TITLE PAGE

Protocol Title:	A Phase 2 Randomized, Double-blind, Placebo-controlled, Adaptive Study to Evaluate the Efficacy, Safety, and Tolerability of 2 Dose Levels of IW-3300 Administered Rectally for 12 Weeks to Treat Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Short Title:	A Phase 2 Study of IW-3300 for the Treatment of Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Protocol Number:	C3300-201
Version Number:	3.0
Approval Date:	26 February 2024
Study Intervention:	IW-3300
Study Phase:	2
Sponsor:	Ironwood Pharmaceuticals, Inc. 100 Summer Street, Suite 2300 Boston, MA 02110 USA
Medical Monitor **Study Contact:**	Medical monitor and study contact information will be provided separately.

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PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 2 (Version 3.0)	26 February 2024
Amendment 1 (Version 2.0)	06 December 2022
Original Protocol (Version 1.0)	07 July 2022

Amendment 2 (26 February 2024)

Overall Rationale for the Amendment:

Additions, clarifications, and corrections were made to improve the protocol.

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1 (Synopsis) and Section 3 (Objectives, Estimands, and Endpoints)	The exploratory endpoint "Weekly change from baseline (CFB) in mean nighttime voiding frequency (nocturia)" will be assessed using data collected from an existing "Urinary Frequency" eDiary question rather than from a "Difficulty Sleeping" eDiary question. Furthermore, a new exploratory endpoint "Monthly CFB in mean nighttime voiding frequency (nocturia)" will be added. This endpoint will be assessed using data collected in the existing O'Leary/Sant Interstitial Cystitis Symptom Index.	The original data assessing nocturia relied on the subject's responses to the "Difficulty Sleeping" eDiary question that featured a qualitative response scale. To allow for more precision in the "nighttime voiding frequency" endpoint analysis, the question that will be used to assess nocturia will be taken from the "Urinary Frequency". eDiary question that captures the actual number of urinations that caused the subject to wake from sleep. In addition to assessing the weekly change from baseline, in "nighttime voiding frequency", this endpoint will also be assessed in terms of the monthly change from baseline using the O'Leary/Sant Interstitial Cystitis Symptom Index that is already included in the protocol. Because this questionnaire is completed on a monthly basis, it will provide another analysis of the effect of IW-3300 on "nighttime voiding frequency" based on a longer time range for comparison.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1 (Synopsis) and Section 3 (Objectives, Estimands, and Endpoints)	An exploratory endpoint was added, "Weekly treatment satisfaction."	The addition of this endpoint reflects the plan to analyze the responses to the existing eDiary question that asks subjects about treatment satisfaction.	Substantial
Section 1.1 (Synopsis) and Section 3 (Objectives, Estimands, and Endpoints)	The estimand describing "Strategy for addressing intercurrent event" was revised to address the intercurrent events of taking rescue medication and premature discontinuation due to lack of treatment efficacy.	This change adds specificity to the types of estimands associated with intercurrent events.	Substantial
Section 1.3 (Schedule of Activities [Table 1])	The time window (≤15 minutes) for the Day 1 predose pharmacokinetic (PK) sample was removed.	The time window was deemed unnecessary. The Day 1 predose PK sample can now be drawn when convenient and ideally with the predose clinical chemistry and hematology blood draws.	Substantial
Section 4.1.1.1 (Screening Period)	The window for the Screening Period was expanded by 15 days to accommodate subjects who need time to complete additional procedures or medication washouts.	This time window expansion will accommodate subjects who need additional time to complete Screening.	Substantial
Section 5.1 (Inclusion Criteria)	Inclusion criterion #1 was revised to increase the upper limit of the eligible age range from 70 to 75 years.	The increase in the acceptable age range will expand study access to more subjects.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #1 was revised to specify that benign prostatic hyperplasia would only be excluded if it is characterized by active symptoms that are difficult to distinguish from IC/BPS.	This change was added to clarify the type of benign prostatic hyperplasia that would lead to study exclusion.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #2 was revised as follows *(new text in **bold** and deleted text in ~~strikethrough~~)*: Subject has **pain due to another medical condition (eg,** pelvic floor dysfunction ~~or~~ vulvodynia**, or endometriosis)** that~~,~~ in the opinion of the investigator~~,~~ would be difficult **for the investigator or the subject** to discriminate from bladder pain **(which can include sensations like burning, pressure, and/or discomfort).**	To minimize potentially confounding background pain, the entry criterion was broadened to exclude subjects with any other medical condition that features pain that cannot be distinguished from bladder pain. Pelvic floor dysfunction, vulvodynia, and endometriosis are listed as possible examples of such conditions, but this is not an exhaustive list. The criterion was also revised to incorporate the necessity of the subject, and not just the investigator, to be able to discriminate their bladder pain from other pain-causing conditions.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #4 was corrected to state that colorectal cancer, and not rectal cancer, is an excluded diagnosis.	Correction	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #12 was revised to exclude subjects who have a planned surgical procedure between screening and the EOS phone call.	Subjects with a planned surgical procedure will be excluded because surgical procedures could confound the results of the study.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #13 was revised to exclude subjects who have a planned cystoscopy for any reason between screening and the EOS phone call.	Subjects with a planned cystoscopy will be excluded because cystoscopy could confound the results of the study.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #14 was revised to exclude subjects who have drug-induced cystitis.	This exclusion criterion will eliminate a potential confounding factor.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #15 was updated to address the permissibility of sacral neuromodulation devices. Subjects will be excluded if they are actively using a sacral neuromodulation device. Note: active use of the device is defined as using it within 12 months of the screening visit. If the subject has not used their device in the last 12 months and agrees to have the device off for the duration of the study, they may be eligible. A discussion with the medical monitor is required.	The exclusion of active sacral neuromodulation device use will eliminate a potential confounding factor.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #16 was modified to state that a positive result for benzodiazepines or barbiturates is allowed if they are being used for a medical condition other than IC/BPS.	This change reflects the permissibility of these medications for indications other than IC/BPS.	Substantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #20 was revised to remove text that excluded subjects who previously entered the Pretreatment Period.	Subjects who enter the Pretreatment Period but are not randomized for any reason ***not*** included in Section 5.4 will be eligible to rescreen. The change preserves access to the study for subjects who may have not been randomized due to a logistical reason.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.4 (Screen Failures)	A statement was added to ensure that subjects who failed screening due to Inclusion Criterion #4 or #5 (ie, they did not meet the eDiary eligibility criteria) will no longer be allowed to rescreen.	Enrollment of subjects who failed to meet the eDiary compliance or average daily pain threshold during their initial Pretreatment evaluation could confound the results of the study.	Substantial
Section 6.5 (Concomitant Therapy)	Subjects were previously required to abstain from any concomitant medication that could interfere with the study, from 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of the Pretreatment Visit until completion of the EOS Follow-up Phone Call. The text was revised to allow subjects to continue taking concomitant medications that pose no risk for interference with the with the study, as long as the subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow up Phone Call.	The change is consistent with the guidance for concomitant medications given in Section 6.5.1, and it simplifies the rules for managing concomitant medications that do not pose a risk for interference with the study.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.5.1 (Prohibited Medications, Medications Requiring a Washout Period, and Medications Permitted Under Select Circumstances)	The washout window for intravesical therapy and pentosan polysulfate (ELMIRON) was changed from 3 months to 30 days. Immunosuppressants/ immunomodulators for IC/BPS (eg, cyclosporine) have been added to the list of prohibited medications that require a washout (30-day period).	The shorter washout period for intravesical therapy and pentosan polysulfate will reduce barriers to study participation and reflects the pharmacokinetic-pharmacodynamic characteristics of pentosan polysulfate. Prohibition of immunosuppressants/ immunomodulators was added based on expert opinion that individuals receiving these medications likely have a different phenotype of IC/BPS (ie, autoimmune driven disease phenotype). Therefore, including these subjects may confound the interpretation of efficacy data based on the mechanism of action of IW-3300.	Substantial
Section 6.5.1 (Prohibited Medications, Medications Requiring a Washout Period, and Medications Permitted Under Select Circumstances)	Benzodiazepines were added to the list of therapies permitted for a medical condition other than IC/BPS (ie, anxiety) if a subject has been on a stable dose/regimen for ≥30 days prior to Pretreatment and plans to continue on the same dose/regimen until after the EOS Follow up Phone Call.	This addition will reduce a barrier to study participation.	Substantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.5.2 (Prohibited Interventions)	This new section defines interventions that are prohibited between screening and the EOS Follow-up Phone Call: • Cystoscopy with or without hydrodistension • Surgical procedures unless specifically discussed and permitted by the medical monitor • Active use of sacral neuromodulation devices (eg, Interstim).	These interventions are prohibited because they could confound the results of the study.	Substantial
Section 1.1 (Synopsis) and Section 3 (Objectives, Estimands, and Endpoints)	A clarification was added to the first exploratory endpoint (Weekly average CFB in daily bladder pain at its worst) to state that the Week 12 timepoint is the primary endpoint.	Clarification	Nonsubstantial
Section 4.4 (End of Study Definition)	The end of study (EOS) definition for the entire study can include the date of either the last visit or EOS Phone Call for the last subject in the study.	This update reflects the possibility that a subject might miss the EOS Phone Call.	Nonsubstantial
Section 5.2 (Exclusion Criteria)	Exclusion criterion #18. The example of painful kidney stones was added to enhance investigators' understanding of the type of acute or chronic conditions that could limit a subject's ability to participate in the study (ie, conditions that may cause pain in the urinary tract). A statement was added to encourage investigators to discuss conditions with the medical monitor.	Clarification. The enrollment criterion has not changed substantially; the additions will enhance the investigators' ability to enroll appropriate subjects.	Nonsubstantial
Section 6.2 (Packaging, Labeling, Storage, and Accountability)	The lower end of Fahrenheit temperature range was changed from 35ºF to 36ºF.	Correction	Nonsubstantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.3.2 (Blinding)	The Ironwood Pharmaceutical Development Manufacturing & Sciences Group was added to the list of study personnel who may be unblinded to treatment assignment for the purposes of supplying study drug.	Clarification. This practice has been in place since study initiation; the text is being added to reflect current practice.	Nonsubstantial
Section 6.4 (Study Intervention Compliance)	The statement that deviations from the prescribed dosage regimen should be documented as a protocol deviation was removed.	Deviations from the prescribed dosing regimen will continue to be captured, but the specific guidelines are described in another document.	Nonsubstantial
Section 6.5.3 (Rescue Medicine)	Text was added to clarify that either ibuprofen or acetaminophen may be used as rescue medicine at any given time. Sites will dispense one of these medicines, not both. If the subject wants to switch from acetaminophen to ibuprofen or from ibuprofen to acetaminophen, and the investigator feels it is medically appropriate to switch, the site may dispense new rescue medication. Text was added to clarify that the investigator and medical monitor will review use of rescue medication during the Treatment Period (and not during the Pretreatment Period) to determine if the subject should continue in the study.	Clarifications. These practices have been in place since study initiation; the text is being revised to reflect current practice.	Nonsubstantial
Section 7.2 (Subject Discontinuation/With drawal from the Study)	Text was revised to correct the statement that subjects who permanently discontinue study intervention will also discontinue from the study *at the same time*.	Correction. The phrase “at the same time” was removed to reflect the EOS Follow-up Phone Call that subjects should complete approximately 2 weeks after they discontinue study intervention.	Nonsubstantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 8.1.2 and Section 10.5.6 (Margolis Body Map for Pain Assessment)	The instructions for completing the Margolis Body Map Pain Assessment were updated to match those that accompany the assessment.	Correction	Nonsubstantial
Section 8.2.5 (Pregnancy Testing) and Section 10.2 (Clinical Laboratory Tests)	For consistency with the Schedule of Assessments, text was added to reflect that pregnancy testing is also required on Day 1 (before the first dose).	Correction	Nonsubstantial
Section 8.2.7 (Suicidal Ideation and Behavior Risk Monitoring), Section 8.6 (Health-related Quality of Life), and Section 9.4.8 (Health-related Quality of Life)	The Hospital Anxiety and Depression Scale is a health-related quality of life assessment and not related to suicidal ideation and behavior monitoring. Thus, it was relocated to the correct section of the protocol.	Correction. The text was moved from Section 8.2.7 to Section 8.6 and reference to HADS scores was added to Section 9.4.8.	Nonsubstantial
Section 9.3 (Populations for Analysis, Table 2)	For the Safety, PK, and Microbiome Analysis Sets, a footnote was added to define "treatment actually received". The Microbiome Analysis Set was revised to reflect that subjects need to have both a baseline sample and at least 1 postbaseline sample.	Clarification	Nonsubstantial
Section 10.1.7 (Data Quality Assurance)	Text was added to describe processes for identifying, monitoring, and reporting on quality tolerance limits (QTLs).	Clarification. These practices were in effect before the start of the study; the text is now being added to reflect the prior and ongoing practice.	Nonsubstantial
Global	Throughout the document, the time window >30 days was changed to ≥30 days.	Correction. The time window now reflects prior and ongoing practice.	Nonsubstantial

Section # (Name)	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Global	Throughout the document, minor edits were made to correct typographical errors and improve readability.	Corrections	Nonsubstantial

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title

A Phase 2 Randomized, Double-blind, Placebo-controlled, Adaptive Study to Evaluate the Efficacy, Safety, and Tolerability of 2 Dose Levels of IW-3300 Administered Rectally for 12 Weeks to Treat Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome

Short Title

A Phase 2 Study of IW-3300 for the Treatment of Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome

Rationale

The aim of this study is to assess the efficacy, safety, and tolerability of IW-3300 for the treatment of interstitial cystitis/bladder pain syndrome (IC/BPS). IC/BPS is a chronic condition characterized by bladder pain that is usually accompanied by urinary urgency, increased frequency, and/or nocturia. The morbidity of IC/BPS is substantial and often leads to poor quality of life for patients, their families, and day-to-day caregivers.(1) It is estimated that 3% to 7% of women and 3% to 4% of men have IC/BPS.(2) At this time, there are no effective treatments targeting bladder pain in IC/BPS. A safe and effective treatment would greatly improve the quality of life for this patient population.

Objectives, Estimands, and Endpoints

Objectives	Estimands	Endpoints
Primary		
Investigate the efficacy of IW-3300, administered as a rectal foam, on bladder pain in subjects with IC/BPS	See table below	Change from baseline (CFB) in weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12
Secondary		
Investigate the efficacy of IW-3300, administered as a rectal foam, on the components of bladder pain in subjects with IC/BPS	--	• CFB in weekly average of a burning sensation in the bladder at its worst at Week 12 • CFB in weekly average of a pressure sensation in the bladder at its worst at Week 12 • CFB in weekly average of discomfort in the bladder at its worst at Week 12 • CFB in Genitourinary Pain Index (GUPI) Pain subscale score at Week 12

Objectives	Estimands	Endpoints
Investigate the safety and tolerability of IW-3300, administered as a rectal foam, in subjects with IC/BPS	--	• Frequency of TEAEs occurring in ≥2% of subjects • Overall frequency of TEAEs by severity grade
Exploratory		
	--	• Weekly average CFB in daily bladder pain at its worst (Note: Weekly average CFB to Week 12 is the primary endpoint.) • Weekly average CFB in daily bladder pain-free days • Weekly average CFB in urinary urgency • Weekly CFB in mean urinary frequency • Weekly CFB in mean nighttime voiding frequency (nocturia) • Monthly CFB in mean nighttime voiding frequency (nocturia) • CFB in O'Leary/Sant Interstitial Cystitis Symptom Index (ICSI) total score • CFB in GUPI Urinary subscale score • CFB in GUPI Quality of Life (QoL) subscale score • CFB in Global Response Assessment (GRA) total score • CFB in the sexual function question score • CFB in sleep difficulty due to bladder pain • CFB in sleep difficulty due to a need to urinate • CFB in the body pain category as assessed using the Margolis body map • CFB in IC/BPS symptom severity • IC/BPS global impression of symptom change • 6/12 Week Adequate Relief of IC/BPS Pain Responder • CFB in EuroQol 5-Dimension 5-Level Version (EQ-5D-5L) utility score and Visual Analog Scale • Weekly treatment satisfaction • CFB in 16s RNA based analysis of gut microbiome composition at the genus level • PK parameters of IW-3300 and metabolites in plasma

The following estimand is a precise description of the primary efficacy treatment effect to be estimated:

Definition	**Attributes**			
	Population	**Variable (or endpoint)**	**Strategy for addressing intercurrent event**	**Population-level summary**
The primary estimand is the effect of IW-3300 compared with placebo at Week 12 in weekly average of daily bladder pain at its worst scores	18 to 75 years of age with IC/BPS defined through inclusion and exclusion criteria as stated in the protocol	Change from baseline in weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12	-Taking rescue medication (Hypothetical strategy) -Premature discontinuation due to lack of treatment efficacy (Hypothetical strategy)	Difference in mean change of weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12 between active (IW-3300) and placebo groups

Overall Design

This is a Phase 2, randomized, placebo-controlled, parallel-assignment, 12-week, adaptive-design study to evaluate the efficacy, safety, and tolerability of IW-3300 in subjects with IC/BPS. Subjects will initially be randomized 1:1:1 to IW-3300 100 µg, IW-3300 300 µg, or matching placebo administered as a rectal foam.

As shown in Figure 1, the study consists of 4 periods: a Screening Period, a Pretreatment Period, a 12-week Treatment Period, and a 2-week Follow-up Period. The assessments conducted during each period are shown in the Schedule of Activities (SoA)/Table 1.

During the Screening Visit, subjects will undergo preliminary screening procedures to ensure that they meet the eligibility criteria for the study. Subjects requiring additional procedures or a medication washout will have up to 30 days to become eligible to enter the Pretreatment Period. The end of the Screening Period coincides with the beginning of the Pretreatment Period. During the Pretreatment Visit, subjects will receive training on how to complete the daily and weekly electronic diary (eDiary) questions at home and starting that night, subjects will answer the eDiary questions to establish a baseline during the study. Eligibility to continue into the Treatment Period of the study will be based on the answers recorded in the eDiary during the 14 days prior to Day 1. Subjects who meet the entry criteria will be randomized on Day 1 and continue into the Treatment Period. On Day 1 of the Treatment Period, randomized subjects will receive the first dose of study drug, formulated as a rectal foam, and administered by the subject under supervision of the site staff. Subjects will also receive training on how to self-administer the study drug at home. For the next 12 weeks, subjects will self-administer study drug at approximately the same time each day. Subjects will also continue to answer the daily and weekly eDiary questions. Subjects will have routine study visits at Week 1, Week 4, Week 8, and Week 12/End of Treatment (EOT) for completion of efficacy, safety, and other assessments.

Two weeks after the last day of dosing, an End of Study (EOS) Follow-up Phone Call will be conducted to assess the subject for AEs and medications taken since the Week 12/EOT Visit.

At least 1 interim analysis (IA) will be performed during the study to provide an early assessment of the efficacy or futility of IW-3300. The purpose of the IA(s) is to stop the study early for efficacy if there is overwhelming evidence of effectiveness, to discontinue a treatment arm if it does not warrant further exploration, or to stop enrollment early for futility. Details will be provided in an IA plan and a DMC Charter.

Disclosure Statement

This is a 12-week, double-blind, parallel-group, 3-arm study to evaluate the efficacy, safety, and tolerability of 2 dose strengths of IW-3300 (100 μg and 300 μg) or matching placebo, administered once daily as a rectal foam.

Number of Subjects

Approximately 300 subjects will be randomly assigned to study intervention. The sample size of approximately 300 subjects represents the maximum number of subjects to be enrolled.

Intervention Groups and Duration

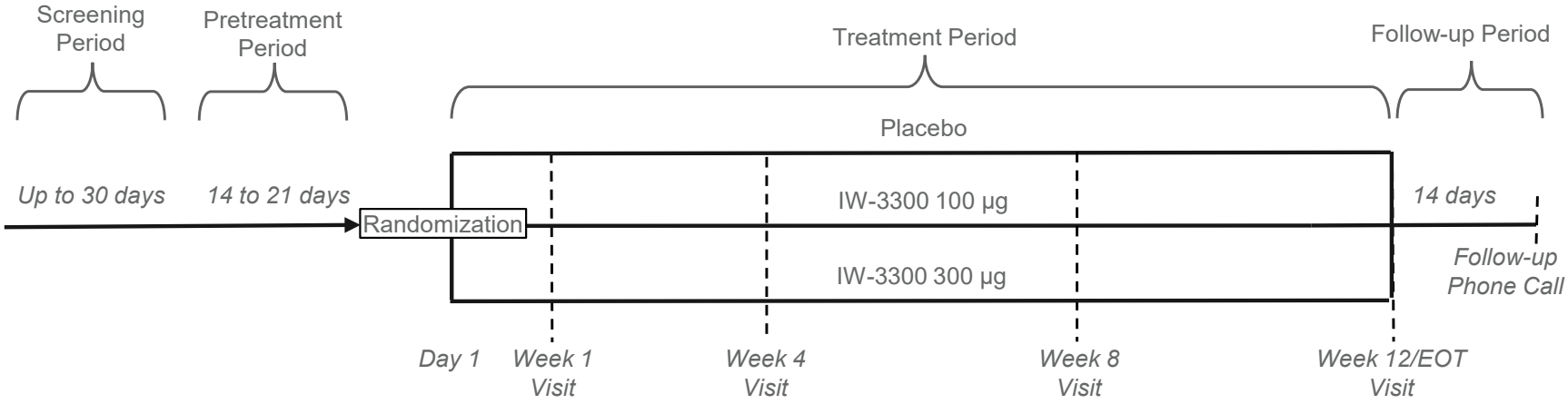
Subjects will initially be randomized in a 1:1:1 ratio to receive IW-3300 100 μg, IW-3300 300 μg, or matching placebo. Study drug will be administered rectally once daily for 12 weeks.

Data Monitoring Committee

An independent DMC will be used to regularly monitor safety and tolerability. Additionally, independent review will be conducted to evaluate efficacy of IW-3300 as part of the IA for the purpose of early stopping or discontinuation of a study arm based on prespecified efficacy or futility decision rules documented in the IA plan. Details of the DMC membership, standard operational procedures for data monitoring/review, frequency of review, and other pertinent details will be provided in a separate DMC Charter.

1.2. Schema

Figure 1: Schematic of Study Design



1.3. Schedule of Activities

Table 1: Schedule of Activities

Study Period→		Screening Period (Up to 30 days)	Pretreatment Period (14 to 21 days)	Treatment Period (12 weeks)					Follow-up Period (2 weeks)
Study Procedure ↓	**Visit/Day →**	**Screening Visit (Day -51 to Day -15)[a]**	**Pretreatment Visit (Day -21 to Day -14)**	**Random-ization Visit/ Day 1**	**Week 1 Visit (Day 7±3)**	**Week 4 Visit (Day 28±3)**	**Week 8 Visit (Day 56±3)**	**Week 12/EOT Visit (Day 84+3)**	**End of Study (EOS) Follow-up Phone Call (Day 98±3)**
Informed consent		X							
Eligibility criteria		X	X	X*					
IWRS registration		X	X	X**	X	X	X	X	X
Medical history and IC/BPS characteristics[b]		X							
Demographics		X							
Body weight & height[c]		X		X	X	X	X	X	
Urine alcohol and drug screen		X							
HIV antibody and hepatitis panel		X							
Physical examination[d]		X		X	X	X	X	X	
Visual rectal examination[e]		X		X	X	X	X	X	
Digital rectal exam		X						X	
Seated vital signs[f]		X		X*	X	X	X	X	
12-lead ECG		X		Post: 1h[g]				X	

Study Period→		Screening Period (Up to 30 days)	Pretreatment Period (14 to 21 days)	Treatment Period (12 weeks)					Follow-up Period (2 weeks)
Study Procedure ↓	**Visit/Day →**	**Screening Visit (Day -51 to Day -15)[a]**	**Pretreatment Visit (Day -21 to Day -14)**	**Random-ization Visit/ Day 1**	**Week 1 Visit (Day 7±3)**	**Week 4 Visit (Day 28±3)**	**Week 8 Visit (Day 56±3)**	**Week 12/EOT Visit (Day 84+3)**	**End of Study (EOS) Follow-up Phone Call (Day 98±3)**
Clinical chemistry, hematology, urinalysis		X		X**	X	X	X	X	
ESR and CRP				X**	X	X	X	X	
Pregnancy test[h]		X		X*		X	X	X	
Hemoccult test[i]		X						X	
Prohibited medications washout instructions		X							
Prior and concomitant medications		X	X	X*	X	X	X	X	X
AE monitoring[j]		X	X	X	X	X	X	X	X
eDiary training[k]			X						
Daily and weekly eDiary assessments[l]			X						
GUPI (weekly)				X**	X				
GRA (weekly)					X				
Monthly eDiary assessment O'Leary/Sant ICSI[m]				X**		X	X	X	

Study Period→		Screening Period (Up to 30 days)	Pretreatment Period (14 to 21 days)	Treatment Period (12 weeks)					Follow-up Period (2 weeks)
Study Procedure ↓	**Visit/Day →**	**Screening Visit (Day -51 to Day -15)[a]**	**Pretreatment Visit (Day -21 to Day -14)**	**Random-ization Visit/ Day 1**	**Week 1 Visit (Day 7±3)**	**Week 4 Visit (Day 28±3)**	**Week 8 Visit (Day 56±3)**	**Week 12/EOT Visit (Day 84+3)**	**End of Study (EOS) Follow-up Phone Call (Day 98±3)**
EQ-5D-5L[n]				X**		X	X	X	
HADS[o]				X**				X	
Margolis body map for pain assessment[p]				X**		X	X	X	
Compliance verification and reminder for the eDiary[q]				X*	X	X	X	X	
Randomization				X**					
Rescue medicine dispensed			X						
Study drug dispensed				X		X	X		
Study drug administration training				X**					
Study drug administration[r]				X					
PK blood draws[s]				Predose. Postdose (1h [±15min])		X	X	X	
Stool collection[t]				X				X	

Study Period→		Screening Period (Up to 30 days)	Pretreatment Period (14 to 21 days)	Treatment Period (12 weeks)					Follow-up Period (2 weeks)
Study Procedure ↓	**Visit/Day →**	**Screening Visit (Day -51 to Day -15)[a]**	**Pretreatment Visit (Day -21 to Day -14)**	**Random-ization Visit/ Day 1**	**Week 1 Visit (Day 7±3)**	**Week 4 Visit (Day 28±3)**	**Week 8 Visit (Day 56±3)**	**Week 12/EOT Visit (Day 84+3)**	**End of Study (EOS) Follow-up Phone Call (Day 98±3)**
Study drug accountability[u]					X	X	X	X	
Follow-up phone call[v]									X

AE=adverse event; CRP=c-reactive protein; ECG=electrocardiogram; eCRF=electronic case report form; eDiary=electronic diary; EOS=end of study; EOT=end of treatment; EQ-5D-5L= EuroQol 5-Dimension 5-Level; ESR=erythrocyte sedimentation rate; GRA=Global Response Assessment; GUPI=Genitourinary Pain Index; h=hour; HADS=Hospital Anxiety and Depression Scale; ICF=informed consent form; ICSI= Interstitial Cystitis Symptom Index; IWRS=interactive web response system; min=minute; PK=pharmacokinetic; Post=postdose; Pre=predose; WOCBP=women of childbearing potential.

* Assessment done prior to randomization; ** Assessment done predose

[a] After discussion with the medical monitor, the Screening Period may be extended by up to 15 days (to Day -66) for subjects requiring additional procedures (eg, cystoscopy, ureter stents) or a medication washout (refer to Section 4.1.1.1).

[b] Site staff will complete the Prior IC/BPS Symptom Management Assessment (refer to Section 8.2.6).

[c] Height will only be measured at the Screening Visit.

[d] A complete physical examination at Screening. At all other visits, physical examinations will be symptom-directed.

[e] The visual rectal examination is a visual inspection of the perianal area. At visits other than Screening and Week 12/EOT (ie, the 2 visits when a digital rectal examination is required), if significant redness, rectal pain, bleeding, or mucus is observed on visual inspection of the rectal (perianal) area, a digital rectal examination and hemoccult test should be performed. If further investigation is warranted, subjects with these signs or symptoms should undergo proctoscopy or recto-sigmoidoscopy. A colonoscopy may be performed instead of the recto-sigmoidoscopy after discussion between the investigator and the sponsor. Refer to Section 8.2.1.

[f] Vital signs must be obtained in the seated position and will include oral temperature, respiratory rate, blood pressure, and pulse rate. Blood pressure and pulse measurements will be taken after the subject has been sitting for 5 minutes in a quiet setting without distractions (eg, television, cell phones) and prior to blood draws, when applicable.

[g] ECGs should be done after the subject has been supine for at least 5 minutes and prior to blood draws, when applicable.

[h] Required for WOCBP. Serum-based test at Screening and Week 12/EOT. Urine-based test at all other timepoints, with a repeat serum-based test if the urine-based test is positive. Refer to Section 8.2.5 for additional details on pregnancy testing.

[i] Stool that is present on the digital rectal exam can be used for the hemoccult test.

[j] All AEs occurring after the subject signs the ICF will be captured.

[k] At the Pretreatment Visit, appropriate site staff will train the subject on use of the eDiary. Refer to the eDiary user manual.
[l] The subject will complete the eDiary, responding to the daily questions (refer to Section 8.1.1.1), general weekly questions (refer to Section 8.1.1.2.1), and weekly GUPI and GRA questionnaires (refer to Section 8.1.1.2.2 and Section 8.1.1.2.3).
[m] The subject will complete the questionnaire via the eDiary, refer to Section 8.1.1.3.1.
[n] The subject will complete the questionnaire via the eDiary, refer to Section 8.6.
[o] The subject will complete the questionnaire via the eDiary; refer to Section 8.2.7.
[p] The subject will complete the assessment at the site; refer to Section 8.1.2.
[q] At the Day 1 Visit and all subsequent visits, appropriate site staff will review the eDiary entries to verify subject compliance with the requirements. After determining the subject's compliance, appropriate site staff will remind subjects to complete the eDiary (except at the Week 12/EOT Visit).
[r] At the Day 1 Visit, study drug will be administered in the clinic by the subject under supervision of appropriate site staff; refer to Section 6.1.1.1 for detailed dosing instructions. On all subsequent days through the Week 12 study visit, the subject will self-administer study drug at home; refer to Section 6.1.1.2 for detailed dosing instructions.
[s] On Weeks 4, 8, and 12, a single PK sample will be drawn during the site visit without respect to the timing of the previous dose administration. The time of the subject's last dose will be recorded.
[t] Subjects in the microbiome substudy will bring in a stool sample from home, collected prior to the study visit (and predose for Day 1), using the sample collection kit issued by the site; refer to Section 8.7.
[u] Study drug accountability will be performed as described in Section 6.2.
[v] Appropriate site personnel will contact subjects by phone for safety follow-up. Any AE or concomitant medication reported during the phone call will be captured on the eCRF. Subjects who report AEs during the call will be provided with instructions on the appropriate follow-up care. At the discretion of the investigator, subjects may be requested to return to the clinic for their follow-up contact.

2. INTRODUCTION

IW-3300 is a novel, 13 amino-acid, guanylate cyclase C (GC-C) agonist peptide being developed for the treatment of bladder pain associated with interstitial cystitis/bladder pain syndrome (IC/BPS).

2.1. Study Rationale

The aim of this study is to assess the efficacy, safety, and tolerability of IW-3300 for the treatment of IC/BPS. IC/BPS is a chronic condition characterized by bladder pain that is usually accompanied by urinary urgency, increased frequency, and/or nocturia. The morbidity of IC/BPS is substantial and often leads to poor quality of life for patients, their families, and day-to-day caregivers. (1) It is estimated that 3% to 7% of women and 3% to 4% of men have IC/BPS. (2) At this time, there are no effective treatments targeting pain associated with IC/BPS. A safe and effective treatment would greatly improve the quality of life for this patient population.

2.2. Background

IC/BPS is a chronic condition characterized by bladder pain that is usually accompanied by urinary urgency, increased frequency, and/or nocturia. IC/BPS is often misdiagnosed as a urinary tract infection (UTI). IC/BPS occurs in both males and females over a broad age range and across ethnic/racial groups. The morbidity of IC/BPS is substantial and often leads to poor quality of life for patients, their families, and day-to-day caregivers. (1) It is estimated that 3% to 7% of women and 3% to 4% of men meet the definition of IC/BPS, yet only a small percentage of these populations have been given an IC/BPS diagnosis. (2) There may be several contributing factors for the cause of IC/BPS, and it is unknown if IC/BPS is a primary condition or the secondary result of another disorder. (2) There are no diagnostic tests for IC/BPS; clinical diagnosis is generally based on urinary symptoms of urgency and frequency accompanied by pain related to the bladder, and exclusion of other diagnoses (eg, UTI). Cystoscopy may show bladder inflammation, including Hunner's lesions (mucosal lesions or ulcerations seen with or without hydrodistension of the bladder), or other pathology, but can also be normal. Diagnosis is generally not made until other diseases that could cause these symptoms are ruled out.

Managing IC/BPS often begins with nonpharmacological treatments (general relaxation, stress management, behavior modification, and physical therapy techniques). A recent Cochrane analysis indicated very low certainty of evidence to establish the effectiveness of medicines, behavioral therapy, physiotherapy, and neuromuscular blockade to treat all, or any, of the symptoms of bladder pain syndrome. (3) In the US, there is currently only 1 approved oral medication available for IC/BPS: ELMIRON (pentosan polysulfate). ELMIRON, a synthetic sulfated polysaccharide, is an orally administered drug that was approved in 1996, but that has since been associated with safety concerns related to macular degeneration (4) and lack of efficacy in a post-approval study. (3) Despite limited evidence of effectiveness, physicians often utilize invasive therapies, including intravesical installations, in an attempt to relieve BPS symptoms. Dimethyl sulfoxide (DMSO) is FDA-approved for intravesical installation; however, the data supporting clinical utility in IC/BPS are marginal. (3)

IW-3300 is being developed for the treatment of bladder pain associated with IC/BPS. IW-3300 is a novel, 13 amino-acid, GC-C agonist peptide. GC-C, the target of IW-3300, is predominantly expressed on the luminal surface of the small and large intestines. When GC-C receptors are stimulated, intracellular cyclic guanosine monophosphate (cGMP) is secreted across the basolateral membrane of colonic epithelial cells in the submucosa by multidrug resistance proteins (MRP)4 and MRP5, decreasing the activity of afferent sensory nerve fibers located in the colonic wall, resulting in reduced visceral pain, which ultimately produces an analgesic effect in other organs of the abdominopelvic region via action mediated through the common afferent pathways. (5, 6)

Experiments conducted in animals indicate that colonic hypersensitivity induces persistent hypersensitivity of bladder afferent pathways in the absence of bladder pathology. (6) Hypersensitivity of bladder afferent pathways resembles the symptoms observed in patients with overactive bladder and IC/BPS. Afferent neurons are known to have peripheral endings in both the distal colon and the bladder, and the axons of colonic and bladder neurons traveling through the same splanchnic and pelvic nerves. These nonclinical observations support the concept of cross-organ sensitization and suggest that GC-C agonists such as IW-3300 may be useful clinically for the treatment of bladder pain associated with IC/BPS.

IW-3300 has shown beneficial effects in reducing visceral pain in several animal models, including models of colonic pain and models of extra-intestinal chronic pelvic pain following intracolonic or intrarectal administration. IW-3300 (3 µg/kg/day), administered either intracolonically or orally to female rats, reduced endometriosis-induced mechanical hind paw allodynia. When administered intracolonically (1, 3, 10 µg/kg/day), IW-3300 reduced endometriosis-induced vulvar pain in a dose-dependent manner. In a rat model of chronic radiation proctopathy induced either by fractionated dose (total dose 48 Gray) or single high dose (25 Gray) of radiation, IW-3300 (3 µg/kg/day) administered intracolonically reversed colorectal and bladder hypersensitivity. IW-3300 (3 µg/kg/day) administered intrarectally reduced vaginal hypersensitivity in adult female mice previously exposed to neonatal vaginal irritation. In a rat model of early life stress, IW-3300 (3 µg/kg/day) administered intracolonically reduced bladder and mechanical hind paw allodynia in adult male rats.

IW-3300 is a new chemical entity that has similar activity to the naturally occurring peptide hormones guanylin and uroguanylin, which are key regulators of electrolytes. Activation of GC-C expressed on the luminal surface of the intestinal epithelium leads to increased intracellular concentrations of the second messenger cGMP, which triggers a signal transduction cascade leading to the activation of the cystic fibrosis transmembrane conductance regulator (CFTR) through its cGMP-dependent protein kinase G type II (PKG II). (7, 8, 9) CFTR activation causes secretion of chloride and bicarbonate into the intestinal lumen, resulting in increased fluid secretion and acceleration of intestinal transit. (10) These effects have been observed following oral administration, whereas no significant effects on intestinal transit have been observed with direct administration of IW-3300 to the colon in preclinical studies. This observation was probably due to the high capacity of the large intestine to reabsorb fluid, which can overcome the secretory effects of GC-C when stimulation is limited to the colon. The cellular function and activity of GC-C stimulation is the same regardless of location in the gut; using ligated loops of stomach, small intestine, and colon, GC-C stimulation has been shown to produce increased fluid and cGMP accumulation in all gut tissue. (11) When stimulation of GC-C is restricted to just the colon, the ability of the colon to reabsorb fluid is greater than the

amount of extra fluid produced by activation of GC-C. This is supported by data generated from MD-7246, an investigational delayed-release formulation of another GC-C agonist, linaclotide, in healthy volunteers (Study MCP-103-105), in subjects with irritable bowel syndrome with constipation (IBS-C) (Study MCP-103-204), and in subjects with irritable bowel syndrome with diarrhea (IBS-D) (Study MCP-103-205).

IW-3300 acts directly on the lumen of the large intestine and has minimal (≤0.2%) systemic bioavailability after oral and intrarectal administration in nonclinical species (rodents and monkeys). Based on pharmacologically active doses in animal models of visceral pain (3 μg/kg to 10 μg/kg), the anticipated human pharmacologically active dose is in the range of 180 μg to 600 μg (human equivalent dose [HED] based on body weight).

The safety of IW-3300 has been evaluated in nonclinical repeat-dose toxicity, safety pharmacology, and genotoxicity studies. In these studies, IW-3300 was well tolerated when administered intrarectally in rats and monkeys at 20 mg/kg/day for 14 days, with no adverse findings of dehydration or decreases in stool consistency, no local irritation, and no histopathological changes. After intrarectal administration of IW-3300 for up to 13 weeks, a non-adverse increase in sporadic abnormal stools was noted in monkeys and no effects were observed in rats. Additional nonclinical data appear in the IW-3300 Investigator's Brochure (IB).

The safety of IW-3300 has also been evaluated in 2 Phase 1 studies in healthy subjects. In Study C3300-101, single doses ranging from 100 μg to 2500 μg (administered rectally as a low-volume [20 mL] enema) were well tolerated compared with placebo (N=6 for each of the IW-3300 dose groups and N=8 for the placebo group). There were no deaths, serious adverse events (SAEs) or treatment-emergent adverse events (TEAEs) leading to study discontinuation. All TEAEs were considered by the investigator to be Grade 1 in severity and there were no notable trends across the IW-3300 dose groups. Clinical laboratory, vital sign, and electrocardiogram (ECG) assessments did not reveal any clinically significant changes from baseline and there were no notable effects observed for bowel function, stool consistency, bladder symptoms, or urinary parameters.

In Study C3300-102, once-daily doses of IW-3300 100 μg and 300 μg (administered rectally as a low-volume [20 mL] enema) were evaluated over a dosing period of 7 days (N=6 for each of the IW-3300 dose groups and placebo group). IW-3300 was well tolerated. There were no deaths or SAEs. On IW-3300, no subject had a TEAE that led to premature study drug discontinuation, and all TEAEs were considered by the investigator to be Common Terminology Criteria for Adverse Events (CTCAE) Grade 1 or Grade 2 severity. In the 100-μg group, 3 subjects (50.0%) had 5 TEAEs: corneal irritation and diarrhea (2 separate events; verbatim term="loose stool" for both events) in 1 subject, electrocardiogram abnormal in 1 subject (not considered clinically significant by the investigator), and testicular pain in 1 subject. In the 300-μg group, 2 subjects (33.3%) had 6 TEAEs: diarrhea (verbatim term="loose stool" for all events), abdominal distension, pruritus, and presyncope in 1 subject, and nausea and acarodermatitis in 1 subject. In the placebo group, 3 subjects (50.0%) had 3 TEAEs: diarrhea in 1 subject (verbatim term="loose stool" for all events), abdominal pain in 1 subject, and rash that led to study drug discontinuation in 1 subject. For the other safety parameters assessed (ie, clinical laboratory assessments, vital signs, ECGs, bladder and bowel function parameters), there were no trends observed across the IW-3300 groups or compared with the placebo group.

This clinical study is a 12-week, randomized, double-blind, placebo-controlled, adaptive study to evaluate the efficacy, safety, and tolerability of IW-3300, administered as a rectal foam, in IC/BPS subjects. This rectal route of administration was selected to target effects of IW-3300 to the colon. Two dosage strengths, 100 µg and 300 µg, will be evaluated in Study C3300-201.

2.3. Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of IW-3300 can be found in the IW-3300 IB. The available nonclinical and clinical data with IW-3300 support a favorable risk-benefit profile.

2.3.1. Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention: IW-3300		
Potential for diarrhea and secondary dehydration	These are class effects of GC-C agonism or effects observed in nonclinical safety pharmacology and toxicology studies of IW-3300. In Phase 1 clinical studies in healthy subjects, IW-3300 administered rectally as a low-volume enema was well tolerated at single doses ranging from 100 µg to 2500 µg and as once-daily doses of 100 µg and 300 µg over 7 days. In these studies, no trends across the IW-3300 groups, or compared with placebo, were noted for any of the safety parameters assessed. Refer to the IW-3300 IB for additional nonclinical and clinical data.	As described in Section 2.2, intrarectal administration will likely decrease the risk of diarrhea and subsequent dehydration. Throughout the Treatment Period, subjects will be monitored at Week 1 and at subsequent monthly visits for AEs, physical examination abnormalities, laboratory abnormalities, and other safety findings. The study design includes safety-based rules for the temporary discontinuation of study drug for individual subjects (Section 7.1.2) and for study suspension and termination (Section 7.1.1).
Abdominal distension	This effect was observed in nonclinical safety toxicology studies of IW-3300. In Phase 1 clinical studies in healthy subjects, IW-3300 administered rectally as a low-volume enema was well tolerated at single doses ranging from 100 µg to 2500 µg and as once-daily doses of 100 µg and 300 µg over 7 days. In these studies, no trends across the IW-3300 groups, or compared with placebo, were noted for any of the safety parameters assessed. Refer to the IW-3300 IB for additional nonclinical and clinical data	Throughout the Treatment Period, subjects will be monitored at Week 1 and at subsequent monthly visits for AEs, physical examination abnormalities, laboratory abnormalities, and other safety findings. The study design includes safety-based rules for the temporary discontinuation of study drug for individual subjects (Section 7.1.2) and for study suspension and termination (Section 7.1.1).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Unknown safety findings with a novel study drug, including rare or unknown side effects such as allergic or life-threatening reactions	This is an early phase study with IW-3300 and, as such, limited clinical data are available. In Phase 1 clinical studies in healthy subjects, IW-3300 administered rectally as a low-volume enema was well tolerated at single doses ranging from 100 µg to 2500 µg and as once-daily doses of 100 µg and 300 µg over 7 days. In these studies, no trends across the IW-3300 groups, or compared with placebo, were noted for any of the safety parameters assessed. This study will use a foam formulation (rather than a low-volume enema) that has not been tested in humans. While it is possible that the IW-3300 foam formulation has different distribution characteristics than the IW-3300 low-volume enema, the site of action and target of delivery is expected to be the same. Refer to the IW-3300 IB for additional nonclinical and clinical data	The first dose will be administered in the clinic on Day 1. Subjects will remain in the clinic for at least 1-hour postdose for observation and completion of safety assessments. Throughout the Treatment Period, subjects will be monitored at Week 1 and at subsequent monthly visits for AEs, physical examination abnormalities, laboratory abnormalities, and other safety findings. The study design includes safety-based rules for the temporary discontinuation of study drug for individual subjects (Section 7.1.2) and for study suspension and termination (Section 7.1.1).
Potential for discomfort, pain, and bleeding related to rectal administration of study drug	There is a risk of discomfort, pain, and bleeding.	Subjects will receive training and instructional materials on how to properly administer the study drug and they will administer the first dose in the clinic under site supervision. A visual rectal examination will be conducted at each study visit to monitor for AEs. Lastly, subjects will be instructed on how to properly administer the study drug and recognize symptoms.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Unknown risks to a pregnancy, embryo, or fetus if pregnancy occurs	There are unknown risks to a pregnancy, embryo, or fetus if a female subject or female partner of a male subject becomes pregnant.	Women of childbearing potential (WOCBP) will be tested for pregnancy at Screening and on a monthly basis during the Treatment Period. Contraceptive requirements for WOCBP and male subjects with female partners are outlined in Section 10.4.2.
Study Procedures		
Venipuncture	There is possible, although unlikely, risk of momentary discomfort and/or bruising. infection, excess bleeding, clotting, or fainting.	Only appropriately qualified personnel will perform these study procedures. Subjects will be monitored for these risks and managed appropriately.
Physical examinations that include a visual inspection of the perianal area and a digital rectal examination at certain visits or if indicated.	There is risk of discomfort due to the uncomfortable nature of the examination. Additional risks may include pain and bleeding.	
ECGs	There is a risk of mild discomfort, some local irritation, redness, or burning in the areas where the leads are attached.	

2.3.2. Benefit Assessment

Benefits to individual subjects may include:

- the chance to receive a study drug that may reduce pain associated with IC/BPS;
- medical evaluations and assessments associated with study procedures (eg, physical examinations, ECGs, laboratory evaluations); and
- contributing to the process of developing new therapies for IC/BPS, a disease without effective treatment.

2.3.3. Overall Benefit/Risk Conclusion

Considering the measures taken to minimize risks to subjects participating in this study, the potential risks identified in association with IW-3300 are considered acceptable given the anticipated benefits that may be afforded to subjects with IC/BPS if this drug is proven to be safe and effective.

3. OBJECTIVES, ESTIMANDS, AND ENDPOINTS

Objectives	Estimands	Endpoints
Primary		
Investigate the efficacy of IW-3300, administered as a rectal foam, on bladder pain in subjects with IC/BPS	See table below	Change from baseline (CFB) in weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12
Secondary		
Investigate the efficacy of IW-3300, administered as a rectal foam, on the components of bladder pain in subjects with IC/BPS	--	• CFB in weekly average of a burning sensation in the bladder at its worst at Week 12 • CFB in weekly average of a pressure sensation in the bladder at its worst at Week 12 • CFB in weekly average of discomfort in the bladder at its worst at Week 12 • CFB in Genitourinary Pain Index (GUPI) Pain subscale score at Week 12
Investigate the safety and tolerability of IW-3300, administered as a rectal foam, in subjects with IC/BPS	--	• Frequency of TEAEs occurring in ≥2% of subjects • Overall frequency of TEAEs by severity grade
Exploratory		
	--	• Weekly average CFB in daily bladder pain at its worst (Note: Weekly average CFB at Week 12 is the primary endpoint.) • Weekly average CFB in daily bladder pain-free days • Weekly average CFB in urinary urgency • Weekly CFB in mean urinary frequency • Weekly CFB in mean nighttime voiding frequency (nocturia) • Monthly CFB in mean nighttime voiding frequency (nocturia) • CFB in O'Leary/Sant Interstitial Cystitis Symptom Index (ICSI) total score • CFB in GUPI Urinary subscale score

Objectives	Estimands	Endpoints
		• CFB in GUPI Quality of Life (QoL) subscale score • CFB in Global Response Assessment (GRA) total score • CFB in the sexual function question score • CFB in sleep difficulty due to bladder pain • CFB in sleep difficulty due to a need to urinate • CFB in the body pain category as assessed using the Margolis body map • CFB in IC/BPS symptom severity • IC/BPS global impression of symptom change • 6/12 Week Adequate Relief of IC/BPS Pain Responder • CFB in EuroQol 5-Dimension 5-Level Version (EQ-5D-5L) utility score and Visual Analog Scale • Weekly treatment satisfaction • CFB in 16s RNA based analysis of gut microbiome composition at the genus level • PK parameters of IW-3300 and metabolites in plasma

The following estimand is a precise description of the primary efficacy treatment effect to be estimated:

Definition	Attributes			
	Population	**Variable (or endpoint)**	**Strategy for addressing intercurrent event**	**Population-level summary**
The primary estimand is the effect of IW- 3300 compared with placebo at Week 12 in weekly average of daily bladder pain at its worst scores	18 to 75 years of age with IC/BPS defined through inclusion and exclusion criteria as stated in the protocol	Change from baseline in weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12	-Taking rescue medication (Hypothetical strategy) -Premature discontinuation due to lack of treatment efficacy (Hypothetical strategy)	Difference in mean change of weekly average of daily bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst at Week 12 between active (IW-3300) and placebo groups

4. STUDY DESIGN

4.1. Overall Design

This is a Phase 2, randomized, placebo-controlled, parallel-assignment, 12-week, adaptive-design study to evaluate the efficacy, safety, and tolerability of IW-3300 in subjects with IC/BPS. Subjects will be randomized 1:1:1 to IW-3300 100 µg, IW-3300 300 µg, or matching placebo administered as a rectal foam.

As shown in Figure 1, the study consists of 4 periods: a Screening Period, a Pretreatment Period, a 12-week Treatment Period, and a 2-week Follow-up Period. The assessments conducted during each period are shown in the Schedule of Activities (SoA)/Table 1.

4.1.1. Study Periods

4.1.1.1. Screening Period

The Screening Period will begin with the signing of the informed consent form (ICF) at the Screening Visit (which can occur from Day -51 to Day -15) and will last up to 30 days. During this time, subjects will undergo preliminary procedures to ensure that they meet the eligibility criteria for the study. After obtaining informed consent, sites with supporting procedures in place may elect to conduct some screening assessments that do not require the subject to be present (eg, collecting demographics, medical history, prior medications) over the phone. After discussion with the medical monitor, the Screening Period may be extended by up to 15 days (to Day -66) for subjects requiring additional procedures (eg, cystoscopy, ureter stents) or a medication washout.

The end of the Screening Period coincides with the beginning of the Pretreatment Period.

4.1.1.2. Pretreatment Period

The Pretreatment Period is defined as the 14 to 21 days immediately before the Day 1/Randomization Visit. At the Pretreatment Visit, subjects will receive training on how to complete the online daily and weekly eDiary questions at home. Subjects will receive rescue medicine as described in Section 6.5.2. Starting on the night of the Pretreatment Visit and continuing throughout the Pretreatment Period, subjects will answer the eDiary questions to establish a baseline during the study. Eligibility to continue into the Treatment Period of the study will be based on the answers recorded in the eDiary during the 14 days prior to Day 1. Subjects who meet the entry criteria will be randomized on Day 1 and continue into the Treatment Period.

4.1.1.3. Treatment Period

On Day 1 of the Treatment Period, randomized subjects will receive the first dose of study drug, formulated as a rectal foam, and administered by the subject under supervision of the site staff. Subjects will also receive training on how to self-administer the study drug at home. For the next 12 weeks, subjects will self-administer study drug at approximately the same time each day. Subjects will also continue to answer the daily and weekly eDiary questions.

Subjects will have routine study visits at Week 1, Week 4, Week 8, and Week 12/End of Treatment (EOT) for completion of efficacy, safety, and other assessments.

4.1.1.4. Follow-up Period

Two weeks after the last day of dosing (Week 12/EOT Visit), an End of Study (EOS) Follow-up Phone Call will be conducted to assess the subject for any AEs and any medications taken since the Week 12/EOT Visit.

4.2. Scientific Rationale for Study Design

A randomized, double-blind, placebo-controlled study design was chosen in accordance with the concepts in ICH E10 (Choice of Control Groups and Related Issues in Clinical Trials) to investigate the effects of IW-3300 administered once daily for 12 weeks as a rectal foam. Subjects will be randomized to ensure that the treatment groups are comparable and to minimize the potential for selection bias. The study will be double-blind to ensure that the subjects and site staff are unaware of the treatment assignment and to minimize the potential for bias in study assessments or AE reporting. Placebo was chosen as the control so that the rate of spontaneously occurring AEs can be determined and to reduce the potential for bias in the reporting of AEs.

This study has a 14- to 21-day Pretreatment Period to establish a baseline on study and to familiarize subjects with data collection methodology (ie, eDiary), and a 12-week Treatment Period to compare IW-3300 with a matching-placebo control.

4.2.1. Subject Input into Design

Patients with IC/BPS were surveyed to gain insights into the burden of disease and the appropriateness of select aspects of the study design including endpoints, assessments, and eligibility criteria.

4.3. Justification for Dose

IW-3300 doses of 100 µg and 300 µg were selected based on nonclinical pharmacology models of visceral hypersensitivity and bladder dysfunction using IW-3300. The pharmacologically active doses of IW-3300 in these nonclinical models are in the range of 3 µg/kg/day to 10 µg/kg/day, with diminishing benefit observed with doses above 10 µg/kg/day. The human equivalent daily dose extrapolated from pharmacologically active dose levels in nonclinical models is 180 µg to 600 µg based on body weight and 15 µg to 100 µg based on body surface area. Choosing 2 doses for investigation in this study should allow for observation of a dose response.

Two completed Phase 1 studies in healthy subjects support the further evaluation of IW-3300 rectally administered at doses of 100 µg and 300 µg in subjects with IC/BPS. Phase 1 Study C3300-101 evaluated single doses of IW-3300 ranging from 100 µg to 2500 µg. Phase 1 Study C3300-102 evaluated once-daily doses of IW-3300 100 µg and 300 µg administered over 7 days. In these studies, no trends across the IW-3300 groups, or compared with placebo, were noted for any of the safety parameters assessed. Additional data appear in the IW-3300 IB.

4.4. End of Study Definition

A subject is considered to have completed the study if they have completed all phases of the study including the EOS/Follow-up Phone Call.

The end of the study is defined as the date of the last visit or EOS Follow-up Phone Call of the last subject in the study.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

Age

1. Subject is 18 to 75 years of age, inclusive, at the time of signing the ICF.

Type of Subject and Disease Characteristics

2. Subject has been diagnosed with IC/BPS by a urologist or other clinician specializing in IC/BPS.
3. Subject has chronic bladder pain associated with filling the bladder over the past 6 months before the Screening Visit.
4. Subject is compliant with eDiary completion by adequately responding to eDiary questions (ie, completing the daily evening report) at least 10 days over Week -1 and Week -2 (14-day period) with at least 5 of those eDiary completion days occurring during Week -1.
5. Subject has a weekly average daily score for bladder pain at its worst of ≥4, as reported in the eDiary using an 11-point numeric rating scale (NRS) during Week -1.
6. Subject has at least 1 of the following urinary symptoms related to IC/BPS during the 6 months before the Screening Visit:
   - nocturia ≥2 voids/night
   - daytime frequency >8×day
   - urgency
7. Subject is willing to use a rectally administered product and able to administer study drug, understand directions, and complete study assessments.
8. If the subject has been treated with nonpharmacologic interventions for IC/BPS (eg, physical therapy, pelvic floor massage, acupuncture, naturopathy, mindfulness exercises, cognitive behavioral therapy), these were unchanged for a minimum of 30 days before the Screening Visit and the subject agrees to continue them unchanged from the Screening Visit until after the EOS Follow-up Phone Call, as outlined in Section 5.3.
9. Subject agrees to refrain from making any new or major lifestyle changes that may affect IC/BPS symptoms (eg, new diet, exercise regimen) from the Screening Visit until after the EOS Follow-up Phone Call, as outlined in Section 5.3.
10. Subject agrees to comply with all other lifestyle restrictions outlined in Section 5.3.

Weight

11. Subject has a body mass index ≤40 kg/m^2 at the Screening Visit.

Sex

12. A female subject is eligible to participate if she is not pregnant or breastfeeding, and one of the following conditions applies:

    a. Is a woman of nonchildbearing potential (WONCBP) as defined in Section 10.4/Appendix 4 (Contraceptive and Barrier Guidance)

    OR

    b. Is a woman of childbearing potential (WOCBP) who

    – Is using a contraceptive method that is highly effective, with a failure rate of <1%, as described in Section 10.4/Appendix 4 (Contraceptive and Barrier Guidance) during the study (ie, from the Pretreatment Visit until the EOS Follow-up Phone Call). The investigator should evaluate the potential for contraceptive method failure (eg, noncompliance, recently initiated) in relationship to the first dose of study drug.

    – Has a negative pregnancy test before the first dose of study intervention on Day 1, refer to Section 8.2.5 (Pregnancy Testing). If a urine test cannot be confirmed as negative (eg, an ambiguous result), a serum pregnancy test is required. In such cases, the subject must be excluded from participation if the serum pregnancy result is positive.

        –Additional requirements for pregnancy testing during study intervention are located in Section 8.2.5 (Pregnancy Testing)

        –The investigator is responsible for review of medical history, menstrual history, and recent sexual activity to decrease the risk for inclusion of a woman with an early undetected pregnancy.

13. A male subject is eligible to participate if he agrees to the following during the study (ie, from the Pretreatment Visit until the EOS Follow-up Phone Call):

    a. Refrain from donating sperm

    PLUS, either:

    b. Be abstinent from heterosexual intercourse as their preferred and usual lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

    OR

    c. Agree to use contraception/barrier method as detailed below

    – Agree to use a male condom and should also be advised of the benefit for a female partner to use a highly effective method of contraception as a condom may break or leak when having sexual intercourse with a woman of childbearing potential who is not currently pregnant.

- Agree to use a male condom when engaging in any activity that allows for passage of ejaculate to another person

14. Subject agrees to refrain from inserting anything other than the study drug applicator into the rectum from the Screening Visit until after the EOS Follow-up Phone Call, as outlined in Section 5.3.

Informed Consent

15. Subject is capable of giving signed informed consent as described in Section 10.1.3 which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

5.2. Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Male subject has a history of bacterial prostatitis or benign prostatic hyperplasia with active symptoms that are difficult to distinguish from IC/BPS.
2. Subject has pain due to another medical condition (eg, pelvic floor dysfunction, vulvodynia, or endometriosis) that in the opinion of the investigator would be difficult for the investigator or the subject to discriminate from bladder pain (which can include sensations like burning, pressure, and/or discomfort).
3. Subject has anal fissure, anal abscess, inflammatory bowel disease, active proctitis, or any other condition that can be a contraindication to using a rectal foam or bias the evaluation of efficacy and safety of the product.
4. Subject has cancer under active treatment or a history of uterine, cervical, pelvic, colorectal, ovarian, or vaginal cancer.
5. Subject has a history of benign or malignant bladder tumors.
6. Subject has an active UTI during the Screening or Pretreatment Period or subject has had ≥2 UTIs within 90 days before the Screening Visit.
7. Subject has a positive hemoccult test at the Screening Visit.
8. Subject has a clinically significant concurrent illness or finding on physical examination or clinical laboratory test after signing the ICF but before receiving the first dose of study drug. (The investigator will determine if a finding is clinically significant. The investigator will consider whether the finding 1) could prevent the subject from performing any study procedure or assessment, 2) represents a condition that would be exclusionary, 3) could represent a safety concern if the subject participated in the study, or 4) could confound any study assessment.)
9. Subject has a hypersensitivity or allergy to any of the ingredients contained in the IW-3300 drug product.

10. Subject has an uncontrolled major psychiatric condition or has made a suicide attempt during the 2 years before the Screening Visit.
11. Subject has a malabsorption syndrome.

Prior/Concomitant Therapy

12. Subject has had surgery in the pelvic or abdominal region within 90 days before the Screening Visit, or is planning pelvic or abdominal region surgical procedures between screening and the EOS Follow-up Phone Call. A subject who has a history of major pelvic or abdominal surgery must be discussed with the medical monitor to confirm the appropriateness of enrollment.
13. Subject has received a cystoscopy with or without hydrodistension for diagnostic purposes (ie, not for pain relief) within 30 days before the Screening Visit, a cystoscopy with therapeutic hydrodistension (ie, for pain relief) within 90 days before the Day 1 Visit, or is planning a cystoscopy for any reason between screening and the EOS Follow-up Phone Call.
14. Subject has history of pelvic irradiation or radiation cystitis, or drug-induced cystitis.
15. Subject is taking or has recently taken a prohibited medication or is not willing to abide by the restrictions outlined in Section 6.5.1, and/or subject is actively using a sacral neuromodulation device (refer to Section 6.5.2). Note: Active use of the device is defined as using it within 12 months of the Screening Visit. If the subject has not used their device in the last 12 months and agrees to have the device off for the duration of the study, they may be eligible. A discussion with the medical monitor is required.
16. Subject has a positive drug screen. A positive result for amphetamines is allowed if the subject has a documented medical need (refer to Section 6.5.1). A positive result for barbiturates or benzodiazepines is allowed if the subject is using these medications for a medical condition other than IC/BPS. A positive result for marijuana is also allowed.

Prior/Concurrent Clinical Study Experience

17. Subject has participated in another interventional clinical study within 6 months before the Screening Visit or is planning to do so at any time during the study.

Other Exclusions

18. Subject has an acute or chronic condition that, in the investigator's opinion, would limit their ability to complete or participate in the study (eg, painful kidney stones). Investigators are encouraged to discuss conditions with the medical monitor.
19. Subject has a recent history (during the 12 months before the Day 1 Visit) of drug or alcohol abuse. (Note: Subjects with a history of drug or alcohol abuse that was diagnosed greater than 12 months before the Day 1 Visit may be enrolled if they have exhibited no actual abuse during the 12 months before the Day 1 Visit.)
20. Subject has already been randomized in this study at another clinical site.
21. Subject is directly or indirectly involved in the conduct and administration of this study as an investigator, subinvestigator, study coordinator, study staff member, or employee of

Ironwood Pharmaceuticals; or the subject is a direct relative of one of the above individuals involved in the study.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

Subjects should not make substantial changes to their diet from the signing of the ICF until after the EOS Follow-up Phone Call.

5.3.2. Caffeine, Alcohol, and Tobacco

Subjects should not make any substantial changes to their consumption habits for caffeine, alcohol, or tobacco from the signing of the ICF until after the EOS Follow-up Phone Call.

5.3.3. Activity

If the subject is using any nonpharmacologic interventions for IC/BPS (eg, physical therapy, pelvic floor massage, acupuncture, naturopathy, mindfulness exercises, or cognitive behavioral therapy), those should continue unchanged for a minimum of 30 days before the Screening Visit until after the EOS Follow-up Phone Call.

Subjects must comply with the contraception requirements outlined in Section 10.4.2.

Subject agrees to refrain from inserting anything other than the study drug applicator into the rectum from the Screening Visit until after the EOS Follow-up Phone Call.

5.4. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screening information is required to ensure transparent reporting of screen failure subjects 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.

Subjects who do not meet 1 or more study entry criteria may be rescreened following consultation with the sponsor, with the following considerations:

- Subjects who fail screening due to Exclusion Criterion #6, the presence of an unresolved UTI, are allowed to rescreen without consultation with the sponsor.
- Subjects who fail screening due to Inclusion Criterion #4 or #5 (ie, they do not meet the eDiary eligibility criteria) are not allowed to rescreen.

Rescreened subjects must be assigned a separate subject number for each screening; once a subject number has been assigned it must not be reused.

6. STUDY INTERVENTION

Study intervention (also referred to as study drug) is defined as any investigational intervention(s), and may include marketed product(s) and placebo, intended to be administered to a study subject according to the study protocol.

The study will evaluate, IW-3300 rectal foam 100 µg and 300 µg, and matching placebo in a double-blind manner. Study drug is formulated as an oil-in-water emulsion filled into an aluminum canister with a metered-dose head and an aerosol propellant for delivery as a foam. Each drug product canister is intended to be used for 22 actuations, 1 priming actuation followed by 21 dosing actuations. The active bulk rectal foam is formulated in 2 concentrations such that a single, metered actuation of 1.15 g±15% of foam corresponds to 1 of the following unit doses: 100 µg or 300 µg.

The IW-3300 rectal foam, 100 µg and 300 µg, contains the active pharmaceutical ingredient with the following excipients: purified water, propylene glycol, sodium phosphate dibasic, sodium dihydrogen phosphate monohydrate, disodium edetate (disodium EDTA), methylparaben, light mineral oil, isopropyl myristate, white petrolatum, polyoxyl 20 cetostearyl ether, cetyl alcohol, stearyl alcohol, propylparaben, and AP-35. AP-35 is a commonly used aerosol propellant which is a mixture of butane, iso-butane, and propane.

Matching placebo will be provided in identical canisters and contain the same excipients as the active formulation.

Study drug will be administered once daily as a rectal foam for 12 weeks.

6.1. Study Interventions Administered

Intervention Name	**IW-3300 100 µg**	**IW-3300 300 µg**	**Matching Placebo**
Type	Drug	Drug	Placebo
Dose Formulation	Rectal Foam	Rectal Foam	Rectal Foam
Unit Dose Strength/Dose Volume	100 µg/1.15 mL (foaming to 20 mL)	300 µg/1.15 mL (foaming to 20 mL)	0 µg/1.15 mL (foaming to 20 mL)
Dosage Level(s)	100 µg once daily	300 µg once daily	0 µg once daily
Route of Administration	Rectally	Rectally	Rectally
Use	Experimental	Experimental	Placebo
Investigational Medicinal Product (IMP)/ Non-IMP (NIMP)	IMP	IMP	IMP
Sourcing	Provided centrally by the sponsor	Provided centrally by the sponsor	Provided centrally by the sponsor

Intervention Name	IW-3300 100 µg	IW-3300 300 µg	Matching Placebo
Packaging and Labeling	Study drug will be provided in canisters. Each canister will be labeled as required per country requirement.	Study drug will be provided in canisters. Each canister will be labeled as required per country requirement.	Study drug will be provided in canisters. Each canister will be labeled as required per country requirement.

6.1.1. Dosing Information

At the Day 1 Visit, study drug will be administered in the clinic by the subject under supervision of the site staff. On all other days during the Treatment Period, subjects will self-administer study drug at home. Subjects will continue dosing until their Week 12 study visit.

6.1.1.1. Dose Administration at the Day 1 Site Visit

On Day 1, subjects will receive a single dose of study drug at the site visit. Subjects will be encouraged to empty their bowels in the morning prior to dosing, if possible.

Before dosing, the site staff will provide the subject with study drug administration training and instructional materials for self-administration at home.

Following training, subjects will administer study drug rectally as per the Directions for Use under the supervision of site staff. Subjects will be instructed to stand, with 1 foot on the floor and the other foot elevated on a flat surface. Subjects will place a prelubricated applicator onto the pump nozzle, shake the canister, unlock the pump dome, and invert the canister. They will then gently insert the applicator into the rectum as far as comfortable, press and hold the pump dome for approximately 5 seconds, then release the pump dome to administer the metered dose into the rectum (metered-dose volume is 1.15 mL, which foams to a volume of approximately 20 mL). After administering the foam, subjects will hold the applicator in the rectum for 10 to 15 seconds, then dispose of the used applicator. Site staff will monitor subjects for leakage of the study drug from the rectum during the initial 30 minutes post administration. Subjects should avoid using the bathroom and try to hold in the foam for at least 1 hour. After subjects complete the Day 1 postdose assessments shown in the SoA/Table 1, they can leave the clinic.

The site staff will record the date and time of study drug administration in the electronic case report form (eCRF) and will also record (as yes/no) if there was study drug leakage from the rectum in the 30 minutes following drug administration.

6.1.1.2. Dose Administration at Home

Following Day 1, subjects will self-administer study drug at home once daily at approximately the same time of day according to their routine. Subjects will be encouraged to empty their bowels prior to dosing, if possible. Dosing at night before bedtime is recommended. Dosing should be done in accordance with study drug administration training received at the Day 1 site visit.

For self-administration of study drug at home, subjects will be instructed to administer the foam in a standing position, with 1 foot on the floor and the other foot elevated. Subjects will place a prelubricated applicator onto the pump nozzle, shake the canister, unlock the pump dome, and invert. They will then gently insert the applicator into their rectum as far as comfortable, press

and hold the pump dome for approximately 5 seconds, then release the pump dome to administer the metered dose into the rectum (metered-dose volume is 1.15 mL, which foams to a volume of approximately 20 mL). After administering the foam, subjects will hold the applicator in their rectum for 10 to 15 seconds, then dispose of the used applicator. Subjects should avoid using the bathroom and try to hold in the foam for at least 1 hour. On a daily basis, subjects will complete the study drug retention and study drug application assessments in the eDiary (Section 8.1.1.1).

6.2. Packaging, Labeling, Storage, and Accountability

IW-3300 rectal foam, 100 µg and 300 µg, and matching placebo will be supplied in metered-dose canisters containing enough rectal foam for 22 actuations, 1 priming actuation followed by 21 dosing actuations. Each canister will be provided with a kit containing 21 prelubricated applicators and disposal bags for used applicators. Canisters will be uniquely numbered and labeled in a double-blind fashion that conforms to regulatory requirements.

All study drug will be provided by Ironwood or designee. IW-3300 rectal foam, 100 µg and 300 µg, and matching placebo canisters will be shipped and stored at 2°C to 8°C (36°F to 46°F). Once the first dose from each canister has been administered, the subject may store the canister at ambient conditions for the remainder of its use. Any deviations from the storage conditions must be reported to Ironwood and use of the study drug suspended until authorization for its continued use has been provided by Ironwood.

Storage and accountability of study drug should adhere to the following:

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug.
2. Only subjects enrolled in the study may receive study drug and only authorized site staff may supply study drug or administer study drug at the Day 1 Visit. All study drug must be stored in accordance with the labeled storage conditions.
3. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

6.3. Measures to Minimize Bias: Randomization and Blinding

6.3.1. Randomization

The subject identification (SID) number will be assigned in an ascending sequential order (eg, beginning with 001, 002, etc.) at the time the subject signs the ICF. The subject will retain the same unique SID number throughout the study.

Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be randomized into the study on Day 1. An adaptive sample size algorithm is utilized for determining the final sample size of the study, with a range of approximately 100 to 300 subjects planned for enrollment. Subjects will initially be randomized in a 1:1:1 ratio to receive IW-3300 100 µg, IW-3300 300 µg, or matching placebo. Randomization numbers encoding the subjects' study intervention assignments will be based on a randomization schedule that is computer generated prior to the study by an independent statistician (who is not otherwise associated with

the study) from a contract research organization (CRO). All subjects will be centrally assigned to randomized study intervention using an interactive web response system (IWRS). Before the study is initiated, the telephone number and call-in directions for the IWRS and/or the log in information and directions for the IWRS will be provided to each site.

6.3.2. Blinding

The investigator and all other site staff, sponsor study personnel, and the subject will remain blinded to individual subject treatment assignments throughout the study, except as noted below.

As described in Section 9.6, an independent Data Monitoring Committee (DMC) will review the results of the interim analysis(es) (IA[s]) of the safety and efficacy data. The designated independent statisticians, who will not be involved in the final study data analysis and interpretation, will have access to the randomization schedule including treatment assignments to conduct the efficacy IA(s) and safety reporting.

Specific designated personnel in the Ironwood Global Patient Safety Group may be unblinded to the treatment assignment of individual subjects for regulatory reporting purposes. Specific designated personnel in the Ironwood Pharmaceutical Development & Manufacturing Sciences Group may be unblinded to treatment assignment for the purpose of supplying study drug kits to the sites. All other sponsor study personnel, except as described, will remain blinded until the study is complete and the database is locked, unless warranted by emerging safety or tolerability issues.

The handling of pharmacokinetic (PK) concentration data that may unblind subject treatment assignments is described in Section 8.5.

6.3.3. Procedures for Unblinding

The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subject's intervention assignment is warranted. Subject safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's intervention assignment unless this could delay emergency treatment of the subject. If a subject's intervention assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and eCRF, as applicable.

6.4. Study Intervention Compliance

On Day 1, subjects will self-administer study drug under the supervision of designated site staff. The date and time of the dose administered in the clinic will be recorded in the source documents and eCRF. The dose of study intervention and study subject identification will be confirmed at the time of dosing by a member of the study-site staff.

Following Day 1, when subjects self-administer study drug at home, compliance with study intervention will be assessed at each visit. Site staff should review the subject's reports in the eDiary (refer to Section 8.1.1.1) and reinforce any compliance concerns with the subject.

A record of the number of canisters dispensed to and returned by each subject (including the dispense/return dates and canister identification numbers) must be maintained and reconciled with study drug and compliance records.

6.5. Concomitant Therapy

Any medication (including over-the-counter or prescription medicine, vitamin, and/or herbal supplement) or vaccine that the subject is receiving at the time of Screening or receives during the study must be recorded along with:

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

Unless, in the opinion of the investigator after consultation with the medical monitor, the concomitant medication will interfere with the study, subjects may continue taking prescription or nonprescription drugs (including vitamins and dietary or herbal supplements) as long as the subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call. The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Section 6.5.1 lists prohibited medications, medications requiring a washout period, and medications permitted under select circumstances. Section 6.5.2 lists prohibited interventions. Section 6.5.3 describes protocol-specific rescue medications for bladder pain associated with IC/BPS and UTI.

6.5.1. Prohibited Medications, Medications Requiring a Washout Period, and Medications Permitted Under Select Circumstances

The following medications are prohibited from the Screening Visit until after the EOS Follow-up Phone Call:

- GC-C agonists (eg, linaclotide, plecanatide)
- Diuretics (eg, furosemide)
- Opioids
- Tricyclic antidepressants

The following medications are prohibited from the Screening Visit until after the EOS Follow-up Phone Call and require a washout period:

- Intravesical therapy: 30-day washout period
- Pentosan polysulfate (ELMIRON): 30-day washout period
- Immunosuppressants/immunomodulators for IC/BPS (eg, cyclosporine): 30-day washout period

The following medications may be permitted under the specific circumstances outlined:

- Antidepressants (other than tricyclic antidepressants): permitted if prescribed for depression (ie, not pain control) and the subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call.
- Antihistamines, antispasmodics, anticholinergics: permitted if subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call. Antihistamines for treatment of allergy symptom flares may be used but only for ≤2 days.
- Amphetamines: permitted if prescribed for a non-IC/BPS-related condition and the subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call.
- Cannabinoids: permitted if subject has been on a stable dose for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call. CBD should be recorded as a concomitant medication regardless of medical need.
- Other pain medications/therapies (eg, pregabalin, gabapentin, barbiturates, benzodiazepines): permitted for a medical condition other than IC/BPS if subject has been on a stable dose/regimen for ≥30 days prior to the Pretreatment Visit and plans to continue on the same dose/regimen until after the EOS Follow-up Phone Call.
- Phenazopyridine hydrochloride (eg, PYRIDIUM): permitted during the time of a confirmed, active UTI only (refer to Section 8.3.9).

6.5.2. Prohibited Interventions

The following therapeutic interventions are prohibited from the Screening Visit until after the EOS Follow-up Phone Call:

- Cystoscopy with or without hydrodistension
- Surgical procedures, unless specifically discussed and permitted by the medical monitor
- Active use of sacral neuromodulation devices (eg, Interstim)

6.5.3. Rescue Medicine

At the Pretreatment Visit, the study site will supply rescue medicine to subjects for bladder pain associated with IC/BPS or active, confirmed UTI (refer to Section 8.3.9).

One of the following rescue medicines may be used:

- Acetaminophen ([325 mg/tablet] administered up to 2 tablets every 4 to 6 hours up to 10 tablets/day)
- Ibuprofen ([200 mg/tablet] administered every 4 to 6 hours up to 6 tablets/day)

Beginning at the Pretreatment Visit, the site will dispense either acetaminophen or ibuprofen to the subject. If the subject wants to switch from acetaminophen to ibuprofen or from ibuprofen to acetaminophen, and the investigator feels it is medically appropriate to switch, the site may

dispense new rescue medication. At any given time, the subject should only be taking either ibuprofen or acetaminophen. The subject must record their use of rescue medicine in the daily eDiary (refer to Section 8.1.1.1).

Throughout the Pretreatment and Treatment Periods, the eDiary will send automated alerts to the investigator if subjects have excessive use of rescue medicine. If a subject reports rescue medicine use >3 days per week for 2 consecutive weeks, the investigator must review the use of rescue medicine with the subject and decide whether the subject should remain in the study or be discontinued. During the treatment period, the investigator should make this decision in consultation with the medical monitor.

6.6. Dose Modification

Not applicable for this study

6.6.1. Retreatment Criteria

Not applicable for this study

6.7. Intervention After the End of the Study

There is no study intervention following the end of the study.

Subjects with ongoing AEs or other safety concerns will be followed until resolution or until no further queries or follow-up actions are required.

7. DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

It may be necessary for a subject to permanently discontinue study drug. A subject will be discontinued from the study in the event of:

- A confirmed pregnancy
- An SAE where the investigator recommends discontinuation

Dosing of study drug for an individual subject may be temporarily discontinued as detailed in Section 7.1.2. If the subject is unable to resume dosing, they should follow the instructions for permanent discontinuation below and in Section 7.2.

If treatment is permanently discontinued, then the subject will return to the clinic to have the Week 12/EOT assessments performed and the EOS Phone Call will also need to be performed. Refer to the SoA/Table 1 for data to be collected at the time of intervention discontinuation and follow-up, and for any further evaluations that need to be completed. If the subject discontinues treatment due to an AE, the subject will be followed until the AE resolves, stabilizes, or can be explained as being unrelated to study drug.

7.1.1. Study Suspension or Termination

The sponsor may stop enrollment prematurely because of a low recruitment rate, a change in the sample size based on the IA, or the study meeting prespecified efficacy criteria based on the IA (refer to Section 9.5).

The sponsor may permanently terminate the study, or a component of the study, at any time.

Reasons for temporary suspension of study drug dosing and/or study termination may include, but are not limited to:

- Death or treatment-emergent SAE considered to be related to IW-3300 that may preclude further dosing, in the opinion of the sponsor
- Change in opinion of the institutional review boards (IRBs), a DMC recommendation based on the results of the IA(s) (refer to Section 9.5 and Section 9.6), or a regulatory authority decision
- Low recruitment rate

In the case of temporary suspension of study drug dosing, dosing may be reinitiated or permanently halted after evaluation by the DMC.

Refer to Section 10.1.9.2 for additional information on site and study termination.

7.1.2. Temporary Discontinuation

The investigator may allow a subject to stop taking study drug for up to 3 days should an intolerable AE occur. If the investigator believes that the subject is unable to resume dosing after 3 days or requires a suspension of dosing on more than 1 occasion, the investigator should contact the medical monitor to discuss the subject's continued participation in the study.

7.1.3. Rechallenge

Not applicable for this study

7.2. Subject Discontinuation/Withdrawal from the Study

- A subject may withdraw from the study at any time at their own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.
- At the time of discontinuing from the study, if possible, an EOT visit should be conducted, as shown in the SoA/Table 1. Refer to the SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.
- If a subject is permanently discontinued from the study intervention, they will also be discontinued from the study.
- If the subject 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 subject withdraws from the study, they may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

7.3. Lost to Follow-up

A subject will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and is unable to be contacted by the study site.

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

- The site representative must attempt to contact the subject, reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, they will be considered to have withdrawn from the study and reason for discontinuation will be "lost to follow-up."

Discontinuation of specific sites or of the study as a whole are handled as described in Section 10.1/Appendix 1.

8. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA/Table 1. Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA/Table 1, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

8.1. Efficacy Assessments

8.1.1. eDiary Assessments

Unless otherwise noted, eDiary assessments will be reported throughout the Pretreatment Period and Treatment Period.

8.1.1.1. Daily eDiary Assessments

Daily assessment of bladder pain at its worst

Subject assessment of bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst will be reported via a daily evening report in the eDiary at baseline (Pretreatment Period) and throughout the Treatment Period. The rating of bladder pain (which can include sensations like burning, pressure, and/or discomfort) at its worst during the previous 24 hours on an 11-point NRS will be provided by the subject answering the following question:

"How would you rate your worst bladder pain (which can include sensations like burning, pressure, and/or discomfort) in the past 24 hours?"

> eDiary presents NRS where 0 is anchored with "None" and 10 is anchored with "Worst possible"

Assessment of a burning sensation in the bladder at its worst

Subject assessment of a burning sensation in the bladder at its worst will be reported via a daily evening report in the eDiary at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. The rating of a burning sensation in the bladder at its worst during the previous 24 hours on an 11-point NRS will be provided by the subject answering the following question:

"How would you rate your worst burning sensation in the bladder in the past 24 hours?"

> eDiary presents NRS where 0 is anchored with "None" and 10 is anchored with "Worst possible"

Assessment of a pressure sensation in the bladder at its worst

Subject assessment of a pressure sensation in the bladder at its worst will be reported via a daily evening report in the eDiary at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. The rating of a pressure sensation in the bladder at its worst during the previous 24 hours on an 11-point NRS will be provided by the subject answering the following question:

"How would you rate your worst pressure sensation in the bladder in the past 24 hours?"

eDiary presents NRS where 0 is anchored with "None" and 10 is anchored with "Worst possible"

Assessment of discomfort in the bladder at its worst

Subject assessment of discomfort in the bladder at its worst will be reported via a daily evening report in the eDiary at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. The rating of discomfort in the bladder at its worst during the previous 24 hours on an 11-point NRS will be provided by the subject answering the following question:

"How would you rate your worst discomfort in the bladder in the past 24 hours?"

eDiary presents NRS where 0 is anchored with "None" and 10 is anchored with "Worst possible"

Urinary frequency

Urinary frequency will be reported at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. Subjects will record each urination in the eDiary on an event-driven basis and indicate if the urination caused them to wake from sleep. The assessment of urinary frequency is based on the dates and times recorded by the subject. If the subject misses an entry, they can enter the urination into the eDiary at a later time and include the actual date and time of the urination.

Urinary urgency

Urinary urgency will be reported at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. Subject assessment of urgency will be collected by the eDiary on an event-driven basis for each urination reported in the eDiary. If the subject misses a urination entry, they can enter the urination and associated assessment of urgency into the eDiary at a later time.

0=No urgency

1=Mild urgency

2=Moderate urgency

3=Severe urgency

4=Urge incontinence

Difficulty sleeping

Difficulty sleeping will be reported via a daily evening report in the eDiary at baseline (Pretreatment Period) and for 7 days before the Week 4, Week 8, and Week 12 timepoints. The ratings of difficulty sleeping will be provided by the subject answering the following 2 questions:

"How much difficulty did you have sleeping because of your bladder pain in the past 24 hours?"

1=None

2=Mild

3=Moderate

4=Severe

5=Very severe

"How much difficulty did you have sleeping because of a need to urinate in the past 24 hours?"

1=None

2=Mild

3=Moderate

4=Severe

5=Very severe

Use of rescue medicine

Subjects will report their use of rescue medicine on a daily basis at baseline (Pretreatment Period) and throughout the Treatment Period by eDiary entry. An evening report will ask whether the subject used acetaminophen or ibuprofen and the number of tablets taken that day.

Study drug administration

Subject will report their use of study drug on a daily basis throughout the Treatment Period by eDiary entry. The daily assessment will be provided by the subject answering the following question:

"Did you administer the study drug today?"

1=Yes

2=No

If the answer is "1=Yes", 2 additional questions will be asked:

Canister-related dosing problems

Subject assessment of canister-related dosing problems will be reported daily by eDiary entry throughout the Treatment Period. The daily assessment of canister-related dosing problems will be provided by the subject answering the following question:

"Did you have any dosing problems related to the canister today?"

1=Yes

2=No

Study drug retention

Subject assessment related to study drug retention will be reported daily by eDiary entry throughout the Treatment Period. The daily assessment of study drug retention will be provided by the subject answering the following question:

"Did you have any foam leakage after taking study drug today?"

1=Yes

2=No

8.1.1.2. Weekly eDiary Assessments

8.1.1.2.1. General Weekly Questions

Weekly assessment of IC/BPS symptom severity

Subject global impression of IC/BPS severity will be reported weekly in the eDiary each week of the Pretreatment and Treatment Periods. The rating of overall IC/BPS severity during the previous 7 days on a 5-point ordinal scale will be provided by the subject answering the following question:

"How would you rate your overall IC/BPS symptoms during the past 7 days?"

1=None

2=Mild

3=Moderate

4=Severe

5=Very severe

Weekly assessment of treatment satisfaction

Subject assessment of treatment satisfaction will be reported weekly by eDiary entry each week of the Treatment Period. Subjects will answer the following question on a 5-point ordinal scale:

"Overall, how satisfied are you with the study drug's ability to relieve your IC/BPS pain during the past 7 days?"

1=Not at all satisfied

2=A little satisfied

3=Moderately satisfied

4=Quite satisfied

5=Very satisfied

Weekly assessment of adequate relief

Subject assessment of adequate relief of IC/BPS pain will be reported by an eDiary entry each week of the Treatment Period. The rating of adequate relief during the previous 7 days on a binary scale will be provided by the subject answering the following question:

"Overall, have you had adequate relief from your bladder pain during the past 7 days?"

1=Yes

2=No

Weekly assessment of IC/BPS symptom change

Subject global impression of IC/BPS severity will be reported weekly in the eDiary each week of the Treatment Period. The rating of overall IC/BPS severity during the previous 7 days on a 7-point ordinal scale will be provided by the subject answering the following question:

"Compared to before you started the study, please rate your overall change in IC/BPS symptoms during the past 7 days."

1=Very much better

2=Moderately better

3=A little better

4=No change

5=A little worse

6=Moderately worse

7=Very much worse

Weekly assessment of sexual function

The sexual function question will be reported weekly in the eDiary each week of the Treatment Period. The answer on a 7-point ordinal scale will be provided by the subject answering the following question:

"Compared to before you started the study treatment, how would you rate your ability to have sexual intercourse?"

-3=Markedly worse

-2=moderately worse

-1=slightly worse

0=no change

1=slightly better

2=moderately better

3=markedly better

99=not applicable, prefer not to answer

8.1.1.2.2. Genitourinary Pain Index (GUPI)

The GUPI assesses the degree of symptoms in both men and women with genitourinary pain complaints over the last week, including experience (Yes/No) of pain or discomfort in various areas, frequency of pain/discomfort (0=Never to 5=Always), average pain/discomfort (11-point NRS), frequency of urinary symptoms (0=Not at all to 5=Almost always), and quality of life (QoL) impact (0=None to 3=A lot; or 1=Pleased to 6=Terrible). Refer to Section 10.5.1 for the full questionnaire.

8.1.1.2.3. Global Response Assessment (GRA)

The GRA measures overall improvement with therapy, asking "As compared to when you started the study [treatment], how would you rate your interstitial cystitis symptoms now?" The response is recorded on a 7-point scale centered at zero (no change): markedly worse (=-3); moderately worse (=-2); slightly worse (=-1); no change (=0); slightly improved (=+1); moderately improved (=+2); and markedly improved (=+3). Refer to Section 10.5.3 for the full questionnaire.

8.1.1.3. Monthly eDiary Assessment

8.1.1.3.1. O'Leary/Sant Interstitial Cystitis Symptom Index (ICSI)

The O'Leary/Sant ICSI assesses the incidence of IC symptoms during the past month. Questions on urgency and frequency are scored on a scale of '0=Not at all' to '5=Almost always'. The question on nocturia is scored on a scale of '0=None' to '5=5 or more times'. The question on pain or burning in the bladder is scored on a scale of '0=Not at all' to '5=Usually'. Refer to Section 10.5.2 for the full questionnaire.

8.1.2. Margolis Body Map for Pain Assessment

At the study visits shown in the SoA/Table 1, pain location will be assessed using the Margolis body map (12); refer to Section 10.5.6 for the full instrument.

Subjects will be asked to circle the number corresponding to each body site on the map where they experienced pain in the past week. Subjects reporting pain in Sites 14, 15, or 16 only will be considered to have "pelvic pain only", a designation used by Nickel et al in a previous pain pattern study in women with IC/BPS. (13)

Using the subject's completed body map and a classification system described by Lai et al (14), their body pain will be categorized in as:

- Pelvic pain only
- Pelvic pain and 1 to 2 other sites
- Pelvic pain and 3 to 7 other sites
- Pelvic pain and 8+ other sites

8.2. Safety Assessments

The planned timepoints for all safety assessments are provided in the SoA/Table 1.

8.2.1. Physical Examinations

Physical examinations will be performed as outlined in the SoA/Table 1, by the investigator or a licensed health professional listed on Form FDA 1572.

- A complete physical examination will include, at a minimum, assessments of the general appearance of the subject and the HEENT (head, eyes, ears, nose, and throat), cardiac, respiratory, GI, musculoskeletal, neurological, and dermatological systems.
- A visual and digital rectal examination, including a hemoccult test, will be done at Screening and Week 12/EOT. Only a visual rectal examination will be done at other visits. However, if significant redness, rectal pain, bleeding, or mucus is observed on visual inspection of the rectal (perianal) area, a digital rectal examination and hemoccult test should be performed. If further investigation is warranted, subjects with these signs or symptoms should undergo proctoscopy or recto-sigmoidoscopy. A colonoscopy may be performed instead of the recto-sigmoidoscopy after discussion between the investigator and the sponsor.
- Height (only at Screening) and weight will also be measured and recorded.
- Investigators should pay special attention to clinical signs related to previous serious illnesses, which will be reason for exclusion from the study.
- Any physical examination abnormality that the investigator considers to be potentially clinically significant and changed from baseline will be reported as an AE.

8.2.2. Vital Signs

Vital signs will be performed as outlined in the SoA/Table 1.

- Oral temperature, respiratory rate, blood pressure, and pulse rate will be assessed.
- Blood pressure and pulse measurements will be taken in the seated position with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements will be taken after the subject has been sitting for 5 minutes in a quiet setting without distractions (eg, television, cell phones).
- When applicable, vital signs measurements will be obtained prior to blood draws.

8.2.3. Electrocardiograms

- Triplicate 12-lead ECG will be obtained as outlined in the SoA/Table 1 using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTcF intervals.
- ECGs should be obtained after the subject has been supine for at least 5 minutes and prior to blood draws, when applicable.
- At each time point at which triplicate ECGs are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart.

8.2.4. Clinical Safety Laboratory Assessments

- Refer to Section 10.2/Appendix 2 for the list of clinical laboratory tests to be performed and to the SoA/Table 1 for the timing and frequency. All protocol-required laboratory assessments must be conducted in accordance with the laboratory manual and the SoA/Table 1.
- 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 eCRF. 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 subject's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 24 hours after the last dose of study intervention 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.
  - If laboratory values from nonprotocol specified laboratory assessments performed at the institution’s local laboratory require a change in subject 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.

8.2.5. Pregnancy Testing

- Refer to Section 5.1, Inclusion Criteria, for screening and Day 1 (before the first dose) pregnancy testing criteria.
- Pregnancy testing must be conducted at the timepoints indicated in the SoA/Table 1.
- Pregnancy testing (urine or serum as required by local regulations) should be conducted at the end of relevant systemic exposure.
- Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator, or required by local regulation, to establish the absence of pregnancy at any time during the subject's participation in the study.

8.2.6. Medical History

In addition to general medical history, baseline data on a subject’s IC/BPS characteristics and prior symptom management will also be collected at the Screening Visit. All of these data will be incorporated within the subject’s source documentation and entered into the eCRF.

For IC/BPS characteristics, the following data will be collected:

- date of first IC/BPS symptom onset
- date of first diagnosis

- findings from prior cystoscopy, if available (eg, date of prior cystoscopy and presence of Hunner's lesions [yes/no/unknown])
- presence of associated conditions (eg, IBS, fibromyalgia, chronic fatigue syndrome, anxiety and depression disorders, migraines).

8.2.7. Suicidal Ideation and Behavior Risk Monitoring

Patients with IC/BPS have an increased risk for depression and may be at increased risk for suicide. (15) Subjects who have an uncontrolled major psychiatric condition or have made a suicide attempt during the 2 years before the Screening Visit are excluded from the study.

IW-3300 is not considered to be a central nervous system-active drug, nor is it related to products with an increased risk of suicidal ideation or behavior. However, consideration should be given to discontinuing the study drug in subjects who experience signs of suicidal ideation or behavior.

8.3. Adverse Events and Serious Adverse Events

The definitions of AEs and SAEs can be found in Section 10.3/Appendix 3.

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

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up on AEs that are serious, considered related to the study intervention or study procedures, or that caused the subject to discontinue study drug (refer to Section 7).

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

All AEs and SAEs will be collected from the signing of the ICF until completion of study participation (ie, EOS Follow-up Phone Call) at the timepoints specified in the SoA/Table 1.

Any medical condition that is present at the time that a randomized subject is screened and does not deteriorate (worsen in severity and/or frequency) should be recorded as medical history and not as an AE. However, if the subject's condition deteriorates at any time during the study, it will be recorded as an AE. AEs characterized as intermittent require documentation of onset and duration of each episode. Pretreatment AEs will be collected and captured in the subject's source documentation from the time the subject signs the ICF until the subject receives study drug. Pretreatment AEs in randomized subjects will additionally be entered on the AE page of the subject's eCRF. Laboratory abnormalities, changes in vital signs, and physical examination findings should be considered AEs and reported on the AE page of the subject's eCRF only if the investigator considers them clinically significant and/or they necessitate intervention.

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.3/Appendix 3. 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 AEs or SAEs after the conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after

a subject has been discharged from the study, and they consider the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3/Appendix 3.

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

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. Subjects with ongoing AEs or other safety concerns will be followed until resolution or until no further queries or follow-up actions are required. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Section 10.3.3/Appendix 3.

8.3.4. 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 subjects and the safety of a study intervention 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 intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/Independent Ethics Committees (IECs), and investigators.
- For all studies except those utilizing medical devices, 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 an 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.

8.3.5. Pregnancy

- Details of all pregnancies that occur in female subjects and female partners of male subjects following study drug exposure will be collected either until completion of the EOS Follow-up Phone Call or until the details regarding the pregnancy outcome are

reported. The investigator should make a reasonable effort to follow any pregnant patients until delivery or end of the pregnancy.

- 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 Section 10.4/Appendix 4.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.3.6. Cardiovascular and Death Events

Cardiovascular and death events should be reported according to the definitions and reporting procedures for AEs and SAEs outlined in Section 10.3/Appendix 3.

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

Not applicable to this study

8.3.8. Adverse Events of Special Interest

Not applicable to this study

8.3.9. Urinary Tract Infections

UTIs must be reported according to the definitions and reporting procedures for AEs and SAEs outlined in Section 10.3/Appendix 3. Additional clinical data on UTIs will be collected. UTIs must be confirmed by a urinalysis and urine culture.

8.4. Treatment of Overdose

For this study, any dose that exceeds the dosage or frequency specified in the protocol will be considered an overdose.

The sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator should:

1. Report any case of overdose to the sponsor and use their clinical judgment to treat the case of overdose as needed with the appropriate general supportive measures.
2. Closely monitor the subject for any AE/SAE and laboratory abnormalities until study drug can no longer be detected systemically.
3. Document the quantity of the excess dose(s) as well as the duration of the overdose in the eCRF.

Decisions regarding dose interruptions will be made by the investigator in consultation with the medical monitor based on the clinical evaluation of the subject.

8.5. Pharmacokinetics

- Plasma will be collected for measurement of plasma concentrations of IW-3300 as specified in the SoA/Table 1. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.
- Samples will be used to evaluate the PK of IW-3300. Each plasma sample will be divided into 2 aliquots.

PK samples may be analyzed for drug concentrations in batches on a periodic basis as the study is being conducted. However, information that may unblind the study will not be reported to investigative sites or other blinded personnel at the site, sponsor, or CRO until after the study has been unblinded.

8.6. Health-related Quality of Life

The EuroQol 5-Dimension 5-Level Version (EQ-5D-5L) questionnaire will be captured at the timepoints noted in the SoA/Table 1. Refer to Section 10.5.4 for the full questionnaire.

The Hospital Anxiety and Depression Scale (HADS) will be captured at the timepoints shown in Table 1/SoA. Refer to Section 10.5.5 for the full questionnaire.

8.7. Microbiome

Gut microbiome will be assessed as an exploratory evaluation in a subset of approximately 60 subjects who complete the study (~20/arm) using stool samples collected in accordance with the SoA/Table 1. At the visits required, subjects in the microbiome substudy will bring in a stool sample from home, collected prior to the study visit (and predose for Day 1), using the sample collection kit issued by the site. The methods for collecting, handling, and processing samples will be provided in a laboratory manual.

8.8. Pharmacodynamics

Not applicable for this study

8.9. Genetics

Genetics are not evaluated in this study; no samples will be collected for genetics research.

8.10. Biomarkers

Biomarkers are not evaluated in this study; no samples will be collected for biomarker research.

8.11. Immunogenicity Assessments

Immunogenicity is not evaluated in this study; no samples will be collected to evaluate antibodies to IW-3300.

8.12. Medical Resource Utilization and Health Economics

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

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

The primary efficacy endpoint of the treatment differences for weekly average CFB in bladder pain at its worst at Week 12 will be assessed to test the following hypothesis:

$$H_0 : \mu_{100\mu g} - \mu_{Placebo} \geq 0 \text{ and } \mu_{300\mu g} - \mu_{Placebo} \geq 0,$$

$$H_a : \mu_{100\mu g} - \mu_{Placebo} < 0 \text{ or } \mu_{300\mu g} - \mu_{Placebo} < 0.$$

The study-wide type I error rate is controlled at 1-sided 0.05. To account for the multiplicity resulting from testing 2 doses, the total 0.05 α is split between the 100 µg and 300 µg doses, 60% to the high dose and 40% to the low dose. This unequal split is intended to prioritize the higher dose based on the hypothesis that efficacy of the high dose is greater or equal to efficacy of the low dose. Thus, the high dose will be allocated 0.03 1-sided α and the low dose will be allocated 0.02 1-sided α. If a null hypothesis is rejected only for a dose group under the initial allocation of the alpha-level, the alpha-level for that dose will be propagated to the remaining nonsignificant dose.

9.2. Sample Size Determination

The sample size and power for the study was estimated based on simulations with Bayesian adaptive design for the primary efficacy endpoint. The assumption of the placebo effect was obtained by augmenting the historical information of effects of placebo treatment from 4 publications of randomized placebo-controlled studies in subjects with IC/BPS, which utilized the same bladder pain endpoint as measured on an 11-point NRS and similar treatment durations; as well as the effects of placebo treatment from 3 previous Ironwood studies conducted with linaclotide (another GC-C agonist) in subjects with IBS-C, which utilized a similar pain endpoint for abdominal pain that was also measured on an 11-point NRS. The effect of the active treatment was obtained through meta-analysis of the 3 previous Ironwood studies in IBS-C with linaclotide. Per the historical data exploratory results, a change from baseline treatment difference of -0.96 with standard deviations of 2.326 (active) and 2.084 (placebo) for the primary efficacy endpoint at Week 12 among the active treatments and placebo arm, respectively, was used as a reference for the simulations.

These simulations showed that the study is powered at 80% or higher to detect an approximate 1-point difference in the primary outcome under various scenarios. In the simulations, the standard deviation (SD) was varied around 2, a 10% attrition rate was assumed, and the maximum sample size was set to 300. The average sample size ranged from approximately 200 to 280.

Based on the outcome(s) of the IA(s), the study may be stopped early for efficacy, a treatment arm may be discontinued if it does not warrant further exploration, or enrollment may be stopped early for futility. Given these scenarios, specifically for the case of a treatment arm being discontinued, enrollment will continue for the remaining 2 arms, for a total number of approximately 300 subjects for the study.

9.3. Populations for Analyses

The analysis populations are defined in Table 2.

Table 2: Analysis Populations

Analysis Set	Description
Screened Analysis Set	The Screened Analysis Set consists of all subjects who have signed informed consent.
Intent-to-Treat (ITT) Analysis Set	The ITT Analysis Set consists of all subjects who are randomized to a treatment regimen. Analysis will be performed according to the treatment assigned at the Day 1 Visit.
Safety Analysis Set	The Safety Analysis Set consists of all subjects who receive any amount of study drug. Analysis will be performed according to the treatment actually received[a] regardless of the allocated treatment.
PK Analysis Set	The PK Analysis Set consists of all subjects who receive any amount of study drug and have at least 1 postdose PK assessment. Analysis will be performed according to treatment actually received[a] regardless of the treatment regimen allocated.
Microbiome Analysis Set	The Microbiome Analysis Set consists of all subjects in the Safety Analysis Set who have both baseline and at least 1 postbaseline microbiome assessment. Analysis will be performed according to the treatment actually received[a] regardless of the allocated treatment.

[a.] Treatment actually received is determined by the majority of the study drug administered. In case of a tie, the highest dose level will be considered the actual study treatment for the subject.

9.4. Statistical Analyses

The statistical analysis plan (SAP) will be finalized prior to database lock. The SAP will include a more technical and detailed description of the statistical analyses. A separate IA plan will be written to cover the details of the IA.

9.4.1. General Considerations

In general, descriptive statistics will be presented by treatment group and by visit, as applicable.

For analysis of continuous parameters (eg, CFB), descriptive statistics (n, mean, SD, median, and range) will be calculated and presented for each visit and treatment. For categorical parameters (eg, responder vs. nonresponder), the number and percentage of each category will be calculated and presented for each visit and treatment.

Baseline values for the efficacy parameters will be generated based on the eDiary data collected in the last 14 days of the Pretreatment Period. Further details will be available in the SAP.

The 2 comparisons of the primary efficacy endpoint among the IW-3300 doses relative to placebo will be adjusted unequally, that is, the total 1-sided 0.05 α is split between the 100 µg and 300 µg doses, 60% to the high dose and 40% to the low dose, and an α-propagation approach will be applied for the analysis. Multiple comparisons will not be adjusted in the conduct of comparisons among the IW-3300 doses relative to placebo for all other efficacy endpoints.

Missing data will not be imputed for the primary efficacy analysis. Statistical analyses will be conducted using SAS version 9.4 (or later).

9.4.2. Primary Endpoint Analysis

All efficacy analysis will be performed on the ITT Analysis Set. The primary efficacy endpoint is the CFB in weekly average of daily bladder pain at its worst at Week 12.

The statistical null hypothesis for the primary endpoint is that the treatment effect of 100 µg and 300 µg is the same as placebo. This hypothesis will be tested on the ITT Analysis Set using a mixed model for repeated measurement. The CFB in weekly average of daily bladder pain at its worst postbaseline assessments up to Week 12 will be used for the analysis. The model will include a covariate of baseline average of daily bladder pain at its worst, and factors of visit, treatment, and interaction of treatment by visit.

A sensitivity analysis will be implemented to confirm the results from the primary analysis. Missing values will be imputed using multiple imputation assuming missing not at random. Analysis of covariance (ANCOVA) model with treatment as a factor and baseline average of daily bladder pain at its worst as a covariate will be applied.

Details of the statistical models and analyses including other sensitivity analyses will be provided in the SAP, and details of the IA will be provided in the IA plan.

9.4.3. Secondary Endpoint Analysis

The secondary efficacy endpoints analyses will be conducted on the ITT population. Statistical comparisons of placebo and active arms on the CFB in weekly average of a burning sensation, pressure sensation, or discomfort in the bladder at its worst at Week 12 will be conducted, respectively. A similar analysis will be performed for the CFB in the GUPI Pain subscale score.

The secondary safety endpoint analyses will be performed on the Safety Analysis Set. The number and percentage of subjects reporting TEAEs with an occurrence of ≥2% (in any IW-3300 dose strength) of subjects in each treatment group will be tabulated by MedDRA system organ class and preferred term. If a subject has >1 TEAE coded to the same preferred term, the subject will be counted only once for that preferred term. Statistical tests will not be performed.

The overall number and percentage of subjects reporting TEAEs in each treatment group will be tabulated by severity grade.

9.4.4. Exploratory Endpoints Analyses

The appropriate analysis set will be applied for each of the exploratory endpoints. Statistical comparisons will be performed whenever they are appropriate. However, as these endpoints are exploratory in nature, no multiplicity adjustment will be performed.

9.4.5. Safety Analyses

All safety analyses will be performed on the Safety Analysis Set. All safety parameters will be summarized using descriptive statistics. For each safety parameter, the last nonmissing assessment made before the first dose of study drug will be used as the baseline for all analyses of that safety parameter.

9.4.6. Pharmacokinetics

The PK analysis will be performed on the PK Analysis Set. If systemic levels of IW-3300 are measurable, plasma concentrations of IW-3300 will be summarized using appropriate descriptive statistics by IW-3300 dose strength and overall at each assessment timepoint.

9.4.7. Microbiome

The analysis of the microbiome will be performed on the Microbiome Analysis Set. This analysis will be conducted in a subgroup of subjects comprising approximately 60 subjects (~20/arm). The relative abundance of microorganisms by different family and genera will be constructed and summarized by treatment at Baseline and Week 12/EOT using descriptive statistics. Changes from baseline will be presented.

9.4.8. Health-related Quality of Life

The EQ-5D-5L utility scores and Visual Analog Scale scores and the HADS scores will be summarized for each visit using descriptive statistics by treatment for the Safety Analysis Set.

9.5. Interim Analyses

At least 1 IA will be performed during the study to provide an early assessment of the efficacy or futility of IW-3300. The purpose of the IA(s) is to stop the study early for efficacy if there is overwhelming evidence of effectiveness, to discontinue a treatment arm if it does not warrant further exploration, or to stop enrollment early for futility. Details will be provided in an IA plan.

The study-wide type I error rate is controlled at 1-sided 0.05. The strategy to control the overall α for the study primary endpoint is described in Section 9.1. The actual α-spending level will be determined by the Kim-Demets α-spending function and an α-propagation approach will be used for the analyses.

Regardless of whether the decision is to stop the study early or to continue to the end of the study, formal statistical comparisons will be conducted at the end of the study based on the prespecified α-spending level. Details of the α-spending level and the operating characteristics of the simulation will be provided in an IA plan.

9.6. Data Monitoring Committee

An independent DMC will be used to regularly monitor safety and tolerability. Additionally, independent review will be conducted to evaluate efficacy of IW-3300 as part of the IA for the purpose of early stopping or discontinuation of a study arm based on prespecified efficacy or futility decision rules documented in the IA plan. Details of the DMC membership, standard operational procedures for data monitoring/review, frequency of review, and other pertinent details will be provided in a separate DMC Charter.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 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 (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 subjects.

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 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2. 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.

10.1.3. Informed Consent Process

The investigator or their representative will explain the nature of the study to the subject or their legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR Part 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) 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 subject 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.

Subjects must be reconsented 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 subject or the subject's legally authorized representative.

Subjects who are rescreened may be required to sign a new ICF.

10.1.4. Data Protection

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of participant data.

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that their personal study-related data will be used by the sponsor in accordance with local data protection laws. The level of disclosure must also be explained to the subject who will be required to give consent for their data to be used as described in the ICF.

The subject must be informed that their 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.

10.1.5. Committee Structure

Key study personnel, including sponsor and CRO representatives, and their contact details will be provided prior to the Site Initiation Visit.

The safety of this study will be closely monitored on an ongoing basis by sponsor representatives. An independent DMC will be implemented as described in Section 9.6. Details of the DMC membership, standard operational procedures for data monitoring/review, frequency of review, and other pertinent details will be provided in a separate DMC Charter.

10.1.6. Dissemination of Clinical Study Data

The sponsor will make study results available through www.ClinicalTrials.gov or other public registries in accordance with applicable local laws and regulations.

10.1.7. Data Quality Assurance

All subject data relating to the study will be recorded on the eCRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

Quality tolerance limits (QTLs) will be predefined in the Centralized Monitoring Plan to identify systematic issues that can impact subject safety and/or reliability of study results. These predefined parameters will be monitored during the study, and important deviations from the QTLs and remedial actions taken will be summarized in the clinical study report.

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 (eg, CROs).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects 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.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for the longer of:

- 2 years after the last marketing authorization for the study drug has been approved or the sponsor has discontinued its research with respect to the study drug, or
- Such longer period as required by applicable US regulatory requirements or by US law

No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8. Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.

Data reported on the eCRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The

investigator may need to request previous medical records or transfer records, depending on the study.

Definition of what constitutes source data can be found in the Monitoring Plan.

Description of the use of the EDC system is documented in the Data Management Plan.

10.1.9. Study and Site Start and Closure

10.1.9.1. First Act of Recruitment and Study Start

The first act of recruitment is the date that the first subject signed the ICF and will be the study start date.

10.1.9.2. Study and Site Termination

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. The study site will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for termination of the study include but are not limited to:

- Discontinuation of further study intervention development

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigator, the IEC/IRB, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the subject and should ensure appropriate subject therapy and/or follow-up.

Study termination is also provided for in the clinical study agreement. If there is any conflict between the contract and this protocol, the contract will control as to termination rights.

10.1.10. Publication Policy

The results of this study may be published or presented at scientific meetings. The sponsor will comply with the requirements for publication of study results. Any publication of data from the study by the investigator will be subject to mutual agreement between the investigator and the sponsor.

10.2. Appendix 2: Clinical Laboratory Tests

All protocol-required laboratory assessments, as defined below, must be conducted in accordance with the laboratory manual and the SoA/Table 1.

- The laboratory tests are detailed in Table 3.
- All blood samples for ESR testing will be analyzed at the local laboratory and entered into the eCRF. Apart from ESR, local laboratory results are only required in the event that the central laboratory results are not available in time for either study intervention 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. (See exceptions below for urinalysis and urine culture performed in the case of a UTI.)
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.
- Repeat or medically necessary tests may be performed at any time during the study, as determined necessary by the investigator or required by local regulations. Laboratory tests outside of those specified in the protocol should be discussed with the medical monitor.
- For pregnancy testing, refer to Section 5.1 for screening and Day 1 (prior to dosing) requirements and Section 8.2.5 for requirements during and after study drug dosing.
- UTIs must be confirmed by urinalysis and urine culture analyzed by the central laboratory; refer to Section 8.3.9. If these tests are performed at the local laboratory, however, no samples need to be collected for central laboratory testing.
- SARS-CoV-2 (ie, COVID) testing is not required as part of this study protocol. Testing should be conducted in accordance with the site's testing policy.

Table 3: Protocol-required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet count RBC count Hemoglobin Hematocrit	RBC indices: Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC) % Reticulocytes		WBC count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils
Clinical Chemistry[a]	Blood urea nitrogen (BUN)	Potassium	Aspartate aminotransferase (AST)	Total and direct bilirubin
	Creatinine	Sodium	Alanine aminotransferase (ALT)	Total protein
	Glucose (random)	Calcium	Alkaline phosphatase	Magnesium
	Chloride	Albumin	Bicarbonate	Phosphate
	Cholesterol	Uric acid		
Inflammatory Markers	Erythrocyte sedimentation rate (ESR) C-reactive protein (CRP)			
Hemoccult	Point-of-care hemoccult test (In addition to the timepoints listed in the SoA/Table 1, if significant redness, rectal pain, bleeding, or mucus is observed on visual inspection of the rectal [perianal] area, a digital rectal examination and hemoccult test should be performed. Stool that is present on the digital rectal exam can be used for the hemoccult test.)			
Routine Urinalysis	Specific gravity pH, glucose, protein, blood, ketones Microscopic examination (if blood or protein is abnormal) Urinalysis and urine culture (performed if UTI is suspected)			
Other Screening Tests	Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, and benzodiazepines) HIV antibody and hepatitis panel (hepatitis B surface antigen [HBsAg] and hepatitis C virus [HCV] antibody)			
Hormone tests for female subjects	Highly sensitive pregnancy test (WOCBP only, refer to Section 10.4.1.1) Follicle-stimulating hormone (FSH; refer to Section 10.4.1.1 and Section 10.4.1.2)			

[a] All events of ALT >3×upper limit of normal (ULN) and bilirubin >2×ULN (>35% direct bilirubin) or ALT >3×ULN and international normalized ratio (INR) >1.5, if INR is measured which may indicate severe liver injury (possible Hy's Law case), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

Investigators must document their review of each laboratory safety report.

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

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE and TEAE

10.3.1.1. AE Definition

- An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study intervention, whether or not considered related to the study intervention.
- NOTE: 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 study intervention.

10.3.1.2. TEAE Definition

- A TEAE is an event that emerges, or a pre-existing event that worsens, any time after the initiation of the first dosing of the investigational product.

10.3.1.3. Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, 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 conditions detected or diagnosed after study intervention 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 intervention 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.
- The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. Also, “lack of efficacy” or “failure of expected pharmacological action” also constitutes an AE or SAE.
- Specific Situations
  - AEs that are secondary to other AEs: In general, AEs that are secondary to other AEs (eg cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. If a medically significant secondary AE that is separated in time from the initiating event occurs,

the event may be recorded as an independent event on the AE eCRF after consultation with the sponsor. All AEs should be recorded separately if it is not clear as to whether the events are associated.

- Persistent AEs: A persistent AE is one that extends continuously, without resolution, between subject evaluation time points. When a persistent AE changes in severity over time, record the AE only once, with initial start date and highest severity reached. Exception: If an AE that started before study drug administration worsens after study drug administration, record 2 AEs. The first AE should have the initial start date and severity, an end date equal to the day before the worsening onset, and an outcome of recovered/resolved. The second AE should have a start date equal to the day the AE worsened. If the AE becomes more severe, the most extreme severity should be recorded within the same AE entry.
- Recurrent AEs: A recurrent AE is one that resolves between subject visits and subsequently recurs. Each recurrence of the AE should be recorded as a separate AE on the AE eCRF.

10.3.1.4. Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject'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.

10.3.2. Definition of SAE

If an event is not an AE per definition above (Section 10.3.1.1), 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:

1. Results in death
2. Is life-threatening
   - The term "life-threatening" in the definition of "serious" refers to an event in which the subject 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.

3. Requires inpatient hospitalization or prolongation of existing hospitalization
    - In general, hospitalization signifies that the subject 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.
4. 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.
5. Is a congenital anomaly/birth defect
6. 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 subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above Definition of SAE. 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.

10.3.3. Recording and Follow-Up of AE and/or SAE

10.3.3.1. AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the eCRF.
- It is not acceptable for the investigator to send photocopies of the subject's medical records to Ironwood Global Patient Safety in lieu of completion of the SAE Form/AE/SAE eCRF page.

- There may be instances when copies of medical records for certain cases are requested by Ironwood Global Patient Safety. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to Ironwood Global Patient Safety.
- 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/SAE.
- For subjects who fail screening and have an AE or SAE, only the SAE will be collected in eCRF. AEs will be documented only in source documentation for screen-failed subjects.

10.3.3.2. Assessment of Intensity/Severity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories, according to the National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE]):

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADLs)
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADLs
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

A semicolon indicates "or" within the description of the grade.

ADLs are defined as follows: Instrumental ADLs refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. Self-care ADLs refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

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.

10.3.3.3. Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult IB and/or Product Information, for marketed products, in their assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that they have reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor.
- The investigator may change their opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

10.3.3.4. Follow-up of AEs and SAEs

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

10.3.4. Reporting of SAEs

10.3.4.1. SAE Reporting to Ironwood Global Patient Safety via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to the sponsor will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (refer to the next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.

- After the study is completed, the electronic data collection tool will be taken offline to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken offline, then the site can report this information on a paper SAE form (refer to the next section) or to the medical monitor/SAE coordinator by telephone.
- Contacts for SAE reporting can be found in the study contact list.

10.3.4.2. SAE Reporting to Ironwood Global Patient Safety via Paper SAE Report Form

- If the electronic data collection system is not available, email transmission of the SAE report form is the preferred method to transmit this information to the medical monitor and SAE coordinator (ICSRoperations@ironwoodpharma.com). If email is not available, the SAE report form should be transmitted via facsimile.
- In rare circumstances and in the absence of email or facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE report form within the designated reporting time frames.
- Contacts for SAE reporting can be found in the study contact list.

10.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

10.4.1. Definitions

10.4.1.1. Women of Childbearing Potential (WOCBP)

Women in the following categories are considered WOCBP (fertile):

1. Following menarche
2. From the time of menarche until becoming postmenopausal unless permanently sterile (refer to the text below)
    - 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 at the discretion of the investigator to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). (The laboratory threshold for defining postmenopausal state by FSH is >30 IU/L.)
        - Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-estrogen 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.
    - Permanent sterilization methods (for the purpose of this study) include:
        - Documented hysterectomy
        - Documented bilateral salpingectomy
        - Documented bilateral oophorectomy
        - For individuals with permanent infertility due to an alternate medical cause other than the above (eg, Mullerian agenesis, androgen insensitivity, gonadal dysgenesis), investigator discretion should be applied for determining study entry.

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

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

10.4.1.2. Women Not Considered Women of Childbearing Potential (WONCBP)

Women in the following categories are NOT considered WOCBP:

1. Premenopausal female with permanent infertility due to one of the following (for the purpose of this study):
    a. Documented hysterectomy
    b. Documented bilateral salpingectomy

c. Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above (eg, Mullerian agenesis, androgen insensitivity), investigator discretion should be applied.

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

2. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
  - A high FSH level in the postmenopausal range may be used at the discretion of the investigator to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). (The laboratory threshold for defining postmenopausal state by FSH is >30 IU/L.)
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen 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.

10.4.2. Contraception Guidance

Table 4: Contraceptives Allowed During the Study

CONTRACEPTIVES[a] ALLOWED DURING THE STUDY INCLUDE:
Highly Effective Methods[b] That Have Low User Dependency **(***Failure rate of <1% per year when used consistently and correctly)*
Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
Intrauterine device (IUD)
Bilateral tubal occlusion
Intrauterine hormone-releasing system (IUS)[c]
Azoospermic partner (vasectomized or due to a medical cause) *Azoospermia is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.* Note: documentation of azoospermia for a male participant can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
Highly Effective Methods[b] That Are User Dependent **(***Failure rate of <1% per year when used consistently and correctly)*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[c] • oral • intravaginal • transdermal • injectable
Progestogen-only hormone contraception associated with inhibition of ovulation[c] • oral • injectable
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 study intervention. 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 participant.*

Note: Periodic abstinence (eg, calendar, symptothermal, post-ovulation methods), withdrawal (eg, coitus interruptus), spermicides only, and lactational amenorrhea method are not acceptable methods of contraception. Male condom and female condom should not be used together due to risk of failure from friction.

[a] Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies.

[b] Failure rate of <1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly.

[c] If locally required, in accordance with Clinical Trial Facilitation Group guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.

10.4.2.1. Collection of Pregnancy Information

10.4.2.1.1. Male Subjects with Partners who Become Pregnant

- The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who have been exposed to study drug and who, upon unblinding were found to have received IW-3300. The site should be notified by the subject as soon as possible when a partner pregnancy is confirmed.
- After obtaining the necessary signed ICF from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

10.4.2.1.2. Female Subjects who Become Pregnant

- The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. The site should be notified by the subject as soon as possible when a subject's pregnancy is confirmed. The initial information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a subject's pregnancy.
- The subject will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. A spontaneous abortion (occurring at <22 weeks gestational age) or still birth (occurring at >22 weeks gestational age) is always considered to be an SAE and will be reported as such. Any poststudy pregnancy-related SAE considered reasonably related to the study intervention by the investigator will be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study subjects, they may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue study intervention or be withdrawn from the study.

10.5. Appendix 5: Questionnaires

10.5.1. Genitourinary Pain Index (GUPI)

Questionnaire begins on the next page.

Male Genitourinary Pain Index

1. In the last week, have you experienced any pain or discomfort in the following areas?

a. Area between rectum and testicles (perineum)	□1 Yes	□0 No
b. Testicles	□1 Yes	□0 No
c. Tip of penis (not related to urination)	□1 Yes	□0 No
d. Below your waist, in your pubic or bladder area	□1 Yes	□0 No

2. In the last week, have you experienced:

a. Pain or burning during urination?	□1 Yes	□0 No
b. Pain or discomfort during or after sexual climax (ejaculation)?	□1 Yes	□0 No
c. Pain or discomfort as your bladder fills?	□1 Yes	□0 No
d. Pain or discomfort relieved by voiding?	□1 Yes	□0 No

3. How often have you had pain or discomfort in any of these areas over the last week?

□0 Never □1 Rarely □2 Sometimes □3 Often □4 Usually □5 Always

4. Which number best describes your AVERAGE pain or discomfort on the days you had it, over the last week?

□	□	□	□	□	□	□	□	□	□	□
0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

5. How often have you had a sensation of not emptying your bladder completely after you finished urinating, over the last week?

□0 Not at all □1 Less than 1 time in 5 □2 Less than half the time □3 About half the time □4 More than half the time □5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

□0 Not at all □1 Less than 1 time in 5 □2 Less than half the time □3 About half the time □4 More than half the time □5 Almost always

7. How much have your symptoms kept you from doing the kinds of things you would usually do, over the last week?

□0 None □1 Only a little □2 Some □3 A lot

8. How much did you think about your symptoms, over the last week?

□0 None □1 Only a little □2 Some □3 A lot

9. If you were to spend the rest of your life with your symptoms just the way they have been during the last week, how would you feel about that?

□0 Delighted
□1 Pleased
□2 Mostly satisfied
□3 Mixed (about equally satisfied and dissatisfied)
□4 Mostly dissatisfied
□5 Unhappy
□6 Terrible

Female Genitourinary Pain Index

1. In the last week, have you experienced any pain or discomfort in the following areas?

a. Entrance to vagina	□1 Yes	□0 No
b. Vagina	□1 Yes	□0 No
c. Urethra	□1 Yes	□0 No
d. Below your waist, in your pubic or bladder area	□1 Yes	□0 No

2. In the last week, have you experienced:

a. Pain or burning during urination?	□1 Yes	□0 No
b. Pain or discomfort during or after sexual intercourse?	□1 Yes	□0 No
c. Pain or discomfort as your bladder fills?	□1 Yes	□0 No
d. Pain or discomfort relieved by voiding?	□1 Yes	□0 No

3. How often have you had pain or discomfort in any of these areas over the last week?

□0 Never □1 Rarely □2 Sometimes □3 Often □4 Usually □5 Always

4. Which number best describes your AVERAGE pain or discomfort on the days you had it, over the last week?

□	□	□	□	□	□	□	□	□	□	□
0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

5. How often have you had a sensation of not emptying your bladder completely after you finished urinating, over the last week?

□0 Not at all □1 Less than 1 time in 5 □2 Less than half the time □3 About half the time □4 More than half the time □5 Almost always

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

□0 Not at all □1 Less than 1 time in 5 □2 Less than half the time □3 About half the time □4 More than half the time □5 Almost always

7. How much have your symptoms kept you from doing the kinds of things you would usually do, over the last week?

□0 None □1 Only a little □2 Some □3 A lot

8. How much did you think about your symptoms, over the last week?

□0 None □1 Only a little □2 Some □3 A lot

9. If you were to spend the rest of your life with your symptoms just the way they have been during the last week, how would you feel about that?

□0 Delighted
□1 Pleased
□2 Mostly satisfied
□3 Mixed (about equally satisfied and dissatisfied)
□4 Mostly dissatisfied
□5 Unhappy
□6 Terrible

Scoring

Pain subscale: Total of items la, lb, lc, ld, 2a, 2b, 2c, 2d, 3, and 4	= _____ (range 0-23)
Urinary subscale: Total of items 5 and 6	= _____ (range 0-10)
QOL Impact: Total of items 7, 8, and 9	= _____ (range 0-12)
Total score: Sum of subscale scores	= _____ (range 0-45)

10.5.2. O'Leary/Sant Interstitial Cystitis Symptom Index (ICSI)

Q1. During the past month, how often have you felt the strong need to urinate with little or no warning?

0. not at all
1. less than 1 time in 5
2. less than half the time
3. about half the time
4. more than half the time
5. almost always

Q2. During the past month, have you had to urinate less than 2 hours after you finished urinating?

0. not at all
1. less than 1 time in 5
2. less than half the time
3. about half the time
4. more than half the time
5. almost always

Q3. During the past month, how often did you most typically get up at night to urinate?

0. none
1. once
2. 2 times
3. 3 times
4. 4 times
5. 5 or more times

Q4. During the past month, have you experienced pain or burning in your bladder?

0. not at all
2. a few times
3. almost always
4. fairly often
5. usually

Add the numeric values of the checked entries; total score: ____

10.5.3. Global Response Assessment (GRA)

-3	Markedly worse
-2	Moderately worse
-1	Slightly worse
0	No change
+1	Slightly improved
+2	Moderately improved
+3	Markedly improved

10.5.4. EuroQol 5-Dimension 5-Level Version (EQ-5D-5L) Questionnaire

EQ-5D-5L	
Health Questionnaire English version for the USA	**Health Questionnaire** **Version (Target Language)** ***Version (English)*** **Subtitle (if applicable)** Description (if applicable)
Please tap the ONE box that best describes your health TODAY.	Instruction
MOBILITY	**MOBILITY**
I have no problems walking	MB1
I have slight problems walking	MB2
I have moderate problems walking	MB3
I have severe problems walking	MB4
I am unable to walk	MB5
SELF-CARE	**SELF-CARE**
I have no problems washing or dressing myself	SC1
I have slight problems washing or dressing myself	SC2
I have moderate problems washing or dressing myself	SC3
I have severe problems washing or dressing myself	SC4
I am unable to wash or dress myself	SC5
USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)* ****	**USUAL ACTIVITIES**
I have no problems doing my usual activities	UA1
I have slight problems doing my usual activities	UA2
I have moderate problems doing my usual activities	UA3
I have severe problems doing my usual activities	UA4
I am unable to do my usual activities	UA5
PAIN / DISCOMFORT	**PAIN / DISCOMFORT**
I have no pain or discomfort	PD1
I have slight pain or discomfort	PD2
I have moderate pain or discomfort	PD3
I have severe pain or discomfort	PD4
I have extreme pain or discomfort	PD5
ANXIETY / DEPRESSION	**ANXIETY / DEPRESSION**
I am not anxious or depressed	AD1
I am slightly anxious or depressed	AD2
I am moderately anxious or depressed	AD3
I am severely anxious or depressed	AD4
I am extremely anxious or depressed	AD5
We would like to know how good or bad your health is TODAY.	VAS Line 1
This scale is numbered from 0 to 100.	VAS Line 2
100 means the **<u>**best**</u>** health you can imagine.	VAS Line 3a
0 means the **<u>**worst**</u>** health you can imagine.	VAS Line 3b
Please tap on the scale to indicate how your health is TODAY.	VAS Line 4
The best health you can imagine	Top Thermometer
The worst health you can imagine	Bottom Thermometer
YOUR HEALTH TODAY	Healthbox
Previous	Button 'Previous'
Next	Button 'Next'
This item is blank, please complete it.	Error message
	Copyright Line
Disclaimer: *This is a preview of the EQ-5D instrument. It demonstrates the text, questions and response options included in this version. This preview does not represent the final product and should not be used as an official EQ-5D instrument.* ****	

10.5.5. Hospital Anxiety and Depression Scale (HADS)

Hospital Anxiety and Depression Scale (HADS)

GL Assessment

Name: _____ Date: _____

FOLD HERE

Clinicians are aware that emotions play an important part in most illnesses. If your clinician knows about these feelings he or she will be able to help you more.

This questionnaire is designed to help your clinician to know how you feel. Read each item below and **underline the reply** which comes closest to how you have been feeling in the past week. Ignore the numbers printed at the edge of the questionnaire.

Don't take too long over your replies, your immediate reaction to each item will probably be more accurate than a long, thought-out response.

FOLD HERE

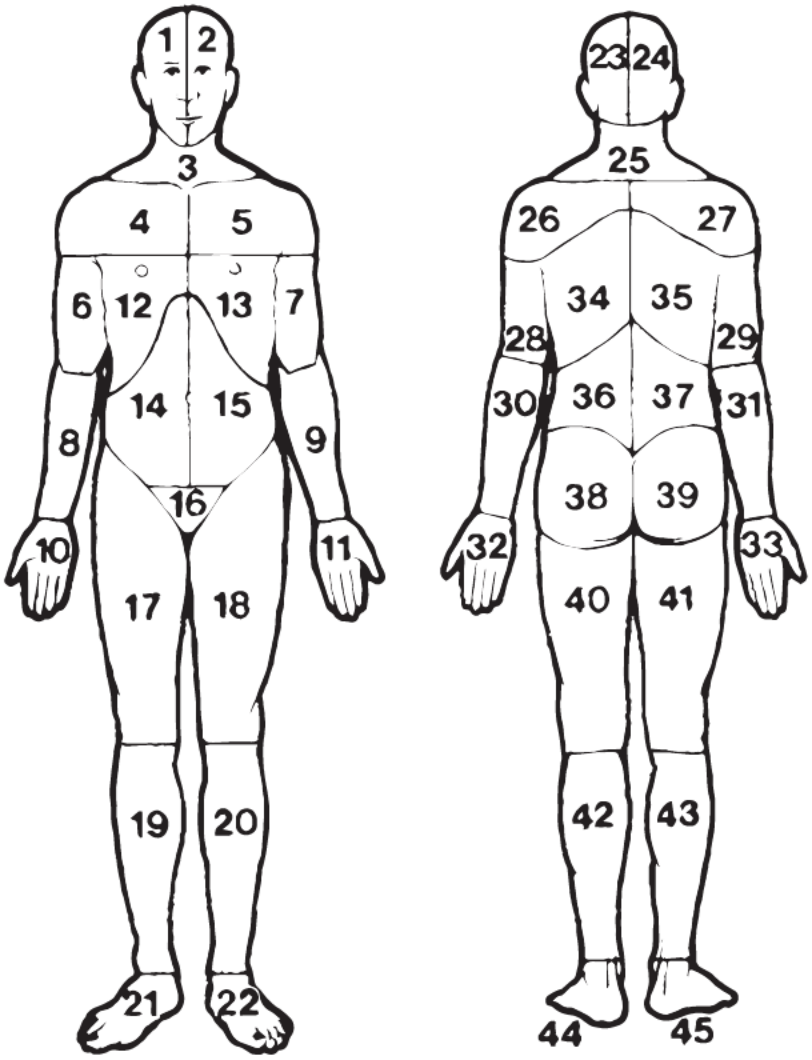
A	D			A	D
		I feel tense or "wound up"	**I feel as if I am slowed down**		
3		Most of the time	Nearly all the time		3
2		A lot of the time	Very often		2
1		From time to time, occasionally	Sometimes		1
0		Never	Never		0
		I enjoy the things I used to enjoy	**I get a sort of anxious feeling like "butterflies" in the stomach**		
	0	Definitely			
	1	Not quite so much	Never	0	
	2	Only a little	Occasionally	1	
	3	Hardly at all	Often	2	
			Very often	3	
		I get a sort of frightened feeling as if something awful is about to happen	**I have lost interest in my appearance**		
3		Very definitely and fairly badly	Definitely		3
2		Yes, but not too badly	Often I don't take as much care as I should		2
1		Sometimes, but it doesn't worry me	Sometimes I don't take as much care as I should		1
0		Never	I take just as much care as ever		0
		I can laugh and see the funny side of things	**I feel restless as if I have to be on the move**		
	0	As much as I always could	Definitely	3	
	1	Not quite so much now	Quite a lot	2	
	2	Definitely not so much now	Not very much	1	
	3	Never	Never	0	
		Worrying thoughts go through my mind	**I look forward with enjoyment to things**		
3		A great deal of the time	As much as I ever have		0
2		A lot of the time	Somewhat less than I used to		1
1		Not too often	Much less than I used to		2
0		Almost never	Rarely		3
		I feel cheerful	**I get sudden feelings of panic**		
	3	Never	Very often	3	
	2	Not often	Often	2	
	1	Sometimes	Not very often	1	
	0	Most of the time	Never	0	
		I can sit at ease and feel relaxed	**I can enjoy a good book, radio or television program**		
0		Always	Often		0
1		Usually	Sometimes		1
2		Not often	Not often		2
3		Never	Very seldom		3

Please make sure you have answered all the questions.

	A	D
TOTAL		

10.5.6. Margolis Body Map for Pain Assessment

Circle the number corresponding to each body site on the map where you experienced pain in the past week.



10.6. Appendix 6: Abbreviations

Table 5: List of Abbreviations

Abbreviation	Definition
ADLs	activities of daily living
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBD	cannabidiol
CFB	change from baseline
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanosine monophosphate
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRO	contract research organization
CRP	c-reactive protein
DMC	data monitoring committee
DMSO	dimethyl sulfoxide
eCRF	electronic case report form
EDC	electronic data capture
eDiary	electronic diary
ESR	erythrocyte sedimentation rate
EOS	end of study
EOT	end of treatment
EQ-5D-5L	EuroQol 5-Dimension 5-Level Version
FSH	follicle-stimulating hormone
GC-C	guanylate cyclase C
GRA	Global Response Assessment
GUPI	Genitourinary Pain Index
HADS	Hospital Anxiety and Depression Scale
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus

Abbreviation	Definition
HED	human equivalent dose
HEENT	head, eyes, ears, nose, and throat
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormonal replacement therapy
IA	interim analysis/analyses
IB	investigator's brochure
IBS-C	irritable bowel syndrome with constipation
IBS-D	irritable bowel syndrome with diarrhea
IC	interstitial cystitis
IC/BPS	interstitial cystitis /bladder pain syndrome
ICF	informed consent form
ICSI	Interstitial Cystitis Symptom Index
IEC	independent ethics committee
IMP	investigational medicinal product
INR	international normalized ratio
IRB	institutional review board
ITT	intent-to-treat
IWRS	interactive web response system
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
min	minute
MedDRA	Medical Dictionary for Regulatory Activities
MRP	multidrug resistance protein
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIMP	noninvestigational medicinal product
NRS	numeric rating scale
PK	pharmacokinetic(s)
PKG II	protein kinase G type II
QoL	quality of life
QTL	quality tolerance limit

Abbreviation	Definition
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SID	subject identification
SoA	schedule of activities
SUSAR	suspected unexpected serious adverse reactions
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
WBC	white blood cell
WOCBP	woman/women of childbearing potential
WONCBP	woman/women of nonchildbearing potential

10.7. Appendix 7: Protocol Signatures

Sponsor Signature

Protocol Title:	A Phase 2 Randomized, Double-blind, Placebo-controlled, Adaptive Study to Evaluate the Efficacy, Safety, and Tolerability of 2 Dose Levels of IW-3300 Administered Rectally for 12 Weeks to Treat Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Short Title:	A Phase 2 Study of IW-3300 for the Treatment of Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Protocol Number:	C3300-201
Version Number:	3.0
Version Date:	26 February 2024

This clinical study protocol was subject to critical review and has been approved by the sponsor.

Signed: *{Electronic signature page appended}* Date:

[redacted]

[redacted]

Investigator Signature

Protocol Title:	A Phase 2 Randomized, Double-blind, Placebo-controlled, Adaptive Study to Evaluate the Efficacy, Safety, and Tolerability of 2 Dose Levels of IW-3300 Administered Rectally for 12 Weeks to Treat Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Short Title:	A Phase 2 Study of IW-3300 for the Treatment of Bladder Pain in Subjects with Interstitial Cystitis/Bladder Pain Syndrome
Protocol Number:	C3300-201
Version Number:	3.0
Version Date:	26 February 2024

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Investigator Name: _____

Signed: _____ Date: _____

10.8. Appendix 8: Protocol Amendment History

The Protocol Amendment Summary of Changes Table summarizing the current amendment is located directly before the Table of Contents. Prior protocol amendments are summarized below.

Amendment 1/Version 2.0 (06 December 2022)

Overall Rationale for the Amendment:

Additions and corrections were made to improve the protocol.

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1 (Synopsis, Objectives, Estimands, and Endpoints) and Section 3 (Objectives, Estimands, and Endpoints)	A precise description of estimands applied to the analysis of the primary efficacy endpoint treatment effect was added.	The description of the estimands was expanded in accordance with Guidance for Industry ICH E9(R1), Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials.	Substantial
Section 1.1 (Synopsis, Number of Subjects)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: Approximately 300 subjects will be randomly assigned to study intervention ~~in order to have approximately 255 evaluable subjects (85 per group)~~. The sample size of approximately 300 subjects represents the maximum number of subjects to be enrolled ~~based on initial assumptions. Based on the outcome(s) of the IA(s), the number of subjects planned for enrollment could change.~~	This change reflects the adaptive-design nature of the study.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.3 (Schedule of Activities; Footnote d)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: The visual rectal examination is a visual inspection of the perianal area. **At visits other than Screening and Week 12/EOT (ie, the 2 visits when a digital rectal examination is required),** ~~I~~**i**f significant redness, rectal pain, bleeding, or mucus is observed on visual inspection of the rectal (perianal) area, a digital rectal examination and hemoccult test should be performed. If further investigation is warranted, subjects with these signs or symptoms should undergo proctoscopy or recto-sigmoidoscopy. ~~If these tests do not identify the source of inflammation, a colonoscopy may be performed after a discussion between the investigator and the sponsor.~~ **A colonoscopy may be performed instead of the recto-sigmoidoscopy after discussion between the investigator and the sponsor. Refer to Section 8.2.1**.	The changes 1) clarify when a digital rectal examination and hemoccult test need to be performed, and 2) allow the investigator, after consultation with the medical monitor, the option to conduct a colonoscopy rather than a recto-sigmoidoscopy followed by a colonoscopy (ie, 2 separate procedures), when signs and symptoms of inflammation warrant further investigation.	Substantial
Section 2.2 (Background)	Data from the completed 13-week toxicology studies in rats and monkeys were added. Data from Study C3300-102, a multiple-ascending dose Phase 1 study in healthy subjects, were added.	New nonclinical and clinical data were added to expand the assessment of potential risks associated with IW-3300 administration.	Substantial
Section 2.3.1 (Risk Assessment)	This text in the risks for "Study Intervention: IW-3300" section of the table was updated with data from Study C3300-102, a multiple-ascending dose Phase 1 study in healthy subjects.	New clinical data were added to expand the assessment of potential risks associated with IW-3300 administration.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 4.3 (Justification for Dose)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: **Two completed Phase 1 studies support the further evaluation of IW-3300 rectally administered at doses of 100 µg and 300 µg in subjects with IC/BPS.** Phase 1 Study C3300-101 evaluated single doses of IW-3300 ranging from 100 µg to 2500 µg. **Phase 1 Study C3300-102 evaluated once-daily doses of IW-3300 100 µg and 300 µg administered over 7 days. In these studies, ~~N~~no ~~dose related~~** trends across the IW-3300 groups, or compared with placebo, were noted for any of the safety parameters assessed. **Additional data appear in the IW-3300 IB.** ~~The currently ongoing Phase 1 Study C3300-102 is evaluating IW-3300 doses of 100 µg and 300 µg. Data from this study will be included in a future update to the IW-3300 IB.~~	The dose justification was updated with results from the now completed Study C3300-102. These data confirm the appropriateness of the originally planned IW-3300 doses, 100 µg and 300 µg.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.1 (Inclusion Criterion #2)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: Subject has been diagnosed with IC/BPS **by a urologist or other clinician specializing in IC/BPS** ~~at least 6 months prior to entering the study and currently meets the American Urological Association (AUA) criteria for IC/BPS. (2)~~	To ensure that subjects have IC/BPS, they will be required to have been diagnosed by a urologist or other clinician specializing in IC/BPS. The requirement for 6 months to have elapsed between diagnosis and eligibility was removed. Subjects are required to have had symptoms for ≥6 months prior to the Screening Visit (as per Inclusion Criteria #3 and #6), but the length of time since diagnosis is not stipulated. The requirement that subjects meet the AUA criteria for IC/BPS was removed because the specific criteria are specifically described in other inclusion criteria.	Substantial
Section 5.1 (Inclusion Criterion #3)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: 3. Subject has chronic bladder pain associated with filling ~~and emptying~~ the bladder over the past 6 months **before the Screening Visit**.	Pain is not required during both filling and emptying to be eligible for the study. The specific timeframe of "before the Screening Visit" was added for consistency with Inclusion Criterion #6.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.1 (Inclusion Criterion #6)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: 6. Subject has at least 1 of the following urinary symptoms related to IC/BPS during the ~~3~~ **6** months before the Screening Visit: • nocturia ≥2 voids/night • daytime frequency >8×day • urgency	For consistency with the Inclusion Criterion #3, the duration of symptoms related to IC/BPS was changed from 3 months to 6 months in accordance with FDA guidance (Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS): Establishing Effectiveness of Drugs for Treatment Guidance for Industry, December 2019).	Substantial
Section 5.2 (Exclusion Criterion #1)	The following text was added (in **bold)**: 1. Male subject has a history of **bacterial** prostatitis or benign prostatic hyperplasia	Male subjects will be excluded if they specifically have bacterial prostatitis.	Substantial
Section 5.2 (Exclusion Criterion #6)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: 6. Subject has an **active** ~~unresolved urinary tract infection~~ **UTI** during the Screening or Pretreatment Period **or subject has had ≥2 UTIs within 90 days before the Screening Visit**.	"Active" replaced "unresolved" urinary tract infection (UTI) because it captures both new and ongoing UTIs. Subjects who are susceptible to frequent UTIs may develop UTIs during the study which could confound the evaluation of efficacy and safety. Therefore, these subjects will be excluded from enrollment.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.2 (Exclusion Criterion #12)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): 12. Subject has **had** ~~any history of~~ surgery in the pelvic ~~region~~ or ~~has had surgery in the~~ abdominal region within 90 days ~~of~~ **before** the Screening Visit. **A subject who has a history of major pelvic or abdominal surgery must be discussed with the medical monitor to confirm the appropriateness of enrollment.**	The exclusion criterion was modified to reflect that the timeline of "90 days before the Screening Visit" will apply to surgeries in both the pelvic and abdominal region. Any subject who has a history of major pelvic or abdominal surgery must be discussed with the medical monitor to confirm the appropriateness of enrollment. Some surgeries could have profound and long-lasting impact on the nerve pathways in the lower abdomen and pelvis and enrollment of subjects could confound the results of the study.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.3.1 (Randomization)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be randomized into the study on Day 1.~~ Approximately 300 subjects will be randomized in a 1:1:1 ratio to receive IW-3300 100 µg (N=100), IW-3300 300 µg (N=100), or matching placebo (N=100).~~ **An adaptive sample size algorithm is utilized for determining the final sample size of the study, with a range of approximately 100 to 300 subjects planned for enrollment. Subjects will initially be randomized in a 1:1:1 ratio to receive IW-3300 100 µg, IW-3300 300 µg, or matching placebo.**	This change was made to reflect the adaptive-design nature of the study.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.4 (Study Intervention Compliance)	The following text was removed (in ~~strikethrough~~) or added (in **bold)**: Following Day 1, when subjects self-administer study drug at home, compliance with study intervention will be assessed at each visit. ~~Compliance will be assessed (by weighing the returned canisters during the site visits and through~~ **Site staff should review the** subject's reports in the daily eDiary **(**~~[~~refer to Section 8.1.1.1~~]~~**)** and ~~documented in the source documents and eCRF~~ **reinforce any compliance concerns with the subject.** Deviations from the prescribed dosage regimen should be ~~recorded in the eCRF~~ **documented as a protocol deviation, as applicable.** A record of the number of canisters dispensed to and returned by each subject (including the dispense/return dates~~,~~ **and** canister identification numbers~~, and returned canister weights~~) must be maintained and reconciled with study drug and compliance records.	This change was made to reflect that compliance with study intervention will only be assessed through a review of the subjects' reports in the daily eDiary entries and not by weighing the canisters.	Substantial
Section 6.5 (Concomitant Therapy)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): Unless, in the opinion of the investigator **after consultation with the medical monitor** ~~and Sponsor~~, the concomitant medication will not interfere…	Clarification was made regarding the decision-making roles for determining the appropriateness of a concomitant medication.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.5.2 (Rescue Medicine)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): Ibuprofen (~~up to 2 tablets [200 mg/tablet] every 4 to 6 hours up to 10 tablets/day~~ **[200 mg/tablet] administered every 4 to 6 hours up to 6 tablets per day**)	The dose of ibuprofen was updated to match the product's over-the-counter Drug Facts Label.	Substantial
Section 7.1.2 (Temporary Discontinuation)	The following text was added (in **bold**): If the investigator believes that the subject is unable to resume dosing **after 3 days** or requires a suspension of dosing on more than 1 occasion, the investigator should contact the medical monitor to discuss the subject's continued participation in the study	A time window of 3 days was added.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 8.2.1 (Physical Examinations)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): • A visual and digital rectal examination, **including a hemoccult test,** will be done at Screening and Week 12/EOT. **Only a** ~~A~~ visual rectal examination will be done at other visits. **However, if** ~~If~~ significant redness, rectal pain, bleeding, or mucus is observed on visual inspection of the rectal (perianal) area, a digital rectal examination and hemoccult test should be performed. If further investigation is warranted, subjects with these signs or symptoms should undergo proctoscopy or recto-sigmoidoscopy. **A colonoscopy may be performed instead of the recto-sigmoidoscopy after discussion between the investigator and the sponsor.** ~~If these tests do not identify the source of inflammation, a colonoscopy may be performed after a discussion between the investigator and the sponsor.~~	The 2nd bullet point was revised to 1) clarify when a digital rectal examination and hemoccult test need to be performed, and 2) allow the investigator, after consultation with the medical monitor, the option to conduct a colonoscopy rather than a recto-sigmoidoscopy followed by a colonoscopy (ie, 2 separate procedures), when signs and symptoms of inflammation warrant further investigation.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 9.1 (Statistical Hypotheses)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): The primary efficacy endpoint of the treatment differences for **weekly average** CFB in bladder pain at its worst at Week 12 will be assessed to test the following hypothesis… ~~Each of the active dose strength arms (100 µg or 300 µg) will be compared with the placebo arm at a 1-sided 0.025 significance level. This multiplicity adjustment for the primary efficacy endpoint will control the α level at 1-sided significance level of 0.05.~~ **The study-wide type I error rate is controlled at 1-sided 0.05. To account for the multiplicity resulting from testing 2 doses, the total 0.05 α is split between the 100 µg and 300 µg doses, 60% to the high dose and 40% to the low dose. This unequal split is intended to prioritize the higher dose based on the hypothesis that efficacy of the high dose is greater or equal to efficacy of the low dose. Thus, the high dose will be allocated 0.03 1-sided α and the low dose will be allocated 0.02 1-sided α. If a null hypothesis is rejected only for a dose group under the initial allocation of the alpha-level, the alpha-level for that dose will be propagated to the remaining nonsignificant dose.**	This change reflects that a statistical approach with different weights for each dose and an alpha propagation method will be used.	Substantial
Section 9.2 (Sample Size Determination)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): The sample size and power for the study was estimated based on simulations with Bayesian	The maximum sample size of approximately 300 subjects remains the same. Details on the sample size calculation were added.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
	~~hierarchical models~~ **adaptive design** for the primary efficacy endpoint. …The effect of the active treatment was obtained through meta-analysis of the~~se~~ 3 previous Ironwood studies **in IBS-C with linaclotide** ~~using linaclotide in subjects with IBS-C~~. **Per the historical data exploratory results, a change from baseline treatment difference of -0.96 with standard deviations of 2.326 (active) and 2.084 (placebo) for the primary efficacy endpoint at Week 12 among the active treatments and placebo arm, respectively, was used as a reference for the simulations.** ~~The overall study-wise type I error of 0.10 is equally split between the 2 comparisons of the primary efficacy endpoint among the IW-3300 doses relative to placebo following Bonferroni correction; an alpha of 0.05 will be spent for each comparison. Based on the simulations, a sample size of at least 85 subjects per arm will yield 81% power to confirm superiority at the 0.05 alpha level. With a 10% to 15% attrition rate, approximately 95 to 100 subjects per arm will need to be randomized.~~ **These simulations showed that the study is powered at 80% or higher to detect an approximate 1-point difference in the primary outcome under various scenarios. In the simulations, the standard deviation (SD) was varied around 2, a 10% attrition rate was assumed, and the maximum sample size was set to 300. The average sample**		

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
	size ranged from approximately 200 to 280. ~~This sample size represents the maximum number of subjects to be enrolled based on initial assumptions.~~ Based on the outcome(s) of the IA(s), the study may be stopped early for efficacy, a treatment arm may be discontinued if it does not warrant further exploration, or enrollment may be stopped early for futility. ~~Given these scenarios, the number of subjects planned for enrollment could change (refer to Section 9.5).~~ **Given these scenarios, specifically for the case of a treatment arm being discontinued, enrollment will continue for the remaining 2 arms, for a total number of approximately 300 subjects for the study.**		
Section 9.4.1 (General Considerations)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): ~~The 2 comparisons of the primary efficacy endpoint among the IW-3300 doses relative to placebo will be adjusted using Bonferroni correction, ie, the type I error of 0.10 will be equally split between the 2 comparisons; an alpha of 0.05 will be spent for each comparison. Multiple comparisons will not be adjusted in the conduct of comparisons among the IW-3300 doses relative to placebo for all other efficacy endpoints. Missing data will be imputed for efficacy analysis. Statistical analyses will be conducted using SAS version 9.4 (or later), and a statistical~~	This change reflects that an alpha propagation approach with different weights for each dose will be used.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
	~~package developed by Berry Consultants.~~ **The 2 comparisons of the primary efficacy endpoint among the IW-3300 doses relative to placebo will be adjusted unequally, that is, the total 1-sided 0.05 α is split between the 100 µg and 300 µg doses, 60% to the high dose and 40% to the low dose, and an α-propagation approach will be applied for the analysis. Multiple comparisons will not be adjusted in the conduct of comparisons among the IW-3300 doses relative to placebo for all other efficacy endpoints. Missing data will not be imputed for the primary efficacy analysis. Statistical analyses will be conducted using SAS version 9.4 (or later).**		
Section 9.4.2 (Primary Endpoint Analysis)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): ~~A frequentist 2-sample t-test will be performed to test the hypotheses; 1-sided 97.5% CIs and the corresponding p-values will be provided for the treatment differences for the CFB in bladder pain at Week 12 between 100 µg vs. Placebo and 300 µg vs. Placebo.~~ ~~As a sensitivity analysis, a Bayesian hierarchical model will be implemented to confirm the results from the frequentist approaches.~~ **The statistical null hypothesis for the primary endpoint is that the treatment effect of 100 µg and 300 µg is the same as placebo. This hypothesis will be tested on the ITT Analysis Set using a mixed model for**	The statistical methods for the primary efficacy endpoint analysis were updated.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
	repeated measurement. The CFB in weekly average of daily bladder pain at its worst postbaseline assessments up to Week 12 will be used for the analysis. The model will include a covariate of baseline average of daily bladder pain at its worst, and factors of visit, treatment, and interaction of treatment by visit. **A sensitivity analysis will be implemented to confirm the results from the primary analysis. Missing values will be imputed using multiple imputation assuming missing not at random. Analysis of covariance (ANCOVA) model with treatment as a factor and baseline average of daily bladder pain at its worst as a covariate will be applied.** Details of the statistical models and analyses **including other sensitivity analyses** will be provided in the SAP**,** and details of the IA will be provided in the IA plan.		

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 9.4.4 (Exploratory Endpoints Analyses)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): The appropriate analysis set will be applied for each of the exploratory endpoints.~~ Statistical comparisons will not be performed as these endpoints are exploratory in nature; a summary of descriptive statistics will be presented.~~ **Statistical comparisons will be performed whenever they are appropriate. However, as these endpoints are exploratory in nature, no multiplicity adjustment will be performed.**	The statistical methods for the exploratory efficacy endpoint analyses were updated.	Substantial
Section 9.4.7 (Microbiome)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): This analysis will be conducted in a subgroup of subjects ~~comprised of~~ **comprising** approximately 60 subjects ~~who complete the study~~ (~20/arm). The relative abundance of **microorganisms by** different family~~,~~ **and genera** ~~genus, and species~~ will be constructed and summarized by treatment at Baseline and Week 12/EOT using descriptive statistics. Changes from baseline will be **presented** ~~calculated~~.	The microorganism analysis will be based on family and genera, and not on other hierarchical categories. Because subjects will have a microbiome sample at the Week 12 or EOT Visit (depending on whether they complete the study), the requirement to perform this analysis on only subjects who complete the study was removed.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 9.5 (Interim Analyses)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): ~~The 1-sided, study-wise type I error will be controlled at 0.05 level for the primary efficacy analysis. Each of the 2 superiority comparisons of the dose strengths (IW-3300 100 µg and 300 µg) will be controlled at a 1-sided 0.025 level against the placebo arm. The actual alpha-spending level will be determined by the Kim-Demets alpha-spending function.~~ **The study-wide type I error rate is controlled at 1-sided 0.05. The strategy to control the overall α for the study is described in Section 9.1. The actual α-spending level will be determined by the Kim-Demets α-spending function and an α-propagation approach will be used for the analyses.**	This change reflects that an alpha propagation approach with different weights for each dose will be used.	Substantial
Section 10.2 (Appendix 2, Clinical Laboratory Tests, Table 2)	Table 2 lists estradiol as a test to be conducted in women of nonchildbearing potential. This was removed.	Estradiol was listed in error and will not be analyzed during the study.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.4.1.1 (Women of Childbearing Potential [WOCBP]) and Section 10.4.1.2 (Women Not Considered Women of Childbearing Potential [WONCBP])	The following text was removed (in ~~strikethrough~~) or added (in **bold**): A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used **at the discretion of the investigator** 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, confirmation with more than 1 FSH measurement is required.~~ (The laboratory threshold for defining postmenopausal state by FSH **is >30 IU/L**) ~~will be based on the local standard combined with investigator judgment)~~.	Women who have less than 12 months of amenorrhea will be considered WOCBP regardless of FSH level. Therefore, the option to test FSH as a means to demonstrate being in a postmenopausal state before having 12 months of amenorrhea was removed. For consistency across sites, a specific threshold (>30 IU/L) was added for FSH.	Substantial
Section 10.4.1.1 (Women of Childbearing Potential [WOCBP])	The following text was removed (in ~~strikethrough~~) or added (in **bold**): **Permanent sterilization methods (for the purpose of this study) include:** • **Documented hysterectomy** • **Documented bilateral salpingectomy** • **Documented bilateral oophorectomy** ~~(Note: Women who have undergone permanent sterilization methods including hysterectomy, bilateral salpingectomy, or bilateral oophorectomy are considered WONCBP. However, women who have had prior surgeries in the pelvic region are not eligible to enroll in the study [Section 5.2]).~~	Prior pelvic surgery is now an exclusion criterion only if it occurs within 90 days before the Screening Visit. This language in the definition of WOCBP was added to reflect that change.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.4.1.2 (Women Not Considered Women of Childbearing Potential [WONCBP])	The following text was removed (in ~~strikethrough~~) or added (in **bold**): ~~1. Premenarchal~~ 2. Premenopausal ~~woman~~ **female** with permanent infertility due to **one of the following (for the purpose of this study):** **a. Documented hysterectomy** **b. Documented bilateral salpingectomy** **c. Documented bilateral oophorectomy** **For individuals with permanent infertility due to** an alternate medical cause other than the above (eg, Mullerian agenesis, androgen insensitivity), investigator discretion should be applied ~~to determining study entry~~. Note: Documentation can come from the site personnel's~~:~~ review of the subject's medical records, medical examination, or medical history interview. ~~Note: Women who have undergone permanent sterilization methods including hysterectomy, bilateral salpingectomy, or bilateral oophorectomy are considered WONCBP. However, women who have had prior surgeries in the pelvic region are not eligible to enroll in the study [Section 5.2]).~~	For consistency with international guidelines, premenarchal women were removed from the list defining WONCBP. Prior pelvic surgery is now an exclusion criterion only if it occurs within 90 days before the Screening Visit. This language was added to reflect that change.	Substantial
Section 10.4.2 (Contraception Guidance, Table 4, Contraceptives Allowed During the Study	**Bilateral tubal occlusion** was added to the list of "Highly Effective Methods That Have Low User Dependency"	Prior pelvic surgery is now an exclusion criterion only if it occurs within 90 days before the Screening Visit. This language was added to reflect this change.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1 (Synopsis, Overall Design)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): During **the Pretreatment Visit** ~~this~~ period, subjects will receive training on how to complete the daily and weekly electronic diary (eDiary) questions at home **and starting that night, subjects.** ~~On subsequent days, subjects~~ will answer the eDiary questions to establish a baseline **during the study**.	Subjects will begin to complete their eDiary questions at home the night of the Pretreatment Visit rather than beginning to complete their eDiary questions on subsequent days after the Pretreatment Visit.	Nonsubstantial
Section 1.3 (Schedule of Activities; Footnote s)	The following text was added (in **bold)**: Subjects in the microbiome substudy will bring in a stool sample **from home, collected prior to the study visit (and predose for Day 1),** using the sample collection kit issued by the site; refer to Section 8.7.	To avoid errors in the timing of the stool sample, text was added to clarify that the Day 1 sample is intended to be collected at home, prior to the Day 1 visit and prior to dosing.	Nonsubstantial
Section 4.1.1.1 (Screening Period)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): **After obtaining informed consent,** ~~S~~**s**ites with supporting procedures in place may elect to ~~obtain informed consent electronically and~~ conduct ~~initial~~ **some screening** assessments that do not require the subject to be present (eg, collecting demographics, medical history, prior medications) over the phone.	Due to logistical concerns, electronic informed consent will not be offered.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 4.1.1.2 (Pretreatment Period)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): **Subjects will receive rescue medicine as described in Section 6.5.2. Starting on the night of the Pretreatment Visit and continuing throughout the Pretreatment Period,** ~~Sites with supporting procedures in place may elect to perform this visit virtually using a video telehealth system. S~~subjects will answer the eDiary questions ~~throughout the Pretreatment Period~~ to establish a baseline **during the study**.	The site will dispense rescue medicine at the Pretreatment Visit. Subjects will begin to complete their eDiary questions at home the night of the Pretreatment Visit rather than beginning to complete their eDiary questions on subsequent days after the Pretreatment Visit.	Nonsubstantial
Section 6.3.2 (Blinding)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): As described in Section 9.6, an independent Data Monitoring Committee (DMC) will ~~conduct~~ **review the results of the** ~~at least 1~~ interim analysis**(es)** (IA**[s]**) of the safety and efficacy data. ~~A~~ **The** designated independent ~~study~~ statistician**s**, who will not be involved in **the final** study data analysis and interpretation, will have access to the randomization schedule including treatment assignments to conduct the **efficacy** IA**(s) and safety reporting.**	This change clarifies the responsibility of the DMC (to review the results of efficacy and safety) versus the independent statisticians (to conduct the efficacy IA[s] and safety reporting).	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.5.1 (Prohibited Medications, Medications Requiring a Washout Period, and Medications Permitted Under Select Circumstances)	The following text was removed (in ~~strikethrough~~): Amphetamines: permitted if prescribed for a non-IC/BPS-related condition and the subject has been on a stable dose for >30 days prior to the Pretreatment Visit and plans to continue on the same dose until after the EOS Follow-up Phone Call. ~~Amphetamines should be recorded as concomitant medications regardless of medical need.~~	This specific guidance for amphetamines was removed; it was deemed unnecessary as all concomitant medications should be recorded.	Nonsubstantial
Section 6.5.1 (Prohibited Medications, Medications Requiring a Washout Period, and Medications Permitted Under Select Circumstances)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): ~~Cannabidiol (CBD)/~~ **Cannabinoids**: permitted if subject has been…	Cannabidiol (CBD) was replaced with the broader category of cannabinoids.	Nonsubstantial
Section 6.7 (Intervention After the End of the Study)	The following text was removed (in ~~strikethrough~~): Subjects with ongoing AEs or other safety concerns will be followed until resolution or until no further queries or follow-up actions are required~~; at the discretion of the investigator, subjects with ongoing safety concerns may have discharge delayed if necessary for continued monitoring~~.	This text was not relevant to this study and removed.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 7.1 (Discontinuation of Study Intervention)	The following text was added (in **bold**): If treatment is permanently discontinued, then the subject will return to the clinic to have the Week 12/EOT assessments performed **and the EOS Phone Call will also need to be performed.**	This text was added to clarify that the End of Study (EOS) phone call would be performed for subjects who discontinue treatment prematurely.	Nonsubstantial
Section 8.1.1.1 (Daily eDiary Assessments/ Difficulty Sleeping)	The 2 daily questions asking about the subject's sleep difficulty were amended with the phrase **"…in the past 24 hours?**"	A 24-hour recall period was intended but not specified in the original protocol. The protocol was modified to reflect the original intent.	Nonsubstantial
Section 8.1.2 (Margolis Body Map for Pain Assessment)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): Pelvic pain and ~~7+~~ **8+** other sites	To avoid an overlap in categories, the last category was revised to "pelvic pain in 8+ sites".	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 8.2.6 (Medical History) and Section 10.5.5 (IC/BPS Prior Symptom Management Assessment)	In Section 8.2.6, the following text was removed (in ~~strikethrough~~) or added (in **bold**): In addition to general medical history, baseline data on a subject's IC/BPS characteristics and prior symptom management will also be collected at the Screening Visit. **All of these data will be incorporated within the subject's source documentation and entered into the eCRF.** For IC/BPS characteristics, the following data will be collected: • **date of first IC/BPS symptom onset** • date of first diagnosis ~~(month, year~~ • findings from prior cystoscopy, if available (eg, date of prior cystoscopy ~~(day/month/year),~~ **and**~~;~~ presence of Hunner's lesions [yes/no/unknown] ~~, presence of glomerulations [yes/no/unknown]~~) … ~~For prior IC/BPS symptom management, the questionnaire in Section 10.5.5 will be administered by the site staff, incorporated within the subject's source documentation, and entered into the eCRF.~~ Section 10.5.5, the IC/BPS symptom management assessment tool, was removed.	Because diagnosis of IC/BPS can often lag behind the onset of IC/BPS symptoms, data on the date of first onset of symptoms will now be collected. Having these data will enable a more detailed characterization of the subjects enrolled in the study. To avoid discrepancy with the electronic data collection (EDC) date format specifications, the date formats (ie, day/month/year) were removed. The presence of glomerulations on prior cystoscopy will not be captured. As part of medical history, IC/BPS symptom management will continue to be collected and entered into the EDC system. However, the specific IC/BPS symptom management assessment tool was removed from the protocol.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 8.3.1 (Time Period and Frequency for Collecting AE and SAE Information)	The following text was removed (in ~~strikethrough~~): All AEs and SAEs will be collected from the signing of the ICF until completion of study participation (ie, ~~early termination or~~ EOS Follow-up Phone Call) at the timepoints specified in the SoA/Table 1.	This text was removed to clarify that the EOS phone call is the planned end of study participation for all subjects.	Nonsubstantial
Section 8.7 (Microbiome)	The following text was added (in **bold**): At the visits required, subjects in the microbiome substudy will bring in a stool sample **from home, collected prior to the study visit (and predose for Day 1),** using the sample collection kit issued by the site.	To avoid errors in the timing of the stool sample, text was added to clarify that the Day 1 sample is intended to be collected at home, prior to the Day 1 visit and prior to dosing.	Nonsubstantial
Section 10.2 (Appendix 2: Clinical Laboratory Tests)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): • **All blood samples for ESR testing will be analyzed at the local laboratory and entered into the eCRF. Apart from ESR, L**~~**l**~~**ocal** laboratory results are only required in the event that the central laboratory results are not available in time for either study intervention 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 a study intervention decision or response evaluation, the results must be entered into the eCRF.~~ **(See exceptions below for urinalysis and urine culture performed in the case of a UTI.)**	The 2nd bullet point was revised to reflect that: 1) erythrocyte sedimentation rate (ESR) will be always analyzed locally and that testing for UTIs have specific and different rules for central versus local analysis. 2) Local laboratory results will not be entered into the eCRF with the exception of ESR. 3) UTI testing (urinalysis and urine culture) will follow separate rules.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.2 (Appendix 2: Clinical Laboratory Tests)	The following text was removed (in ~~strikethrough~~) or added (in **bold**): • ~~Additional tests, including~~ **r**Repeat or **medically necessary** tests may be performed at any time during the study, as determined necessary by the investigator or required by local regulations. **Laboratory tests outside of those specified in the protocol should be discussed with the medical monitor.**	The 4th bullet point was revised to state that any laboratory tests outside of those specified in the protocol should be discussed with the medical monitor.	Nonsubstantial
Section 10.2 (Appendix 2: Clinical Laboratory Tests)	The following text was added (in **bold**): • ~~Urinary tract infection~~ **UTIs** must be confirmed by urinalysis and urine culture **analyzed by the central laboratory**; refer to Section 8.3.9. **If these tests are performed at the local laboratory, however, no samples need to be collected for central laboratory testing.**	The 6th bullet point was revised to reflect the specific rules for central versus local laboratory testing for UTIs.	Nonsubstantial
Section 10.3.3.1 (AE and SAE Recording)	The following text was added (in **bold**): • For subjects who fail screening and have an **AE or** SAE, only the SAE will be collected in eCRF. **AEs will be documented only in source documentation for screen-failed subjects.**	In the 6th bullet point, the rule for the documentation of AEs that occur in subjects who fail screening was clarified.	Nonsubstantial
Section 10.3.4.2 (SAE Reporting to Ironwood Global Patient Safety via Paper SAE Report Form)	The following text was added (in **bold**): **I**CSRoperations@ironwoodpharma.com	The email address was updated.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.4.2.1.1 (Males Subjects with Partners who Become Pregnant)	The following text was added (in **bold**): **The site should be notified by the subject as soon as possible when a partner pregnancy is confirmed.**	Clarification was made on the timing for the reporting of a partner pregnancy.	Nonsubstantial
Section 10.4.2.1.2 (Female Subjects who Become Pregnant)	The following text was added (in **bold**): **The site should be notified by the subject as soon as possible when a subject's pregnancy is confirmed.**	Clarification was made on the timing for the reporting of a subject's pregnancy.	Nonsubstantial
Section 10.5.2. (O'Leary/Sant Interstitial Cystitis Symptom Index [ICSI])	Minor wording changes were made to the questionnaire (eg, the phrase "**During the past month…**" is repeated for each question and not just as the main lead-in listed once before all questions; the responses no longer have the first word capitalized; and the periods at the end of the responses were removed).	Minor wording discrepancies between the questionnaire version in the protocol and the questionnaire version incorporated into the eDiary were corrected.	Nonsubstantial
Section 10.5.4 (EuroQol 5-Dimension 5-Level Version [EQ-5D-5L] Questionnaire)	The UK version of the EQ-5D-5L was replaced with the American version. Minor changes were made to the wording to reflect cultural expressions.	Minor wording discrepancies between the version in the protocol and the questionnaire version incorporated into the eDiary were corrected.	Nonsubstantial
Section 10.5.5 (old Section 10.5.6, Hospital Anxiety and Depression Scale [HADS])	Minor wording changes were made to the questionnaire (eg, "I enjoy the things I used to enjoy" vs. "I still enjoy the things I used to enjoy").	Minor wording discrepancies between the version in the protocol and the questionnaire version incorporated into the eDiary were corrected.	Nonsubstantial
Global	Throughout the document, minor editorial changes were made.	Minor editorial changes were made to improve readability.	Nonsubstantial

11. REFERENCES

1. Mullins C, Bavendam T, Kirkali Z, Kusek JW. Novel research approaches for interstitial cystitis/bladder pain syndrome: thinking beyond the bladder. Transl Androl Urol. 2015;4(5):524-33.
2. Hanno PM, Erickson D, Moldwin R, Faraday MM, American Urological A. Diagnosis and treatment of interstitial cystitis/bladder pain syndrome: AUA guideline amendment. J Urol. 2015;193(5):1545-53.
3. Imamura M, Scott N, Wallace S, Ogah J, Ford A, Dubos Y, et al. Interventions for treating people with symptoms of bladder pain syndrome: a network meta-analysis. Cochrane Database Syst Rev. 2020;7(7).
4. Jain N, L A, Yu Y, VanderBeek B. Association of macular disease with long-term use of pentosan polysulfate sodium: findings from a US cohort. Br J Ophthalmol. 2020;104(8):1093-7.
5. Castro J, Harrington AM, Hughes PA, Martin CM, Ge P, Shea CM, et al. Linaclotide Inhibits Colonic Nociceptors and Relieves Abdominal Pain via Guanylate Cyclase-C and Extracellular Cyclic GMP. Gastroenterology. 2013;145(6):1334-46.
6. Grundy L, Harrington AM, Castro J, Garcia-Caraballo S, Deiteren A, Maddern J, et al. Chronic linaclotide treatment reduces colitis-induced neuroplasticity and reverses persistent bladder dysfunction. JCI Insight. 2018;3(19):e121841.
7. Pfeifer A, Aszódi A, Seidler U, Ruth P, Hofmann F, Fässler R. Intestinal secretory defects and dwarfism in mice lacking cGMP-dependent protein kinase II. Science. 1996;274(5295):2082-6.
8. Forte, LR. Uroguanylin and guanylin peptides: pharmacology and experimental therapeutics. Pharmacol Ther. 2004;104(2):137-62.
9. Schlossmann J, Feil R, Hofmann F. Insights into cGMP signalling derived from cGMP kinase knockout mice. Frontiers in Bioscience. 2005;10:1279-89.
10. Forte LR. Guanylin regulatory peptides: structures, biological activities mediated by cyclic GMP and pathobiology. Regul Pept. 1999;81(1-3):25-39.
11. London RM, Krause WJ, Fan X, Eber SL, Forte LR. Signal transduction pathways via guanylin and uroguanylin in stomach and intestine. Am J Physiol. 1997;273(1 Pt 1):G93-105.
12. Margolis RB, Tait RC, Krause SJ. A rating system for use with patient pain drawings. Pain. 1986;24(1):57-65.
13. Nickel JC, Tripp DA. International Interstitial Cystitis Study Group. Clinical and psychological parameters associated with pain pattern phenotypes in women with interstitial cystitis/bladder pain syndrome. The Journal of Urology. 2015;193:138-44.
14. Lai HH, Jemielita T, Sutcliffe S, Bradley CS, Bruce Naliboff, Williams DA, et al. For the MAPP Research Network. Characterization of whole body pain in urologic chronic pelvic pain syndrome at baseline – a MAPP Research Network study. Journal of Urology. 2017;198(3):622–31.

15. Hepner KA, Watkins KE, Elliott MN, Clemens JQ, Hilton LG, Berry SH. Suicidal ideation among patients with bladder pain syndrome/interstitial cystitis. Urology. 2012;80(2):280-5.

The data and information in this file that pertain to my line function are truthful and accurate.

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