Pre-Treatment Phase** (up to 4 weeks), in which all patients will be assessed for eligibility. The pre-treatment phase will consist of initial screening assessments (Visit 1) and a Run-in period (Visit 2 through Visit 3).

2. A **Treatment Phase** (4 weeks), in which all patients will return to the clinical for randomization and start of treatment (Visit 3 – Day 1) followed by weekly visits (Visit 4 – Day 8 [± 1 day], Visit 5 – Day 15 [± 1 day], Visit 6 – Day 22 [± 1 day]) until Visit 7 – Day 29 (± 1 day), and

3. A **Follow-up Phase** (2 weeks), in which patients who complete Visit 7, will be asked to return for an End-of-Study Follow-up visit (Visit 8 – Day 43 [± 3 days]) for assessment of treatment outcome (e.g., safety, durability of effect).

8.2 Screening Assessments

After signing the ICF(s), the patient will undergo the initial screening assessments and procedures as described in the Schedule of Activities (Section 1.3) to determine eligibility.

Eligible patients will enter the Run-in period. At the beginning of the Run-in period, patients will receive instructions for completing an electronic diary to collect daily information related to their IBS-D symptoms, their bowel function, and rescue medication use.

Patients who are:

- Compliant in completing the screening diary on a daily basis on at least 6 of the 7 days during the week prior to randomization AND on at least 11 of the 14 days during the 2 weeks prior to randomization, and

- Have an average WAP score in the past 24 hours of between 4.0 and 8.0 on a 0 to 10 scale over the week prior to randomization, and

- Have an average daily BSFS of ≥ 5.0 and at least 5 days with a BSFS score ≥ 5.0 on a 1 to 7 scale over the week prior to randomization, and

- Have an average daily IBS-GS score of ≥ 2.0 on a 0 to 4 scale over the week prior to randomization, and

- Who have not used any loperamide rescue medication in the 2 weeks prior to randomization

will be eligible for participation and immediate randomization into the double-blind treatment phase (i.e., all 5 diary conditions must be met to qualify for randomization).

Eligible patients will be instructed to return to the study site for the Baseline visit on Day 1.

If after 2 weeks, patients do not meet all of the 5 diary conditions required for entry, the run-in may be extended by up to one week in accordance with investigator judgement (for a total run-in period of up to 3 weeks).

8.3 Efficacy Assessments

The efficacy objectives of the study are to evaluate the effect of BOS-589, relative to PBO after 4 weeks of therapy, on pain, defecation, and key IBS-D related signs and symptoms.

During the 4 weeks of the double-blind treatment phase, patients will be required to access their electronic diary every day, preferably at the same time each day, to record IBS-D symptom data and information related to their bowel function and rescue medication use.

8.3.1 Worst Abdominal Pain

Throughout the 4 weeks of the double-blind treatment phase, patients will be asked to rate their WAP in the past 24 hours. The patient-reported WAP in the past 24 hours will be recorded on a 0 to 10 scale, where 0 corresponds to no pain and 10 corresponds to worst imaginable pain.

8.3.2 Bristol Stool Form Scale

Patients will be asked to record daily stool consistency according to the BSFS most representative of the past 24 hours. The patient-reported BSFS consistency score is based on a 1 to 7 scale where 1 corresponds to a hard stool and 7 corresponds to watery diarrhea (CCI [redacted]). Please refer to Appendix 3.

8.3.3 IBS-D Global Symptom Score

Patients will be asked to record daily their overall IBS-D global symptoms in the prior 24 hours. The patient-reported daily IBS-GS is based on a 0 to 4 scale where:

- 0 corresponds to no symptoms;
- 1 corresponds to mild symptoms;
- 2 corresponds to moderate symptoms;

- 3 corresponds to severe symptoms; and
- 4 corresponds to very severe symptoms.

8.3.4 IBS Severity Score System

Patients will be asked to complete 5 questions regarding the severity of their IBS. Each of the 5 questions generate a maximum score of 100, leading to a total possible score of 500.

8.4 Safety and Other Assessments

8.4.1 Adverse Events and Serious Adverse Events

See Section 8.5.

8.4.2 Physical Examinations

A complete physical examination (PE) will be performed at Screening. A symptom-directed PE will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) and as clinically indicated, and results recorded in the source document. Any clinically significant PE finding noted at Screening will be recorded as medical history. Any clinically significant PE finding noted at after enrollment will be reviewed for reporting as an AE (see Section 8.5).

A complete PE will include, at a minimum, assessment of the cardiovascular, respiratory, GI, and neurological systems. A targeted PE will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

Height and weight will also be measured and BMI will be calculated and the data recorded at the timepoints specified in the Schedule of Activities (Section 1.3).

Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.4.3 Vital Signs

At the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection, vital signs will be measured in a supine position after 5 minutes rest and will include oral temperature, systolic and diastolic blood pressure, pulse, and respiratory rate. Any clinically significant abnormal vital sign value will be recorded as an AE (see Section 8.5).

8.4.4 Electrocardiograms

Single 12-lead ECGs will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection. Electrocardiogram interval measurements and interpretation (normal, abnormal not clinically significant,

abnormal clinically significant) will be recorded in the source document. Any clinically significant change in ECG interpretation will be recorded as an AE (see Section 8.5).

8.4.5 Clinical Safety Laboratory Assessments

See Appendix 1 for the list of clinical laboratory tests to be performed and to the Schedule of Activities (Section 1.3) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record in the AE section of the CRF any clinically relevant changes occurring during the study. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 1, must be conducted in accordance with the laboratory manual and the Schedule of Activities.

If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the Investigator (e.g., SAE, or AE, or dose modification), then the results must be recorded in the CRF.

8.5 Adverse Events and Serious Adverse Events

Adverse events will be reported by the patient (or, when appropriate, by a caregiver, surrogate, or the patient's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting and documenting events that meet the definition of an AE or SAE and are responsible for following all AEs.

8.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of investigational product.

8.5.2 Events of Special Interest

Not applicable.

8.5.3 Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death because of progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life threatening;

  The term 'life threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization;

  In general, hospitalization signifies that the subject or patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.

  Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE;

- Results in persistent disability/incapacity;

  The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised when deciding if SAE reporting is appropriate in other situations such as important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the

patient or may require medical or surgical intervention to prevent any of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.5.4 Classification of an Adverse Event

8.5.4.1 Assessment of Severity

The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities;
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities;
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as ‘serious’ when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

8.5.4.2 Assessment of Causality

All AEs must have their relationship to the investigational product and/or study participation assessed by the Investigator who examines and evaluates the patient based on temporal relationship and his or her clinical judgment. Alternative causes, such as underlying disease, concomitant therapy, and other risk factors, as well as the temporal relationship of the event to investigational product administration will be considered and investigated. The Investigator will also consult the IB in his or her assessment.

The degree of certainty about causality will be graded using the categories below:

- **Related** – The AE is known to occur with the investigational product, there is a reasonable possibility that the investigational product caused the AE, or there is a temporal relationship between the investigational product and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the investigational product and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the investigational product caused the event, there is no temporal relationship

between the investigational product and event onset, or an alternate etiology has been established.

For each AE, the Investigator must document in the medical notes that he or she has reviewed the event and has provided an assessment of causality. The Investigator may change his or her opinion of causality in light of follow-up information and updated causality assessment reported.

8.5.4.3 Expectedness

The Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the investigational product.

8.5.5 Time Period and Frequency for Event Assessment and Follow-up

All AEs will be collected from the signing of the ICF until the End-of-Study Follow-up visit.

Whenever possible, all AEs should be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the patient is stable.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, whether considered investigational product related or not, and must include an assessment of if there is a reasonable possibility that the investigational product caused the event. The Investigator will also submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he or she considers the event to be reasonably related to the investigational product or study participation, the Investigator must promptly notify the sponsor.

8.5.6 Adverse Event Reporting

At each study visit, patients will be evaluated for new AEs and the status of existing AEs. Care will be taken not to introduce bias when evaluating for AEs. The Investigator should use open-ended questions when soliciting information from a patient regarding AEs, followed by appropriate questions that clarify the patient’s verbatim description of AEs or change in concomitant medications.

When an AE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE.

The Investigator will then record all relevant AE information in the CRF. It is not acceptable for the Investigator to send photocopies of the patient's medical records in lieu of completion of the CRF page. New or updated information will be recorded in the originally completed CRF. However, there may be instances when copies of medical records for certain cases are requested. In this case, all patient identifiers, with the exception of the patient number, will be redacted on the copies of the medical records before submission.

If a patient dies during participation in the study or during a recognized follow-up period, the Investigator will provide a copy of any postmortem findings, including histopathology when available.

If a site receives report of a new SAE from a study patient or receives updated data on a previously reported SAE after database lock of the electronic data capture (EDC) system, then the site can report this information on a paper SAE form or to the sponsor by telephone. See the Study Manual for additional information on SAE reporting.

The study sponsor, or designee, will be responsible for notifying all applicable regulatory authorities of any required safety events in compliance with country-specific regulatory requirements. In addition, the sponsor must notify applicable regulatory authorities and all participating Investigators of suspected unexpected serious adverse reactions (SUSARs), from clinical trials or any other source.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the Institutional Review Board/ Independent Ethics Committee (IRB/IEC), if appropriate according to local requirements.

8.5.7 Reporting of Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. Pregnancy in a patient's partner is not considered an AE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs. Elective abortions without complications should not be handled as AEs; however, an induced therapeutic abortion to terminate a pregnancy because of complications or medical reasons must be reported as an SAE. The underlying medical diagnosis for this procedure should be reported as the SAE term. A spontaneous abortion in a study patient is always considered an SAE.

All pregnancies in female patients and, if indicated, pregnancies in female partners of male patients, will be collected after the start of investigational product and until 5 weeks after the last dose. Pregnancy outcomes, defined as the first well baby visit for live births, or spontaneous or induced abortion, will be documented even after the patient is withdrawn from or completes the study.

If a pregnancy is reported, the Investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 2.

8.6 Treatment of Overdose

There are no data on overdose because this is the first study of BOS-589 in patients and, in a prior study in healthy human volunteers, no evidence of overdose was reported. There is no definition of what constitutes an overdose and no known antidote. Any patient who receives a higher dose than that intended (i.e., more than 200 mg per dose; more than 400 mg in a given day) should be monitored closely, managed with appropriate supportive care, and followed up appropriately. If possible, a blood sample for PK should be collected as soon as is feasible from any patient who takes a higher dose than that intended.

If AEs or SAEs are reported and are considered related to a patient receiving a higher dose than intended, dosing should be interrupted if deemed necessary by the Investigator and the case discussed further with the Medical Monitor.

8.7 Pharmacokinetics

Plasma samples of approximately 4 mL will be collected for measurement of plasma concentrations of BOS-589 at the following times and as specified in the Schedule of Activities (Section 1.3).

- On Day 1 at predose and at 0.5, 1, 2, and 4 hours postdose.
- On Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If samples cannot be obtained on Day 15, then samples should be obtained on Day 22.
- A predose sample will be obtained on all other Treatment Study Visits.

Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for the 0.5 and 1 hour postdose timepoints, and ± 10 minutes for the 2 and 4 hours postdose timepoints.

Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual time of dosing and actual date and time (24-hour clock time) of each sample will be recorded.

Drug concentration information that may unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

Any changes in the timing or addition of timepoints for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

The following PK parameters will be estimated from the plasma concentrations of BOS-589 using either traditional noncompartmental methods or a population-PK model:

AUC_{0-t} Area under the plasma concentration versus time curve from time zero to the last quantifiable sample;

AUC_{0-4} Area under the plasma concentration versus time curve from time zero to 4 hours postdose;

C_{max} Maximum observed plasma concentration;

C_{min} Minimum observed plasma concentration.

In addition to parameters above, the AUC from time zero to 4 hours postdose (AUC_{0-12}) may be calculated using predose concentrations other than Day 1 predose to impute a 12-hour concentration. Details will be provided in the PK analysis plan.

8.8 Biomarkers/Pharmacodynamics

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9 STATISTICAL CONSIDERATIONS

9.1 Timing of Analyses

Formal hypothesis testing will occur at the end of the study and post database lock. There are no planned Data, Safety, or Adjudication Committees for this study and no planned statistical interim analyses.

9.2 Sample Size Determination

This is a phase 2a study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint. The primary endpoint is defined as a change in the 24-hour WAP score at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and compared between treatment groups in a hierarchical testing order using a t-test of equal variances and a two-tailed alpha of 0.05.

The sample size was based on the first hypothesis test in the hierarchical plan, with 80% power, two-sided alpha of 0.05, and a standard deviation (SD) of 3 points on the NRS to detect a 1.6 minimum change between treatment groups (N = 120 patients). To account for attrition and a potential higher PBO rate, a total of 132 patients will be randomized to one of 3 treatment groups in a 1:1:1 ratio (44 patients per treatment group).

9.3 Populations for Analyses

1. The Intention-to-Treat (ITT) analysis dataset will comprise all randomized patients;

2. The Per-protocol Analysis Dataset will comprise patients in the ITT Analysis Dataset who received at least 1 dose of investigational product and did not have any major protocol deviations;

3. The Safety Analysis Dataset will comprise all patients who received at least 1 dose of investigational product;

4. The Pharmacokinetic Analysis Dataset will comprise all randomized patients who received the active study drug and have available serum time concentration data.

9.4 Statistical Analyses

Detailed methodology for descriptive and inferential statistical analyses of the data collected in the study will be documented in the Statistical Analysis Plan (SAP). The SAP will be prepared by the Study Biostatistician and agreed upon by the Sponsor. The SAP will be finalized and approved prior to database lock. The SAP will take precedence over the protocol for details regarding the statistical analyses to be conducted for the study. In addition to the SAP, other graphical representations of the results may be produced after review of the data (*post hoc*). Any major modifications of the Primary Endpoint's definition and/or its analysis will be reflected in a protocol amendment.

In general, descriptive statistical methods will be used to summarize the data from this study. Unless stated otherwise, the term "descriptive statistics" refers to number of patients (n), mean, median, SD, minimum, and maximum for continuous data, and frequencies and percentages for categorical data.

All statistical analyses will be performed using Statistical Analysis System (SAS®) software Version 9.4 or higher.

9.4.1 Efficacy Analyses

9.4.1.1 Primary Endpoint

This is a proof-of-principle study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change in the 24-hour WAP score at

Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and compared between treatment groups in the following testing order using a t-test of equal variances and a two-tailed alpha of 0.05:

Hypothesis 1:

Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 2.

Hypothesis 2:

Cohort 1 (High Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 3.

Hypothesis 3:

Cohort 2 (Low Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4.

Hypothesis 4:

Cohort 1 (High Dose) versus Cohort 2 (Low Dose).

9.4.1.2 Secondary Endpoint

Mean daily BSFS score (stool type) and mean daily frequency of bowel movements will be tabulated and summarized by study day for each treatment for the ITT and Per-protocol Analysis Dataset populations. Time profiles and box plots will also be presented by study day and by treatment group. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.3 Other Efficacy Endpoints

Scores from IBS-GS and IBS-SS questionnaires will be summarized for the ITT and Per-protocol Analysis Dataset populations. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.4 Missing Data

Study implementation procedures and training will assist in limiting the amount of missing data in the trial. However, sensitivity analyses may be performed when indicated for the study endpoint and the amount of missing data to be handled. Details of the statistical methods for handling missing data will be described in the SAP.

9.4.2 Safety Analyses

9.4.2.1 Adverse Events

Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All SAEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced.

All AEs will be listed for individual patients showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs (TEAEs) related to investigational product will be generated.

Any event reported on the CRF that occurs on at the time of or after the initiation of investigational product is defined as treatment-emergent. Additionally, an AE that is reported to have started on Day 1 without an associated onset time will be assumed to have occurred after the initiation of investigational product. Hence, AEs occurring on Day 1 with no associated onset time will be assumed to be treatment emergent.

Serious AEs associated with a protocol-specified procedure and occurring after the time of consent but before administration of first dose of investigational product will be defined as non-treatment-emergent AEs (NTEAEs).

All safety analyses will be performed in the Safety Analysis Dataset.

9.4.2.2 Clinical Laboratory Evaluations

Laboratory data will be listed for all patients. Laboratory data will be reviewed by the Investigator as they become available. All abnormal laboratory results will be evaluated by the Investigator as either clinically significant or not clinically significant.

Descriptive summaries of clinical laboratory results will be presented by date and time of collection. The number and percentage of patients experiencing treatment-emergent graded toxicities will be summarized by treatment arm and severity grade. Laboratory toxicity shifts from baseline to post-baseline assessments will be summarized by treatment arm. Changes from baseline in laboratory tests will be summarized for each treatment arm.

9.4.2.3 Twelve-lead Electrocardiograms, Vital Signs, and Physical Examinations

Twelve-lead ECG and vital signs data will be listed and summarized.

Physical examination findings will be captured and listed as part of a patient’s medical history or as AEs as applicable.

9.4.3 Other Analyses

9.4.3.1 Pharmacokinetic Analysis

A full description of the methods for evaluating the PK of BOS-589 in this patient population will be provided in a PK data analysis plan. The PK data from this study may be combined with data from other studies to complete a population-PK analysis.

A full list of PK parameters to be calculated is included in Section 8.7.

9.4.4 Baseline Descriptive Statistics

Demographic and baseline characteristics will be summarized for all patients overall and by treatment arm. Summary statistics (e.g., number of patients, mean, median, SD, and range) will be generated for continuous variables (e.g., age and weight) and the number and percentage of patients within each category will be presented for categorical variables (e.g., gender, ethnicity, race).

A detailed description of patient disposition will be provided. It will include:

- A summary of overall patient enrollment status (consented, screened, screen failures, replacements, randomized);
- A summary of patients who discontinued the study;
- An account of identified protocol deviations.

All patients who are consented for the study will be accounted for in the summation. The number of patients who do not qualify for certain analysis populations will be summarized.

9.4.5 Subgroup Analyses

No subgroup analyses are powered for the study. Subgroup analyses may be performed for descriptive purposes as described in the SAP.

9.5 Planned Interim Analyses

No formal efficacy interim analysis is planned for the purposes of statistically comparing the data prior to the completion of the study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines;
- Applicable International Conference on/Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines;
- Applicable laws and regulations.

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted, and reviewed and approved as necessary, to the relevant Competent Authorities, IRBs and IECs before the study is initiated.

Any amendments to the protocol will require regulatory approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC;
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures;
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Informed Consent/Assent Process

An initial sample ICF is provided for the Investigator to prepare the informed consent document to be used at his or her site. Updates to the sample ICF are to be communicated formally in writing from the Sponsor or Sponsor’s designee to the Investigator. The written ICF is to be prepared in the language of the potential patient population.

The Investigator or his/her representative will explain the nature of the study to the patient or his/her legally authorized representative and answer all questions regarding the study.

Patients must be informed that their participation is voluntary. Patients or their legally authorized representative will be required to sign a statement of informed consent, and/or assent, that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Patients must be re-consented to the most current version of the ICF(s) during their participation in the study if one becomes available.

A copy of the ICF(s) must be provided to the patient or the patient's legally authorized representative.

The Investigator is also responsible for asking the patient if the patient has a primary care physician and if the patient agrees to have his/her primary care physician informed of the patient's participation in the clinical study if it is a local requirement. If the patient agrees to such notification, the Investigator is to inform the patient's primary care physician of the patient's participation in the clinical study. If the patient does not have a primary care physician and the Investigator will be acting in that capacity, the Investigator is to document such in the patient's medical record.

If a patient is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written ICF and any other written information to be provided to patients, is read and explained to the patient or the patient's legally acceptable representative, and after the patient or the patient's legally acceptable representative has orally consented to the patient's participation in the trial and, if capable of doing so, has signed and personally dated the ICF, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the patient or the patient's legally acceptable representative, and that informed consent was freely given by the patient or the patient's legally acceptable representative (Refer to ICH GCP guideline, Section 4.8.9).

10.1.3 Confidentiality and Privacy

Patient confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, and the sponsor and its designees. This confidentiality is

extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to patients. Therefore, the study documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the study data will be released to any unauthorized third party without prior written approval of the sponsor.

Study patient research data will not include the patient's contact or identifying information. Rather, individual patients and their research data will be identified by a unique study identification number. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred. Research data will be used in accordance with local data protection laws.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/IEC or regulatory agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

10.1.4 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices, Good Manufacturing Practices).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.5 Data Handling and Record Keeping

10.1.5.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site, under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

The Investigator must maintain adequate source documentation. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into a 21 CFR Part 11-compliant EDC system or transmitted electronically (e.g., laboratory data). The data system(s) includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Data recorded in the CRF should be consistent with the data recorded on the source documents or the discrepancies must be explained.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data. The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.5.2 Study Records Retention

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations.

No records will be destroyed without the written consent of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

It is the responsibility of the sponsor to inform the Investigator when these documents no longer need to be retained.

10.1.6 Study and Site Discontinuation and Closure

The sponsor or its designee reserves the right to suspend or prematurely terminate a study site or study at any time for any reason and at the sole discretion of the sponsor.

An Investigator may initiate site closure at any time, provided there is reasonable cause and sufficient notice is given to the sponsor in advance of the intended termination.

Written notification, documenting the reason for site or study suspension or termination, will be provided by the suspending or terminating party to study participants, Investigator, funding agency, the Investigational New Drug sponsor, and regulatory authorities.

If the study is prematurely terminated or suspended, the Investigator will promptly inform study participants, the IRB/IEC, and sponsor, and will provide the reason(s) for

the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants;
- Demonstration of efficacy that would warrant stopping;
- Insufficient compliance to the protocol, regulatory requirements, or GCP guidelines;
- Data that are not sufficiently complete and/or evaluable;
- Determination that the primary endpoint has been met;
- Determination of futility;
- Inadequate recruitment of patients.

10.1.7 Dissemination of Clinical Study Data

A Clinical Study Report (CSR) will be prepared in accordance with the ICH guideline on structure and contents of CSRs and any applicable regulatory and legal requirements.

10.1.8 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

11 REFERENCES

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12 APPENDICES

Appendix 1. Clinical Laboratory Tests

The tests detailed in Table 2 will be performed by the central laboratory with the exception of erythrocyte sedimentation rate and urine pregnancy tests. The central laboratory will provide kits and instructions to perform these assessments on site.

Local laboratory results are only required in the event that the central laboratory results are not available in time for either investigational product administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either an investigational product decision or response evaluation, the results must be entered into the CRF.

Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Pregnancy testing: Refer to Section *5.1* for Screening pregnancy criteria.

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count Red blood cell (RBC) count Hemoglobin Hematocrit		RBC Indices: Mean corpuscular volume Mean corpuscular Hemoglobin % Reticulocytes	White blood cell count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical Chemistry	Blood urea nitrogen	Potassium	Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase	Total and direct bilirubin
	Creatinine	Sodium	Alanine aminotransferase/Serum glutamic pyruvic transaminase	Total protein
	Glucose (fasted)	Calcium	Alkaline phosphatase	
CCI [redacted]	CCI [redacted]	CCI [redacted]	CCI [redacted]	
Routine Urinalysis	Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Microscopic examination (if blood or protein is abnormal)			

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Other Screening Tests	Hemoglobin A1c Follicle-stimulating hormone and estradiol (as needed in women of nonchildbearing potential only) Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential) Serology (HIV antibody, hepatitis B surface antigen, and hepatitis C virus antibody) serum tissue transglutaminase IgA antibody (tTG-IgA) and IgA (if applicable)

Investigators must document their review of each laboratory safety report.

Laboratory results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

Appendix 2. Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories **are not considered WOCBP**

1. Premenarchal
2. Premenopausal female with 1 of the following:
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy
    c. Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's review of the patient's medical records, medical examination, or medical history interview.

3. Postmenopausal female
    a. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
    b. Females on HRT and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Male patients.

Male patients with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the treatment period and for at least 14 weeks after the last dose of investigational product:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year, as described in Table 3, when having penile-vaginal intercourse with a WOCBP who is not currently pregnant

In addition, male patients must refrain from donating sperm for the duration of the study and for 14 weeks after the last dose of investigational product.

Male patients with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration for the duration of the study and for 14 weeks after the last dose of investigational product.

Female patients.

Female patients of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception, as described in Table 3, consistently and correctly.

Table 3. Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.* Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal Progestogen only hormonal contraception associated with inhibition of ovulation • Oral • Injectable
Highly Effective Methods That Are User Independent[a] Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
Vasectomized Partner *A vasectomized partner is a highly effective contraception method, provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual Abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the investigational product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the patient.*

NOTES:

[a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for patients participating in clinical studies.

[b] Hormonal contraception may be susceptible to interaction with the investigational product, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 5 weeks after the last dose of investigational product.

Pregnancy Testing:

- Any WOCBP should only be included after a negative pregnancy test.
- Additional urine pregnancy testing should be performed at the Day 29 visit, and the Day 43 End-of-Study Follow-up visit.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected

Collection of Pregnancy Information:

- All occurrences of pregnancies must be reported on a Pregnancy Notification and Outcome Form (within 24 hours of awareness of any such pregnancy that occurs for either the study patient or for the female partner of a male patient); all pregnancies will be followed to outcome, which means until either the first well-baby visit for live births or until documented spontaneous or induced abortion.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

- Pregnancies that occur beyond 90 days after the last dose of study drug will only need to be reported if there is an associated SAE that the Investigator assessed as related to study drug.

- Safety Team will follow-up with the Investigator every 3 months regarding pregnancy outcome. The Investigator must follow the pregnancy to conclusion in order to determine the outcome. If the case is beyond 30 days from the expected due date and no information has been received regarding the outcome, the Safety Team will contact the Investigator to request outcome.

Male Patients With Partners Who Become Pregnant

- The Investigator will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to male patients who receive BOS-589.

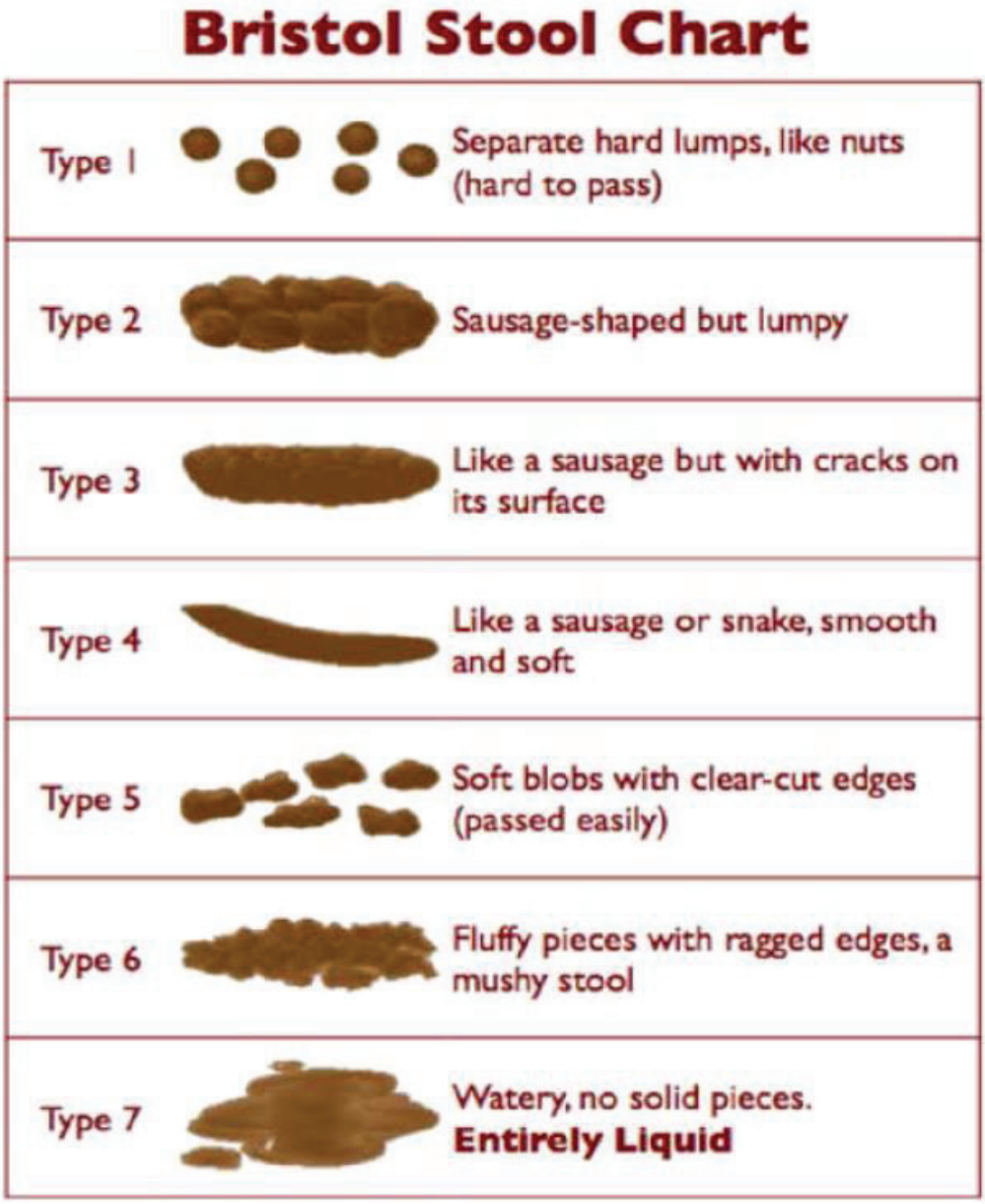
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the sponsor as outlined above.

Female Patients Who Become Pregnant

- The Investigator will collect pregnancy information on any female patient who becomes pregnant while participating in this study. Information will be recorded on the appropriate form as noted above.

- Any female patient who becomes pregnant while participating in the study will be withdrawn from the study.

Appendix 3. The Bristol Stool Form Scale



(CCI [redacted])

Appendix 4. Concomitant Medications

If the study site/investigator has any questions regarding permitted or excluded medications, the medical monitor should be contacted for further discussion.

Medications permitted during the course of the study:

- Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or supplements) that in the opinion of the Investigator, should not interfere with study procedures, compromise safety, or the scientific integrity of the data;
- Stable doses of medications for allergies, migraines (with the exception of opioids for acute treatment), anxiety (e.g., as-needed use of benzodiazepines), depression or other chronic conditions at the discretion of the Investigator;
- Stable doses of antidepressants. As-needed use of buspirone and benzodiazepines for anxiety;
- Loperamide can be used during study as rescue medication based on protocol-specified guidelines (see Section 6.7.1) for allowable on-study rescue medications);
- Acetaminophen, up to 2 g/day for up to 3 consecutive days;
- The Investigator should monitor closely for potential side effects related to any of the following medicines which are considered moderate to highly sensitive substrates of CYP3A4.

alprazolam	dronedarone	nisoldipine
aprepitant	ebastine	pimozide
atorvastatin	eletriptan	rivaroxaban
avanafil	felodipine	sildenafil
budesonide	lomitapide	simvastatin
buspirone	lovastatin	tadalafil
colchicine	midazolam	triazolam
darifenacin	naloxegol	vardenafil

Medications excluded during the course of the study:

- 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron);
- Strong inhibitors of P-glycoprotein:

amiodarone	dronedarone	quinidine
carvedilol	itraconazole	ranolazine
clarithromycin	propafenone	verapamil

- Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
- Narcotic- or opioid-containing agents;
- Cannabis-containing products;
- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents);
- Planned use of rifaximin or oral antibiotics, with the exception of topical antibiotics or a 1-day course with an antibiotic); a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomized;
- Any investigational drug.

Appendix 5. Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is provided as a separate document.

DOCUMENT HISTORY	
Document	**Date of Issue**
Amendment 1, v.2.0	09 May 2019
Original, v.1.0	25 January 2019

CLINICAL TRIAL PROTOCOL

Study Title:	A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)
Study Number:	BOS-589-201
Regulatory Identification Number(s):	IND 142421
Study Phase:	2a
Investigational Product:	BOS-589
Indication:	Diarrhea-predominant Irritable Bowel Syndrome
Sponsor:	Boston Pharmaceuticals, Inc. 55 Cambridge Parkway, Suite 400 Cambridge, Massachusetts 02142 USA

Version	**Date**
Final v.1.0	25-January 2019

Confidentiality Statement

SPONSOR SIGNATURE PAGE

Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

Protocol Number: BOS-589-201

Version Number and Date: v.1.0, 25-January 2019

I, the undersigned, have approved the final version of this Clinical Trial Protocol.

_____ _____
Date

_____ _____
Date

INVESTIGATOR AGREEMENT

Protocol Title: A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)

Protocol Number: BOS-589-201

Version Number and Date: v.1.0, 25-January 2019

I, the undersigned, have read the protocol and agree to conduct the trial in compliance with the International Conference on/Council for Harmonization (ICH) guidelines and any other applicable regulatory requirements; as well as Good Clinical Practice (GCP) standards (CPMP/ICH/135/95).

I will provide copies of the protocol and all pertinent information to all individuals who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the investigational product and the conduct of the study.

I will use only the Informed Consent Form approved by the sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this study.

I agree that the sponsor or its representatives will have access to any source documents from which case report form information may have been generated.

Investigator's Signature	Date

Name of Investigator *(typed or printed)*

Institution Name

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ABBREVIATIONS

AE	adverse event
AKT	protein kinase B
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration versus time curve
AUC_{0-4}	area under the concentration versus time curve from time zero to 4 hours postdose
AUC_{0-12}	area under the concentration versus time curve from time zero to 12 hours postdose
AUC_{0-t}	area under the concentration versus time curve from time zero to the last measurable concentration
bid	twice daily
BSFS	Bristol Stool Form Scale
BMI	body mass index
CFR	Code of Federal Regulations
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
CNS	central nervous system
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CSR	clinical study report
CYP	cytochrome P450
ECG	electrocardiogram
EDC	electronic data capture
ENS	enteric nervous system
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
FDR	first-degree relative
GCP	Good Clinical Practice
GDNF	glial cell line-derived neurotrophic factor
GFR-α	glial cell line-derived neurotrophic factor family receptor-alpha
GI	gastrointestinal
GLP-1	glucagon-like peptide-1
HBV	hepatitis B virus
HCV	hepatitis C virus

HDPE	high-density polyethylene
hERG	human ether-à-go-go related gene
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
IB	Investigator's Brochure
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome-constipation
IBS-GS	Irritable Bowel Syndrome Global Scale
IBS-D	irritable bowel syndrome-diarrhea
IBS-M	irritable bowel syndrome-mixed
IBS-SS	Irritable Bowel Syndrome Severity Score
IBS-U	irritable bowel syndrome-unclassified
IC_{50}	half-maximal inhibitory concentration
ICF	Informed Consent Form
ICH	International Conference on/Council for Harmonisation
IEC	Independent Ethics Committee
ILC3	group 3 innate lymphoid cells
IRB	Institutional Review Board
ITT	intention-to-treat
IV	intravenous
IWRS	interactive web response system
JAK	Janus kinase
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	no observed adverse effect level
NRS	numeric rating scale
NTEAE	non-treatment-emergent adverse event
PBO	placebo
PD	pharmacodynamic(s)
PE	physical examination
P-gp	P-glycoprotein
PI3K	phosphoinositide 3-kinase
PK	pharmacokinetic(s)
PRO	patient-reported outcome
PYY	pancreatic peptide YY
QC	quality control
QTcB	QT interval corrected by Bazett's formula

QTcF	QT interval corrected by Fridericia's formula
RAS	rat sarcoma
RBC	red blood cell
RET	REarranged during Transfection
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous
SD	standard deviation
SK-N-AS	a human neuroblastoma cell line
STAT	signal transducer and activator of transcription proteins
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
TT	a human thyroid medullary carcinoma cell line
ULN	upper limit of normal
WAP	worst abdominal pain
WOCBP	woman of childbearing potential

1 PROTOCOL SUMMARY

1.1 Synopsis

<table>
<tr><td>Sponsor:</td><td colspan="2">Boston Pharmaceuticals Inc.</td></tr>
<tr><td>Title:</td><td colspan="2">A Phase 2a, Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy, Safety, and Tolerability of BOS-589 in the Treatment of Patients with Diarrhea-predominant Irritable Bowel Syndrome (IBS-D)</td></tr>
<tr><td>Protocol Number:</td><td colspan="2">BOS-589-201</td></tr>
<tr><td>Phase:</td><td colspan="2">2a</td></tr>
<tr><td rowspan="5">Objectives and Endpoints:</td><td>OBJECTIVES</td><td>ENDPOINTS</td></tr>
<tr><td colspan="2">Primary</td></tr>
<tr><td>• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.</td><td>• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• Incidence of adverse events (AEs), serious adverse events (SAEs), discontinuations because of AEs, and any treatment-related severe AEs.</td></tr>
<tr><td colspan="2">Secondary</td></tr>
<tr><td>• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.</td><td>• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• Change in stool frequency, measured by the total number of</td></tr>
</table>

		spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
	• To evaluate the steady-state pharmacokinetics (PK) of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).
Study Design:	A phase 2a, randomized, double-blind, PBO-controlled, multicenter trial to provide proof-of-principle efficacy of BOS-589 in IBS-D patients and to inform dose selection for subsequent development. The study will comprise 3 phases: A **Pre-treatment Phase** of up to 4 weeks, in which all patients will be assessed to determine eligibility. This phase will consist of initial screening assessments after which eligible patients will enter a Run-in Period of up to 3 weeks. During the Run-in period the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medication use, to confirm disease activity, and diary compliance.	

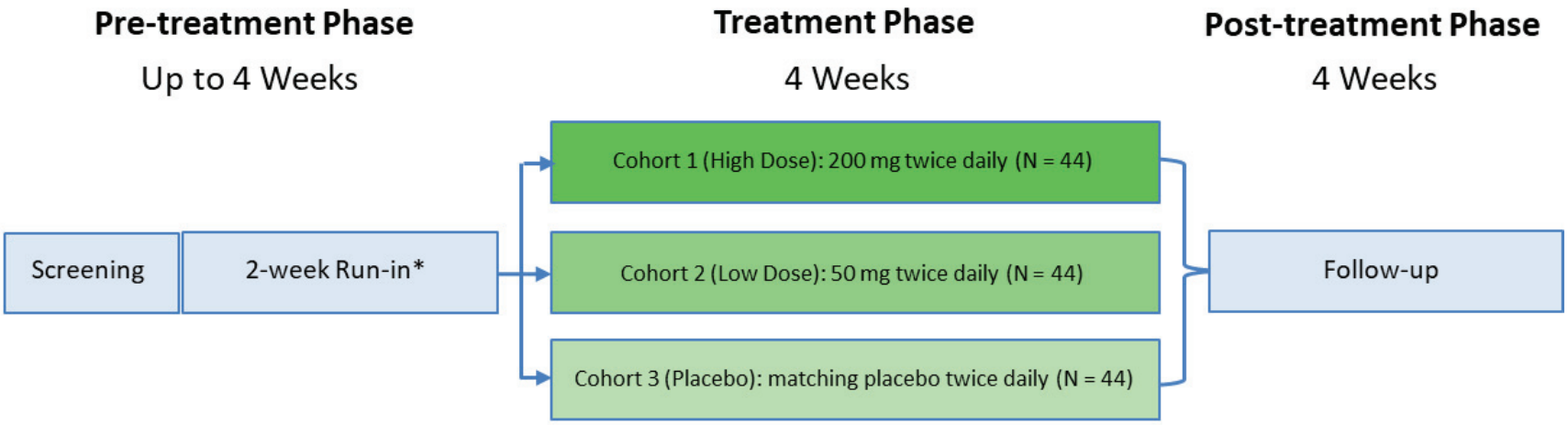
	Upon completion of the Run-in Period, patients will return to the study site to confirm eligibility for randomization into the double-blinded Treatment Phase. A **Treatment Phase**, in which eligible patients will be randomized (1:1:1) to receive BOS-589 or PBO twice daily (bid) for a total of 4 weeks. BOS-589 will be administered at 1 of 2 dose levels. Patients will be randomized to the following cohorts: • Cohort 1 (High Dose): 200 mg BOS-589 bid orally; • Cohort 2 (Low Dose): 50 mg BOS-589 bid orally; • Cohort 3 (PBO): matching PBO oral tablets bid. During the treatment phase, patients will continue to complete the electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine. A **Post-treatment Phase**, in which all patients who complete 4 weeks of treatment will return to the clinical for a 2-week follow-up visit. Patients who discontinue treatment early will be asked to return to the clinic for safety assessments.
Study Population:	Male and female patients 18 to 65 years of age, inclusive, with a diagnosis of IBS-D.
Number of Patients Planned:	Approximately 300 patients will be screened with the intent of randomizing 132 patients for the study.
Duration of Patient Participation and Study:	The duration of patient participation is anticipated to be up to 10 weeks. The duration of the study is anticipated to be approximately 12 months.
Study Sites:	Up to 66 sites in the United States.
Investigational Product:	BOS-589 (oral doses bid).
Reference Treatment:	Matching PBO (oral doses bid).
Concomitant Product:	Not applicable.

Statistical Methods:	**Efficacy:** This is a phase 2a proof-of-principle study. Descriptive and inferential statistical methods will be employed. A standalone Statistical Analysis Plan will be developed and approved prior to database lock. A hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change from baseline to Day 29 in the patient-reported outcome for WAP measured daily on an 11-point numeric rating scale. Mean change from baseline will be measured as a continuous variable and compared between treatment groups in the following testing order using a t-test of equal variances with a two-tailed alpha of 0.05: Hypothesis 1: Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3); If statistically significant at $P < 0.05$, then proceed to Hypothesis 2. Hypothesis 2: Cohort 1 (High Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$, then proceed to Hypothesis 3. Hypothesis 3: Cohort 2 (Low Dose) versus Cohort 3 (PBO); If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4. Hypothesis 4: Cohort 1 (High Dose) versus Cohort 2 (Low Dose). **Safety:** Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for

	Regulatory Activities Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All serious AEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced. All AEs will be listed for individual patients, showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs related to investigational product will be generated.

1.2 Study Schema

Figure 1. Study Schematic



* Run-in may be extended by 1 week to achieve for patient-reported outcomes requirements

1.3 Schedule of Activities

Table 1. Schedule of Activities

	Screening Days -29 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Informed consent	X							
Demographics	X							
Medical and surgical history	X							
Rome IV assessment	X							
Concomitant medication review	X	X	X	X	X	X	X	X
Adverse event review and evaluation		X- continuous - X						
Physical examination[c]	X		X				X	
Height, weight, and body mass index	X[d]		X		X		X	X
Vital signs	X		X	X	X	X	X	X
12-lead electrocardiogram	X						X	
Clinical safety laboratory tests[e]	X		X		X		X	X
HIV, HCV/HBV testing	X							
Urine pregnancy test	X		X				X	X
C-reactive protein and erythrocyte sedimentation rate tests	X		X		X		X	X
Biomarker sample collection			X		X		X	X
Pharmacokinetic sample collection[f]			X	X	X	X	X	
Worst abdominal pain	X	X[g]	X- [h] - X					X[h]
Bristol Stool Form Scale	X	X[g]	X- [h] - X					X[h]

	Screening Days -29 to -1, Study Visit 1	Run-in Period[a] Days -15 to -1, Study Visit 2	Baseline Day 1, Study Visit 3	Day 8 (± 1 day) Study Visit 4	Day 15 (± 1 day) Study Visit 5	Day 22 (± 1 day) Study Visit 6	Day 29 (± 1 day) End of Treatment /Early Termination[b] Study Visit 7	Day 43 (± 3 days) End of Study Study Visit 8
Procedures	**Pre-treatment**		**Treatment**					**Follow-up**
Spontaneous bowel movements (total number of stools)		X[g]	X- -[h]- X					X[h]
IBS-GS		X[g]	X- -[h]- X					X[h]
Rescue medications		X[g]	X- -[h]- X					X[h]
IBS-SS			X				X	X
Eligibility review	X		X					
Randomization			X					
Administration of investigational product			Day 1 - Day 28					

HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IBS-GS = Irritable Bowel Syndrome Global Scale; IBS-SS = Irritable Bowel Syndrome Severity Score.

[a] Run-in may be extended by up to one week with Sponsor approval and thus may begin on Day -22.

[b] Procedures performed at Visit 7/End of Treatment or upon Early Termination. Early Termination procedures are to be performed in close proximity to discontinuation of dosing for patients who are withdrawn from the study. Other procedures may be performed at Investigator or Sponsor discretion.

[c] Full physical exam to be conducted at Screening. Symptom-directed physical examinations should be conducted at subsequent visits.

[d] Height and body mass index to be collected at Screening Visit only.

[e] Hematologic, complete serum chemistry, urinalysis panels and fecal calprotectin to be completed as noted in Appendix 1.

[f] Pharmacokinetic (PK) serial blood samples are to be collected on Day 1 and Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If PK serial blood samples cannot be collected on Day 15, PK serial blood samples should be collected on Day 22. A predose sample will be obtained on all other Treatment Study Visits. Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for 0.5 and 1 hour postdose, and ± 10 minutes for 2 and 4 hours postdose.

[g] Data will be collected daily for entire Run-in Period.

[h] Data will be collected daily until Early Termination or until End-of-Study Visit, whichever is later.

2 INTRODUCTION

2.1 Study Rationale

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) illness with a prevalence of approximately 5% to 20% globally and characterized by a constellation of clinical symptoms. Establishing a diagnosis and assessing response to treatment remains challenging because there are no biomarkers that reliably correlate with disease state.

BOS-589 is an oral agent with limited systemic absorption that inhibits RET (REarranged during Transfection), a receptor tyrosine kinase that is hypothesized to play a key role in the maintenance of a healthy enteric nervous system (ENS). Inhibition of RET may represent a novel therapeutic strategy for the treatment of IBS by attenuating visceral hypersensitivity and/or colonic motility.

BOS-589 has been evaluated in healthy human subjects at doses up to 200 mg twice daily (bid) for 2 weeks. Consistent with its low oral bioavailability, BOS-589 was safe and well tolerated with no significant safety signals identified. The purpose of this study is to evaluate the efficacy, safety, and tolerability of BOS-589 in the treatment of patients with diarrhea-predominant IBS (IBS-D).

2.2 Background

2.2.1 Disease Under Study

Irritable bowel syndrome is a relatively common GI illness characterized by a number of clinical symptoms, including abdominal pain and discomfort, abnormal bowel habits, and bloating (CCI). Subtypes of IBS, based on the predominant bowel habit(s) reported by IBS patients, include diarrhea (IBS-D), constipation (IBS-C), mixed (IBS-M), or unclassified (IBS-U). Currently approved medications for IBS address the restoration of patients' bowel habits and are minimally effective in addressing abdominal pain and discomfort. Because the etiology of the disease has not been clearly established, diagnosis is difficult and relies primarily on the presence of a specific symptom complex occurring in the absence of an alternative explanation. The development of criteria by expert panels, with recent iterations as recently as 2016, has improved the diagnosis and management of IBS patients (CCI).

It is generally believed that the sensory inputs/outputs in the ENS and central nervous system (CNS) are altered in patients with IBS and this contributes to the signs and symptoms they experience. For example, patients with IBS have a heightened and disproportionate sensory experience (visceral hypersensitivity) for a given stimulus (CCI). Visceral hypersensitivity and abnormal bowel habits may result from visceral afferent neurons or increased nerve fiber density and sprouting that have been observed in the intestinal mucosal tissues of IBS patients (CCI ; CCI). The sensitizing event causing visceral hypersensitivity may be transient or chronic; however, its impact on the CNS and ENS

may be long lasting (CCI). Supernatants from these intestinal tissues can stimulate nerve outgrowth and increase neuronal signaling *in vitro*, suggesting that neurotrophic factors and/or inflammatory cytokines might be mediators of neuronal plasticity in IBS (CCI ; CCI). Given the central role the ENS may play in mediating signals between the CNS and the intestine, ENS modulation may provide therapeutic benefit to IBS patients by impacting visceral hypersensitivity and intestinal motility.

The only drugs currently approved by the Food and Drug Administration (FDA) for the treatment of IBS are alosetron (for women only), rifaximin, and eluxadoline for refractory IBS-D, and plecanatide, linaclotide, and lubiprostone for IBS-C. The majority of these drugs, and those used off label to treat IBS, target patients' symptoms by altering GI motility and are minimally effective in addressing abdominal pain and discomfort. Of the FDA-approved agents, only alosetron is hypothesized to modulate the CNS and ENS (CCI). Therefore, an agent such as BOS-589, which may ameliorate visceral hypersensitivity and potentially impact intestinal motility, would address a significant unmet medical need for IBS-associated pain.

2.2.2 The "RET" ("REarranged During Transfection") Gene and the Enteric Nervous System

The RET gene, localized on human chromosome 10q11.2, encodes a receptor-type tyrosine kinase with an extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain (CCI). The ligands for RET have been identified as neurotrophic factors of the glial cell line-derived neurotrophic factor (GDNF) family, including GDNF, neurturin, artemin, and persephin. Ligand binding to its corresponding GDNF family receptor-alpha (GFRα) co-receptor triggers RET dimerization and subsequent transphosphorylation of intracellular tyrosines (CCI) and leads to the activation of different intracellular signaling cascades, including the Janus kinase/signal transducer and activator of transcription proteins (JAK/STAT), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), and rat sarcoma/extracellular signal-regulated kinase (RAS/ERK) pathways.

Mice deficient in the GDNF ligand, its coreceptor GFRα1, or the RET protein itself, exhibit severe defects in kidney and ENS development. This implicates RET signaling as critical to the development of normal kidneys and the ENS (CCI). The role of RET in the development of the ENS is also apparent in patients with Hirschsprung's disease, who frequently suffer from colonic obstruction because of a lack of normal colonic innervation. In Hirschprung's disease, different loss-of-function mutations that occur in the *RET* gene account for the highest proportion of both familial and sporadic cases of the disease (CCI).

While its role during the development of the ENS has been well established, recent reports also implicate a significant role for RET in the maintenance and plasticity of the adult ENS. Neurons within the submucosal and myenteric plexus of the adult human colon have been shown to express RET and its coreceptors, GFRα1 and GFRα2, while the GDNF ligand is expressed in the muscularis mucosa and in circular and longitudinal

muscle tissue (CCI). Systemic administration of GDNF in adult rodents results in significant increases in submucosal neuron density in both the small intestine and colon and altered function (CCI). Furthermore, a conditional knockout of the RET co-receptor, GFRα3, results in decreased colonic hypersensitivity implicating a role for RET signaling in visceral nociception (CCI). Therefore, by reducing RET signaling, inhibition of RET may modulate ENS activity.

2.2.3 Investigational Product

BOS-589, formerly GSK3352589, is a potent and selective inhibitor of RET that has been shown to reduce visceral hypersensitivity in an animal model of IBS and inhibit cholinergic-induced increases in colonic motility (details are provided in the BOS-589 Investigator's Brochure [IB]). The results from these preclinical studies suggest that inhibition of RET with a potent, selective, and gut-restricted small molecule may represent a novel therapeutic strategy for the treatment of abdominal pain and defecation abnormalities (i.e., diarrhea) in patients with IBS through the attenuation of visceral hypersensitivity and/or cholinergically mediated ion transport and colonic motility. The patient population likely to derive the greatest benefit would comprise individuals with IBS-D with increased GI motility.

2.2.3.1 Nonclinical Summary

A range of *in vitro* and *in vivo* studies have been conducted to investigate the primary, secondary, and safety pharmacology of BOS-589. Details are provided in the BOS-589 IB.

2.2.3.1.1 *In Vitro* Studies

CCI

CCI

CCI

CCI

2.2.3.1.2 *In Vivo* Studies

CCI

2.2.3.1.3 Pharmacokinetics and Product Metabolism in Animals

CCI

CCI

CCI

CCI

2.2.3.1.4 Toxicology and Nonclinical Assessment of Safety

2.2.3.2 Clinical Experience

Study CCI evaluated the safety, tolerability, pharmacokinetics (PK), and exploratory pharmacodynamics (PD) of escalating single and multiple oral doses of BOS-589 ranging from 2 to 400 mg in the fasted state, and 25 mg in the fed state. All doses in the single ascending-dose portion of the study were generally safe and well tolerated, with a safety profile similar to that of placebo (PBO). Similarly, repeat-dose administration of BOS-589 for 14 days at doses ranging from 5 to 200 mg bid in the fed state was generally safe and well tolerated. There were no drug-related clinically significant changes in safety laboratory tests, vital signs, ECGs, or stool patterns as assessed by the Bristol Stool Form Scale (BSFS) in this study. There were no serious

adverse events (SAEs) considered related to the administration of BOS-589. A review of all GI adverse events (AEs) demonstrated that the occurrence of GI AEs was similar in the PBO- and BOS-589-treatment groups. There was no pattern or trend with dose escalation suggestive of a treatment effect.

The PK profile of BOS-589 demonstrated limited oral bioavailability and systemic exposure, with plasma concentrations generally less than 2 ng/mL, when measured utilizing a sensitive analytical method to detect concentrations as low as 5 pg/mL. Exposures were dose dependent and less than dose proportional, although box plots of dose-normalized parameters suggested dose proportionality between 15 mg and 100 mg. Dosing was escalated to the highest possible dose because predicted mean systemic exposures did not exceed the defined plasma toxicokinetic limits ($AUC_{0\text{-}t}$ and C_{max} of 40.4 ng*h/mL and 26.7 ng/mL, respectively) and there was no evidence of dose-limiting toxicities. There was accumulation of BOS-589 with repeat dosing, but systemic exposures remained very low after 14 days of dosing, with respective geometric mean (CV%) $AUC_{0\text{-}t}$ and C_{max} values of 24.1 (45.9) h*ng/mL and 1.53 (43.6) ng/mL for the highest dose of 200 mg bid; BOS-589 was likely at steady state before that time. Accumulation was lower for doses below 100 mg bid (1.3 to 1.8 fold) and highest for the highest dose of 200 mg bid (2.0 to 2.7 fold).

The study included a pilot food-effect group to evaluate the magnitude of a food challenge on the bioavailability of single-dose BOS-589. Following administration of a single dose of BOS-589 25 mg in the fed state, there was a small decrease in exposure with food, with decreases in mean C_{max} and AUC values in the range of 20% to 35%. The time to C_{max} (T_{max}) remained the same with or without food. These food-effect results were deemed not clinically important; dosing BOS-589 with or without food is not anticipated to affect future evaluation of safety or efficacy.

Because RET is expressed in enteroendocrine cells lining the intestinal mucosa, an exploratory objective of the study was to explore the effect of BOS-589 on glucagon-like peptide-1 (GLP-1) and pancreatic peptide YY (PYY) excursions in plasma; however, no clear relationship or impact on BOS-589 administration and peptide secretion was identified.

2.3 Risk/Benefit Assessment

Summaries of findings from nonclinical and clinical studies conducted with BOS-589 can be found in the IB (refer to the IB for additional details). The following section outlines the risk assessment and mitigation strategy for this protocol.

The current study, BOS-589-201, represents the first administration of BOS-589 to patients with IBS-D. Considerations for safety monitoring are derived primarily from the literature regarding RET expression in the intestine, nonclinical data, and clinical experience dosing BOS-589 to normal healthy volunteers (Study CCI [redacted]), in which no clinically relevant risks were identified that would preclude dosing a RET inhibitor for up to 4 weeks in patients with IBS-D.

2.3.1 Risk Assessment

2.3.2 Benefit Assessment

Patients randomized to the active treatment arms may potentially experience improvement in their IBS-D during the course of the study. Those randomized to the PBO arm are not expected to obtain any benefit beyond that of their background treatment.

2.3.3 Overall Risk/Benefit Conclusion

On the basis of nonclinical and clinical study results to date, limited effective alternative treatments, and the strength of the scientific hypothesis under evaluation, BOS-589 is considered to have a favorable benefit-risk profile for patients with IBS-D.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
• To evaluate in patients with IBS-D the abdominal pain response to BOS-589 after 4 weeks of treatment, relative to placebo (PBO).	• 24-hour worst abdominal pain scores (WAP) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the overall safety and tolerability of BOS-589 in the treatment of IBS-D during 4 weeks of treatment, relative to PBO.	• Incidence of adverse events (AEs) serious adverse events (SAEs), discontinuations because of AEs, and any treatment-related severe AEs.
Secondary	
• To evaluate the treatment effect of BOS-589 on defecation after 4 weeks, relative to PBO.	• Change in stool consistency, measured by the daily Bristol Stool Form Score (BSFS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). • Change in stool frequency, measured by the total number of spontaneous bowel movements in 24 hours at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the treatment effect of BOS-589 on IBS-related signs and symptoms.	• Change in the IBS Severity Score (IBS-SS) at Day 29 compared to baseline. • Change in the IBS Global Scale (IBS-GS) at Day 29 compared to baseline (averaged over the week prior to each respective timepoint).
• To evaluate the steady-state pharmacokinetics of BOS-589.	• Maximum observed plasma concentration (C_{max}); time to C_{max} (T_{max}); minimum plasma concentration (C_{min}); area under the concentration versus time curve (AUC) from time zero to 4 hours postdose (AUC_{0-4}); AUC from time zero to the last quantifiable concentration (AUC_{0-t}).

4 STUDY DESIGN

4.1 Overall Design

This study is a phase 2a, randomized, double-blind, PBO-controlled, multicenter trial designed to provide the first proof-of-principle efficacy of BOS-589 in IBS-D patients, and to inform dose selection for subsequent development. The study will consist of a pre-treatment phase, a 4-week double-blind treatment phase, and a 2-week post-treatment follow-up period.

Pre-Treatment Phase: During the pre-treatment phase, patients will be evaluated for up to 4 weeks to assess eligibility. The pre-treatment phase will consist of initial screening assessments and a Run-in period.

After the initial screening assessments have been performed, eligible patients will enter a Run-in period of up to 3-weeks. During the Run-in period, the patients will complete an electronic diary to collect daily information related to their IBS-D symptoms, bowel function, and rescue medicine use, to confirm disease activity and diary compliance. Patients will also be requested to discontinue any prohibited medications during this phase of the study.

Upon completion of the Run-in period, patients will return to the study site to confirm eligibility for randomization into a 4-week double-blinded Treatment Phase.

Treatment Phase: Eligible patients will be randomized (1:1:1) into the following cohorts:

- Cohort 1 (High Dose): 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 50 mg BOS-589 bid orally
- Cohort 3 (PBO): matching PBO oral tablets bid orally

During the 4 weeks of double-blind treatment, patients will continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary, as described in Section 8.3.

Post-treatment Phase: A 2-week post-treatment follow-up visit will occur for patients who complete the Treatment Phase. During the 2 weeks of follow-up, patients should continue to record their daily IBS-D symptoms, bowel function, and rescue medicine use in the electronic diary.

Patients who prematurely discontinue treatment should return to the study center to complete the early termination assessments as soon as possible after stopping the treatment.

Data analyses will occur after all patients in the trial have completed the last visit or procedure shown in the Schedule of Activities, Section 1.3.

Post-study Access to Therapy: No post-treatment access to therapy will be provided to patients randomized in the study.

4.1.1 Study Duration for Patients

Unless shortened by intolerable AEs or rapid disease progression, each patient's participation in this study is anticipated to last up to 10 weeks.

The total duration of this study is anticipated to be approximately 12 months, including patient enrollment, treatment, and follow-up.

4.1.2 Number of Patients

Approximately 300 patients will be screened to randomize approximately 132 patients with IBS-D (44 patients per cohort).

4.1.3 Replacement of Patients

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who have received at least 1 dose of investigational product and are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

4.1.4 Number of Sites

Up to 66 sites in the United States may participate in this study.

4.2 Rationale for Study Design

Key aspects of the study (e.g., eligibility, cohort size, stopping criteria, safety data collection, efficacy assessments, use of PBO as control, and use of patient-reported outcome [PRO] tools) are based upon generally accepted clinical trial methodologies for phase 2a efficacy studies and prior studies conducted in patients with IBS.

The Rome IV (CCI [redacted]) diagnostic criteria for IBS is the best accepted tool for standardized IBS diagnosis.

A Run-in Phase is designed to account for fluctuations in symptoms and the potential for wide variations in bowel habits; patient diaries that record frequency and severity of daily symptoms will be used to ensure that symptom severity fluctuations are identified and taken into account for subject eligibility. Patients will also be assessed on their ability to record their disease-related information in the required manner.

The use of PBO as a control comparator in IBS clinical trials is acceptable given the lack of consistently effective treatments. Placebo is an important component of IBS clinical trials given the high PBO effect in this population.

The key primary and secondary endpoints in this study are based on PRO assessments. These assessments evaluate pain, defecation, and IBS signs and symptoms. Abdominal pain intensity is measured using an 11-point (i.e., 0 to 10) numeric rating scale (NRS) that asks patients daily to rate their *worst abdominal pain over the past 24-hours* as recommended by the FDA guidance on IBS (FDA, May 2012). The use of the NRS for pain has been validated in IBS clinical studies (CCI). The IBS Severity Score (IBS-SS) (CCI) and IBS Global Scale (IBS-GS) (CCI) are validated and standard methods of assessing IBS symptoms in clinical trials.

Although treatment durations longer than 4 weeks will be required for true assessment of efficacy in IBS patients, in this initial phase 2a study, 4 weeks should be sufficient to identify any efficacy signal that warrants further clinical invention.

The use of multiple enrolling sites is aimed at maximizing external validity and to minimizing the potential influence of regional variations in diet, exercise habits, and ethnicity.

4.3 Justification for Dose

CCI

CCI

CCI

CCI

CCI [redacted]

4.4 End of Study Definition

The end of the study is defined as completion of the last visit or procedure shown in the Schedule of Activities in the trial globally. A patient will be considered to have completed the study if he or she has completed all phases of the study, including the last visit or the last scheduled procedure shown in the Schedule of Activities, Section 1.3.

5 STUDY POPULATION

Before any study-specific activities/procedures, the appropriate written informed consent must be obtained. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

Age

1. Male and female patients must be 18 to 65 years of age, inclusive, at the time of signing informed consent.

Run-in eDiary Compliance

2. Patient has completed the daily electronic diary on at least 6 of the 7 days during the week prior to randomization AND at least 11 of the 14 days during the 2 weeks prior to randomization. Patients have up to 3 weeks to meet these criteria.

Type of Patient and Disease Characteristics

3. Patient meets the diagnosis of IBS based on the Rome IV diagnostic criteria (CCI):*

    - Recurrent abdominal pain occurring, on average, at least 1 day per week and associated with 2 or more of the following:
        - Related to defecation;
        - Associated with a change in frequency of bowel movements;
        - Associated with a change in form (appearance) of stool;

    * These criteria must be fulfilled for the last 3 months prior to randomization and onset must have occurred at least 6 months prior to randomization.

4. Patient meets the diagnosis of IBS-D subtype based on Rome IV diagnostic criteria (CCI). On days when the patient experiences IBS symptoms:

    - At least 25% of stools are loose or watery; AND
    - Fewer than 25% of stools are hard.

5. Over the week prior to randomization, the patient has an average of worst abdominal pain (WAP) scores in the prior 24 hours of 4.0 to 8.0 (on a 0 to 10 numerical rating scale, where 0 indicates no pain and 10 indicates worst pain imaginable).

6. Over the week prior to randomization, the patient has an average daily BSFS score ≥ 5.0 (on a 1 to 7 scale, where 1 = hard, lumpy stools, and 7 = watery, liquid stools) and at least 5 days with a BSFS score ≥ 5.0.

7. Over the week prior to randomization, the patient has an average daily IBS-GS score of ≥ 2.0 (on a 0 to 4 scale, where 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe).

8. Patient is not planning to change his or her usual diet and lifestyle during the course of the study.

Diagnostic Assessments

9. Patient must undergo or previously have undergone (a) an appropriate evaluation for their IBS symptoms, including an evaluation for organic/structural etiologies (if in the presence of alarm symptoms); and (b) age-appropriate screening for colorectal cancer, if applicable.

    a) If at least one of the following alarm features are present, then the patient must have had a colonoscopy since the onset of symptoms or within the past 5 years (whichever is less):

        - Documented and unexplained weight loss of ≥ 10% within the past 6 months;
        - Nocturnal diarrhea;
        - Blood mixed with stool (except hemorrhoidal bleeding, defined as occasional blood found on the toilet paper only or limited dripping of blood into the toilet bowl after defecation;
        - Unexplained iron-deficiency anemia.

    b) If no alarm features are present, then the patient must have had a colonoscopy or other appropriate exam, based on criteria as outlined below:

        - Age ≥ 50 (≥ 45 if African-American): Colonoscopy since onset of symptoms or within past 5 years (whichever is less);
        - First-degree relative (FDR) diagnosed with colorectal cancer under age 60 OR 2 FDRs diagnosed with colorectal cancer at any age: Colonoscopy within past 5 years, *beginning* 10 years before age of youngest FDR (at time of diagnosis);

- Age ≥ 40 AND FDR diagnosed with colorectal cancer (at any age): Colonoscopy within past 10 years;

10. Patient is negative for serum tissue transglutaminase IgA antibody (tTG-IgA) plus has evidence of detectable serum IgA within the normal reference range.

Weight

11. Body mass index (BMI) within the range 16 to 39 kg/m^2 (inclusive).

Gender

12. Male and female patients are eligible if:

a. Male patients:

A male patient must agree to use contraception as detailed in Appendix 2 of this protocol during the treatment period and for at least 14 weeks after the last dose of investigational product. Patients must also agree to refrain from donating sperm during this period.

b. Female patients:

A female patient is eligible to participate if she is not pregnant (see Appendix 2), not breastfeeding, and at least 1 of the following conditions applies:

i. Not a woman of childbearing potential (WOCBP) as defined in Appendix 2; OR

ii. A WOCBP who agrees to follow the contraceptive guidance in Appendix 2 during the treatment period and for at least 5 weeks after the last dose of investigational product.

Informed Consent

13. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the Informed Consent Form (ICF) and in this protocol.

14. Patient is willing to be compliant with study procedures, including completing the daily electronic diary during the Run-in Period and throughout the study.

5.2 Exclusion Criteria

An individual for whom any of the following criteria apply will be excluded from participation in this study:

Gastrointestinal-related Medical Conditions

1. At the time of screening, patient has a diagnosis of an IBS subtype other than IBS-D, based on Rome IV criteria (CCI [redacted]). Based on stool patterns on days that the patient experiences symptoms, other IBS subtypes are defined as follows:

    a. IBS-C: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements;

    b. IBS-M: hard or lumpy stools ≥ 25% of bowel movements and loose or watery stools ≥ 25% of bowel movements;

    c. IBS-U: hard or lumpy stools ≤ 25% of bowel movements and loose or watery stools ≤ 25% of bowel movements.

2. Patient has a history of inflammatory or immune-mediated GI disorders including (but not limited to) inflammatory bowel disease (i.e., Crohn's disease, ulcerative colitis, microscopic colitis, and celiac disease).

3. Patient has had an episode of diverticulitis within 3 months prior to Screening.

4. Patient has a history of intestinal obstruction, stricture, toxic megacolon, GI perforation, fecal impaction, gastric banding, bariatric surgery, adhesions, ischemic colitis, or impaired intestinal circulation (e.g., aortoiliac occlusive disease).

5. Patient has any of the following surgical history:

    a. Cholecystectomy with ANY history of post-cholecystectomy biliary tract pain. A successful cholecystectomy with no postoperative biliary tract pain is not exclusionary;

    b. Any abdominal surgery within the 3 months prior to Screening;

    c. Major gastric, esophageal, hepatic, pancreatic, or intestinal surgery (appendectomy, hemorrhoidectomy, or polypectomy) within the 3 months prior to Screening.

6. Patient has a history or current evidence of laxative abuse.

Other Medical Conditions

7. Patient has a history of a cardiovascular event, including stroke, myocardial infarction, congestive heart failure, or transient ischemic attack within 6 months prior to Screening.

8. Patient has a history of malignancy within 5 years prior to Screening (except squamous and basal cell carcinomas and cervical carcinoma *in situ*).

9. Patient has a history of alcohol abuse or binge drinking.

10. Patient has uncontrolled hypertension, defined as systolic blood pressure > 180 mmHg or a diastolic blood pressure > 100 mmHg at the time of Screening.

11. Patient a history of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.

Laboratory Assessments

12. Fecal calprotectin ≥ 50µg/g.

13. Hemoglobin A1c level ≥ 8.0% or a confirmed fasting plasma glucose level ≥ 180 mg/dL.

14. Confirmed alanine aminotransferase (ALT) ≥ 5 upper limit of normal (ULN).

15. Confirmed total bilirubin ≥ 3 mg/dL (≥ 51.3 mM/L), unless the patient has a documented history of Gilbert's syndrome.

16. Confirmed QT interval corrected by Fridericia's formula (QTcF) or QT interval corrected by Bazett's formula (QTcB) > 500 msec.

17. Evidence of active hepatitis B virus (HBV) infection, based on a positive hepatitis B surface antigen (HBsAg) screen.

18. Evidence of hepatitis C virus (HCV) infection based on a positive HCV antibody screen (patients who have been successfully treated for HCV are eligible if an undetectable HCV viral load at least 6 months after completion of treatment can be demonstrated).

19. Human immunodeficiency virus (HIV)-1 or HIV-2 antibody positive.

Prior/Concomitant Therapy

20. Within 14 days of randomization, patient has used 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron).

21. Within 14 days of randomization, patient has used any of the following:

    - Loperamide; (Note: loperamide can be used during the study as rescue medication based on protocol specified guidelines [see Section 6.7.1]);
    - Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
    - Narcotic- or opioid-containing agents;
    - Cannabis-containing products;

- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents).

22. Within 14 days of randomization, receipt of any prescribed or over-the-counter, systemic, herbal (including St. John's wort), or topical medication, or any expectation of requiring use of such medication while participating in the study that, in the opinion of the Investigator, would interfere with study procedures, compromise safety, or the scientific integrity of the data.

23. Patient has used, or is expected to use, the following antibiotics:

    - Rifaximin within 90 days prior to randomization;
    - Other oral antibiotics within 28 days of randomization (with the exception of topical antibiotics or a 1-day course with an antibiotic).

    However, a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomly assigned to study drug.

24. Within 3 months prior to randomization, patient has had significant changes to his or her antidepressant regimen (i.e., addition of a new agent, discontinuation of a prior agent, significant modifications to the dose of a current medication). Patients on chronic stable doses of antidepressants will be allowed to participate in the study. As-needed use of buspirone and benzodiazepines for anxiety is permitted during the study;

25. Within 30 days prior to randomization (or 5 half-lives, if known), the patient has received an investigational drug, or is currently enrolled in an investigational study.

Other Exclusions

26. Patient is unable to swallow solid oral dosage forms whole with the aid of liquid (patients may not chew, divide, dissolve, or crush the study drug).

27. Patient has an elective surgery planned or expects to need elective surgery at any time during the study.

28. Patient is pregnant or breastfeeding.

29. Patient has any medical or psychological disorder or condition that, in the opinion of the Investigator, would compromise the wellbeing of the patient or the study or prevent the patient from meeting or performing study requirements.

30. Patient has poor peripheral venous access.

31. Patient is an employee of the Investigator or study center with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members of the employees or the Investigator.

5.3 Lifestyle Considerations

During this study, patients will be asked to abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests.

Only on days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities; Section 1.3), patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study. Investigational product should be taken with approximately 240 mL water.

5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened with Medical Monitor approval.

6 INVESTIGATIONAL PRODUCT

6.1 Investigational Products

6.1.1 Investigational Product Description

Investigational Product Name:	BOS-589	Placebo
Dosage Formulation:	Tablet	Tablet
Unit Dose Strengths/Dosage Levels:	50 mg (2 x 25-mg tablets) and 200 mg (2 x 100-mg tablets)	Visually matching placebo tablets across both BOS-589 dose levels
Route of Administration:	Oral	Oral
Dosing Instructions:	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush	Swallow whole with a glass of water, do not chew, divide, dissolve, or crush
Packaging and Labeling:	Investigational product will be provided in white, opaque, high-density polyethylene (HDPE) bottles with a child-resistant closure. Each bottle will be labeled as per country requirement	Investigational product will be provided in white, opaque, HDPE bottles with a child-resistant closure. Each bottle will be labeled as per country requirement
Manufacturer:	WuXi STA, Shanghai, China	WuXi STA, Shanghai, China

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all investigational product received and any discrepancies are reported and resolved before use of the investigational product.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for investigational product accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

6.2.2 Product Storage and Stability

All investigational product must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

Further guidance and information for the final disposition of unused investigational products are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias

This is a randomized, double-blinded, PBO-controlled study. Patients should be randomized as close as possible to the time of the planned first dose of study treatment.

Randomization will be conducted via an interactive web response system (IWRS) in a 1:1:1 ratio for patients to receive BOS-589 200 mg, BOS-589 50 mg, or PBO. Patients will be assigned to the treatment arms using a permuted block randomization stratified by site (44 patients per group). The randomization schedule will be generated by the Study Biostatistician and will be transferred to the IWRS team for loading into the system. The randomization file will be held by the Study Biostatistician until the end of the trial and when the database has been locked.

The sponsor, site staff, and patients will be blinded to the treatment assignment. BOS-589 and PBO will be similar in appearance, and provided in white, opaque, high-density polyethylene (HDPE) bottles, each with a child-resistant closure. Each bottle will be labeled as per country requirement.

Only in a medical emergency will a patient's treatment assignment be unblinded, and this process will be performed and documented in the IWRS. Every effort should be made to contact the Medical Monitor to discuss unblinding prior to breaking the blind.

6.4 Dosing and Administration

Only patients enrolled in the study may receive investigational product and only authorized site staff may supply or administer investigational product.

Eligible patients will be randomized (1:1:1) to receive for a total of 4 weeks BOS-589 at 1 of 2 dose levels or PBO bid. Patients will be randomized to the following cohorts:

- Cohort 1 (High Dose): 2 x 100 mg tablets for the 200 mg BOS-589 bid orally
- Cohort 2 (Low Dose): 2 x 25 mg tablets for the 50 mg BOS-589 bid orally
- Cohort 3 (PBO): 2 x visually matched PBO tablets bid orally

For each dose level and at each dosing timepoint, the 2 tablets are to be swallowed with approximately 240 mL room temperature water and are to be swallowed whole (i.e., not divided, crushed, dissolved, or chewed). On days when postdose PK blood samples are to be taken (see footnote f in the Schedule of Activities, Section 1.3, patients will be asked to refrain from consumption of food and water for 1 hour prior to and for 2 hours after administration of investigational product. Food and water may be consumed *ad libitum* at all other times during the study.

Patients will be instructed to take the investigational product approximately every 12 hours at approximately the same time each day. If a patient misses a dose at a given timepoint, and the time is within 4 hours of the regularly scheduled dosing time, the patient should be instructed to take the investigational product. If it has been longer than

4 hours, the patient should skip the missed dose at that timepoint and resume the regularly scheduled dosing schedule at the next scheduled timepoint.

In the event of vomiting following administration, BOS-589 should not be taken until the next scheduled dose.

6.5 Dose modification

Study treatment should be interrupted if the patient reports any treatment-related severe AE. Therapy should only be restarted after approval by the Medical Monitor.

If therapy is restarted, the same dose should be administered as that prior to occurrence of the AE. If the patient is still having tolerability issues and an AE reoccurs, the dose may be reduced from 2 tablets of study treatment bid to 1 tablet of study treatment bid, without breaking the study blind.

If another AE occurs after the study treatment dose reduction, study treatment will be permanently discontinued and the subject followed until the end of the study.

6.6 Investigational Product Compliance

All patients in this study will commence therapy on site on Study Day 1; oral investigational product will be administered under clinic staff supervision. After patients are discharged to continue therapy at home, compliance will be assessed on subsequent visits by returned tablet count. Administration of investigational product and any deviation(s) from the prescribed dosage regimen should be recorded in the case report form (CRF) and Drug Accountability Record.

6.7 Concomitant Therapy

Refer to Appendix 4 for more details on which concomitant medications are permitted and which are excluded while patients are on study.

Any prior or concomitant therapy (including over the counter or prescription medicines, vitamins and/or supplements) taken 28 days prior to the first dose of investigational product through the End-of-Study Visit must be recorded, along with:

- Reason for use;
- Dates of administration including start and end dates;
- Dosage information including dose and frequency.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported

in the CRF are concomitant prescription medications, over-the-counter medications, vaccines, vitamins, and supplements.

6.7.1 Rescue Medicine

During the double-blind Treatment Phase of the study, patients will be allowed to take loperamide rescue medication for the acute treatment of uncontrolled diarrhea. Loperamide at a unit dose of 2 mg may be taken once approximately every 6 hours with the following guidelines:

- No more than 4 unit doses over a continuous 24-hour time period (8 mg/day);
- No more than 7 unit doses over a continuous 48-hour time period (14 mg over 2 days);
- No more than 11 unit doses over a continuous 7-day time period.

The use of loperamide rescue medication should be recorded electronically.

6.8 Intervention After the End of the Study

BOS-589 will not be provided to study patients after the end of the study.

7 DISCONTINUATION / WITHDRAWAL

7.1 Discontinuation of Investigational Product

A patient may discontinue from study treatment at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons. Discontinuation from the investigational product does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to, changes from baseline) after enrollment, the Investigator or qualified designee will determine if any change in patient management is needed. Any new clinically relevant finding will be reported as an AE.

Upon investigational product discontinuation, patients should complete all procedures collected in the Day 43/End-of-Study Follow-up visit as applicable per the Schedule of Activities (Section 1.3).

7.2 Discontinuation/Withdrawal from the Study

A patient may withdraw from the study at any time at his or her own request or may be withdrawn at any time at the discretion of the Investigator for the following reasons:

- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the patient;
- Disease progression that requires discontinuation of the investigational product;
- Pregnancy;
- If the patient meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation after consultation with the Medical Monitor;
- Significant investigational product noncompliance;
- Termination of the study by the sponsor.

If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a patient withdraws from the study, he or she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

See Schedule of Activities, Section 1.3, for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The reason for patient discontinuation or withdrawal from the study will be recorded on the CRF.

Patients who sign the ICF and are randomized but do not receive the investigational product may be replaced. Once randomized, patients who are withdrawn from therapy before completing 28 days of dosing or are discontinued from the study for any reason will not be replaced.

7.3 Lost to Follow-up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site will attempt to contact the patient and reschedule the missed visit as soon as possible; counsel the patient on the importance of maintaining the assigned visit schedule; and ascertain if the patient wishes to and/or should continue in the study.

- Before a patient is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods. These contact attempts should be documented in the patient's medical record or study file.

- Should the patient continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

Planned timepoints for all assessments are provided in the Schedule of Activities (Section 1.3).

8.1 Study Periods

The study will comprise 3 phases:

1. **Pre-Treatment Phase** (up to 4 weeks), in which all patients will be assessed for eligibility. The pre-treatment phase will consist of initial screening assessments (Visit 1) and a Run-in period (Visit 2 through Visit 3).

2. A **Treatment Phase** (4 weeks), in which all patients will return to the clinical for randomization and start of treatment (Visit 3 – Day 1) followed by weekly visits (Visit 4 – Day 8 [± 1 day], Visit 5 – Day 15 [± 1 day], Visit 6 – Day 22 [± 1 day]) until Visit 7 – Day 29 (± 1 day), and

3. A **Follow-up Phase** (2 weeks), in which patients who complete Visit 7, will be asked to return for an End-of-Study Follow-up visit (Visit 8 – Day 43 [± 3 days]) for assessment of treatment outcome (e.g., safety, durability of effect).

8.2 Screening Assessments

After signing the ICF(s), the patient will undergo the initial screening assessments and procedures as described in the Schedule of Activities (Section 1.3) to determine eligibility.

Eligible patients will enter the Run-in period. At the beginning of the Run-in period, patients will receive instructions for completing an electronic diary to collect daily information related to their IBS-D symptoms, their bowel function, and rescue medication use.

Patients who are:

- Compliant in completing the screening diary on a daily basis on at least 6 of the 7 days during the week prior to randomization AND on at least 11 of the 14 days during the 2 weeks prior to randomization, and

- Have an average WAP score in the past 24 hours of between 4.0 and 8.0 on a 0 to 10 scale over the week prior to randomization, and

- Have an average daily BSFS of ≥ 5.0 and at least 5 days with a BSFS score ≥ 5.0 on a 1 to 7 scale over the week prior to randomization, and

- Have an average daily IBS-GS score of ≥ 2.0 on a 0 to 4 scale over the week prior to randomization, and

- Who have not used any loperamide rescue medication in the 2 weeks prior to randomization

will be eligible for participation and immediate randomization into the double-blind treatment phase (i.e., all 5 diary conditions must be met to qualify for randomization).

Eligible patients will be instructed to return to the study site for the Baseline visit on Day 1.

Patients who do not meet all of the 5 diary conditions required for entry in the preceding 2 weeks may be granted an extra week of screening time in which to satisfy all of the criteria (for a total screening period of 3 weeks).

8.3 Efficacy Assessments

The efficacy objectives of the study are to evaluate the effect of BOS-589, relative to PBO after 4 weeks of therapy, on pain, defecation, and key IBS-D related signs and symptoms.

During the 4 weeks of the double-blind treatment phase, patients will be required to access their electronic diary every day, preferably at the same time each day, to record IBS-D symptom data and information related to their bowel function and rescue medication use.

8.3.1 Worst Abdominal Pain

Throughout the 4 weeks of the double-blind treatment phase, patients will be asked to rate their WAP in the past 24 hours. The patient-reported WAP in the past 24 hours will be recorded on a 0 to 10 scale, where 0 corresponds to no pain and 10 corresponds to worst imaginable pain.

8.3.2 Bristol Stool Form Scale

Patients will be asked to record daily stool consistency according to the BSFS most representative of the past 24 hours. The patient-reported BSFS consistency score is based on a 1 to 7 scale where 1 corresponds to a hard stool and 7 corresponds to watery diarrhea (CCI [redacted]). Please refer to Appendix 3.

8.3.3 IBS-D Global Symptom Score

Patients will be asked to record daily their overall IBS-D global symptoms in the prior 24 hours. The patient-reported daily IBS-GS is based on a 0 to 4 scale where:

- 0 corresponds to no symptoms;
- 1 corresponds to mild symptoms;
- 2 corresponds to moderate symptoms;

- 3 corresponds to severe symptoms; and
- 4 corresponds to very severe symptoms.

8.3.4 IBS Severity Score System

Patients will be asked to complete 5 questions regarding the severity of their IBS. Each of the 5 questions generate a maximum score of 100, leading to a total possible score of 500.

8.4 Safety and Other Assessments

8.4.1 Adverse Events and Serious Adverse Events

See Section 8.5.

8.4.2 Physical Examinations

A complete physical examination (PE) will be performed at Screening. A symptom-directed PE will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) and as clinically indicated, and results recorded in the source document. Any clinically significant PE finding noted at Screening will be recorded as medical history. Any clinically significant PE finding noted at after enrollment will be reviewed for reporting as an AE (see Section 8.5).

A complete PE will include, at a minimum, assessment of the cardiovascular, respiratory, GI, and neurological systems. A targeted PE will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).

Height and weight will also be measured and BMI will be calculated and the data recorded at the timepoints specified in the Schedule of Activities (Section 1.3).

Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.4.3 Vital Signs

At the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection, vital signs will be measured in a supine position after 5 minutes rest and will include oral temperature, systolic and diastolic blood pressure, pulse, and respiratory rate. Any clinically significant abnormal vital sign value will be recorded as an AE (see Section 8.5).

8.4.4 Electrocardiograms

Single 12-lead ECGs will be performed at the timepoints specified in the Schedule of Activities (Section 1.3) and before any blood sample collection. Electrocardiogram interval measurements and interpretation (normal, abnormal not clinically significant,

abnormal clinically significant) will be recorded in the source document. Any clinically significant change in ECG interpretation will be recorded as an AE (see Section 8.5).

8.4.5 Clinical Safety Laboratory Assessments

See Appendix 1 for the list of clinical laboratory tests to be performed and to the Schedule of Activities (Section 1.3) for the timing and frequency.

The Investigator must review the laboratory report, document this review, and record in the AE section of the CRF any clinically relevant changes occurring during the study. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the sponsor notified.

All protocol-required laboratory assessments, as defined in Appendix 1, must be conducted in accordance with the laboratory manual and the Schedule of Activities.

If laboratory values from non-protocol-specified laboratory assessments performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the Investigator (e.g., SAE, or AE, or dose modification), then the results must be recorded in the CRF.

8.5 Adverse Events and Serious Adverse Events

Adverse events will be reported by the patient (or, when appropriate, by a caregiver, surrogate, or the patient's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting and documenting events that meet the definition of an AE or SAE and are responsible for following all AEs.

8.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of investigational product, whether or not considered related to the investigational product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of investigational product.

8.5.2 Events of Special Interest

Not applicable.

8.5.3 Definition of Serious Adverse Events

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death because of progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life threatening;

  The term 'life threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization;

  In general, hospitalization signifies that the subject or patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred or was necessary, the AE should be considered serious.

  Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE;

- Results in persistent disability/incapacity;

  The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised when deciding if SAE reporting is appropriate in other situations such as important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the

patient or may require medical or surgical intervention to prevent any of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.5.4 Classification of an Adverse Event

8.5.4.1 Assessment of Severity

The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities;
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities;
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as ‘serious’ when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

8.5.4.2 Assessment of Causality

All AEs must have their relationship to the investigational product and/or study participation assessed by the Investigator who examines and evaluates the patient based on temporal relationship and his or her clinical judgment. Alternative causes, such as underlying disease, concomitant therapy, and other risk factors, as well as the temporal relationship of the event to investigational product administration will be considered and investigated. The Investigator will also consult the IB in his or her assessment.

The degree of certainty about causality will be graded using the categories below:

- **Related** – The AE is known to occur with the investigational product, there is a reasonable possibility that the investigational product caused the AE, or there is a temporal relationship between the investigational product and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the investigational product and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the investigational product caused the event, there is no temporal relationship

between the investigational product and event onset, or an alternate etiology has been established.

For each AE, the Investigator must document in the medical notes that he or she has reviewed the event and has provided an assessment of causality. The Investigator may change his or her opinion of causality in light of follow-up information and updated causality assessment reported.

8.5.4.3 Expectedness

The Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the investigational product.

8.5.5 Time Period and Frequency for Event Assessment and Follow-up

All AEs will be collected from the signing of the ICF until the End-of-Study Follow-up visit.

Whenever possible, all AEs should be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the patient is stable.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, whether considered investigational product related or not, and must include an assessment of if there is a reasonable possibility that the investigational product caused the event. The Investigator will also submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he or she considers the event to be reasonably related to the investigational product or study participation, the Investigator must promptly notify the sponsor.

8.5.6 Adverse Event Reporting

At each study visit, patients will be evaluated for new AEs and the status of existing AEs. Care will be taken not to introduce bias when evaluating for AEs. The Investigator should use open-ended questions when soliciting information from a patient regarding AEs, followed by appropriate questions that clarify the patient’s verbatim description of AEs or change in concomitant medications.

When an AE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE.

The Investigator will then record all relevant AE information in the CRF. It is not acceptable for the Investigator to send photocopies of the patient's medical records in lieu of completion of the CRF page. New or updated information will be recorded in the originally completed CRF. However, there may be instances when copies of medical records for certain cases are requested. In this case, all patient identifiers, with the exception of the patient number, will be redacted on the copies of the medical records before submission.

If a patient dies during participation in the study or during a recognized follow-up period, the Investigator will provide a copy of any postmortem findings, including histopathology when available.

If a site receives report of a new SAE from a study patient or receives updated data on a previously reported SAE after database lock of the electronic data capture (EDC) system, then the site can report this information on a paper SAE form or to the sponsor by telephone. See the Study Manual for additional information on SAE reporting.

The study sponsor, or designee, will be responsible for notifying all applicable regulatory authorities of any required safety events in compliance with country-specific regulatory requirements. In addition, the sponsor must notify applicable regulatory authorities and all participating Investigators of suspected unexpected serious adverse reactions (SUSARs), from clinical trials or any other source.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the Institutional Review Board/ Independent Ethics Committee (IRB/IEC), if appropriate according to local requirements.

8.5.7 Reporting of Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. Pregnancy in a patient's partner is not considered an AE. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs. Elective abortions without complications should not be handled as AEs; however, an induced therapeutic abortion to terminate a pregnancy because of complications or medical reasons must be reported as an SAE. The underlying medical diagnosis for this procedure should be reported as the SAE term. A spontaneous abortion in a study patient is always considered an SAE.

Details of all pregnancies in female patients and, if indicated, pregnancies in female partners of male patients will be collected after the start of investigational product and until 5 weeks after the last dose.

If a pregnancy is reported, the Investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 2.

8.6 Treatment of Overdose

There are no data on overdose because this is the first study of BOS-589 in patients and, in a prior study in healthy human volunteers, no evidence of overdose was reported. There is no definition of what constitutes an overdose and no known antidote. Any patient who receives a higher dose than that intended (i.e., more than 200 mg per dose; more than 400 mg in a given day) should be monitored closely, managed with appropriate supportive care, and followed up appropriately. If possible, a blood sample for PK should be collected as soon as is feasible from any patient who takes a higher dose than that intended.

If AEs or SAEs are reported and are considered related to a patient receiving a higher dose than intended, dosing should be interrupted if deemed necessary by the Investigator and the case discussed further with the Medical Monitor.

8.7 Pharmacokinetics

Plasma samples of approximately 4 mL will be collected for measurement of plasma concentrations of BOS-589 at the following times and as specified in the Schedule of Activities (Section 1.3).

- On Day 1 at predose and at 0.5, 1, 2, and 4 hours postdose.
- On Day 15 at predose and at 0.5, 1, 2, and 4 hours postdose. If samples cannot be obtained on Day 15, then samples should be obtained on Day 22.
- A predose sample will be obtained on all other Treatment Study Visits.

Allowable sampling windows for PK blood draws will be 30 minutes prior to dosing for the predose sample, then ± 5 minutes for the 0.5 and 1 hour postdose timepoints, and ± 10 minutes for the 2 and 4 hours postdose timepoints.

Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual time of dosing and actual date and time (24-hour clock time) of each sample will be recorded.

Drug concentration information that may unblind the study will not be reported to investigative sites or blinded personnel until the study has been unblinded.

Any changes in the timing or addition of timepoints for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.

The following PK parameters will be estimated from the plasma concentrations of BOS-589 using either traditional noncompartmental methods or a population-PK model:

AUC_{0-t} Area under the plasma concentration versus time curve from time zero to the last quantifiable sample;

AUC_{0-4} Area under the plasma concentration versus time curve from time zero to 4 hours postdose;

C_{max} Maximum observed plasma concentration;

C_{min} Minimum observed plasma concentration.

In addition to parameters above, the AUC from time zero to 4 hours postdose (AUC_{0-12}) may be calculated using predose concentrations other than Day 1 predose to impute a 12-hour concentration. Details will be provided in the PK analysis plan.

8.8 Biomarkers/Pharmacodynamics

CCI

CCI

9 STATISTICAL CONSIDERATIONS

9.1 Timing of Analyses

Formal hypothesis testing will occur at the end of the study and post database lock. There are no planned Data, Safety, or Adjudication Committees for this study and no planned statistical interim analyses.

9.2 Sample Size Determination

This is a phase 2a study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint. The primary endpoint is defined as a change in the 24-hour WAP score at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and compared between treatment groups in a hierarchical testing order using a t-test of equal variances and a two-tailed alpha of 0.05.

The sample size was based on the first hypothesis test in the hierarchical plan, with 80% power, two-sided alpha of 0.05, and a standard deviation (SD) of 3 points on the NRS to detect a 1.6 minimum change between treatment groups (N = 120 patients). To

account for attrition and a potential higher PBO rate, a total of 132 patients will be randomized to one of 3 treatment groups in a 1:1:1 ratio (44 patients per treatment group).

9.3 Populations for Analyses

1. The Intention-to-Treat (ITT) analysis dataset will comprise all randomized patients;

2. The Per-protocol Analysis Dataset will comprise patients in the ITT Analysis Dataset who received at least 1 dose of investigational product and did not have any major protocol deviations;

3. The Safety Analysis Dataset will comprise all patients who received at least 1 dose of investigational product;

4. The Pharmacokinetic Analysis Dataset will comprise all randomized patients who received the active study drug and have available serum time concentration data.

9.4 Statistical Analyses

Detailed methodology for descriptive and inferential statistical analyses of the data collected in the study will be documented in the Statistical Analysis Plan (SAP). The SAP will be prepared by the Study Biostatistician and agreed upon by the Sponsor. The SAP will be finalized and approved prior to database lock. The SAP will take precedence over the protocol for details regarding the statistical analyses to be conducted for the study. In addition to the SAP, other graphical representations of the results may be produced after review of the data (*post hoc*). Any major modifications of the Primary Endpoint's definition and/or its analysis will be reflected in a protocol amendment.

In general, descriptive statistical methods will be used to summarize the data from this study. Unless stated otherwise, the term "descriptive statistics" refers to number of patients (n), mean, median, SD, minimum, and maximum for continuous data, and frequencies and percentages for categorical data.

All statistical analyses will be performed using Statistical Analysis System (SAS®) software Version 9.4 or higher.

9.4.1 Efficacy Analyses

9.4.1.1 Primary Endpoint

This is a proof-of-principle study in which a hierarchical hypothesis test will be employed for the primary efficacy endpoint, defined as a change in the 24-hour WAP score at Day 29 compared to baseline (averaged over the week prior to each respective timepoint). Mean change from baseline will be measured as a continuous variable and

compared between treatment groups in the following testing order using a t-test of equal variances and a two-tailed alpha of 0.05:

Hypothesis 1:

Active Treatment Group (Cohort 1 + Cohort 2) versus PBO (Cohort 3);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 2.

Hypothesis 2:

Cohort 1 (High Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$, then proceed to Hypothesis 3.

Hypothesis 3:

Cohort 2 (Low Dose) versus Cohort 3 (PBO);

If statistically significant at $P < 0.05$ then, proceed to Hypothesis 4.

Hypothesis 4:

Cohort 1 (High Dose) versus Cohort 2 (Low Dose).

9.4.1.2 Secondary Endpoint

Mean daily BSFS score (stool type) and mean daily frequency of bowel movements will be tabulated and summarized by study day for each treatment for the ITT and Per-protocol Analysis Dataset populations. Time profiles and box plots will also be presented by study day and by treatment group. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.3 Other Efficacy Endpoints

Scores from IBS-GS and IBS-SS questionnaires will be summarized for the ITT and Per-protocol Analysis Dataset populations. Pooled active data may be compared to PBO for the analyses, and also by cohort.

9.4.1.4 Missing Data

Study implementation procedures and training will assist in limiting the amount of missing data in the trial. However, sensitivity analyses may be performed when indicated for the study endpoint and the amount of missing data to be handled. Details of the statistical methods for handling missing data will be described in the SAP.

9.4.2 Safety Analyses

9.4.2.1 Adverse Events

Adverse events will be recorded from the time of consent until the Day 43/End-of-Study Follow-up visit. The number and percentage of patients with AEs will be displayed by system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) Version 20.0 or higher, by study treatment. Summaries in terms of severity and relationship to investigational product will also be provided. All SAEs will be summarized in a similar manner. Patient listings of all AEs causing discontinuation of investigational product and all SAEs will be produced.

All AEs will be listed for individual patients showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs (TEAEs) related to investigational product will be generated.

Any event reported on the CRF that occurs on at the time of or after the initiation of investigational product is defined as treatment-emergent. Additionally, an AE that is reported to have started on Day 1 without an associated onset time will be assumed to have occurred after the initiation of investigational product. Hence, AEs occurring on Day 1 with no associated onset time will be assumed to be treatment emergent.

Serious AEs associated with a protocol-specified procedure and occurring after the time of consent but before administration of first dose of investigational product will be defined as non-treatment-emergent AEs (NTEAEs).

All safety analyses will be performed in the Safety Analysis Dataset.

9.4.2.2 Clinical Laboratory Evaluations

Laboratory data will be listed for all patients. Laboratory data will be reviewed by the Investigator as they become available. All abnormal laboratory results will be evaluated by the Investigator as either clinically significant or not clinically significant.

Descriptive summaries of clinical laboratory results will be presented by date and time of collection. The number and percentage of patients experiencing treatment-emergent graded toxicities will be summarized by treatment arm and severity grade. Laboratory toxicity shifts from baseline to post-baseline assessments will be summarized by treatment arm. Changes from baseline in laboratory tests will be summarized for each treatment arm.

9.4.2.3 Twelve-lead Electrocardiograms, Vital Signs, and Physical Examinations

Twelve-lead ECG and vital signs data will be listed and summarized.

Physical examination findings will be captured and listed as part of a patient’s medical history or as AEs as applicable.

9.4.3 Other Analyses

9.4.3.1 Pharmacokinetic Analysis

A full description of the methods for evaluating the PK of BOS-589 in this patient population will be provided in a PK data analysis plan. The PK data from this study may be combined with data from other studies to complete a population-PK analysis.

A full list of PK parameters to be calculated is included in Section 8.7.

9.4.4 Baseline Descriptive Statistics

Demographic and baseline characteristics will be summarized for all patients overall and by treatment arm. Summary statistics (e.g., number of patients, mean, median, SD, and range) will be generated for continuous variables (e.g., age and weight) and the number and percentage of patients within each category will be presented for categorical variables (e.g., gender, ethnicity, race).

A detailed description of patient disposition will be provided. It will include:

- A summary of overall patient enrollment status (consented, screened, screen failures, replacements, randomized);
- A summary of patients who discontinued the study;
- An account of identified protocol deviations.

All patients who are consented for the study will be accounted for in the summation. The number of patients who do not qualify for certain analysis populations will be summarized.

9.4.5 Subgroup Analyses

No subgroup analyses are powered for the study. Subgroup analyses may be performed for descriptive purposes as described in the SAP.

9.5 Planned Interim Analyses

No formal efficacy interim analysis is planned for the purposes of statistically comparing the data prior to the completion of the study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines;
- Applicable International Conference on/Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines;
- Applicable laws and regulations.

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted, and reviewed and approved as necessary, to the relevant Competent Authorities, IRBs and IECs before the study is initiated.

Any amendments to the protocol will require regulatory approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC;
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures;
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2 Informed Consent/Assent Process

An initial sample ICF is provided for the Investigator to prepare the informed consent document to be used at his or her site. Updates to the sample ICF are to be communicated formally in writing from the Sponsor or Sponsor’s designee to the Investigator. The written ICF is to be prepared in the language of the potential patient population.

The Investigator or his/her representative will explain the nature of the study to the patient or his/her legally authorized representative and answer all questions regarding the study.

Patients must be informed that their participation is voluntary. Patients or their legally authorized representative will be required to sign a statement of informed consent, and/or assent, that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Patients must be re-consented to the most current version of the ICF(s) during their participation in the study if one becomes available.

A copy of the ICF(s) must be provided to the patient or the patient's legally authorized representative.

The Investigator is also responsible for asking the patient if the patient has a primary care physician and if the patient agrees to have his/her primary care physician informed of the patient's participation in the clinical study if it is a local requirement. If the patient agrees to such notification, the Investigator is to inform the patient's primary care physician of the patient's participation in the clinical study. If the patient does not have a primary care physician and the Investigator will be acting in that capacity, the Investigator is to document such in the patient's medical record.

If a patient is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written ICF and any other written information to be provided to patients, is read and explained to the patient or the patient's legally acceptable representative, and after the patient or the patient's legally acceptable representative has orally consented to the patient's participation in the trial and, if capable of doing so, has signed and personally dated the ICF, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the patient or the patient's legally acceptable representative, and that informed consent was freely given by the patient or the patient's legally acceptable representative (Refer to ICH GCP guideline, Section 4.8.9).

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The Investigator or authorized designee will explain to each patient the objectives of the exploratory research. Patients will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to

document a patient's agreement to allow any remaining specimens to be used for exploratory research. Patients who decline to participate in this optional research will not provide this separate signature.

10.1.3 Confidentiality and Privacy

Patient confidentiality and privacy is strictly held in trust by the participating Investigators, their staff, and the sponsor and its designees. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to patients. Therefore, the study documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the study data will be released to any unauthorized third party without prior written approval of the sponsor.

Study patient research data will not include the patient’s contact or identifying information. Rather, individual patients and their research data will be identified by a unique study identification number. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred. Research data will be used in accordance with local data protection laws.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/IEC or regulatory agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

10.1.4 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices, Good Manufacturing Practices).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.5 Data Handling and Record Keeping

10.1.5.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site, under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

The Investigator must maintain adequate source documentation. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into a 21 CFR Part 11-compliant EDC system or transmitted electronically (e.g., laboratory data). The data system(s) includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Data recorded in the CRF should be consistent with the data recorded on the source documents or the discrepancies must be explained.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data. The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.5.2 Study Records Retention

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations.

No records will be destroyed without the written consent of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

It is the responsibility of the sponsor to inform the Investigator when these documents no longer need to be retained.

10.1.6 Study and Site Discontinuation and Closure

The sponsor or its designee reserves the right to suspend or prematurely terminate a study site or study at any time for any reason and at the sole discretion of the sponsor.

An Investigator may initiate site closure at any time, provided there is reasonable cause and sufficient notice is given to the sponsor in advance of the intended termination.

Written notification, documenting the reason for site or study suspension or termination, will be provided by the suspending or terminating party to study participants, Investigator, funding agency, the Investigational New Drug sponsor, and regulatory authorities.

If the study is prematurely terminated or suspended, the Investigator will promptly inform study participants, the IRB/IEC, and sponsor, and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants;
- Demonstration of efficacy that would warrant stopping;
- Insufficient compliance to the protocol, regulatory requirements, or GCP guidelines;
- Data that are not sufficiently complete and/or evaluable;
- Determination that the primary endpoint has been met;
- Determination of futility;
- Inadequate recruitment of patients.

10.1.7 Dissemination of Clinical Study Data

A Clinical Study Report (CSR) will be prepared in accordance with the ICH guideline on structure and contents of CSRs and any applicable regulatory and legal requirements.

10.1.8 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

11 REFERENCES

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12 APPENDICES

Appendix 1. Clinical Laboratory Tests

The tests detailed in Table 2 will be performed by the central laboratory.

Local laboratory results are only required in the event that the central laboratory results are not available in time for either investigational product administration and/or response evaluation. If a local sample is required, it is important that the sample for central analysis is obtained at the same time. Additionally, if the local laboratory results are used to make either an investigational product decision or response evaluation, the results must be entered into the CRF.

Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Pregnancy testing: Refer to Section 5.1 for Screening pregnancy criteria.

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count Red blood cell (RBC) count Hemoglobin Hematocrit		RBC Indices: Mean corpuscular volume Mean corpuscular Hemoglobin % Reticulocytes	White blood cell count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical Chemistry	Blood urea nitrogen	Potassium	Aspartate aminotransferase/Serum glutamic oxaloacetic transaminase	Total and direct bilirubin
	Creatinine	Sodium	Alanine aminotransferase/Serum glutamic pyruvic transaminase	Total protein
	Glucose (fasted)	Calcium	Alkaline phosphatase	
CCI [redacted]	CCI [redacted]	CCI [redacted]	CCI [redacted]	
Routine Urinalysis	Specific gravity pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick Microscopic examination (if blood or protein is abnormal)			

Table 2. Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Other Screening Tests	Hemoglobin A1c Follicle-stimulating hormone and estradiol (as needed in women of nonchildbearing potential only) Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential) Serology (HIV antibody, hepatitis B surface antigen, and hepatitis C virus antibody) serum tissue transglutaminase IgA antibody (tTG-IgA) and IgA (if applicable)

Investigators must document their review of each laboratory safety report.

Laboratory results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

Appendix 2. Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories **are not considered WOCBP**

1. Premenarchal
2. Premenopausal female with 1 of the following:
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy
    c. Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's review of the patient's medical records, medical examination, or medical history interview.

3. Postmenopausal female
    a. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
    b. Females on HRT and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Male patients.

Male patients with female partners of childbearing potential are eligible to participate if they agree to ONE of the following during the treatment period and for at least 14 weeks after the last dose of investigational product:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of < 1% per year, as described in Table 3, when having penile-vaginal intercourse with a WOCBP who is not currently pregnant

In addition, male patients must refrain from donating sperm for the duration of the study and for 14 weeks after the last dose of investigational product.

Male patients with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration for the duration of the study and for 14 weeks after the last dose of investigational product.

Female patients.

Female patients of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception, as described in Table 3, consistently and correctly.

Table 3. Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.* Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal Progestogen only hormonal contraception associated with inhibition of ovulation • Oral • Injectable
Highly Effective Methods That Are User Independent[a] Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
Vasectomized Partner *A vasectomized partner is a highly effective contraception method, provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual Abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the investigational product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the patient.*

NOTES:

[a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for patients participating in clinical studies.

[b] Hormonal contraception may be susceptible to interaction with the investigational product, which may reduce the efficacy of the contraceptive method. In this case, 2 highly effective methods of contraception should be utilized during the treatment period and for at least 5 weeks after the last dose of investigational product.

Pregnancy Testing:

- Any WOCBP should only be included after a negative pregnancy test.
- Additional urine pregnancy testing should be performed at the Day 29 visit, and the Day 43 End-of-Study Follow-up visit.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected

Collection of Pregnancy Information:

- All pregnancies must be reported on a Pregnancy Notification and Outcome Form (within 24 hours of awareness of any such pregnancy that occurs for either the study patient or for the female partner of a male patient); all pregnancies will be followed to outcome.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

- Pregnancies that occur beyond 90 days after the last dose of study drug will only need to be reported if there is an associated SAE that the Investigator assessed as related to study drug.

- Safety Team will follow-up with the Investigator every 3 months regarding pregnancy outcome. The Investigator must follow the pregnancy to conclusion in order to determine the outcome. If the case is beyond 30 days from the expected due date and no information has been received regarding the outcome, the Safety Team will contact the Investigator to request outcome.

Male Patients With Partners Who Become Pregnant

- The Investigator will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to male patients who receive BOS-589.

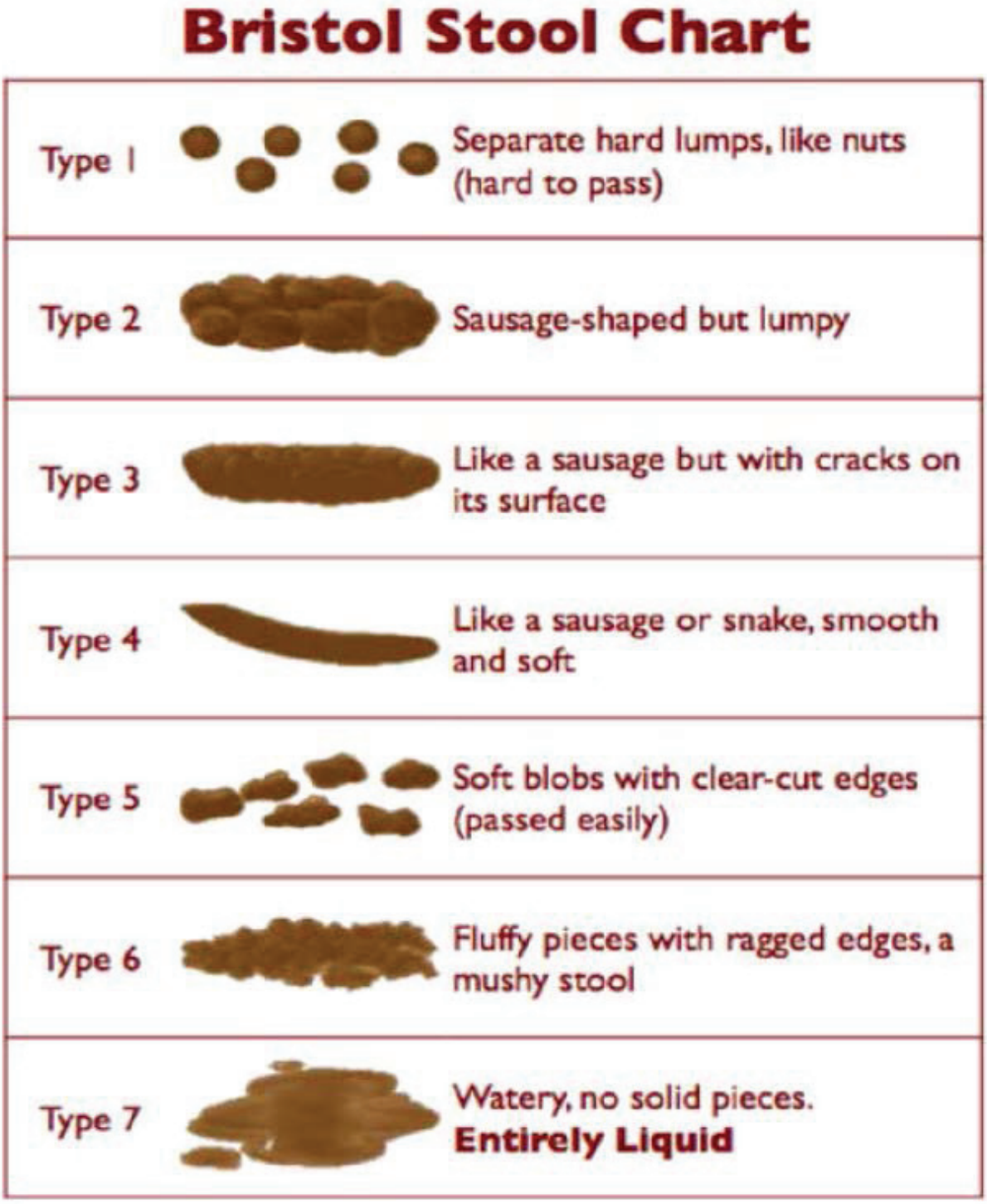
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the sponsor as outlined above.

Female Patients Who Become Pregnant

- The Investigator will collect pregnancy information on any female patient who becomes pregnant while participating in this study. Information will be recorded on the appropriate form as noted above.

- Any female patient who becomes pregnant while participating in the study will be withdrawn from the study.

Appendix 3. The Bristol Stool Form Scale



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Appendix 4. Concomitant Medications

Medications permitted during the course of the study:

- Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or supplements) that in the opinion of the Investigator, should not interfere with study procedures, compromise safety, or the scientific integrity of the data;
- Stable doses of medications for allergies, migraines (with the exception of opioids for acute treatment), anxiety (e.g., as-needed use of benzodiazepines), depression or other chronic conditions at the discretion of the Investigator;
- Stable doses of antidepressants. As-needed use of buspirone and benzodiazepines for anxiety;
- Loperamide can be used during study as rescue medication based on protocol-specified guidelines (see Section 6.7.1) for allowable on-study rescue medications);
- Acetaminophen, up to 2 g/day for up to 3 consecutive days;
- Any drugs or substances known to be sensitive substrates of CYP3A4 enzymes or known to be P-gp inhibitors should be discussed with the Medical Monitor.

Medications excluded during the course of the study:

- 5-hydroxytriptamine $(5\text{-HT})_3$ or 5-HT_4 receptor antagonists (e.g., alosetron);
- Aspirin or aspirin-containing medications (> 325 mg of aspirin per day) or nonsteroidal anti-inflammatory drugs, when taken specifically for the symptoms of IBS;
- Narcotic- or opioid-containing agents;
- Cannabis-containing products;
- Docusate;
- Enemas;
- GI preparations (including antacids containing aluminum or magnesium, antidiarrheal agents, antispasmodic agents, bismuth, peppermint oil, IBgard, FDgard, or prokinetic agents);

- Planned use of rifaximin or oral antibiotics, with the exception of topical antibiotics or a 1-day course with an antibiotic); a patient will be allowed to remain in the study should unplanned use of antibiotics other than rifaximin occur after the patient has been randomized;

- Any investigational drug.