TITLE PAGE

Protocol Title:	A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered Single Ascending Doses of IW-3300 in Healthy Volunteers
Short Title:	A Phase 1 Study of Single Ascending Doses of IW-3300 in Healthy Volunteers
Protocol Number:	C3300-101
Version Number /Amendment Number:	Version 4/Amendment 3
Final Date:	10 January 2022
Study Intervention:	IW-3300
Study Phase:	1
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.

Confidentiality Statement

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Date**
Amendment 3	10 January 2022
Amendment 2	24 November 2021
Amendment 1	22 September 2021
Original Protocol	06 August 2021

Amendment 3 (10 January 2022)

Overall Rationale for the Amendment:

Changes were made at the request of the US FDA, the Institutional Review Board (IRB), and the Clinical Research Unit (CRU).

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1.5, (Synopsis/Overall Design), Section 1.1.8 (Synopsis/ Intervention Groups and Duration), Section 2.3.1 (Risk Assessment, Row #2: Unknown Adverse Events [AEs], laboratory abnormalities, or other safety findings with a novel study drug), Section 4.1 (Overall Design), Section 4.2 (Scientific Rationale for Study Design), and Section 6 (Study Intervention)	Sentinel dosing was added. As such, in Cohort 1 only, the first 2 subjects will be randomized in a 1:1 ratio to IW-3300 or placebo and dosed at least 4 hours before the remaining subjects in the cohort.	The IRB requested that sentinel dosing be incorporated in Cohort 1 only.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.3 (Schedule of Activities/ Table 1) and Section 10.2 (Appendix 2: Clinical Laboratory Tests; Table 2)	The following laboratory tests were added at Check-in (Day -1) (predose) and Day 2 (Discharge): erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and hemoccult testing. All laboratory tests will now be analyzed at a local laboratory with the exception of the point-of-care hemoccult tests and the ESR which will be analyzed at an outside laboratory. Because all urine tests will now be analyzed at the local laboratory, the requirement to enter select urine-based screening test results into the CRF was deleted from Table 2.	The FDA requested these additional laboratory assessments. The CRU now has the ability to run several laboratory assays locally.	Substantial
Section 1.3 (Schedule of Activities/ Table 1) and Section 10.2 (Appendix 2: Clinical Laboratory Tests; Table 2)	Alcohol testing at Check-in (Day -1) will be done via a urine test. References to the alcohol screen being done by breath test were deleted.	The CRU now has the ability to do this screen via a urine test.	Substantial
Section 2.3.1 (Risk Assessment, Row # 1: Potential for diarrhea and secondary dehydration)	The Bristol Stool Form Scale (BSFS) score of 7 (watery stools) was added in the evaluation of the severity of diarrhea.	The FDA requested that the definition of diarrhea that would result in temporary dose suspension or trial discontinuation include a criterion for stool consistency as measured by BSFS.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 5.1.4 (Inclusion Criteria/Sex)	Inclusion Criterion #7b was removed. Criterion #7a was promoted to Criterion #7.	The FDA requested this change. Inclusion Criterion #7b was considered redundant as 1) only women of nonchildbearing potential are eligible for enrollment and these women would not be taking hormonal contraceptives, and 2) subjects who are taking any prescription medications (eg, hormonal contraceptives) are excluded from enrollment as per Exclusion Criterion #5.	Nonsubstantial
Section 8.2.5 (Suicidal Ideation and Behavior Risk Monitoring)	Section was deleted.	This protocol section was part of Ironwood's standard protocol template language and did not apply to IW-3300 as IW-3300 is a guanylate cyclase C (GC-C) agonist that is rectally administered and acts locally in the gastrointestinal tract.	Nonsubstantial
Section 8.3.1 (Time Period and Frequency for Collecting Adverse Event [AE] and Serious Adverse Event [SAE] Information) and Section 10.3.3.1 (AE and SAE Recording)	The guidance for categorizing and recording a subject's medical history versus their pretreatment adverse events was clarified. For subjects who have an SAE and fail screening, only their SAE information will be recorded in the CRF.	This change was made to comport with the Sponsor's data handling guidelines.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.2 (Appendix 2: Clinical Laboratory Tests; Table 2)	Bullet point #4 was added: • *The investigator may consider repeating a laboratory test for any subject who has an abnormal laboratory test result (eg, positive hemoccult test, out-of-range chemistry test, abnormal urine culture) at Discharge (Day 2). The decision to repeat a laboratory test would be based on the investigator's clinical judgment after discussion with the sponsor's medical monitor and depending on the clinical context.*	This addition provides guidance on repeating laboratory tests.	Nonsubstantial
Section 10.3.4.2 (SAE Reporting to Ironwood Global Patient Safety via Paper SAE Report Form)	The email address was updated to: ICSRoperations@ironwoodpharma.com.	The change reflects the update.	Nonsubstantial
Section 10.4.2 (Contraception Guidance)	The reference to hormonal contraceptives was removed.	Because only women of nonchildbearing potential are eligible for enrollment and the reference to hormonal contraceptives was removed.	Nonsubstantial
Throughout	Minor editorial and document formatting revisions	Minor corrections and clarifications	Nonsubstantial

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1. PROTOCOL SUMMARY

1.1. Synopsis

1.1.1. Protocol Title

A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered Single Ascending Doses of IW-3300 in Healthy Volunteers

1.1.2. Short Title

A Phase 1 Study of Single Ascending Doses of IW-3300 in Healthy Volunteers

1.1.3. Rationale

IW-3300 is being developed for the treatment of bladder pain associated with interstitial cystitis/bladder pain syndrome (IC/BPS). IW-3300 is a novel, 13-amino-acid, guanylate cyclase C (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 MRP4 and MRP5, decreasing the activity of afferent 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.(1, 2)

Experiments conducted in animals indicate that colonic hypersensitivity induces persistent hypersensitivity of bladder afferent pathways in the absence of bladder pathology.(2) Hypersensitivity of bladder afferent pathways resembles the symptoms observed in patients with interstitial cystitis/bladder pain syndrome (IC/BPS). Afferent neurons are known to have peripheral endings in both the colon and the bladder, and the axons of colonic and bladder neurons travel 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 (PKGII).(3-5) CFTR activation causes secretion of chloride and bicarbonate into the intestinal lumen, resulting in increased fluid secretion and acceleration of intestinal transit.(6) These effects occur following oral administration, whereas no significant effects on intestinal transit were observed with direct administration of IW-3300 to the colon in preclinical studies. This 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.(7) 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 (Study MCP-103-204), and in subjects with irritable bowel syndrome with diarrhea (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). [redacted]

The safety of IW-3300 has been evaluated in nonclinical repeat dose toxicity, safety pharmacology, and genotoxicity studies and supports evaluation of IW-3300 in humans. 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.

This clinical study is the first-in-human, single-ascending-dose, safety, and tolerability study with IW-3300. IW-3300 will be administered rectally as a low-volume (20 mL) enema; this route of administration was selected to target effects to the colon. Dosing for rectal administration will be initiated at 100 µg and will not go higher than 2500 µg, which is approximately 1/10 of the human equivalent dose (HED) of the lowest no-observed-adverse-effect level (NOAEL; 24,000 µg).

1.1.4. Objectives and Endpoints

Objective	Endpoints
Primary	
To assess the safety and tolerability of single ascending doses of IW-3300 administered rectally via enema in healthy volunteers.	Incidence of treatment-emergent adverse events (TEAEs) Incidence of treatment-emergent serious adverse events (TESAEs)
Exploratory	
To summarize the single-dose pharmacokinetics (PK) of IW-3300 administered rectally via enema in healthy volunteers.	PK parameters Total amount of IW-3300 in stool (concentration and percent recovery)
To summarize the single-dose pharmacodynamics (PD) of IW-3300 administered rectally via enema in healthy volunteers based on bladder and bowel assessments.	Change from baseline in bowel movement (BM) frequency during the Clinic Period Change from baseline in stool consistency (Bristol Stool Form Scale [BSFS] score) during the Clinic Period Change from baseline in urinary frequency during the Clinic Period for a 24-hour duration Change from baseline in urinary urgency during the Clinic Period for a 24-hour duration Change from baseline in nocturia during the Clinic Period for a 24-hour duration Change from baseline in pain or burning in bladder or pelvic area during the Clinic Period for a 24-hour duration

1.1.5. Overall Design

This is a Phase 1, single-center, randomized, double-blind, placebo-controlled, single-ascending-dose study assessing the safety, tolerability, and PK of IW-3300 administered rectally as a low-volume enema in healthy adult volunteers. Each cohort will progress through 3 study periods: (1) Screening Period, (2) Clinic Period, and (3) Follow-up Period; these periods and the progression between cohorts are illustrated in the study schematic (Figure 1).

This first-in-human study in healthy adult volunteers will assess all subjects for safety, PK, and effect on stool frequency/form and urination.

In Cohort 1, sentinel dosing will be implemented. If no safety signals are noted in the first 2 subjects (randomized 1:1 to IW-3300 or placebo) following an observation period of at least 4 hours postdose, the remaining 6 subjects in Cohort 1 will be dosed. For all subsequent cohorts, a Dose Escalation Committee will conduct blinded reviews of all the safety/tolerability parameters through Discharge (Day 2) from the prior dosing cohort in order to make decisions regarding dose escalation. The Dose Escalation Committee will include sponsor and contract research organization (CRO) representatives.

1.1.6. Disclosure Statement

This is a single-ascending-dose safety and tolerability study with up to 6 treatments (placebo and up to 5 dose levels of IW-3300) that is subject- and investigator-blinded.

1.1.7. Number of Subjects

A maximum of 40 subjects will be randomized in the study (up to 5 cohorts of 8 subjects each); within each cohort, 6 subjects will be randomized to IW-3300 and 2 subjects will be randomized to placebo.

1.1.8. Intervention Groups and Duration

The study will evaluate single ascending doses of IW-3300 in a double-blind manner. The 8 subjects within each cohort will be randomized to receive a single dose of IW-3300 (6 subjects) or placebo (2 subjects), administered rectally (as a low-volume [20 mL] enema) following a fast of at least 6 hours (refer to the Schedule of Activities [SoA] for additional details regarding dosing instructions). The planned cohorts are:

- Cohort 1: 100 µg IW-3300 or matching placebo single rectal dose
- Cohort 2: 300 µg IW-3300 or matching placebo single rectal dose
- Cohort 3: 900 µg IW-3300 or matching placebo single rectal dose
- Cohort 4: 2500 µg IW-3300 or matching placebo single rectal dose
- Cohort 5 (optional): ≤2500 µg IW-3300 or matching placebo single rectal dose (specific dose level to be determined after safety reviews of previous cohorts; doses higher than 2500 µg will not be tested)

In Cohort 1, sentinel dosing will be implemented. The first 2 subjects will be randomized in a 1:1 ratio to IW-3300 or placebo and dosed at least 4 hours before the remaining subjects in the cohort. If no safety signals are noted following an observation period of at least 4 hours postdose, the remaining 6 subjects in Cohort 1 will be dosed. Dosing in Cohorts 2 through 5 will only proceed following a review of the prior dosed cohort(s), including adverse events (AEs), clinical laboratory test results, vital signs, and 12-lead ECGs. The determination of dose escalation will be made at a meeting of the Dose Escalation Committee.

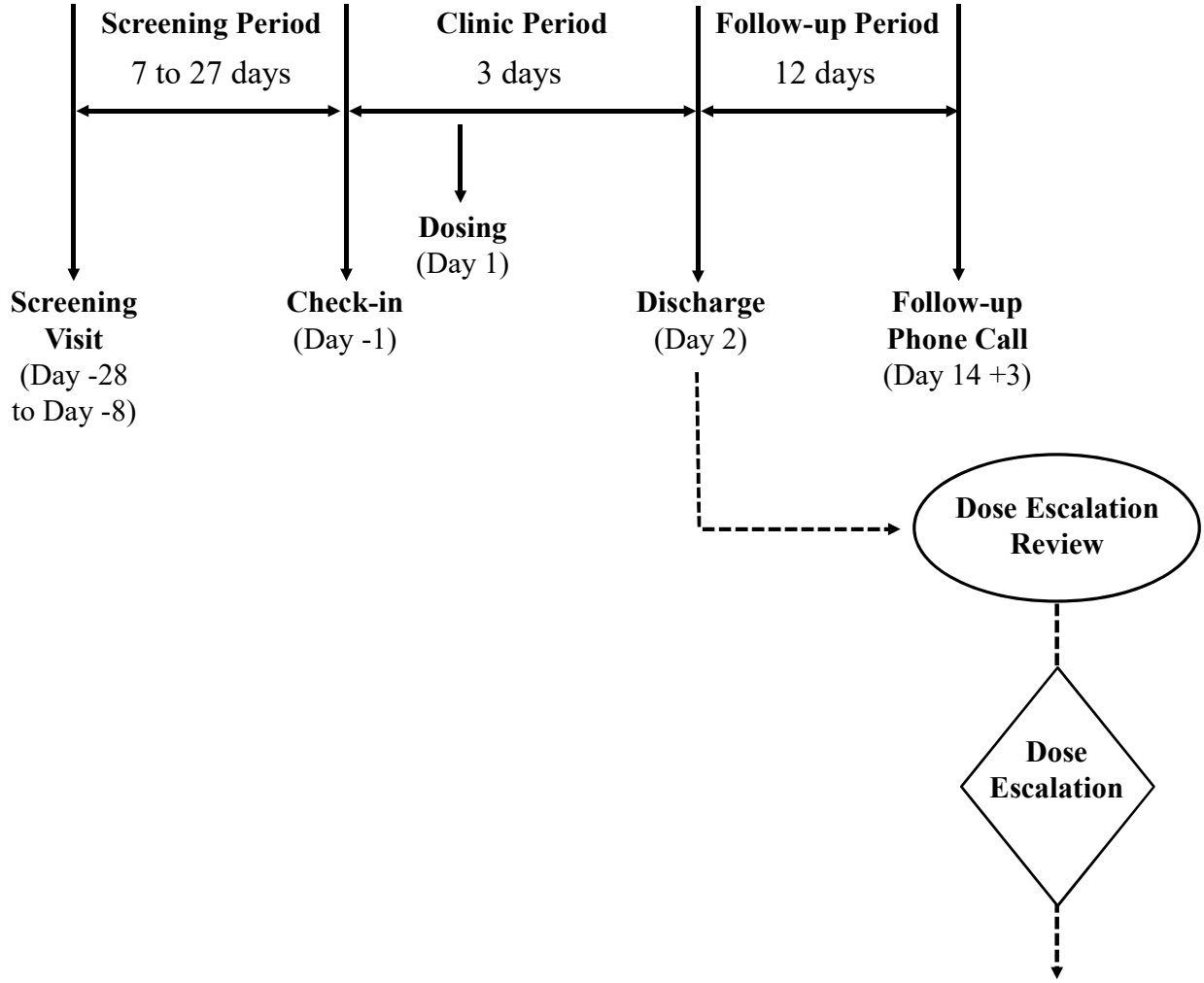
Treatment duration will be 1 day; subjects will be followed in the Phase 1 clinical research unit (CRU) for approximately 24 hours after dosing and contacted by phone for follow-up approximately 2 weeks after dosing. Total subject participation will be 22 to 45 days, including the Screening, Clinic, and Follow-up Periods.

1.1.9. Data Monitoring Committee: No

The study will utilize a Dose Escalation Committee to review safety data for each cohort in order to make decisions about dose escalation.

1.2. Schema

Figure 1: Schematic of Study Design



1.3. Schedule of Activities

Table 1: Schedule of Activities

Study Period →		Screening Period	Clinic Period			Follow-up Period
Study Procedure ↓	**Visit/Day →**	**Screening Visit Day -28 to Day -8**	**Check-in Day -1**	**Dosing Day 1**	**Discharge Day 2[a]**	**Follow-up Phone Call Day 14 (+3 days)**
Informed consent		X				
Eligibility criteria		X	X			
Medical history		X				
Demographics		X				
Body weight & height[b]		X	X		X	
Urine drug & alcohol screen		X	X			
Urine cotinine test		X				
Physical examination[c]		X	X		X	
Vital signs[d]		X		Pre: 0 (≤25m) Post: 0.5, 1, 2, 4, 6, and 8h (±10m)	24h post-Day 1 dose (±25m)	
Triplicate 12-lead ECG[e]		X	X	Pre 1h (±30m) Post: 2h (±30m)	X[f]	
Clinical chemistry, hematology, urinalysis		X	X	Pre: 0 (≤15m) Post: 6h (±30m)	24h post-Day 1 dose (±15m)	
ESR and CRP			X		24h post-Day 1 dose (±15m)	

Study Period → / Study Procedure ↓ / Visit/Day →	Screening Period	Clinic Period			Follow-up Period
	Screening Visit Day -28 to Day -8	**Check-in Day -1**	**Dosing Day 1**	**Discharge Day 2[a]**	**Follow-up Phone Call Day 14 (+3 days)**
SARS-CoV-2 testing	X	X			
HIV & hepatitis panel	X				
BBMD training	X				
BBMD dispensed	X	X			
Daily BBMD completion[g]	Day -8 through Day -2	X	X	X	
BBMD collection and review[h]		X		X	
Concomitant medications	X	X	X	X	X
AE monitoring	X	X	X	X	X
Subject confinement to clinic[i]		X	X	X	
Start of predose fast[j]			Pre: ≥6h		
Randomization			X		
Study drug administration[k]			X		
PK blood draws[l]			Pre: 0 (≤15m) Post: 0.5 (±2m), 1h (±10m), 2, 4, 6, and 8h (±5m)	24h post-Day 1 dose (±15m)	
Stool collection[m]			Post: 0h to Discharge		
Hemoccult testing		X	Post: 0h to Discharge		

Study Period →		Screening Period	Clinic Period			Follow-up Period
Study Procedure ↓	**Visit/Day →**	**Screening Visit Day -28 to Day -8**	**Check-in Day -1**	**Dosing Day 1**	**Discharge Day 2[a]**	**Follow-up Phone Call Day 14 (+3 days)**
Follow-up phone call[n]						X

AE=adverse event; BBMD=bladder and bowel movement diary; CRP=C-reactive protein; CRU=clinical research unit; ECG=electrocardiogram; ESR=erythrocyte sedimentation rate; HIV=human immunodeficiency virus; PK=pharmacokinetic; Post=postdose; Pre=predose; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2

[a] Subjects will be discharged on Day 2 unless they have physical examination findings or laboratory abnormalities that are considered by the investigator to be clinically meaningful. In the event that a subject discontinues from the study, an early termination visit will be performed prior to discharge from the CRU. The early termination assessments will be the same as those conducted at Discharge (Day 2). Every effort should be made and documented to ensure that safety procedures scheduled for Discharge are performed at the early termination visit.

[b] Height will only be measured at the Screening Visit. At Check-in (Day -1) and Discharge (Day 2), subjects will be weighed in the morning upon awakening and before they ingest any water or food.

[c] Rectal examination should be performed at each physical examination; the rectal examination at all timepoints should include a digital rectal examination and inspection of the perianal area for redness or irritation.

[d] Vital signs will include oral temperature, blood pressure, and pulse rate. Side-lying blood pressure and pulse will be obtained at the 0.5 hour postdose timepoint on Day 1 (consistent with the position required following dosing). Blood pressure and pulse at the 2, 4, and 8 hour postdose timepoints should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones). At all other timepoints (ie, predose and 1, 6, and 24 hours postdose), orthostatic blood pressure and pulse measurements will be obtained; subject must lie quietly for ≥5 minutes before supine/semisupine blood pressure and pulse measurements are taken, then assume standing position for 3 minutes before standing blood pressure and pulse measurements are taken. If a subject has orthostatic symptoms upon standing (eg, palpitation, dizziness), they will be assisted and asked to lie down without waiting the 3 minutes for vital sign assessment. Orthostatic measurements may be taken at other vital signs collection timepoints if clinically indicated, at the discretion of the investigator. When applicable, vital sign measurements will be obtained immediately before blood draws.

[e] ECGs should be obtained after the subject has been supine for at least 5 minutes. At each timepoint required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.

[f] Procedure will be done in the morning shortly after the subject awakens but before the subject eats breakfast.

[g] The BM portion of the BBMD will be completed by the subject on an event-driven basis. For subjects with Screening Visits earlier than Day -9, CRU staff will call subjects on Day -9 to remind them to begin recording their BMs in the BBMD the following day, answer any questions, and provide replacement BBMD pages via email, if needed. Bladder and urination information in the BBMD will be collected on Day 1 predose and Day 2, with a 24-hour recall period at each timepoint.

[h] CRU staff will review the subject's Screening Period BBMD at Check-in to confirm that subjects have at least 3 bowel movements during the 7 days prior to Check-in (Days -8 through -2) and no more than 3 bowel movements per day to be eligible for the study; CRU staff will collect the subject's Clinic Period BBMD at Discharge.

[i] Subjects will remain at the CRU from Check-in (Day -1) through Discharge (Day 2).

[j] Subjects will fast for at least 6 hours prior to dosing on Day 1 and for at least 1 hour following dosing.

[k] Study drug (IW-3300 or placebo low-volume enema) will be administered rectally after a fast of at least 6 hours. Subjects will be encouraged to empty their bowels in the morning prior to dosing, if possible. Subjects will be instructed to lie on their left side with their left leg extended and their right leg slightly bent. CRU staff will remove the cap from the applicator tip and gently insert the tip into the subject's rectum, then slowly squeeze the bottle to empty the contents into the rectum. After using the enema, subjects will lie on their left side for at least 30 minutes to allow the liquid to distribute throughout their intestines, followed by at least 30 additional minutes in the semisupine position. CRU 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 hold in the enema for at least 1 hour.

[l] PK blood draws at 0.5 and 1 hour postdose should be collected while the subject maintains the position noted in the dosing instructions above.

[m] Beginning immediately after dosing on Day 1, all stool passed up to the time of Discharge on Day 2 will be collected and the wet weight will be measured, to calculate IW-3300 concentrations in stool.

[n] CRU staff will contact subjects by phone for safety follow-up (at the discretion of the investigator, subjects may be requested to return to the CRU for their follow-up contact).

2. INTRODUCTION

IW-3300 is being developed for the treatment of bladder pain associated with IC/BPS.

2.1. Study Rationale

This clinical study is the first-in-human, single-ascending-dose, safety, and tolerability study with IW-3300. IW-3300 will be administered rectally as a low-volume (20 mL) enema; this route of administration was selected to target effects to the colon. Dosing for rectal administration will be initiated at 100 μg and will not go higher than 2500 μg, which is approximately 1/10 of the HED of the lowest NOAEL (24,000 μg).

2.2. Background

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 cGMP is secreted across the basolateral membrane of colonic epithelial cells in the submucosa by multidrug resistance proteins MRP4 and MRP5, decreasing the activity of afferent nerve fibers located in the colonic wall, resulting in reduced visceral pain This ultimately produces an analgesic effect in other organs of the abdominopelvic region via action mediated through the common afferent pathways.(1, 2)

Experiments conducted in animals indicate that colonic hypersensitivity induces persistent hypersensitivity of bladder afferent pathways in the absence of bladder pathology.(2) Hypersensitivity of bladder afferent pathways resembles the symptoms observed in patients with IC/BPS. Afferent neurons are known to have peripheral endings in both the colon and the bladder, and the axons of colonic and bladder neurons travel through the same splanchnic and pelvic nerves. These sensory afferents have cell bodies located within the thoracolumbar (TL) and lumbosacral (LS) dorsal root ganglia (DRG) and central projections in the dorsal horn of the corresponding regions of the spinal cord.(8) In animal models, daily administration of GC-C agonist linaclotide reversed persistent afferent bladder hypersensitivity. GC-C is restricted to the gastrointestinal (GI) tract and is not expressed in the bladder. 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 CFTR through its cGMP-dependent PKGII.(3-5) CFTR activation causes secretion of chloride and bicarbonate into the intestinal lumen, resulting in increased fluid secretion and acceleration of intestinal transit.(6) These effects occur following oral administration, whereas no significant effects on intestinal transit were observed with direct administration of IW-3300 to the colon in preclinical studies. This 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.(7) 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 the 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 (Study MCP-103-204), and in subjects with irritable bowel syndrome with diarrhea (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). [redacted]

The safety of IW-3300 has been evaluated in nonclinical repeat dose toxicity, safety pharmacology, and genotoxicity studies and supports evaluation of IW-3300 in humans. IW-3300 was well tolerated when administered intrarectally in rats and monkeys at 20 mg/kg/day once daily for 14 days; no adverse findings of dehydration, decreases in stool consistency, local irritation, or any histopathological changes were noted. The observed effects of IW-3300 administered orally in mice included decreases in stool consistency and dehydration resulting in mortality; these adverse GI effects (related to increased fluid secretion) after oral administration in mice were not observed after intrarectal administration of IW-3300 in rats. Decreases in stool consistency after oral and intrarectal administration of IW-3300 were observed in monkeys, but were not considered to be adverse.

Refer to the Investigator's Brochure (IB) for a more detailed description of the chemistry and pharmacology of IW-3300.

2.3. Benefit/Risk Assessment

While there were no clinical data available for IW-3300 at the outset of this first-in-human study, available nonclinical data with IW-3300 support a favorable risk/benefit profile. When administered orally, GC-C agonists may cause diarrhea and dehydration. This should be minimized in this study as IW-3300 is being administered intrarectally as a low-volume enema. More detailed information about the known and expected benefits and potential risks of IW-3300 may be found in the IB.

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.	Intrarectal administration will likely decrease the risk of diarrhea and subsequent dehydration. The study design includes dose escalation to closely monitor subject safety and tolerability at each dose level before dosing with a higher dose; a Dose Escalation Committee will conduct blinded reviews of all the safety parameters of the dosing cohort through Discharge (Day 2) in order to make decisions regarding escalation to the next dose. Orthostatic vital sign measurements will be taken to evaluate the potential for volume depletion. The daily BM frequency and stool consistency will be monitored in real time to evaluate changes in bowel habits and the potential risk of diarrhea. Stopping rules will also be in place to evaluate continued dosing (Section 7.1.1); dosing within a cohort may be temporarily suspended in the event of a diarrhea or dehydration serious adverse event (SAE); ≥1 subject with Grade 3 diarrhea (increase from baseline of >7 stools per day and/or watery stools [BSFS score of 7] resulting in a limiting of self-care activities) or dehydration (hospitalization indicated); or ≥2 subjects with Grade 2 diarrhea (increase from baseline of 4 to 6 stools per day and/or watery stools [BSFS score of 7] resulting in a limiting of instrumental activities) or dehydration (intravenous fluids indicated).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Unknown AEs, laboratory abnormalities, or other safety findings with a novel study drug	This is a first-in-human study with IW-3300 and, as such, there are no clinical data available.	The first cohort will feature sentinel dosing, whereby the first 2 subjects in Cohort 1 will be randomized in a 1:1 ratio to IW-3300 or placebo and dosed at least 4 hours before the remaining subjects in the cohort. Following from there, the study design includes dose escalation to closely monitor subject safety at each dose level before dosing with a higher dose; a Dose Escalation Committee will conduct blinded reviews of all the safety parameters of the dosing cohort through Discharge (Day 2) in order to make decisions regarding escalation to the next dose. Stopping rules are also in place to evaluate continued dosing within a cohort (Section 7.1.1); dosing within a cohort may be temporarily suspended in the event of an SAE considered related to study drug; ≥1 subject with a Grade 3 (severe or medically significant) or higher AE considered related to study drug; or ≥2 subjects with Grade 2 (moderate) or higher AEs considered related to study drug.
Study drug will be administered rectally.	Rectally administered medications may potentially be associated with AEs such as local trauma/discomfort, irritation, or bleeding.	The study excludes subjects for whom rectal administration would be anticipated to be a safety concern. The low-volume enema used in this study is expected to be a lower risk than higher volume enemas. Nonclinical toxicology studies of IW-3300 have shown no signs of local trauma following intrarectal administration.
Study Procedures		
Venipuncture will be performed during the study.	There is the risk of bleeding, bruising, hematoma formation, and infection at the venipuncture site.	Only appropriately qualified personnel will perform the blood draw. Subjects will be monitored for these risks and managed appropriately.

2.3.2. Benefit Assessment

Benefits to individual subjects may include:

- Medical evaluations/assessments associated with study procedures (eg, physical examinations, ECGs, laboratory evaluations, SARS-CoV-2 (COVID-19) diagnostic testing)
- Contributing to the process of developing new therapies in an area of unmet need

2.3.3. Overall Benefit: Risk Conclusion

Taking into account the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with IW-3300 are justified by the anticipated benefits.

3. OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

The primary objective is to assess the safety and tolerability of single ascending doses of IW-3300 administered rectally via enema in healthy volunteers.

3.1.2. Exploratory Objectives

The exploratory objectives are:

- To summarize the single-dose PK of IW-3300 administered rectally via enema in healthy volunteers
- To summarize the single-dose PD of IW-3300 administered rectally via enema in healthy volunteers based on bladder and bowel assessments

3.2. Estimands

Not applicable for this Phase 1 study.

3.3. Endpoints

3.3.1. Primary Endpoints (Safety)

The primary endpoints are:

- Incidence of TEAEs
- Incidence of TESAEs

3.3.2. Exploratory Endpoints (Pharmacokinetics and Pharmacodynamics)

The exploratory endpoints are:

- PK parameters
- Total amount of IW-3300 in stool (concentration and percent recovery)
- Change from baseline in BM frequency during the Clinic Period
- Change from baseline in stool consistency (BSFS score) during the Clinic Period
- Change from baseline in urinary frequency during the Clinic Period for a 24-hour duration
- Change from baseline in urinary urgency during the Clinic Period for a 24-hour duration
- Change from baseline in nocturia during the Clinic Period for a 24-hour duration
- Change from baseline in pain or burning in bladder or pelvic area during the Clinic Period for a 24-hour duration

4. STUDY DESIGN

4.1. Overall Design

This is a Phase 1, single-center, randomized, double-blind, placebo-controlled, single-ascending-dose study assessing the safety, tolerability, and PK of IW-3300 administered rectally as a low-volume enema in healthy adult volunteers. Each cohort will progress through 3 study periods: (1) Screening Period, (2) Clinic Period, and (3) Follow-up Period; these periods and the progression between cohorts are illustrated in the study schematic (Figure 1).

This first-in-human study in healthy adult volunteers will assess all subjects for safety, PK, and effect on stool frequency/form and urination.

The study will include up to 5 cohorts with 8 subjects per cohort; within each cohort, subjects will be randomized to receive a single dose of IW-3300 (6 subjects) or placebo (2 subjects), administered rectally (as detailed in Section 6.1.1). The planned cohorts are:

- Cohort 1: 100 µg IW-3300 or matching placebo single rectal dose
- Cohort 2: 300 µg IW-3300 or matching placebo single rectal dose
- Cohort 3: 900 µg IW-3300 or matching placebo single rectal dose
- Cohort 4: 2500 µg IW-3300 or matching placebo single rectal dose
- Cohort 5 (optional): ≤2500 µg IW-3300 or matching placebo single rectal dose (specific dose level to be determined after safety reviews of previous cohorts; doses higher than 2500 µg will not be tested)

In Cohort 1 only, sentinel dosing will be implemented. If no safety signals are noted in the first 2 subjects (randomized 1:1 to IW-3300 or placebo) following an observation period of at least 4 hours postdose, the remaining 6 subjects in Cohort 1 will be dosed. For all subsequent cohorts, a Dose Escalation Committee will conduct blinded reviews of all the safety/tolerability parameters through Discharge (Day 2) from the prior dosing cohort in order to make decisions regarding dose escalation. The Dose Escalation Committee will include sponsor and CRO representatives. Dose escalation is detailed in Section 6.6.

4.1.1. Study Periods

4.1.1.1. Screening Period

The Screening Period will begin with the signature of the informed consent form (ICF) at the Screening Visit (which can occur from Day -28 to Day -8) and will last 7 to 27 days. At the Screening Visit, subjects will undergo preliminary screening procedures to ensure that they meet the inclusion and exclusion criteria for the study. Eligible healthy volunteers will receive the bladder and bowel movement diary (BBMD), a paper-based diary to complete on an event-driven basis (ie, for each BM) at home, and training on how to complete it to record their bladder and bowel habits. Subjects will complete the BBMD (BM portion only) daily only for the 7 days before Check-in on Day -1. The end of the Screening Period will coincide with the beginning of the Clinic Period at Check-in (Day -1).

4.1.1.2. Clinic Period

The Clinic Period will begin at Check-in to the Phase 1 CRU on Day -1 (the day before dosing), and will end at the time of Discharge on Day 2 (the day after dosing). Following confirmation of eligibility at Check-in, subjects will be admitted to the CRU and will undergo baseline procedures. On the morning of Day 1 (note that there is no Day 0), subjects in each cohort will undergo predose assessments and will be randomized in a 3:1 ratio to receive IW-3300 or matching placebo after a fast of at least 6 hours. In Cohort 1 only, the first 2 subjects will be randomized in a 1:1 ratio to IW-3300 or placebo and dosed at least 4 hours before the remaining subjects in the cohort.

Blood and stool samples for PK assessments will be collected at specified times predose (blood) and postdose (blood and stool) during the Clinic Period (see SoA in Table 1). Throughout the Clinic Period, safety assessments will be performed and subjects will continue to enter their BM information into the BBMD on an event-driven basis. Bladder and urination information in the BBMD will be collected on Day 1 predose and Day 2, with a 24-hour recall period at each timepoint. Subjects will be confined to the CRU from Check-in (Day -1) through 1 day of dosing until Discharge (Day 2), which will occur after completion of the assessments (at least 24 hours after administration of study drug) and at the investigator's discretion.

4.1.1.3. Follow-up Period

The Follow-up Period will start immediately after subjects are discharged from the Phase 1 CRU on Day 2 and will last for 12 (+3) days. At the conclusion of the Follow-up Period (Day 14+3), the Phase 1 CRU will contact subjects for the Follow-up Phone Call (at the discretion of the investigator, subjects may be requested to return to the Phase 1 CRU for their follow-up contact). The Follow-up Phone Call will be considered the end of the study for that subject.

4.2. Scientific Rationale for Study Design

A randomized, double-blind, placebo-controlled study design was chosen to investigate the safety, PK, and PD of IW-3300, and to determine the tolerability of a range of doses of IW-3300 administered rectally as a low-volume enema. Subjects will be randomized within each cohort 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 CRU 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.

Subjects will complete the BM portion of the BBMD during the last 7 days of the Screening Period to establish a baseline without study drug. Subjects will be confined to the CRU for the duration of the Clinic Period, from Check-in (Day -1) through 1 day of dosing until Discharge (Day 2), which will occur after completion of the assessments (at least 24 hours after administration of study drug) and at the investigator's discretion. CRU staff will contact subjects for the Follow-up Phone Call 12 (+3) days after Discharge for safety follow-up; at the discretion of the investigator, subjects may be requested to return to the CRU for their follow-up contact.

Because this is the first-in-human study with IW-3300, Cohort 1 will feature sentinel dosing whereby the first 2 subjects will be randomized in a 1:1 ratio to receive IW-3300 or placebo, and

they will be dosed at least 4 hours before the remaining subjects in the cohort. Subsequently, the cohorts will be enrolled sequentially, following a safety review of prior dosed cohorts. In addition, stopping criteria have been established to ensure that dosing at the same or higher dose levels (a lower dose could be used) will stop should a safety signal be detected.

4.2.1. Subject Input into Design

There was no subject involvement in the design of this clinical study.

4.3. Justification for Dose

This study is intended to evaluate the safety, tolerability, PK, and PD of single doses of IW-3300 when administered rectally as a low-volume (20 mL) enema. This route of administration was selected to target effects of IW-3300 to the colon. The nonclinical toxicology data support the proposed IW-3300 Phase 1 starting dose of 100 µg/subject/day with an approximate 240-fold safety factor compared to the lowest nonclinical NOAEL, resulting in the lowest HED. The 100 µg and 300 µg doses were also selected based on nonclinical pharmacology models of visceral hypersensitivity and bladder dysfunction using IW-3300. [redacted] The higher doses, 900 µg and 2500 µg, are supported by IW-3300 toxicology assessments and allow for assessment of safety and tolerability at a range of doses. Dosing will not go higher than 2500 µg, which is approximately 1/10 of the HED of the lowest NOAEL (24,000 µg).

4.4. End of Study Definition

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

The end of the study is defined as the date of the last 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:

5.1.1. Age

1. Subject must be 18 to 60 years of age, inclusive, at the time of signing the ICF.

5.1.2. Type of Subject and Disease Characteristics

2. Subject is medically healthy with no clinically significant findings during medical evaluation including physical examination, 12-lead ECG, and clinical laboratory tests (serum chemistry, hematology, urinalysis, and urine drug and alcohol screen). (NOTE: In making this determination, the investigator or designee will consider whether any finding could prevent the subject from performing any of the protocol-specified assessments, could represent a condition that would exclude the subject from the study, could represent a safety concern if the subject participates in the study, or could confound the study-specified assessments).
3. Subject has normal formed bowel movements (≥3 per week and ≤3 per day; average BSFS score of >2 and <6, as reported in the BBMD during the 7 days before Check-in [Day -1]).
4. Subject agrees to refrain from making any new or major lifestyle changes (eg, new diet or exercise regimen) from the Screening Visit until after the Follow-up Phone Call on Day 14.
5. If subject is ≥45 years of age, subject is compliant with colorectal cancer screening guidelines according to the ACG Clinical Guidelines: Colorectal Cancer Screening 2021. (9)

5.1.3. Weight

6. Body mass index (BMI) within the range 18.5 to 35.0 kg/m^2 (inclusive) at the Screening Visit.

5.1.4. Sex

7. Female subjects must be of nonchildbearing potential (ie, women who are postmenopausal [greater than 12 months without menses] or who have had a bilateral oophorectomy, bilateral salpingectomy, or hysterectomy).
8. Male subjects and female partners are willing to use double-barrier method of contraception during the study (eg, diaphragm with spermicide plus a condom, condom with spermicide plus a diaphragm or cervical cap), from the Screening Visit until after the Follow-up Phone Call on Day 14.

5.1.5. Informed Consent

9. Capable of giving signed informed consent as described in Appendix 1/Section 10.1 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:

5.2.1. Medical Conditions

1. Subject has evidence or history of clinically significant acute or chronic disease, or clinically significant illness within 30 days of the Screening Visit.
2. Subject has a history of clinically significant hypersensitivity or allergies to any of the inactive ingredients contained in the active or placebo drug products.
3. Subject has a history of any condition that would interfere with their ability to receive an enema.
4. Subject has a recent history of anal fissure, anal abscess, complicated hemorrhoids, or presence or history of inflammatory bowel disease.

5.2.2. Prior/Concomitant Therapy

5. Subject has used a prescription medication during the 14 days before Check-in (Day -1). Use of prescription medication, unless necessary to treat a medical emergency, is excluded during the study until after the Follow-up Phone Call on Day 14.
6. Subject has used any over-the-counter medications, including laxatives, during the 7 days before Check-in (Day -1). Use of over-the-counter medication, including laxatives, is not permitted during the study until after Discharge on Day 2. (Note: Acetaminophen may be used at the investigator's discretion, in consultation with the sponsor.)
7. Subject has used herbal supplements from 7 days before Check-in (Day -1). Use of these supplements is not permitted during the study until after Discharge on Day 2.
8. Subject has received a licensed or investigational vaccine during the 30 days before Check-in (Day -1) or is planning to receive any vaccine during the study (up to the Follow-up Phone Call on Day 14).
9. Subject has received blood products during the 2 months before Check-in (Day -1).
10. Subject has undergone a surgical procedure during the 30 days before Check-in (Day -1), other than minor dermatologic procedures, or has a history of surgery involving the GI tract or anal canal (with the exception of endoscopic procedures, appendectomy, and cholecystectomy).

5.2.3. Prior/Concurrent Clinical Study Experience

11. Subject has received any investigational drug during the 30 days or 5 half-lives of that investigational drug (whichever is longer) before the Screening Visit, or is planning to receive another investigational drug at any time during the study.

5.2.4. Diagnostic Assessments

12. Subject has a 12-lead ECG demonstrating severe bradycardia (heart rate <40 beats per minute) or average QTcF ≥450 msec for males and ≥470 msec for females at the Screening Visit or at Check-in on Day -1.
13. Subject has elevated (>1.5 times the upper limit of normal as defined by the laboratory) levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, or bilirubin at the Screening Visit or at Check-in on Day -1.
14. Subject has confirmed or suspected infection with SARS-CoV-2 (COVID-19) at the Screening Visit or Check-in on Day -1, or was diagnosed with COVID-19 or had contact with a COVID-19 patient within 14 days of the Screening Visit.
15. Subject has positive serology for human immunodeficiency virus (HIV)-1, HIV-2, or hepatitis B surface antigen (HBsAg), or positive for anti-HIV-1, anti-HIV-2, or anti-hepatitis C virus (HCV) antibodies at the Screening Visit.
16. Identification of an anatomical abnormality upon Screening rectal examination which will make enema administration difficult.

5.2.5. Other Exclusions

17. Subject has a history of difficulty receiving an enema.
18. Subject has a history of alcohol or drug addiction during the year before the Screening Visit, or has a positive drug or alcohol screen at the Screening Visit or Check-in on Day -1.
19. Subject has ingested any alcohol-containing foods or beverages during the 48 hours before Check-in (Day -1). Ingestion of alcohol-containing foods or beverages is excluded during the study until after the Follow-up Phone Call on Day 14.
20. Subject has donated blood products during the 6 weeks before Check-in (Day -1).
21. Subject is involved in the conduct and administration of this study as an investigator, subinvestigator, study coordinator, other study staff, or sponsor staff.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

1. Subjects agree to refrain from starting a new diet during the study as described in Section 5.1.
2. Subjects will fast for at least 6 hours prior to dosing on Day 1 and for at least 1 hour following dosing.

5.3.2. Caffeine, Alcohol, and Tobacco

1. Subjects will abstain from ingesting any alcohol-containing foods or beverages from 48 hours before Check-in (Day -1) until after the Follow-up Phone Call as described in Section 5.2.

2. Subjects are permitted to ingest caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate), but should limit intake to ≤3 cups per day.
3. Subjects who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches) will not be permitted while they are in the CRU.

5.3.3. Activity

1. Subjects agree to refrain from making any new or major lifestyle changes (eg, new diet or exercise regimen) as described in Section 5.1.
2. Subjects must follow contraception requirements outlined in Section 5.1.
3. Prior to dosing, subjects will be encouraged to empty their bowels, if possible.
4. After dosing, subjects will lie on their left side for at least 30 minutes (including for vital signs collection), followed by at least 30 additional minutes in the semisupine position as outlined in Section 6.1.1. Subjects should avoid using the bathroom and hold in the enema for at least 1 hour.
5. For vital signs collection at 2, 4, and 8 hours postdose, subjects should rest for at least 5 minutes prior to pulse and blood pressure measurement, in a quiet setting without distractions (eg, television, cell phones).
6. For vital signs collection predose and 1, 6, and 24 hours postdose, subjects must lie quietly for ≥5 minutes before supine/semisupine blood pressure and pulse measurements are taken, then assume a standing position for 3 minutes before standing blood pressure and pulse measurements are taken, as described in Section 8.2.2. If a subject has orthostatic symptoms upon standing (eg, palpitation, dizziness), they will be assisted and asked to lie down without waiting the 3 minutes for vital sign assessment. At the discretion of the investigator, orthostatic measurements may be taken at other vital signs collection timepoints if clinically indicated.

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 screen failure 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.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened following consultation with the sponsor. Rescreened subjects should 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), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

The study will evaluate single ascending doses of IW-3300 in a double-blind manner. The 8 subjects within each cohort will be randomized to receive a single dose of IW-3300 (6 subjects) or placebo (2 subjects), administered rectally (as a low-volume [20 mL] enema) following a fast of at least 6 hours. The planned cohorts are:

- Cohort 1: 100 µg IW-3300 or matching placebo single rectal dose
- Cohort 2: 300 µg IW-3300 or matching placebo single rectal dose
- Cohort 3: 900 µg IW-3300 or matching placebo single rectal dose
- Cohort 4: 2500 µg IW-3300 or matching placebo single rectal dose
- Cohort 5 (optional): ≤2500 µg IW-3300 or matching placebo single rectal dose (specific dose level to be determined after safety reviews of previous cohorts; doses higher than 2500 µg will not be tested)

In Cohort 1 only, sentinel dosing will be implemented. The first 2 subjects will be randomized in a 1:1 ratio to IW-3300 or placebo and dosed at least 4 hours before the remaining subjects in the cohort. If no safety signals are noted following an observation period of at least 4 hours, the remaining 6 subjects in Cohort 1 will be dosed.

Dosing in Cohorts 2 through 5 will only proceed following a review of the prior dosed cohort(s), including AEs, clinical laboratory test results, vital signs, and 12-lead ECGs. The determination of dose escalation will be made at a meeting of the Dose Escalation Committee.

6.1. Study Interventions Administered

Intervention Name	**IW-3300**	**Placebo**
Type	Drug	Placebo
Dose Formulation	Solution	Solution
Unit Dose Strengths (Solution Concentrations)	[redacted]	N/A
Dosage Levels	100 µg, 300 µg, 900 µg, 2500 µg, and (optional) ≤2500 µg dose TBD	N/A
Route of Administration	Rectal enema	Rectal enema
Use	Experimental	Placebo
IMP and NIMP	IMP	IMP
Sourcing	Compounded in the pharmacy at the clinical site	Compounded in the pharmacy at the clinical site

Packaging and Labeling	Study Intervention will be provided in filled syringes. Each syringe will be labeled as required per country requirement.	Study Intervention will be provided in filled syringes. Each syringe will be labeled as required per country requirement.

IMP=investigational medical product; NIMP=non-investigational medical product; TBD=to be determined

6.1.1. Dosing Instructions

Subjects will receive a single dose of study drug (IW-3300 or placebo low-volume enema) in the CRU on Day 1. Study drug will be administered rectally after a fast of at least 6 hours. Subjects will be encouraged to empty their bowels in the morning prior to dosing, if possible. Subjects will be instructed to lie on their left side with their left leg extended and their right leg slightly bent. CRU staff will remove the cap from the applicator tip and gently insert the tip into the subject's rectum, then slowly squeeze the bottle to empty the contents into the rectum. After using the enema, subjects will lie on their left side for at least 30 minutes to allow the liquid to distribute throughout their intestines, followed by at least 30 additional minutes in the semisupine position. CRU 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 hold in the enema for as long as possible (at least 1 hour).

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

6.2. Preparation/Handling/Storage/Accountability

All study drug substance will be stored in the locked, limited access, temperature-controlled pharmacy accessible only to pharmacy staff. IW-3300 drug substance will be protected from moisture and light and stored between -25°C to -15°C. Prior to compounding, IW-3300 drug substance will be thawed for a minimum of 30 minutes prior to use.

The investigational drug product will be compounded on the day of administration and stored at room temperature prior to dosing (continuous mixing is not required). Compounding must occur in accordance with the instructions provided in the Pharmacy Manual. Specifically designated unblinded CRU pharmacy staff will be responsible for preparing dosing for each cohort and providing doses to the study coordinator to administer to subjects (see Section 6.1.1 for dosing instructions).

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 or administer study drug. 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).

4. Further guidance and information for the final disposition of unused study drug are provided in the Pharmacy Manual.

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 Clinic and Follow-up Periods.

Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be randomized into the study on Day 1. A total of up to 40 subjects (up to 5 cohorts of 8 subjects each) will be randomized in a 3:1 ratio to receive IW-3300 or placebo, for each cohort, as detailed in Section 6. Randomization numbers encoding the subjects' treatment assignments will be based on a randomization schedule that is computer-generated prior to the study, by an independent statistician from the CRO who is not otherwise associated with the study.

6.3.2. Blinding

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

The Dose Escalation Committee, which will include sponsor and CRO representatives, will conduct blinded reviews of all the safety/tolerability parameters of the dosing cohort through Discharge (Day 2) in order to make decisions regarding dose escalation.

Specific designated personnel in the Ironwood may be unblinded to the treatment assignment of individual subjects for regulatory reporting purposes. 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 designated independent study statistician, who will not be involved in study data analysis and interpretation, will have access to the randomization schedule including treatment assignments.

Specifically designated unblinded CRU pharmacy staff will be responsible for preparing dosing for each cohort and providing doses to the study coordinator to administer to subjects. The investigator (except as detailed here) and the remaining CRU staff will be blinded as to treatment. In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded study intervention records at the CRU to verify that randomization/dispensing has been done accurately.

Site unblinding of a subject's treatment assignment is restricted to emergency situations that necessitate identifying the study drug for the welfare of the subject. Subject safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator, or person designated by the investigator, should contact the sponsor's medical monitor directly to discuss the need for emergency unblinding. Individual sealed unblinding envelopes, which can be opened to identify the treatment assignment for an individual subject in an emergency, will be provided to and retained by the CRU pharmacist. The reason for breaking the blind will be recorded.

6.4. Study Intervention Compliance

Subjects will receive study drug directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study drug and study subject identification will be confirmed at the time of dosing by a member of the CRU staff other than the person administering the study intervention.

6.5. Concomitant Therapy

Subjects must abstain from taking prescription medications during the 14 days before Check-in (Day -1) until after completion of the Follow-up Phone Call on Day 14; over-the-counter medications (including laxatives, vitamins, and dietary or herbal supplements) during the 7 days before Check-in (Day -1) until after Discharge on Day 2; and vaccines during the 30 days before Check-in (Day -1) until after completion of the Follow-up Phone Call on Day 14.

Acetaminophen at doses of ≤3 grams/day is permitted for use during the study at the investigator's discretion, in consultation with the medical monitor.

Any medication (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) 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

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1. Rescue Medicine

Not applicable to this study of healthy volunteers.

6.6. Dose Modification

The decision to proceed to the next dose level of IW-3300 (either an increase or a decrease) will be made by the Dose Escalation Committee, based on blinded safety and tolerability data obtained through Discharge on Day 2 in all subjects at the prior dose level.

Dosing at a given dose level may be temporarily suspended for any of the reasons listed in Section 7.1.1.

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; at the discretion of the investigator, subjects with ongoing safety concerns may have discharge delayed if necessary for continued monitoring.

7. DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Intervention

It is not anticipated that it will be necessary for a subject to permanently discontinue (definitive discontinuation) study intervention during this single-dose study. In rare instances, a subject may withdraw from the study prior to completion of the Clinic Period; see the SoA (Table 1) for data to be collected at the time of discontinuation and follow-up and for any further evaluations that need to be completed. If a subject is discontinuing due to an AE, the AE must be followed until it is resolved, stable, or judged by the investigator to be not clinically significant.

7.1.1. Study Suspension or Termination

Dosing at a given dose level may be temporarily suspended if any of the following occur within a cohort:

- ≥1 subject with an SAE considered related to study drug
- ≥1 subject with a Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or higher AE considered related to study drug (CTCAE v5.0 will be used; grading criteria are summarized in Appendix 3/Section 10.3.3)
- ≥2 subjects with CTCAE Grade 2 or higher AEs considered related to study drug

The Dose Escalation Committee should consider the benefit-risk ratio in their determination to suspend dosing temporarily or permanently. If the benefit-risk is considered unacceptable and the effect is considered related to active study drug by the Dose Escalation Committee, dosing at a given dose level will be stopped, and no additional cohorts will be dosed at the same or higher dose level (a lower dose could still be studied).

The sponsor may permanently terminate the study, or a component of the study, at any time. Reasons for termination may include, but are not limited to:

- Death, SAE, or other safety finding considered to be related to IW-3300 that may preclude further dosing.

7.2. Subject Discontinuation/Withdrawal from the Study

- A subject may withdraw from the study at any time at his/her 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 discontinuation from the study, if possible, an early discontinuation visit should be conducted, as shown in the SoA (Table 1). See 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.
- The subject will be permanently discontinued both from the study intervention and from the study at that time.

- 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, he/she 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 he or she repeatedly fails 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 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, he/she will be considered to have withdrawn from the study and reason for discontinuation will be "lost to follow-up."

Discontinuation of the site or of the study as a whole are handled as part of Appendix 1/Section 10.1.

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 drug.
- Adherence to the study design requirements, including those specified in the SoA, 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

Efficacy will not be evaluated in this Phase 1 study in healthy volunteers.

8.2. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA (Table 1).

8.2.1. Physical Examinations

A complete physical examination will be performed as outlined in the SoA, 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. Rectal examination should be performed at each physical examination; the rectal examination at all timepoints should include a digital rectal examination and inspection of the perianal area for redness or irritation.
- 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, pulse rate, and blood pressure will be assessed.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse at 0.5 hours postdose on Day 1 will be assessed in a side-lying position (consistent with the position required following dosing).
- Blood pressure and pulse at the 2, 4, and 8 hour postdose timepoints should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones).
- At all other timepoints, blood pressure and pulse measurements will be assessed in a supine and standing position (for calculation of orthostatic measurements). For orthostatic blood pressure and pulse measurements, the subject must lie quietly for ≥5 minutes before supine/semisupine blood pressure and pulse measurements are taken, then assume a standing position for 3 minutes before standing blood pressure and pulse measurements are taken. If a subject has orthostatic symptoms upon standing (eg, palpitation, dizziness), they will be assisted and asked to lie down without waiting the 3 minutes for vital sign assessment. At the discretion of the investigator, orthostatic measurements may be taken at other vital signs collection timepoints if clinically indicated.
- When applicable, vital signs measurements will be obtained immediately before 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.
- At each time point at which triplicate ECG are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes.

8.2.4. Clinical Safety Laboratory Assessments

- See Appendix 2/Section 10.2 for the list of clinical laboratory tests to be performed and 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.
- 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

case report form (CRF). The laboratory reports must be filed with the source documents.

- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 14 days after the dose of study drug 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 non-protocol-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.

8.3. Adverse Events and Serious Adverse Events

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

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 AEs that are serious, considered related to the study intervention or study procedures, or that caused the subject to discontinue study drug (see 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, early termination or 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 Appendix 3/Section 10.3. 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 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 he/she considers 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 Appendix 3/Section 10.3.

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 Appendix 3/Section 10.3.3.

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, institutional review boards (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

This study is enrolling males and females of non-childbearing potential.

- Details of all pregnancies in female subjects and female partners of male subjects will be collected after the start of study intervention and either until completion of the Follow-up Phone Call or until the details regarding the pregnancy outcome are reported. The CRU 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 Appendix 4/Section 10.4.
- 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 Appendix 3/Section 10.3.

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

Not applicable to this study in healthy volunteers.

8.3.8. Adverse Events of Special Interest

Not applicable.

8.4. Treatment of Overdose

For this study, any dose of study drug greater than 1 dose of study drug within a 24-hour time period 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. Subjects will be monitored for potential development of diarrhea and dehydration and, if present, will be managed accordingly.
2. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

8.5. Pharmacokinetics

Blood and feces for the determination of IW-3300 in plasma and stool, respectively, will be collected from all dosed subjects during the Clinic Period at timepoints 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.

The following PK parameters will be calculated using a noncompartmental approach:

- Area under the plasma concentration time curve from time zero to the time at which the last measurable concentration is observed (AUC_{0-t})
- Area under the plasma concentration time curve from time zero to time infinity ($AUC_{0-\infty}$)
- Maximum plasma concentration (C_{max})
- Time to maximum plasma concentration (T_{max})
- Terminal elimination half-life ($t_{1/2}$)

The wet weight of stool collected during the postdose collection period prior to Discharge will be measured and the total amount of IW-3300 in stool will be calculated, including concentration of IW-3300 in stool (ng/g), and percent recovery.

Genetic analyses will not be performed on these samples. Subject confidentiality will be maintained. At visits during which blood samples for the determination of multiple aspects of IW-3300 will be taken, 1 sample of sufficient volume can be used.

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

8.6. Pharmacodynamics

Subjects will enter BM-related information into a paper diary, the BBMD, on an event-driven basis (ie, following each BM) in accordance with the instructions in the SoA (Table 1).

The BM-related information will include the day and time of BMs and a report of stool consistency for each BM using the BSFS (1=Separate hard lumps like nuts [difficult to pass] to 7=Watery, no solid pieces [entirely liquid]).

Subjects will enter bladder information into the BBMD, which will include the following questions:

In the past 24 hours…

- Did you have to urinate more frequently than normal? (yes/no)
- How many times did you urinate? (0 to 2; 3 to 6; 7 to 10; or 10+ times)
- Did you feel the strong need to urinate with little or no warning? (yes/no)
- Did you have to get up to urinate during the night more frequently than usual? (yes/no)
- How many times did you have to get up at night to urinate? (0, 1, 2, 3+ times)
- Did you have pain or burning in your bladder or pelvic area? (yes/no)

8.7. Genetics

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

8.8. Biomarkers

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

8.9. Immunogenicity Assessments

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

8.10. 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

No hypothesis testing is planned.

9.2. Sample Size Determination

No statistical sample size determination process was performed. The sample size chosen for each cohort (8 subjects) is considered sufficient for evaluation of safety, tolerability, PK, and PD.

9.3. Populations for Analyses

The following populations are defined:

Population	Description
Screened Analysis Set	The Screened Analysis Set consists of all subjects who have signed informed consent.
ITT Set	The ITT Set consists of all subjects in the Screened Analysis Set who have been randomized to a treatment regimen. Analysis will be performed according to the allocated treatment regimen regardless of the treatment regimen actually received.
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 regardless of the allocated treatment.
PK Analysis Set	The PK Analysis Set consists of all subjects in the Safety Analysis Set who have at least 1 postdose PK assessment. Analysis will be performed according to the treatment actually received regardless of the allocated treatment.
PD Analysis Set	The PD Analysis Set consists of all subjects in the Safety Analysis Set who have at least 1 postdose BBMD entry in the Clinic Period. Analysis will be performed according to the treatment actually received regardless of the allocated treatment.

9.4. Statistical Analyses

The statistical analysis plan (SAP) will be finalized prior to the clinical database lock and will include a more technical and detailed description of the statistical analyses described in this section.

9.4.1. General Considerations

For summaries by treatment group, data from subjects who receive placebo in different cohorts can be combined and presented as one treatment group (referred to as “treatment” and “placebo”), as appropriate.

Continuous variables will be summarized using descriptive statistics (N, mean, SD, median, minimum, and maximum). Categorical variables will be summarized using the count and

proportion of subjects in each category. Furthermore, PK data will be summarized using descriptive statistics, geometric mean, and coefficient of variation (CV%) of the geometric mean.

9.4.2. Primary Endpoints Analyses

The primary endpoint analysis will be based on the Safety Analysis Set.

AE verbatim terms will be coded in the electronic data capture (EDC) system against the most current version of the Medical Dictionary for Regulatory Activities (MedDRA) available at the start of the study.

TEAEs are defined in Section 10.3.1. The TEAEs and TESAEs for 1 calendar day postdose will be summarized by system organ class, preferred term, and treatment (or cohort).

9.4.3. Secondary Endpoint Analysis

Not applicable.

9.4.4. Exploratory Endpoints Analyses

PK analyses will be based on the PK Analysis Set.

Plasma concentrations of IW-3300 will be summarized by treatment (or cohort) at each assessment timepoint; additionally, a listing of plasma concentrations will be provided. PK parameters (as described in Section 8.5) will also be calculated for each subject and summarized by treatment, if systemic levels of IW-3300 are measurable/as data permit (≥1 plasma concentration above the lower limit of quantitation [LLOQ] is necessary for calculation of C_{max} and T_{max}; ≥3 plasma concentrations above LLOQ are necessary for calculation of AUC_{0-t}, $AUC_{0-\infty}$, and $t_{1/2}$).

The total amount of IW-3300 in stool collected during the postdose collection period prior to Discharge will be summarized by treatment, including the concentration (ng/g; based on the wet weight of stool) and the percent recovery. Subjects who do not have a BM during the stool collection period will not be included in the analysis of IW-3300 in stool.

The analysis of the BBMD parameters will be based on the PD Analysis Set.

BBMD parameters (BM frequency, stool consistency, urinary frequency, urinary urgency, and nocturia) will be summarized by treatment (or cohort) for each study period (Screening Period and Clinic Period) using descriptive statistics and changes from baseline will be calculated whenever it is appropriate.

Baseline BM values will be derived from the BBMD data collected in the Screening Period, specifically, the period of time from 7 days prior to Check-in up to the time of randomization on Day 1. The baseline BM frequency rate (BMs per day) will be derived based on the number of BMs the subject reports during this period. The baseline stool consistency will be calculated as the average of the nonmissing BSFS scores associated with the BMs reported by the subject during this period.

The subjects reported assessments for urination category parameters (urinary frequency, urinary urgency, and nocturia) in the 24-hour period prior to drug administration on Day 1 will be used to establish the baseline for each urinary parameter. The subjects reported assessment of pain or

burning in the bladder or pelvic area in the 24-hour period prior to drug administration on Day 1 will be used to establish the baseline for this parameter.

The Clinic Period values will be derived from the BBMD data collected after randomization in the Clinic Period, specifically, the period of time from Day 1 (postrandomization) through Day 2 (Discharge). The Clinic Period BM frequency rate will be derived based on the number of BMs the subject reports during this period. The Clinic Period stool consistency score is the mean of the nonmissing BSFS scores associated with the BMs reported by the subject during this period.

The subjects reported assessments for urination category parameters (urinary frequency, urinary urgency, and nocturia) in the 24-hour period after dosing on Day 1 will be used to establish the postbaseline assessments for each parameter. Subjects reported assessment of pain or burning in the bladder or pelvic area in the 24-hour period after dosing on Day 1 will be used to establish the postbaseline assessments for this parameter.

For the continuous variables, change from baseline will be defined as the Clinic Period value minus the baseline value. For the categorical variables, category shift from baseline to the Clinic Period will be assessed.

9.4.5. Safety Analyses

All safety analyses will be made on the Safety Analysis Set.

Summaries for the safety parameters corresponding to the primary endpoints (including TEAEs and TESAEs) are described in Section 9.4.2 above. Additionally, listings will be provided for severe AEs, drug-related AEs, SAEs, and AEs leading to study discontinuation.

Descriptive statistics and changes from baseline at each assessment timepoint will be presented by treatment (or cohort) for the Clinic Period for:

- Clinical laboratory values (in standard units) for each hematology, chemistry, and urinalysis parameter
- Vital signs (ie, pulse rate, systolic and diastolic blood pressure, oral temperature, and body weight)
- ECG parameters
  - Additionally, the overall 12-lead ECG interpretation (normal, abnormal not clinically significant, or abnormal clinically significant) will be summarized.

9.4.6. Other Analysis/Analyses

Not applicable.

9.5. Interim Analyses

No interim analysis is planned for the study. Interim safety reviews are detailed in Section 6.6.

9.6. Data Monitoring Committee (DMC)

This study will not utilize a DMC. A Dose Escalation Committee will be utilized to review safety data for each cohort in order to make decisions about dose escalation, as described in Section 6.6.

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 his/her representative will explain the nature of the study to the subject or his/her 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 are 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 his/her 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 his/her medical records may be examined by clinical quality assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

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. A Dose Escalation Committee composed of sponsor and CRO representatives will make decisions about dose escalation, as detailed in Section 6.6.

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.

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 CRF 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 CRF 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

The first act of recruitment is the date of first subject consent and will be the study start date.

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 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 Schedule of Activities.

- The tests detailed in Table 2 will be performed by the local laboratory, with the exception of the point-of-care hemoccult tests and ESR which will be sent to an outside laboratory.
- Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.
- The investigator may consider repeating a laboratory test for any subject who has an abnormal laboratory test result (eg, positive hemoccult test, out-of-range chemistry test, abnormal urine culture) at Discharge (Day 2). The decision to repeat a laboratory test would be based on the investigator's clinical judgment after discussion with the sponsor's medical monitor and depending on the clinical context.
- Pregnancy testing is not required, as women of childbearing potential are not eligible for the study (Section 5).

Table 2: Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	**Parameters**			
Hematology	Platelet count RBC count Hemoglobin Hematocrit	<u>RBC Indices</u>: MCV MCH MCHC % Reticulocytes	<u>WBC Count with Differential</u>: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[a]	Blood urea nitrogen (BUN)	Potassium	AST	Total and direct bilirubin
	Creatinine	Sodium	ALT	Total protein
	Glucose	Calcium	Alkaline phosphatase	Magnesium
	Chloride	Albumin	Bicarbonate	Phosphate
	Cholesterol	Uric acid		
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, and ketones, by dipstick • Microscopic examination (if blood or protein is abnormal) • Urine culture (if urinary tract infection is suspected)			
Inflammatory markers	• ESR (analyzed at an outside laboratory) • CRP			
Hemoccult testing	Point-of-care hemoccult test			
Other Screening Tests	Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, and benzodiazepines). Urine cotinine test SARS-CoV-2 testing (Screening and Check-in) HIV antibody and hepatitis panel (HBsAg, and HCV antibody)			

[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 measured which may indicate severe liver injury (possible Hy's Law), 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 CRU staff 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.
- 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. If the severity changes throughout the duration of the persistent AE, record the severity as it changes.
- 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), 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. 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 CRF.
- 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 CRF 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 SAE, only the SAE will be collected in CRF.

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 (ADL).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
- Grade 4: Life threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

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

ADL are defined as follows: Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. Self care ADL 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 his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has 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 his/her 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 CRF.
- 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 (see 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 the 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 (see 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)

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

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

Women in the following categories are not considered WOCBP:

1. Premenarchal
2. Premenopausal female with 1 of the following:
    - 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), 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.
3. Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - Females on hormonal replacement therapy (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

Male subjects and female partners must use double-barrier method of contraception (ie, a combination of male condom with either cervical cap, diaphragm, or sponge with spermicide) during the study, until after the Follow-up Phone Call on Day 14.

Women of childbearing potential are excluded from the study.

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 receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor 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 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, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study be withdrawn from the study.

10.5. Appendix 5: Abbreviations

Table 3: List of Abbreviations

Abbreviation	Definition
ADLs	Activities of Daily Living
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
$AUC_{0-\infty}$	Area under the plasma concentration time curve from time zero to time infinity
AUC_{0-t}	Area under the plasma concentration time curve from time zero to the time at which the last measurable concentration is observed
BBMD	bladder and bowel movement diary
BM	bowel movement
BMI	body mass index
BSFS	Bristol Stool Form Scale
BUN	blood urea nitrogen
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanosine monophosphate
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRO	contract research organization
CRP	C-reactive protein
CRU	clinical research unit
CTCAE	Common Terminology Criteria for Adverse Events
CV%	coefficient of variation
DMC	data monitoring committee
DRG	dorsal root ganglia
eCRF	electronic case report form
EDC	electronic data capture
ESR	erythrocyte sedimentation rate
GC-C	guanylate cyclase C

Abbreviation	Definition
GI	Gastrointestinal
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
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
IB	investigator's brochure
IC/BPS	interstitial cystitis/bladder pain syndrome
ICF	informed consent form
IEC	independent ethics committee
IMP	investigational medical product
INR	international normalized ratio
IRB	institutional review board
LLOQ	lower limit of quantitation
LS	Lumbosacral
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIMP	noninvestigational medical product
NOAEL	no-observed-adverse-effect level
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PKGII	protein kinase G type II
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SID	subject identification

Abbreviation	Definition
SoA	schedule of activities
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TL	Thoracolumbar
T_{max}	time to maximum plasma concentration
ULN	upper limit of normal
WBC	white blood cell
WOCBP	woman/women of childbearing potential

10.6. Appendix 6: Protocol Signatures

10.6.1. Sponsor Signature

Protocol Title:	A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered Single Ascending Doses of IW-3300 in Healthy Volunteers
Short Title:	A Phase 1 Study of Single Ascending Doses of IW-3300 in Healthy Volunteers
Protocol Number:	C3300-101
Version Number /Amendment Number:	Version 4/Amendment 3
Final Date:	10 January 2022

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

{Electronic signature page appended}

[redacted]

Date

10.6.2. Investigator Signature

Protocol Title:	A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered Single Ascending Doses of IW-3300 in Healthy Volunteers
Short Title:	A Phase 1 Study of Single Ascending Doses of IW-3300 in Healthy Volunteers
Protocol Number:	C3300-101
Version Number /Amendment Number:	Version 4/Amendment 3
Final Date:	10 January 2022

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: _____

10.7. Appendix 7: 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 (22 September 2021)

Overall Rationale for the Amendment:

Updates to the safety and pharmacodynamic (PD) assessments were made based on FDA comments and recommendations.

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
1.3 (Schedule of Activities); 5.3.3 (Activity); 8.2.2 (Vital Signs)	Timepoints for vital signs collection on Days 1 and 2 were changed to align with PK blood sample collection timepoints: Day 1 Predose: 0 (≤25m) Day 1 Postdose: 0.5, 1, 2, 4, 6, and 8h (±10m) Day 2: 24h post-Day 1 dose (±25m) Corresponding footnote specifies that the 0.5 hour postdose assessment on Day 1 will be obtained in the side-lying position (consistent with the position required following dosing); the 2, 4, and 8 hour postdose timepoints should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones); and at all other timepoints (or if clinically indicated), orthostatic measurements will be obtained (supine/semisupine measurements after lying quietly for ≥5 minutes, then standing measurements after 3 minutes in the standing position).	Change to vital signs collection times was requested by FDA. Granularity regarding position for collection of blood pressure and pulse was added to account for additional collection times, as orthostatic measurements were not needed at every timepoint in order to evaluate the potential for volume depletion, unless clinically indicated.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
1.3 (Schedule of Activities)	Timepoints for clinical chemistry, hematology, and urinalysis on Days 1 and 2 were updated to add a Day 1 predose (0 [≤15m]) timepoint and clarify the Day 2 timing (24h post-Day 1 dose [±15m]).	Change to clinical chemistry and urinalysis collection times was requested by FDA; addition of Day 1 predose timepoint establishes a clear baseline for laboratory assessments.	Substantial
1.3 (Schedule of Activities); 8.5 (Pharmacokinetics); 9.4.4 (Exploratory Endpoints Analysis)	The end of the stool collection period was changed from 24h postdose to the time of Discharge on Day 2. The corresponding analysis was clarified to note that subjects who do not have a bowel movement (BM) during the stool collection period will not be included in the analysis of IW-3300 concentration in the stool.	Clarification on the handling of subjects with no stool sample for assessment of IW-3300 in stool was requested by FDA; the collection timing was changed in order to minimize missing data.	Substantial
8.6 (Pharmacodynamics)	The following bladder assessments were added to the PD assessments: day and time of urination, urinary urgency using a 5-point scale (0=no urgency to 4=urge incontinence), and nocturia (the number of awakenings to urinate each night).	Addition of bladder assessments was requested by FDA.	Substantial
1 (Synopsis, Objectives and Endpoints); 3.1.2 (Exploratory Objectives); 3.3.2 (Exploratory Endpoints); 9.4.4 (Exploratory Endpoints Analysis)	Edited exploratory PD objective to specify that it is based on bladder and bowel assessments. Added exploratory PD endpoints: Change from baseline in urinary frequency during the Clinic Period Change from baseline in urinary urgency during the Clinic Period Change from baseline in nocturia during the Clinic Period	To reflect addition of bladder assessments	Substantial
9.4.4 (Exploratory Endpoints Analysis)	Described the derivation/ calculation of baseline and Clinic Period values for bladder assessments.	To reflect addition of bladder assessments and endpoints	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Throughout	The bowel movement diary (BMD) was renamed the bladder and bowel movement diary (BBMD). Descriptions of the diary specify that bladder symptoms will be collected along with BMs/bowel habits.	To reflect addition of bladder assessments	Substantial
1.3 (Schedule of Activities); 4.1.1.2 (Clinic Period); 8.6 (Pharmacodynamics)	BBMD will be completed by the subject throughout the Screening and Clinic Periods, rather than by the subject during the Screening Period and clinical research unit (CRU) staff during the Clinic Period. Added BBMD dispensed at Check-in and BBMD collection at Discharge to account for the subject completing the BBMD during the Clinic Period.	To reflect addition of bladder assessments, which cannot be assessed by CRU staff	Substantial
1.3 (Schedule of Activities); 8.2.1 (Physical Examination)	The physical examination instructions were updated to require a digital rectal examination as part of each physical examination (rather than at the Screening Visit only).	FDA recommended performing digital rectal examination during all physical examinations.	Substantial
1.3 (Schedule of Activities); 5.3.1 (Meals and Dietary Restrictions)	The predose fast instructions were updated to change completion of fasting on Day 1 from 4 hours postdose to at least 1 hour postdose.	Mitigate risk of volume depletion and potential AEs due to prolonged fasting.	Substantial
1.3 (Schedule of Activities); 5.3.3 (Activity); 6.1.1 (Dosing Instructions)	Additional detail was added to the study drug administration instructions to note that subjects will be encouraged to empty their bowels in the morning prior to dosing, if possible.	May help subjects avoid using the bathroom and hold in the enema for at least 1 hour postdose.	Substantial
1.3 (Schedule of Activities)	The window for the pharmacokinetic (PK) blood draw at 1 hour postdose was changed to (±10m).	To allow for orthostatic vital signs to be taken after the hour of restricted activity postdose (30 minutes lying on side followed by 30 minutes in semisupine position), but before the PK blood draw	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
5.2 (Exclusion Criteria)	Exclusion #4 was updated to specifically exclude subjects with presence or history of inflammatory bowel disease.	FDA recommended excluding subjects with presence or history of inflammatory bowel disease.	Substantial
10.2 (Appendix 2: Clinical Laboratory Tests)	Under Routine Urinalysis, added urine culture (if urinary tract infection is suspected).	FDA recommended collecting data for urinary tract infections (including urine culture).	Substantial
10.3.4 (Reporting of SAEs)	Serious adverse event (SAE) reporting method was changed from paper via email (primary) or fax (secondary) to the electronic data collection tool as primary method with paper (SAE Report Form) as secondary.	Correction	Substantial
10.2 (Appendix 2: Clinical Laboratory Tests)	Clinical laboratory tests will be performed by a central laboratory, with the exception of the Check-in (Day -1) laboratory assessment, which will be performed at a local laboratory	Correction (original protocol stated that all tests would be performed at the local laboratory)	Nonsubstantial
9.3 (Populations for Analyses)	An Intent-to-treat (ITT) Set was added, consisting of all subjects in the Screened Analysis Set who have been randomized to a treatment regimen. Analysis will be performed according to the allocated treatment regimen regardless of the treatment regimen actually received.	Preference to present disposition table based on randomized subjects.	Nonsubstantial
10.1.6 (Dissemination of Clinical Study Data)	Removed EudraCT from the list of public registries where results will be made available.	The Sponsor does not plan to post this study on EudraCT.	Nonsubstantial
Throughout	Minor editorial and document formatting revisions	Minor corrections and clarifications	Nonsubstantial

Amendment 2 (24 November 2021)

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1.5 (Overall Design) and Section 4.1 (Overall Design)	The following sentence was deleted: Following blinded review, safety data for individual subjects may be unblinded as described below.	The Dose Escalation Committee will remain blinded to the treatment assignments of individual subjects throughout the study. In accordance with Section 6.3.2, the treatment assignments of individual subjects will only be unblinded to a sponsor representative for regulatory reporting purposes or if warranted by emerging safety or tolerability issues.	Substantial
Section 1.1.4 (Objectives and Endpoints), Section 3.3.2 (Exploratory Endpoints), and Section 9.4.4 (Exploratory Endpoints Analyses)	The 3 exploratory endpoints related to bladder function were modified to specify the time period as a *24-hour duration* during the Clinic Period: • Change from baseline in urinary frequency during the Clinic Period *for a 24-hour duration* • Change from baseline in urinary urgency during the Clinic Period for *a 24-hour duration* • Change from baseline in nocturia during the Clinic Period for a 24-hour duration An additional exploratory endpoint was added: *Change from baseline in pain or burning in bladder or pelvic area during the Clinic Period for a 24-hour duration* To reflect these changes, the statistical analysis sections related to bladder function were updated.	This change adds additional specificity to the exploratory endpoint description and allows for the evaluation of bladder pain and burning in addition to function.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.3 (Schedule of Activities/Table 1 [Footnote a])	The following sentence was added: *The early termination assessments will be the same as those conducted at Discharge (Day 2).*	Clarification was made to specify which assessments would be performed in the event of a subject's early termination.	Substantial
Section 1.3 (Schedule of Activities/Table 1 [Footnote c]) and Section 10.2 (Appendix 2: Clinical Laboratory Tests/Table 2)	In Table 1, the "urine drug and alcohol screen" was renamed "drug and alcohol screen". A new table footnote (Footnote c) was added: *The alcohol screen at Check-in (Day -1) will be via a breath test, but all other drug and alcohol screens will be via urine tests.* In Table 2, the following text was added: *Note: The alcohol screen at Check-in (Day -1) will be via a breath test, but all other drug and alcohol screens will be via urine tests.*	This change was made due to operational feasibility.	Substantial
Section 1.3 (Schedule of Activities/Table 1 [Footnote h], Section 4.1.1.1 (Screening Period), Section 4.2 (Scientific Rationale for Study Design), and Section 8.6 (Pharmacodynamics)	1. The bladder and urination portion of the Bladder and Bowel Movement Diary (BBMD) will not be completed on Days -8 through -1. This bladder and urination portion of the BBMD will be collected on Day 1 (predose) and Day 2 with a 24 -hour recall period at each timepoint. 2. In the BBMD, the specific questions related to bladder function were updated.	This change was made to better characterize any potential changes in bladder function and/or potential urinary symptoms following dosing and reduce subject burden.	Substantial
Section 5.1 (Inclusion Criteria)	The following inclusion criterion was added: *Subject is compliant with colorectal cancer screening guidelines according to the ACG Clinical Guidelines: Colorectal Cancer Screening 2021.*	This change was made to ensure that subjects are in compliance with current colorectal cancer screening guidelines and to avoid potential confounding factors.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 7.1.1 (Study Suspension or Termination)	The following sentence was deleted: *Treatment assignments for the affected subjects will be unblinded to a designated sponsor representative not otherwise involved in the study, to evaluate whether or not to continue dosing in that cohort or to proceed with any further dose escalation in the remaining cohorts.*	In accordance with Section 6.3.2, the treatment assignments of individual subjects will only be unblinded to a sponsor representative for regulatory reporting purposes. The Dose Escalation Committee will remain blinded to the treatment assignments of individual subjects throughout the study.	Substantial
Section 7.1.1 (Study Suspension or Termination)	The following phrase in ~~strikethrough~~ was removed: *The sponsor may permanently terminate the study, or a component of the study, at any time. Reasons for termination may include, but are not limited to:* • *Death, SAE, or other safety finding considered to be related to IW-3300 that may preclude further dosing~~., in the opinion of the sponsor~~*	The rules for permanently terminating the study were modified. The determination to terminate the study will be based on the predefined stopping rules and evaluation of relatedness and balance risk benefit by the dose escalation committee.	Substantial
Section 6.3.2 (Blinding)	The last sentence in the second paragraph was deleted: *Following blinded review, safety data for individual subjects may be unblinded as described in Section 7.1.*	In accordance with the change in Section 1.1.5/Overall Design, Section 4.1, and Section 7.1.1, this sentence is no longer relevant.	Substantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 6.1.1 (Dosing Instructions) and Section 1.3 (Schedule of Activities/Table 1 [Footnote l])	The following information was added: Following study drug administration, clinical research unit (CRU) staff will monitor subjects for leakage of the study drug from the rectum during the initial 30 minutes post administration. CRU staff will record (as yes/no) if there was study drug leakage from the rectum in the 30 minutes following drug administration.	Leakage following dose administration will be monitored and recorded in order to more fully characterize study drug exposure.	Substantial
Section 9.3 (Populations for Analyses)	The Pharmacokinetic (PK) and Pharmacodynamic (PD) Analysis Set definitions were changed. The PK and PD analyses will be performed according to the treatment *actually received regardless of the allocated treatment, rather than according to the allocated treatment regimen regardless of the treatment regimen actually received.*	These changes correct errors in the definitions.	Substantial
Section 1.1.5 (Overall Design), Section 4.1 (Overall Design), Section 6.3.2 (Blinding), and Section 10.1.5 (Committee Structure)	The text in ~~strikethrough~~ was deleted form the description of the Dose Escalation membership: *The Dose Escalation Committee will include sponsor and contract research organization (CRO) representatives ~~(including the sponsor medical monitor, sponsor clinical operations lead, sponsor safety physician, the principal investigator, and others as necessary)~~.*	This change allows for additional flexibility in staffing the committee.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 1.1.3 (Rationale) and Section 2.2 (Background)	The text in underline was added: *This is supported by the 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 (Study MCP-103-204), and in subjects with irritable bowel syndrome with diarrhea (Study MCP-103-205).*	Two additional studies were referenced to provide additional context and to support the potential lower likelihood of diarrhea with colonic release formulations of GC-C agonists.	Nonsubstantial
Section 2.3.1 (Risk Assessment)	Under the risk mitigation for "Potential for diarrhea and secondary dehydration diarrhea", the CTCAE criteria for Grade 2 and Grade 3 diarrhea were corrected.	The change corrects an error in the description.	Nonsubstantial
Section 7.1.1 (Study Suspension and Termination)	The following text in italic was added: *The Dose Escalation Committee should consider the benefit-risk ratio in their determination to suspend dosing temporarily or permanently.* If *the benefit-risk is considered unacceptable and the effect is* considered related to active study drug by the Dose Escalation Committee, dosing at a given dose level will be stopped, and no additional cohorts will be dosed at the same or higher dose level (a lower dose could still be studied).	This addition further describes the intention of the Dose Escalation Committee review.	Nonsubstantial
Section 9.3 (Populations for Analyses)	The following sentence was deleted: *If any subjects are found to have incomplete data, a decision will be made on a case-by-case basis as to their inclusion in the analysis, but they will be presented in the listings.*	The specific rules for handling incomplete data will be described in the Statistical Analysis Plan that will be finalized before database lock.	Nonsubstantial

Section # and Name	Description of Change	Brief Rationale	Substantial/ Nonsubstantial
Section 10.7 (Protocol Amendment History)	The contents of Amendment # 1 were moved to this new section showing the protocol amendment history.	This section was created as per the document template.	Nonsubstantial
Throughout	Minor editorial and document formatting revisions	Minor corrections and clarifications were made.	Nonsubstantial

11. REFERENCES

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

Approval	[redacted] 14-Jan-2022 19:33:37 GMT+0000

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