Objectives and Endpoints

The primary and secondary study objectives and their corresponding endpoints are listed in the table below.

Objectives	Endpoints
Primary	
• To evaluate the efficacy of eluxadoline 100 mg BID in IBS-D participants with evidence of BAM compared to IBS-D participants without evidence of BAM	• Change from baseline in average BSFS score over 4 weeks of treatment period
• To evaluate the safety and tolerability of eluxadoline 100 mg BID in IBS-D participants with evidence of BAM and in IBS-D participants without evidence of BAM	• Evaluation of adverse events, clinical laboratory tests, vital signs, physical examinations
Secondary	
• To further evaluate the efficacy of eluxadoline 100 mg BID in IBS-D participants with evidence of BAM compared to IBS-D participants without evidence of BAM	• Change from baseline in the 4-week average of daily bowel movement frequency during the treatment period • Change from baseline in the 4-week average of daily worst abdominal pain scores during the treatment period • Change from baseline in the 4-week average of daily bloating during the treatment period • Change from baseline in the 4-week average of number of daily urgent bowel movements during the treatment period • The proportion of participants with any fecal incontinence during the treatment period • Change from baseline in IBS-QOL score at the end of the treatment period • Change from baseline in serum 7αC4 levels at the end of the treatment period
• To evaluate the population PK of eluxadoline in IBS-D patients with and without evidence of BAM	• Population pharmacokinetic parameters of eluxadoline determined from plasma concentration data extrapolated from dried blood sample analysis

7αC4 = 7a-hydroxy-4-cholesten-3-one; BAM = bile acid malabsorption; BID = twice daily; BSFS = Bristol Stool Form Scale; IBS-D = irritable bowel syndrome with diarrhea; IBS-QOL = irritable bowel syndrome-quality of life; PK = pharmacokinetics.

Overall Study Design:

This Phase IV study will use an open-label, parallel-group, cohort-controlled design in participants meeting the Rome IV criteria for IBS-D with and without evidence of BAM. Participants will be assigned to 1 of 2 cohorts based on their BAM status. As far as possible the 2 cohorts will be matched by age, gender and severity of symptoms.

1. Cohort 1: IBS-D participants with evidence of BAM treated with eluxadoline 100 mg oral tablets twice daily (BID) with food.

[redacted]

eluxadoline will have similar pharmacodynamic effects to increase stool consistency in IBS-D participants with and without evidence of BAM.

The actual eDiary data entered by the participants will not be provided to the investigative site staff at any time during the study to prevent any potential bias in subsequent participant entries. However, periodic notifications will be generated to inform the investigator of participants' ongoing compliance with eDiary entries and to alert investigators if participants have experienced episodes of constipation or have required excessive loperamide rescue medication for acute treatment of uncontrolled diarrhea.

The total duration of the study is up to 11 weeks, which includes: screening period (0-2 weeks), pretreatment period (2-3 weeks), 4-week open-label treatment period, and 2-week post-treatment follow-up period. A total of 6 study visits are planned for each participant:

- Screening, Week -3 to -5 (Visit 1) prior to Baseline visit
- Pretreatment, Week -3 to -2 (Visit 2) prior to Baseline visit
- Day 1 (Visit 3; first administration of study drug)
- Week 2 (Visit 4; Week 2 of treatment), may be done by telephone
- Week 4 (Visit 5; end of treatment/early termination)
- Week 6 (Visit 6; post-treatment follow-up)

The study design is presented in Table 5–1.

Table 5–1 Study Design

Period	**Screening[a]**	**Pretreatment[a]**	**Open-Label Treatment**			**Post-Treatment**
Duration	0-2 weeks	2-3 weeks	4 weeks			2 weeks
Week	Screening	Pretreatment	Baseline	Week 2[b]	Week 4 (EOT/ET)	Week 6[b] (Follow-up)
Study Visit	1	2	3	4	5	6
Study Day			1	15 (±2)	29 (±2)	Visit 5 + 14 days (±7)

7αC4 = 7a-hydroxy-4-cholesten-3-one; BA = bile acid

[a] Screening and pretreatment periods may be combined if results of fasting serum 7αC4 test or 48-hour fecal BA collection conducted within 1 calendar year from screening are available at the time of the combined visit or results from fasting serum 7αC4 test are available prior to the start of study treatment.

[b] May be done over the phone.

5.2. Participant and Study Completion

A maximum of 24 participants, 12 participants with evidence of BAM and 12 participants without evidence of BAM will receive study treatment such that approximately 24 evaluable participants complete the study.

5.3. End of Study Definition

The end of the study is defined as the date of Visit 6 (post-treatment follow-up visit).

A participant is considered to have completed the study if he/she has completed all periods of the study including post-treatment follow-up visit.

5.4. Scientific Rationale for Study Design

This is an open-label, cohort controlled study designed to evaluate the comparative effects of eluxadoline on the altered bowel function in IBS-D participants with and without evidence of BAM. This non-randomized design is considered most appropriate since the cohort assignment will be based on the determination of BAM status.

A primary factor in the design of this study is the mechanism by which evidence of BAM will be established and documented in the patient population. Both the Rome IV criteria and EMA guideline for IBS suggest that the results from a therapeutic study with a bile acid binding agent can be an appropriate surrogate given that these agents have been shown to improve stool passage and stool consistency in patients with BAM (Wong 2012; Bajor 2015). While empiric studies of sequestrants such as cholestyramine or colesevelam have the advantage of being clinically applicable, they provide only indirect evidence of BAM since these agents can have nonspecific effects to ameliorate diarrhea of other causes (Camilleri 2015). Additionally, therapeutic studies of cholestyramine are hampered by tolerability and compliance issues owing to its poor palatability and potential side effects, while studies of the newer agent colesevelam are hampered by its expense (Camilleri 2014).

Most potentially direct diagnostic tests of BAM are limited in their availability and/or clinical practicality. The most definitive test is the measurement of fecal bile acids in a 48-hour stool collection. Measurement of fecal bile acids is a direct measure of excess bile acids entering the colon. Primary fecal bile acids [Chenodeoxycholic acid (CDCA) and cholic acid (CA)] are significantly higher in patients with BAM and correlate with stool frequency and consistency (Shin 2013, Wong 2012). A potential alternative, the nuclear medicine SeHCAT test which measures bile acid retention is more clinically practical but does require 2 separate clinical visits to determine SeHCAT retention after a week. While SeHCAT testing has been the primary measure by which the overlap of BAM and IBS-D has been identified, it is not approved for use in the United States and is not widely available in the rest of the world (Islam 2012). Total fecal bile acids $\geq$ 2337 µmol/48 hr is the gold standard for BAM diagnosis in countries where SeHCAT retention is not available (Shin 2013).

The typical proportion of primary fecal bile acids in healthy volunteers is ~0.2%. Patients with BAM have higher stool weight compared to those with chronic diarrhea without BAM or healthy volunteers (Vijayvargiya 2018). The sensitivity and specificity of the fecal biomarkers of BAM are listed in Table 5-2. Therefore, elevated primary BA alone or in combination with total fecal bile acids of $\geq$ 1000 µmol/48 hr have a similar diagnostic accuracy as total fecal BA alone of $>$ 2337 µmol/48 hr in detecting elevated fecal weight in patients with BAM. Statistical analysis has demonstrated no significant difference between the ROC curve to estimate elevated fecal weight for either total BA or elevated primary fecal BA (p=0.13). After increasing the primary

BA cutoff to 10% or higher with total fecal BA ≥1000 µmol/48 hr , there was no significant gain in either sensitivity (41%) or specificity (97%) compared to the lower cutoff of primary BA ≥ 4% with total fecal BA ≥ 1,000 µmol/48 hr (Vijayvargiya 2018).

Therefore, even at the lower cutoff values of ≥4%, primary fecal bile acids are indicative of BAD even though the total fecal BA may be only 1000 µmol/48 hr.

However, in an analysis of 986 patients who underwent 48 hour fecal BA evaluation for chronic diarrhea, 26% of patients had elevated total fecal bile acids whereas 46% of patients had fecal primary bile acids ≥10%, indicating that measuring total fecal bile acids alone will miss a subgroup of patients who have features of BAM (Vijayvargiya 2018).

Evidence has suggested that measurement of serum 7α-hydroxy-4-cholesten-3-one (7αC4 test) may also be an effective indicator of BAM. Serum 7αC4 is a surrogate for hepatic BA synthesis rate and has been demonstrated to be elevated in patients with BAM (Eusufzai 1993) and in some patients with IBS-D (Odunsi-Shiyanbade 2010; Wong 2012). The use of serum 7αC4 to diagnose BAM has been validated against SeHCAT testing and was demonstrated to have approximately 90% sensitivity and 79% specificity (Sauter 1999). The fasting (6-10 am) serum 7αC4 test is considered most ideal because of its simplicity, as collection of blood for 7αC4 analysis can occur as part of the routine screening process for the study. The analysis of serum 7αC4 levels requires a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method which is currently available (Camilleri 2009; Camilleri 2014a). Further, serum 7αC4 was demonstrated to have high positive and negative predictive values making it attractive as a diagnostic test of BAM. Therefore, in this study, BAM status will be determined by the presence of at least one of the parameters listed in Table 5-2.

The sample size is based primarily on clinical considerations, including the desire to minimize the number of potential clinical sites given the possible need to utilize a research laboratory for analysis of screening serum 7αC4 samples. While not statistically powered, the study sample is considered sufficient to characterize the effects of eluxadoline on the altered bowel function of IBS-D patients with BAM and to qualitatively evaluate whether these patients respond differently to eluxadoline treatment.

The duration of treatment was chosen to be consistent with clinical practice in IBS, where a 1-month therapeutic study of a new agent is common and is supported by results of the Phase 3 studies which showed that a patient's response to eluxadoline treatment over the first month is reasonably predictive of responsiveness over longer durations. The post-treatment period is intended to allow for an off-treatment assessment of safety.

The study will enroll an IBS-D population similar to that enrolled in the Phase 3 studies of eluxadoline. Participants will meet the Rome IV criteria for IBS-D, which differs from the Rome III criteria used in the Phase 3 studies primarily in eliminating abdominal discomfort as a distinct entity from abdominal pain in the definition of IBS-D (Lacy 2016). Evidence of BAM will be determined by a screening serum 7αC4 value ≥ 52.5 ng/mL, which has previously been utilized as a diagnostic cutoff for BAM based on this value being greater than the 95th percentile of serum 7αC4 values from 184 healthy controls (Vijayvargiya 2017).

A serum pregnancy test will also be performed for all women unless they are surgically sterile or there is a documented history of their postmenopausal status. If the pregnancy test is positive, the participant is not eligible to enter the study.

Eligible participants will be instructed to return to the study site in 1 to 2 weeks for the pretreatment visit.

Pretreatment (Visit 2)

This visit may be combined with the screening visit if results from a fasting serum 7αC4 test or 48-hour fecal BA collection, conducted within 1 calendar year prior to screening, are available at the time of the combined visit or results from fasting serum 7αC4 test are available prior to the start of study treatment. After screening procedures have been performed, eligible participants will enter the pretreatment period. Participants will undergo the pretreatment visit and procedures listed in the SoA. The pretreatment visit will occur approximately 2 to 3 weeks before the first dose of study drug.

The inclusion and exclusion criteria, medical history, concomitant medications, and AEs will be reviewed, vital signs and weight will be measured and recorded. Participants will receive instructions on the use of rescue loperamide.

Participants will be provided with the eDiary device and instructed on the use of the eDiary system to record their IBS-D symptoms and information related to their bowel functioning (eg, bowel movement frequency, urgency and episodes of incontinence) on a daily basis. Additionally, participants will be instructed to record in the eDiary their use of loperamide rescue medication for the acute treatment of uncontrolled diarrhea.

Participants will be eligible for participation in the study to receive an open-label study treatment if they meet all 3 of the following requirements:

- Compliant in completing the eDiary on at least 10 of the 14 days immediately prior to enrollment into open-label treatment period of the study; and
- Have an average daily BSFS score ≥ 5.0 or at least 25% of days of diary entry with a BSFS score of 6 or 7 during the 14 days prior to Day 1 of study treatment, and
- The use of rescue medication on no more than 3 days during the 14 days prior to enrollment in the open-label treatment period as detailed in Section 7.7.3.

[redacted]

9.1.1. Daily IBS Symptoms

Participants will record the following IBS-D signs and symptoms during their daily eDiary entry during the pretreatment period and throughout the 4 weeks of the open-label treatment period:

- Stool Consistency: Participants will be asked to use the BSFS to rate their stool consistency most representative of the past 24 hours. The participant-reported BSFS is a 1 to 7 scale where 1 corresponds to a hard stool and 7 corresponds to watery stool (Appendix 11, Section 12.11; O'Donnell 1988):

  1=Separate hard lumps like nuts (difficult to pass)

  2=Sausage shaped but lumpy

  3=Like a sausage but with cracks on surface

  4=Like a sausage or snake, smooth and soft

  5=Soft blobs with clear-cut edges (passed easily)

  6=Fluffy pieces with ragged edges, a mushy stool

  7=Watery, no solid pieces (entirely liquid)

- Worst Abdominal Pain: Participants will be asked to rate their worst abdominal pain in the past 24 hours. The participant-reported worst abdominal pain 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.
- Abdominal Bloating: Participants will be asked to rate their abdominal bloating in the past 24 hours. The participant-reported worst abdominal bloating in the past 24 hours will be recorded on a 0 to 10 scale, where 0 corresponds to no bloating and 10 corresponds to worst imaginable bloating.
- Frequency, Urgency and Incontinence: Participants will be asked to record the number of bowel movements, number of urgent bowel movements, and number of episodes of fecal incontinence, over the past 24 hours.

9.1.2. Irritable Bowel Syndrome-Quality of Life Questionnaire

The impact of IBS on participants' quality of life will be assessed using the IBS-Quality of Life (IBS-QoL) questionnaire (Drossman 2000; Patrick 1998; Appendix 12, Section 12.12). The IBS-QoL questionnaire will be completed by participants at the study site at Baseline (Day 1) prior to the administration of study drug, and Week 4 (or ET visit). The questionnaire will be completed at the beginning of the applicable study visits before all other evaluations, especially discussion of AEs or the participant's medical condition.

[redacted]

[redacted]

9.2. Adverse Events

The definitions of an AE or SAE can be found in Appendix 4, Section 12.4.

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

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the participant to discontinue the study (see Section 8).

9.2.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs/SAEs from the start of treatment until the follow-up visit will be collected at the timepoints specified in the SoA (Section 2), and as observed or reported spontaneously by study participants. Any clinically significant abnormalities persisting at the end of the study will be followed until the AE has resolved.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the eCRF, not the AE section.

All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Appendix 4, Section 12.4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 4, Section 12.4.

9.2.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

9.2.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AEs/SAEs and non-serious AEs of special interest (as defined in Section 9.2.8) will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3).

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any postmortem findings including histopathology.

New or updated information will be recorded in the originally completed CRF.

The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

9.2.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, institutional review boards (IRBs)/independent ethics committees (IECs), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

9.2.5. Cardiovascular and Death Events

Not applicable

9.2.6. Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

Not applicable

9.2.7. Pregnancy

- Details of all pregnancies in female participants will be collected after the start of study treatment and until 30 days after the last dose.

[redacted]

9.2.8. Adverse Events of Special Interest

9.2.8.1. Adverse Events of Special Interest

AEs of special interest are defined as AEs of sphincter of Oddi spasm, pancreatitis, and severe constipation. These events must be reported to the sponsor expeditiously, as described below. Given the μ-opioid receptor agonism of eluxadoline, there is a potential for increased risk of sphincter of Oddi spasm, resulting in pancreatitis or hepatic enzyme elevation associated with acute abdominal pain (eg, biliary-type pain), especially during the first few weeks of treatment, in participants taking eluxadoline, particularly in participants without a gallbladder. Pancreatitis, with or without sphincter of Oddi spasm has been reported in patients taking eluxadoline, including serious cases resulting in hospitalization, primarily in patients without a gallbladder. Fatal cases have also been reported in patients without a gallbladder.

Participants must be instructed to stop the study treatment and seek medical attention if they experience symptoms suggestive of sphincter of Oddi spasm such as acute worsening of abdominal pain (eg, acute epigastric or biliary [ie, right upper quadrant] pain) that may radiate to the back or shoulder, with or without nausea and vomiting and/or if they experience symptoms suggestive of pancreatitis such as acute abdominal or epigastric pain radiating to the back.

Constipation was the most frequent AE reported in studies of eluxadoline for IBS-D. Per the US Prescribing Information, eluxadoline is to be discontinued in participants who develop severe constipation. To ensure the investigator is made aware of severe constipation (ie, the participant is experiencing severe discomfort that severely limits or prevents his/her performance of normal activities, or represents a definite hazard to health that has or could result in hospitalization and prescription drug therapy) episodes, an alert will be sent to the investigator if a participant fails to record a bowel movement for 3 consecutive days in his/her eDiary. Once notified, the investigator must contact the participant to review the participant's status as soon as possible to assess the severity of his/her constipation or the presence of any sequelae of constipation. Every attempt should be made to reach the study participant within 24 hours. An unscheduled visit to

further evaluate the participant's status should be arranged if deemed warranted by the investigator. The study treatment must be discontinued if the investigator determines that severe constipation is present.

If a participant is suspected of experiencing pancreatitis, sphincter of Oddi spasm, or severe constipation, the AE must be reported to the sponsor's SAE mailbox: IR-Clinical-SAE@allergan.com within 24 hours on an SAE Form for Clinical Trials; in the absence of scanning or emailing, the SAE form may be faxed to +1-714-796-9504.

[redacted]

- Blood pressure and pulse measurements will be assessed in a sitting position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Vital signs will be measured after at least 5 minutes rest for the participant in a quiet setting without distractions (eg, television, cell phones). One reading each of pulse, respiratory rate, and blood pressure will be taken.
- Height and weight will also be measured and recorded as specified in the SoA.

9.4.3. Clinical Safety Laboratory Assessments

- See Appendix 2, Section 12.2 for the list of clinical laboratory tests to be performed and the SoA for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 2 weeks after the last dose of study treatment 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 2, Section 12.2, must be conducted in accordance with the SoA.
    - If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

9.4.4. Suicidal Risk Monitoring

Suicidal Risk Monitoring is not applicable to this study.

9.5. Pharmacokinetics

[redacted]

[redacted]

9.6. Pharmacodynamics

The pharmacodynamic effects of eluxadoline on the altered bowel habits of IBS-D will be assessed via efficacy assessments previously described.

9.7. Genetics

Genetics are not evaluated in this study.

9.8. Biomarkers

Biomarkers are not evaluated in this study.

9.9. Health Economics or Medical Resource Utilization and Health Economics

Health economics/Medical resource utilization and health economics parameters are not evaluated in this study.

Endpoint	Description	Timing	Methodology
	score over 4 weeks of treatment period		summary

Note: Analysis visits will be in the SAP.

BSFS = Bristol Stool Form Scale

Table 10–4 Secondary Efficacy Endpoints

Endpoint	Description	Timing	Methodology
	Change from baseline in the 4-week average of daily bowel movement frequency during the treatment period	Treatment Period	Descriptive summary
	Change from baseline in the 4-week average of daily worst abdominal pain scores during the treatment period	Treatment Period	Descriptive summary
	Change from baseline in the 4-week average of daily worst abdominal pain scores during the treatment period	Treatment Period	Descriptive summary
	Change from baseline in the 4-week average of urgency-free days in a week during the treatment period	Treatment Period	Descriptive summary
	Change from baseline in the 4-week average of daily bloating scores during the treatment period	Treatment Period	Descriptive summary
	Change from baseline in IBS-QOL score at the end of the treatment period	Treatment Period	Descriptive summary
	Change from baseline in serum 7αC4 levels at the end of the treatment period	Treatment Period	Descriptive summary
	Proportion of participants with any fecal incontinence during the treatment period	Treatment Period	Descriptive summary

Note: Analysis visits will be in the SAP.

7αC4 = 7a-hydroxy-4-cholesten-3-one; IBS-QOL = irritable bowel syndrome-quality of life

10.3.3. Safety Analyses

The following safety categories will be summarized as appropriate (eg, categorical or continuous descriptives, shift tables) for the safety population and will be fully defined in the SAP.

- Serious Adverse Events
- Adverse events
- Clinical laboratory assessments
- Potential Hy's law cases
- Potential Drug Induced Liver Injury Cases

- Vital signs including weight
- Physical examinations

10.3.4. Pharmacokinetic Analyses

[redacted]

10.3.5. Other Analyses

None

10.3.6. Interim Analyses

No interim analysis will be conducted.

FSH	follicle stimulating hormone
GCP	Good Clinical Practice
GI	gastrointestinal
HRT	hormonal replacement therapy
IB	Investigator's Brochure
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome with constipation
IBS-D	irritable bowel syndrome with diarrhea
IBS-M	irritable bowel syndrome with constipation and diarrhea
IBS-QOL	irritable bowel syndrome-quality of life
IBS-U	irritable bowel syndrome (unspecified)
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
LC-MS/MS	liquid chromatography tandem-mass spectrometry
mITT	modified intent-to-treat
NCI	National Cancer Institute
NONMEM	nonlinear mixed effects modeling
PK	pharmacokinetic
PT	prothrombin time
Rome	Rome Diagnostic Criteria for Functional Gastrointestinal Disorders
SAE	serious adverse event
SAP	statistical analysis plan
SeHCAT	^{75}Selenium-homocholic acid taurine
SO	sphincter of Oddi
SoA	schedule of activities
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	half-life
t_{max}	time to C_{max}
ULN	upper limit of normal
V	apparent volume of distribution
WOCBP	woman of childbearing potential

12.3. Appendix 3: Study Governance Considerations

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 (CIOMS) international ethical guidelines
- Applicable ICH GCP guidelines
- Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
    - 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 overall conduct of the study at the site and adherence to requirements of applicable local regulations, for example 21 CFR, ICH guidelines, the IRB/IEC, and European regulation 536/2014 for clinical studies (if applicable)

Financial Disclosure

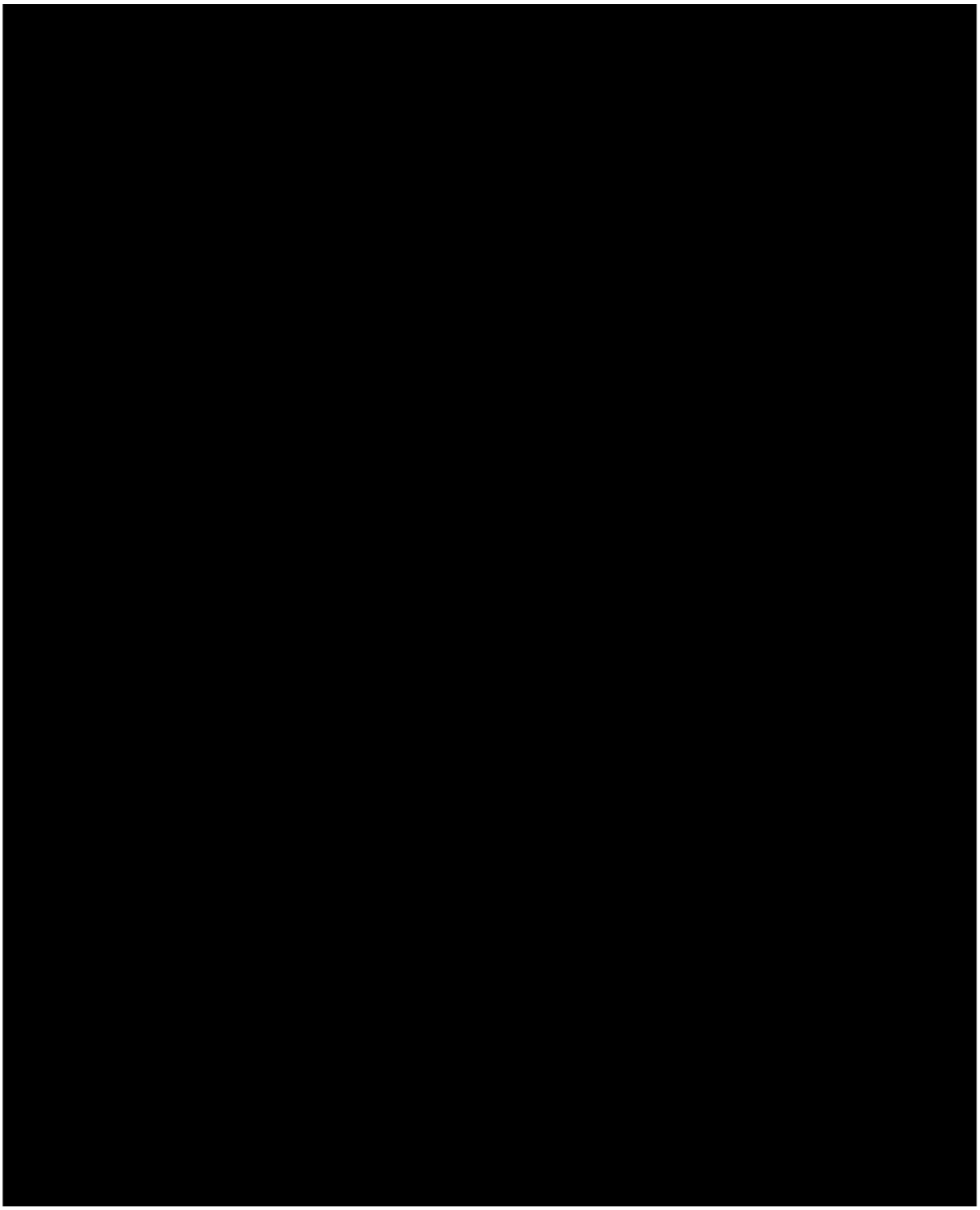
Investigators and subinvestigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, electrocardiograms [ECGs], radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease) • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition • New condition detected or diagnosed after study treatment administration even though it may have been present before the start of the study • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • Lack of efficacy or failure of expected pharmacological action per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AEs or SAEs if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition • The disease/disorder being studied or expected progression, signs, or symptoms (clearly defined) of the disease/disorder being studied, unless more severe than expected for the participant's condition • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital) • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen



[redacted]

2. Cohort 2: IBS-D participants without evidence of BAM treated with eluxadoline 100 mg oral tablets BID with food.

Study Design

Period	Screening[a]	Pretreatment[a]	Open-Label Treatment			Post-Treatment
Duration	0-2 weeks	2-3 weeks	4 weeks			2 weeks
Week	Screening	Pretreatment	Baseline	Week 2[b]	Week 4 (EOT/ET)	Week 6[b] (Follow-up)
Study Visit	1	2	3	4	5	6
Study Day			1	15 (±2)	29 (±2)	Visit 5 + 14 days (±7)

7αC4 = 7a-hydroxy-4-cholesten-3-one; BA = bile acid

[a] Screening and pretreatment periods may be combined. if results of fasting serum 7αC4 test or 48-hour fecal BA collection conducted within 1 calendar year from screening are available at the time of the combined visit or results from fasting serum 7αC4 test are available prior to the start of study treatment..

[b] May be done over the phone.

The total duration of study participation for each participant will be up to 11 weeks.

Key inclusion criteria:

1. Adult men or women aged 18 to 75 years inclusive with a diagnosis of IBS-D per Rome IV criteria.
2. Participants with evidence of BAM must have at least one of the following at screening or within 1 calendar year prior to screening:
   - fasting serum 7a-hydroxy-4-cholesten-3-one (7αC4) level ≥ 52.5 ng/mL
   - total fecal bile acids > 2337 micromoles/48 hours
   - primary bile acids (fecal CA and CDCA) ≥ 10% in a 48h fecal collection
   - primary bile acids (fecal CA and CDCA) ≥ 4% with total fecal bile acid ≥ 1,000 micromoles/48hr
3. .Participants without BAM must have at least one of the following at screening or within 1 calendar year prior to screening:
   - fasting serum 7αC4 level ≤ 47.1 ng/mL
   - fasting serum 7αC4 levels > 47.1 ng/mL but < 52.5 ng/mL with fecal bile acids that are negative for bile acid malabsorption (i.e. do not meet criteria 2.03 based on fecal bile acids level)
   - total fecal bile acids (BA) ≤2337 micromoles/48 hours

[redacted]

5. Study Design

5.1. Overall Design

This is a Phase 4, open-label, parallel-group, cohort controlled study to evaluate the efficacy, safety, tolerability and pharmacokinetics of eluxadoline in participants meeting the Rome IV criteria for IBS-D with and without evidence of BAM. This study will be comprised of a 0 to 2-week screening period, a 2 to 3-week pretreatment period, a 4-week open-label treatment period during which time participants will receive eluxadoline 100 mg BID, and a 2-week post-treatment safety follow-up.

There will be 24 participants enrolled in the study, 12 with evidence of BAM and 12 without evidence of BAM.

- Cohort 1: IBS-D participants with evidence of BAM treated with eluxadoline 100 mg oral tablets BID with food
- Cohort 2: IBS-D participants without evidence of BAM treated with eluxadoline 100 mg oral tablets BID with food

As far as possible, the 2 cohorts will be matched by age, gender and severity of historic symptoms based on the investigator's discretion. Evaluation of IBS-D symptoms during the pretreatment period will establish the baseline of IBS-D severity and will continue for the duration of the study. Measurements of stool consistency scores and other IBS-D signs and symptoms will be measured daily using an electronic diary (eDiary).

After screening procedures have been performed (up to 2 weeks), eligible participants will enter a pretreatment period of 2 to 3 weeks. At the beginning of the pretreatment period participants will receive instructions for completing the eDiary to collect daily information related to their IBS-D symptoms including stool consistency (BSFS), worst abdominal pain, abdominal bloating, bowel movement frequency, rescue medication usage, number of episodes of urgency in a day, and number of episodes of fecal incontinence, if any. At the conclusion of the pretreatment period, participants who meet the study entry criteria related to stool consistency, eDiary compliance, and rescue loperamide use will be enrolled to receive a 4-week open-label eluxadoline 100 mg BID treatment.

Participants enrolled in the treatment period will return to the study site for study visits at Week 2, Week 4 (end-of-treatment study visit), and for a post-treatment follow-up study visit at Week 6. Telephone contacts may be made to participants in lieu of an on-site visit at Week 2. A complete schedule of activities (SoA) is provided in Section 2. Participants who discontinue from the study before the Week 4 visit should return to the study site to complete the early termination assessments as soon as possible after stopping the study drug.

During the open-label treatment period, participants will record via the eDiary their daily IBS-D symptoms including stool consistency (BSFS), worst abdominal pain, abdominal bloating, bowel movement frequency, rescue medication usage, number of episodes of urgency in a day, and number of episodes of fecal incontinence, if any.

In order to have comparable treatment groups, the cohorts will be matched to the highest extent possible by age, gender and severity of historic symptoms based on the investigator's discretion.

Improvements in bowel symptoms will be measured during the study. Stool consistency is a highly sensitive efficacy marker, making it ideally suitable to detect potential differences in responsiveness to treatment among participants with and without evidence of BAM. The primary outcome measure for this study will therefore be changes from baseline in stool consistency scores over the 4-week treatment period. Changes from baseline are considered more appropriate than monthly responder definitions given the small pilot nature of this study.

Table 5-2 Current and future and bile acid diarrhea (BAD) diagnostic tests

Diagnostic Test	Fasting serum C4	Total fecal BA	Primary BA >4% + total fecal BA	Fecal primary BA >10%
What does it measure	Hepatic bile acid synthesis	Total fecal bile acid excreted from the colon	Amount of bile acids with secretory potential with total fecal BA excretion	Amount of bile acids that are directly synthesized from the liver with secretory potential
Diagnostic Cutoffs	$\geq$52.5 ng/mL	$\geq$ 2,337 µmol/48h	Primary BA $\geq$ 4% + total fecal BA $\geq$ 1,000 µmol/48h	$\geq$ 10% Primary BA
Sensitivity relative to fecal weight >400g/48h	15%	59%	46%	49%
Specificity relative to fecal weight >400g/48h	86%	92%	97%	91%

Source: Vijayvargiya 2018

5.5. Justification for Dose

The 100 mg BID dose selected for this study is consistent with US and global labeling for eluxadoline in IBS-D.

Baseline

Day 1 (Visit 3)

On Day 1, participants who were deemed eligible during the pretreatment period will be dispensed study treatment (eluxadoline 100 mg BID).

[redacted]

Open-Label Treatment Period

During the treatment period, participants will undergo the assessments and procedures listed in the SoA.

[redacted]

9.2.9. Medication Errors

Medication error refers to any unintended error in the dosing and/or administration of the study treatment as per instructions in the protocol. Medication errors generally fall into 4 categories as follows:

- Wrong study drug/device
- Wrong dose (including dosing regimen, strength, form, concentration, amount)
- Wrong route of administration
- Wrong participant (ie, not administered to the intended participant)

9.3. Treatment of Overdose

Please refer to the US prescribing information (VIBERZI PI 2017) for the treatment to be given in case of overdose.

[redacted]

9.4. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA.

9.4.1. Physical Examinations

- A physical examination will consist of a full review of all body systems (excluding rectal and pelvic examinations) systems.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.4.2. Vital Signs

- Pulse, respiratory rate, and blood pressure will be assessed.

10. Statistical Considerations

10.1. Sample Size Determination

The total sample size for this study is 24 participants (12 participants with BAM and 12 participants without BAM). No formal sample size estimation will be calculated because the sample size for this study is not based on statistical consideration.

10.2. Populations for Analyses

The analysis populations will consist of participants as defined in Table 10–1.

Table 10–1 Analysis Populations

Population	Definition	Study Treatment
Screened	All screened participants who sign informed consent	—
Enrolled	All participants in Screened Population who meet the eligibility criteria and enter the open-label treatment period	Assignment
Modified intent-to-treat (mITT)	All participants in Enrolled Population with ≥ 1 postbaseline assessment for BSFS	Assignment
Safety	All participants who received ≥ 1 administration of study treatment	Actual received

BSFS = Bristol Stool Form Scale

10.3. Statistical Analyses

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

10.3.1. Key Statistical Methodology

The methodologies defined in Table 10–2 apply as specified to individual endpoints. All efficacy endpoints will be summarized by participant cohort (BAM or Non-BAM) descriptively.

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δOR	delta-opioid receptor
κOR	kappa-opioid receptor
μOR	mu-opioid receptor

21 CFR Part 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study site.

- The medical record must include a statement that written informed consent was obtained before the participant 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.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

Participants who are rescreened are required to sign a new ICF.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Committees Structure

Publication Policy

- Allergan as the sponsor has proprietary interest in this study. Authorship and manuscript composition will reflect joint cooperation between the investigator and Allergan personnel. Authorship will be established prior to the writing of the manuscript.
- 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.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Definition of SAE

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

An SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life threatening The term *life threatening* in the definition of *serious* refers to an event in which the participant 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.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant 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.
d. 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 (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: • Medical or scientific judgment should be exercised in deciding whether 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 participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

[redacted]