TITLE PAGE

Protocol Title:	A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered, Multiple-ascending Doses of IW-3300 in Healthy Subjects
Short Title:	A Phase 1 Study of Multiple-ascending Doses of IW-3300 in Healthy Subjects
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Medical Monitor **Study Contact:**	Medical monitor and study contact information will be provided separately.

Confidentiality Statement

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title

A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered, Multiple-ascending Doses of IW-3300 in Healthy Subjects

Short Title

A Phase 1 Study of Multiple-ascending Doses of IW-3300 in Healthy Subjects

Rationale

The aim of this study is to assess the effects of IW-3300 administered rectally, once-daily for 7 days in healthy subjects. 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. Preclinical models provide preliminary evidence which suggests that 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. This decreases 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) Because of these analgesic effects, IW-3300 is being developed as a rectally-administered therapeutic for the treatment of interstitial cystitis/bladder pain syndrome (IC/BPS), a chronic and debilitating condition for which there are no safe, effective treatment options. Data from this current study will be used to enable a Phase 2 safety, tolerability, and efficacy study of IW-3300 in subjects with IC/BPS. More information on the pharmacology and safety of IW-3300 is provided in Section 2.

Objectives and Endpoints

Objective	Endpoints
Primary	
To assess the safety and tolerability of 7-day dosing with multiple-ascending doses of IW-3300 administered rectally via enema in healthy subjects	Incidence of treatment-emergent adverse events (TEAEs) Incidence of treatment-emergent serious adverse events (TESAEs)
Exploratory	
To summarize the pharmacokinetics (PK) of 7-day dosing with IW-3300 administered rectally via enema in healthy subjects	PK parameters (IW-3300 and metabolites in plasma)
To explore potential changes to the microbiome with 7-day dosing with IW-3300 administered rectally via enema in healthy subjects	Change in 16s RNA based analysis of gut microbiome composition at the genus level from baseline to 7 days post IW-3300 administration

Overall Design

This is a Phase 1, single-center, randomized, double-blind, placebo-controlled, multiple-ascending-dose study of IW-3300 administered rectally as a low-volume enema in healthy adult subjects. This study will assess the effect of IW-3300 on safety, tolerability, and PK, and also explore the potential effect of IW-3300 on gut microbiome composition.

Subjects in 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).

A Dose Escalation Committee will conduct blinded reviews of all the safety/tolerability parameters of the IW-3300 100-µg dosing cohort through Discharge (Day 8) in order to make a decision regarding dose escalation to the next IW-3300 dosing cohort. The Dose Escalation Committee will include sponsor and contract research organization (CRO) representatives.

Disclosure Statement

This is a multiple-ascending-dose safety and tolerability study with 4 possible treatments (placebo, 2 dose levels of IW-3300, and an optional 3rd dose level of IW-3300) that is subject- and investigator-blinded.

Number of Subjects

A total of up to 27 subjects will be randomized in the study (2 cohorts of 9 subjects each, and an optional 3rd cohort of 9 additional subjects). Within each cohort, 6 subjects will be randomized to IW-3300 and 3 subjects will be randomized to placebo.

Intervention Groups and Duration

The study will evaluate multiple-ascending doses of IW-3300 in a double-blind manner. The 9 subjects within each cohort will be randomized to receive IW-3300 (6 subjects) or placebo (3 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; Table 1] for additional details regarding dosing instructions) once daily for 7 days. The planned cohorts are:

- Cohort 1: IW-3300 100 µg or matching placebo rectal dose once daily
- Cohort 2: IW-3300 300 µg or matching placebo rectal dose once daily
- Cohort 3 (optional): IW-3300 >100 µg but <300 µg or matching placebo rectal dose once daily (decision to enroll this cohort will be determined after safety reviews of previous cohorts, in the case of intolerability at 300 µg but tolerability at 100 µg)

Dosing in Cohort 2 and optional Cohort 3 will only proceed following a review of safety data from the previous 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 7 days; subjects will be followed in the Phase 1 clinical research unit (CRU) for the duration of dosing, until at least 24 hours after the last dose of study drug and

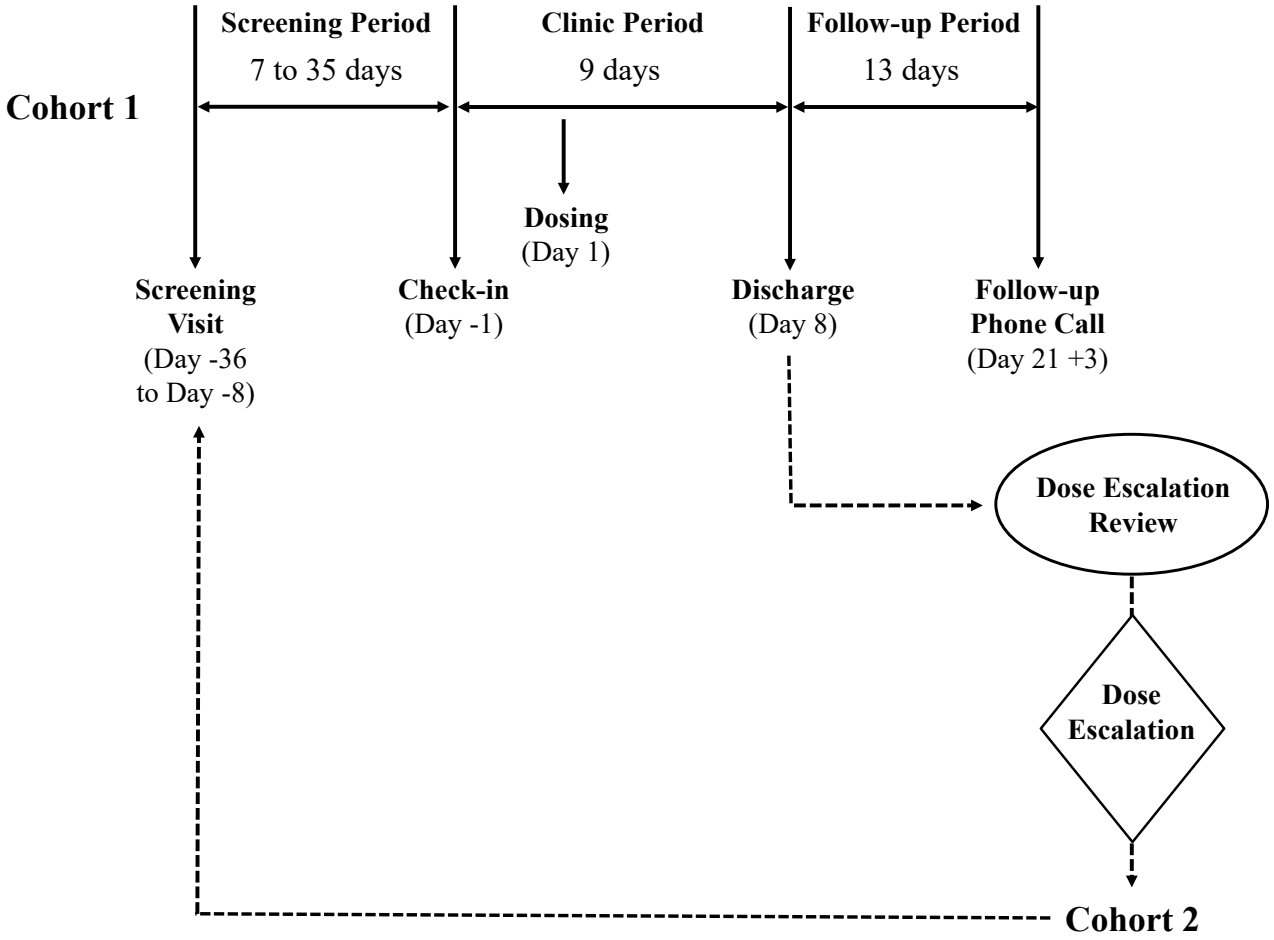
contacted by phone for follow-up approximately 2 weeks after the last dose. Total subject participation will be 29 to 57 days, including the Screening, Clinic, and Follow-up Periods.

Data Monitoring Committee: No

The study will utilize a Dose Escalation Committee to review safety data for Cohort 1 in order to make decisions about dose escalation to Cohort 2. An optional cohort, Cohort 3, may be enrolled based on a review of safety data from Cohorts 1 and 2.

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 -36 to Day -8**	**Check-in Day -1**	**Dosing Day 1**	**Days 2 through 6**	**Day 7**	**Discharge Day 8[a]/ ET**	**Follow-up Phone Call Day 21 (+3 days)**
Informed consent		X						
Eligibility criteria		X	X					
Medical history		X						
Demographics		X						
Body weight & height[b]		X		Pre			X	
Urine drug & alcohol screen		X	X					
Physical examination[c]		X	X	Pre	Pre	Pre	X	
Rectal examination[d]		X	X	Pre	Pre	Pre	X	
Vital signs[e]		X	X	Post: 0.5, 2, 4, and 8h (±10m)	Days 3 through 6 Pre: 0 (≤25m)	Post: 0.5, 2, 4, and 8h (±10m)		
Orthostatic blood pressure and pulse[f]				Pre: 0 (≤25m) Post: 1 and 6h (±20m)	Day 2 only Pre: 0 (≤25m)	Pre: 0 (≤25m) Post: 1 and 6h (±20m)	24h post-Day 7 dose (±20m)	
Triplicate 12-lead ECG[g]		X	X	Pre: 1h (±30m) Post: 2h (±30m)	Pre: 1h (±30m) Post: 2h (±30m)	Pre: 1h (±30m) Post: 2h (±30m)		

Study Period→		**Screening Period**	**Clinic Period**					**Follow-up Period**
Study Procedure↓	**Visit/Day→**	**Screening Visit Day -36 to Day -8**	**Check-in Day -1**	**Dosing Day 1**	**Days 2 through 6**	**Day 7**	**Discharge Day 8[a]/ ET**	**Follow-up Phone Call Day 21 (+3 days)**
Clinical chemistry, hematology, urinalysis		X	X	Pre: 0 (≤15m, ≤30m for UA) Post: 6h (±30m)	Day 4 only Pre: 0 (≤15m, ≤30m for UA)		X	
ESR and CRP blood draw[h]			X				X	
FSH		X						
HCG[i]		X	X					
SARS-CoV-2 testing		X	X					
HIV & hepatitis panel		X						
BBMD training		X						
BBMD dispensed		X	X					
Daily BBMD completion[j]		Day -8 through Day -2	X	X	X	X	X	
BBMD collection and review[k]			X				X	
Concomitant medications		X	X	X	X	X	X	X
AE monitoring		X	X	X	X	X	X	X
Subject confinement to clinic[l]			X	X	X	X	X	
Start of predose fast[m]				Pre: ≥6h	Pre: ≥6h	Pre: ≥6h		
Randomization				X				

Study Period→		Screening Period	Clinic Period					Follow-up Period
Study Procedure↓	**Visit/Day→**	**Screening Visit Day -36 to Day -8**	**Check-in Day -1**	**Dosing Day 1**	**Days 2 through 6**	**Day 7**	**Discharge Day 8[a]/ ET**	**Follow-up Phone Call Day 21 (+3 days)**
Study drug administration[n]				X	X	X		
PK blood draws[o]				Pre: 0 (≤15m) Post: 0.5 (±2m), 1, 2, 4, 6, and 8h (±20m)	Pre: 0 (≤15m)	Pre: 0 (≤15m) Post: 0.5 (±2m), 1, 2, 4, 6, and 8h (±20m)	24h post-Day 7 dose (±20m)	
Stool collection[p]			Check-in to Pre: 0h			1 sample collected between Day 7 (postdose) and Day 8 (predischarge)		
Hemoccult testing[q]			X			X		
Follow-up phone call[r]								X

[a] Subjects will be discharged on Day 8 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 ET Visit will be performed prior to discharge from the CRU. Every effort should be made and documented to ensure that safety procedures scheduled for Discharge are performed at the ET visit.

AE=adverse event; BBMD=bladder and bowel movement diary; CRP=C-reactive protein; CRU=clinical research unit; ECG=electrocardiogram; ESR=erythrocyte sedimentation rate; ET=Early Termination; FSH=follicle-stimulating hormone; HCG: human chorionic gonadotropin; HIV=human immunodeficiency virus; PK=pharmacokinetic; Post=postdose; Pre=predose; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; UA=urinalysis

[b] Height will only be measured at the Screening Visit. On Day 1 (predose) and Discharge (Day 8), subjects will be weighed in the morning upon awakening and before they ingest any water or food.

[c] A complete physical examination will be conducted except on Days 1 through 7 when the physical examination will be symptom-directed and conducted prior to study drug administration.

[d] Rectal examination at all timepoints should include a digital rectal examination and visual inspection of the perianal area for redness or irritation. If significant redness, rectal pain, fever, bleeding, or mucus is observed, anoscopy may be performed as part of the rectal examination.

[e] Vital signs will include oral temperature, blood pressure, and pulse rate. In a semisupine position, blood pressure and pulse will be obtained at the 0.5-hour postdose timepoint on Days 1 and 7 (consistent with the position required following dosing). Blood pressure and pulse at the 2-, 4-, and 8-hour postdose timepoints on Days 1 and 7, as well as predose on Days 3 through 6, should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones). When applicable, vital sign measurements will be obtained before blood draws.

[f] 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 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 before blood draws.

[g] 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.

[h] Elevated inflammatory markers (ESR, CRP), and/or relevant symptoms (eg, rectal pain, hematochezia) may be suggestive of colonic inflammation; subjects with these signs or symptoms should undergo recto-sigmoidoscopy. If this is negative, a colonoscopy may be performed after a discussion between the investigator and the Sponsor.

[i] HCG at Screening will be a blood-based test. HCG at Check-in/Day -1 will be a urine-based test.

[j] 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 additional BM pages via email, if needed. Bladder information in the BBMD will be collected predose on each dosing day, with a 24-hour recall period at each timepoint.

[k] 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.

[l] Subjects will remain at the CRU from Check-in (Day -1) through Discharge (Day 8).

[m] Subjects will fast for at least 6 hours prior to dosing on Days 1 through 7 and for at least 1 hour following dosing. During these times, water is permitted.

[n] Study drug (IW-3300 or placebo low-volume enema) will be administered rectally once daily, after a fast of at least 6 hours; water is permitted. 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. Study drug will be dispensed in capped syringes with a separately packaged colon tip applicator. The CRU dosing staff will remove the cap from the syringe, attach the colon tip applicator, prime the syringe and applicator to 20 mL, and slowly administer by inserting the contents of the syringe into the rectum. After the full dose has been administered, 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).

[o] 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.

[p] At Check-in/Day -1, the subject will bring in a stool sample using the sample collection kit issued by the site. Between Day 7 (postdose) and Day 8 (predischarge), the first stool passed will be collected. If no stool is passed during this time, the subject will be discharged with a stool collection kit and asked to return the sample to the CRU.

[q] The predose hemoccult testing may be performed using a stool sample obtained any time from Check-in (Day -1) to predose. If the subject does not produce a stool sample, stool that is present on the Day 1 (predose) digital rectal examination may be used. The Discharge (Day 8) hemoccult testing may be performed using a stool sample obtained any time from postdose on Day 7 to Discharge (Day 8). If the subject does not produce a stool sample, stool that is present on the Discharge (Day 8) digital rectal examination may be used.

[r] 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 interstitial cystitis/bladder pain syndrome (IC/BPS).

2.1. Study Rationale

This clinical study is the second clinical study designed as a multiple-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 the effects of IW-3300 to the afferent nerve fibers in the colonic wall to reduce visceral pain in the pelvic region while minimizing the risk of diarrhea. Dosing for rectal administration will be initiated at 100 µg and will not go higher than 300 µg, which is approximately 80-fold lower than the human-equivalent dose [HED] based on body weight conversion of the lowest no adverse effect level (NOAEL) (24,000 µg) identified in nonclinical toxicology studies.

2.2. Background

IW-3300 is being developed for the treatment of bladder pain associated with 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. Preclinical models provide preliminary evidence which suggests that 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. This decreases 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 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, or 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 (DR) formulation of another GC-C agonist, linaclotide, directly into the distal colon in healthy subjects and in patients with irritable bowel syndrome with constipation (IBS-C) or irritable bowel syndrome with diarrhea (IBS-D). In Study MCP-103-105, multiple doses of linaclotide DR (at doses up to 3000 µg) were given once daily to healthy subjects and no diarrhea was reported. In Study MCP-103-205, linaclotide DR doses of 300, 600, or 1200 µg were administered to subjects with IBS-D. The rate of diarrhea TEAEs was low (1.0% to 2.1% in the linaclotide DR dose groups compared with 0% in the placebo group). No severe diarrhea TEAEs were reported.

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.

IW-3300 is currently under investigation in a Phase 1 single-ascending-dose study in healthy subjects, Study C3300-101. To date, no safety issues have been noted.

Additionally, 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

Available 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 8) 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 bowel movement (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 [Bristol Stool Form Scale {BSFS} score of 7] resulting in a limiting of self-care activities) or dehydration (hospitalization indicated); or ≥3 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 an early phase study with IW-3300 and, as such, limited clinical data are available.	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 8) 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 ≥3 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 (20 ml) 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 C3300-101, a single-ascending dose clinical study testing IW-3300 doses up to 2500 µg in healthy subjects is currently ongoing.
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 access to medical evaluations/assessments associated with study procedures (eg, physical examinations, ECGs, laboratory evaluations, SARS-CoV-2 [severe acute respiratory syndrome coronavirus 2; COVID-19] diagnostic testing).

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 to patients with IC/BPS.

3. OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

To assess the safety and tolerability of 7-day dosing with multiple-ascending doses of IW-3300 administered rectally via enema in healthy subjects

3.1.2. Exploratory Objectives

The exploratory objectives are:

- To summarize the PK of 7-day dosing with IW-3300 administered rectally via enema in healthy subjects
- To explore potential changes to the microbiome with 7-day dosing with IW-3300 administered rectally via enema in healthy subjects

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

The exploratory endpoints are:

- PK parameters (IW-3300 and metabolites in plasma)
- Change in 16s RNA based analysis of gut microbiome composition at the genus level from baseline to 7 days post IW-3300 administration

4. STUDY DESIGN

4.1. Overall Design

This is a Phase 1, single-center, randomized, double-blind, placebo-controlled, multiple-ascending-dose study of IW-3300 administered rectally, once-daily, for 7 days as a low-volume enema in healthy adult subjects. This study will assess the effect of IW-3300 on safety, tolerability, and PK, and also explore the potential effect of IW-3300 on gut microbiome composition.

Subjects in each dosing 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). Treatment duration will be 7 days; subjects will be followed in the Phase 1 clinical research unit (CRU) for the duration of dosing, until at least 24 hours after the last dose of study drug and contacted by phone for follow-up approximately 14 days after the last dose. Total subject participation will be 29 to 57 days, including the Screening, Clinic, and Follow-up Periods.

The study will evaluate multiple-ascending doses of IW-3300 in a double-blind manner. The 9 subjects within each cohort will be randomized to receive IW-3300 (6 subjects) or placebo (3 subjects), administered rectally, once-daily for 7 days (as a low-volume [20 mL] enema) following a fast of at least 6 hours (refer to the SoA [Table 1] for additional details regarding dosing instructions). The planned cohorts are:

- Cohort 1: IW-3300 100 µg or matching placebo rectal dose once daily
- Cohort 2: IW-3300 300 µg or matching placebo rectal dose once daily
- Cohort 3 (optional): IW-3300 >100 µg but <300 µg or matching placebo rectal dose once daily (decision to enroll this cohort will be determined after safety reviews of previous cohorts, in the case of intolerability at 300 µg but tolerability at 100 µg)

A Dose Escalation Committee will conduct blinded reviews of all the safety/tolerability parameters of the IW-3300 100-µg dosing cohort through Discharge (Day 8) in order to make a decision regarding dose escalation to the next IW-3300 dosing cohort. The Dose Escalation Committee will include sponsor and contract research organization (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 -36 to Day -8) and will last 7 to 35 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 subjects will receive the BM portion of 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 bowel habits. Subjects will complete the BM portion of the BBMD 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 8 (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 2:1 ratio to receive IW-3300 or matching placebo after a fast of at least 6 hours.

The assessments conducted during the Clinic Period are shown in the SOA (Table 1). Daily safety assessments will be performed. Subjects will continue to enter their BM information into the BBMD on an event-driven basis. The questions related to bladder/urinary symptoms in the BBMD will be collected predose on each dosing day, with a 24-hour recall period at each timepoint to ascertain any undesirable effects on the bladder or potential urinary symptoms. Blood samples for PK assessments will be collected at specified times predose (blood) and postdose (blood and stool). Subjects will be confined to the CRU from Check-in (Day -1) through Discharge (Day 8), which will occur after completion of the assessments (at least 24 hours after the last 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 8 and will last until Day 21 (+3). On Day 21 (+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 effects of IW-3300 administered once daily for 7 days 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.

To ensure a thorough assessment of safety, subjects will be confined to the CRU for the duration of the Clinic Period, from Check-in (Day -1) through until Discharge (Day 8), 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 on Day 21 (+3), conducted 14 days after the last dose, for safety follow-up; at the discretion of the investigator, subjects may be requested to return to the CRU for their follow-up contact.

To evaluate any effects of IW-3300 on bowel and bladder function, subjects will complete the BM portion of the BBMD on 7 consecutive days during the Screening Period to establish a

baseline for bowel function without study drug. Similarly, subjects will provide answers to the bladder/urinary-symptom-related questions in the BBMD predose on Day 1, with a 24-hour recall, to establish a baseline without study drug.

Because this is the first multiple-dose study with IW-3300 in humans, cohorts will be enrolled sequentially, following a safety review of prior dosed cohort(s) by a Dose Escalation Committee (Section 6.6). In addition, stopping criteria have been established to ensure that dosing at the same or higher dose levels will stop should a safety signal be detected (Section 7.1.1). In this case, a lower dose of IW-3300 could be used.

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 effects of multiple 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 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 highest dose level evaluated in this study will be 300 µg, which is approximately 80-fold lower than the HED based on body weight conversion of the lowest NOAEL (24,000 µg) identified in nonclinical toxicology studies.

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:

Age

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

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 [eg, incontinence, overactive bladder, prostatitis, urinary tract infection]).
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 21.
5. If subject is ≥45 years of age, subject is compliant with colorectal cancer screening guidelines according to the American College of Gastroenterology (ACG) Clinical Guidelines: Colorectal Cancer Screening 2021. (8)

Weight

6. Subject's body mass index (BMI) is within the range 18.5 to 35.0 kg/m2 (inclusive) at the Screening Visit.

Sex

7. Male and female subjects
    a. Female subject is of nonchildbearing potential (ie, women who are postmenopausal [greater than 12 months without menses] or who have had a procedure such as a bilateral oophorectomy, bilateral salpingectomy, or hysterectomy).
    b. Subject is willing to abstain from sexual intercourse during the period of confinement in the CRU.

c. Male subject and female partner(s) are willing to use a 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 21.

Informed Consent

8. Subject is 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:

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, active proctitis, or presence or history of ulcerative colitis or Crohn's disease.

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 21.
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 8. (Note: Acetaminophen may be used at the investigator's discretion, in consultation with the sponsor, as per Section 6.5.)
7. Subject has used vitamins, dietary, or 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 8.
8. Subject has received a licensed vaccine during the 14 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 21).
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 any surgery involving the

GI tract or anal canal (with the exception of endoscopic procedures, appendectomy, and cholecystectomy).

Prior/Concurrent Clinical Study Experience

11. Subject has received any investigational drug or vaccine 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 (up to the Follow-up Phone Call on Day 21).

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 person with COVID-19 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.

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 21.
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 as described in Section 6. During this time, water is permitted.

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 on Day 21 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, 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 sign collection during the Clinic Period, positional and activity restrictions at each timepoint are noted in the SoA (Table 1) and Section 8.2.2.

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) or placebo, intended to be administered to a study subject according to the study protocol.

The study will evaluate multiple-ascending doses of IW-3300 in a double-blind manner. The 9 subjects within each cohort will be randomized to receive multiple doses of IW-3300 (6 subjects) or placebo (3 subjects), administered rectally (as a low-volume [20 mL] enema) following a fast of at least 6 hours. The planned cohorts are:

- Cohort 1: IW-3300 100 μg or matching placebo rectal dose once daily
- Cohort 2: IW-3300 300 μg or matching placebo rectal dose once daily
- Cohort 3 (optional): IW-3300 >100 μg but <300 μg or matching placebo rectal dose once daily (decision to enroll this cohort will be determined after safety reviews of previous cohorts, in the case of intolerability at 300 μg but tolerability at 100 μg)

Dosing in Cohort 2 and optional Cohort 3 will only proceed following a review of safety data from the previous 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.

Treatment duration will be 7 days; subjects will be followed in the Phase 1 CRU for the duration of dosing, until at least 24 hours after the last dose of study drug and contacted by phone for follow-up approximately 2 weeks after the last dose. Total subject participation will be 29 to 57 days, including the Screening, Clinic, and Follow-up Periods.

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, and (optional) <300 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=noninvestigational 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 through Day 7. Study drug will be administered rectally after a fast of at least 6 hours (water is permitted). 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. Study drug will be dispensed in capped syringes with a separately packaged colon tip applicator. The CRU dosing staff will remove the cap from the syringe, attach the colon tip applicator, prime the syringe and applicator to 20 mL, and slowly administer by inserting the contents of the syringe into the rectum. After the full dose has been administered, 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 postadministration. 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 may be compounded on the day of administration and stored at room temperature prior to dosing (continuous mixing not required). If the investigational drug product is stored for more than 24 hours, it must be stored refrigerated (2 C to 8°C) for no longer than the product shelf life. Refrigerated drug product must be equilibrated to room temperature for at least 30 minutes prior to dosing. Compounding and storage of the drug product 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 27 subjects (up to 3 cohorts of 9 subjects each) will be randomized in a 2:1 ratio to receive IW-3300 (N=6) or placebo (N=3), for each cohort, as detailed in Section 6.1. 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. The randomized treatment allocation for each subject will be contained in a sealed, opaque envelope opened at the time of the subject's randomization.

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 8) in order to make decisions regarding subsequent dosing.

Specific designated personnel in the Ironwood Global Patient Safety Group 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.

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 in the next paragraph) 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 21; 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 8; and licensed vaccines during the 14 days before Check-in (Day -1) until after completion of the Follow-up Phone Call on Day 21.

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 subjects

6.6. Dose Modification

The decision to proceed to the 300-µg dose level of IW-3300 (Cohort 2) will be made by the Dose Escalation Committee, based on blinded safety and tolerability data obtained through Discharge on Day 8 in all subjects at the 100-µg dose level (Cohort 1). The decision to proceed with an optional 3rd cohort will be made by the Dose Escalation Committee based on blinded safety and tolerability data for both Cohorts 1 and 2. The dose will be IW-3300 >100 µg but <300 µg.

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

- A confirmed pregnancy
- A SAE considered by the investigator or Sponsor to be related to study drug

Dosing of study drug for an individual subject may be suspended temporarily as detailed in Section 7.1.1; if the subject is unable to resume dosing, they should follow the instructions for permanent discontinuation below.

If study drug is permanently discontinued, then the subject will have the safety evaluations scheduled for Discharge (Day 8) performed. 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 study drug 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 may be temporarily suspended for either dose level if any of the following occur within a cohort:

- ≥1 subject with an SAE considered related to study drug
- ≥1 subject with an National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-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)
- ≥3 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 ratio is considered unacceptable and the effect 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 ET visit should be conducted, as shown in the SoA (Table 1). See the SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.
- 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 (Table 1), is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

8.1. Efficacy Assessments

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

8.2. Safety Assessments

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

8.2.1. Physical Examinations

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

- A complete physical examination will include, at a minimum, assessments of the general appearance of the subject and the HEENT (head, eyes, ears, nose, and throat), cardiac, respiratory, gastrointestinal, musculoskeletal, neurological, and dermatological systems.
- Rectal examination at all timepoints should include a digital rectal examination and visual inspection of the perianal area for redness or irritation. If significant redness, rectal pain, fever, bleeding, or mucus is observed, anoscopy may be performed as part of the rectal examination.
- On Days 1 through 7, the physical examination will be symptom-directed and conducted prior to study drug administration.
- Height and weight will also be measured and recorded at the timepoints noted in the SoA (Table 1).
- 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).

- Vital signs will include oral temperature, blood pressure, and pulse rate.
- 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.
- In a semisupine position, blood pressure and pulse will be obtained at the 0.5-hour postdose timepoint on Days 1 and 7 (consistent with the position required following dosing).
- Blood pressure and pulse at the 2-, 4-, and 8-hour postdose timepoints on Days 1 and 7, as well as predose on Days 3 through 6, 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, Day 1 predose and 1- and 6-hours postdose, Day 2 predose, Day 7 predose and 1- and 6-hours postdose, and Day 8 [24-hours post-Day 7 dose]), orthostatic blood pressure and pulse measurements will be obtained. Subjects 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 signs measurements will be obtained 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 of 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 ECGs 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 eCRF. 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 last 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.

8.2.5. Bladder and Bowel Movement Diary

The BBMD will be completed in accordance with the instructions in the SoA (Table 1).

Subjects will enter BM-related information into a paper diary, the BBMD, on an event-driven basis (ie, following each BM). The BM-related information will include the day and time of BMs and a report of stool consistency for each BM using the BSFS (8); 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)

8.3. 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 on Day 21 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 subjects.

8.3.8. Adverse Events of Special Interest

Not applicable to this study

8.4. Treatment of Overdose

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

The sponsor does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator should:

1. Report any case of overdose to the sponsor and use their clinical judgment to treat the case of overdose as needed with the appropriate general supportive measures. 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 for the determination of IW-3300 and metabolites in plasma 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 if a sufficient number of plasma samples exist above lower limit of quantification:

- 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 ($t_{1/2}$)

Blood samples will be analyzed in a single batch after all subjects have completed the study. No individual drug concentration information will be shared until the study has been unblinded.

8.6. Microbiome

Gut microbiome will be assessed using stool samples collected in accordance with the SoA (Table 1). At Check-in/Day -1, the subject will bring in a stool sample using the sample collection kit issued by the site. Between Day 7 (postdose) through Day 8 (predischarge), the first stool passed will also be collected. If no stool is passed during this time, the subject will be discharged with a stool collection kit and asked to return the sample to the CRU.

The stool collection kit is a tube that contains DNA stabilizer. An aliquot of stool (approximately 1 teaspoonful) will be added to the tube and frozen for analysis.

8.7. Pharmacodynamics

Pharmacodynamics are not evaluated in this study.

8.8. Genetics

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

8.9. Biomarkers

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

8.10. Immunogenicity Assessments

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

8.11. 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 (9 subjects) is considered sufficient for evaluation of safety, tolerability, PK, and microbiome.

9.3. Populations for Analyses

The following populations are defined:

Population	Description
Screened Set	The Screened Set consists of all subjects who have signed informed consent.
Intent-to-treat Set	The Intent-to-treat Set consists of all subjects in the Screened 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.
Microbiome Analysis Set	The Microbiome Analysis Set consists of all subjects in the Safety Analysis Set who have at least 1 postdose microbiome assessment. 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 post last dose will be summarized by system organ class, preferred term, and treatment (or cohort).

9.4.3. Secondary Endpoint Analysis

Not applicable to this study

9.4.4. Exploratory Endpoints Analyses

PK analyses will be based on the PK Analysis Set.

Plasma concentrations of IW-3300 and metabolites will be summarized by treatment (or cohort) at each assessment timepoint; additionally, a listing of plasma concentrations will be provided. PK parameters (listed in Section 8.5) will also be calculated for each subject and summarized by treatment, if systemic levels of IW-3300 and/or metabolites 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 $t_{1/2}$). Otherwise, the levels of IW-3300 and/or metabolites will not be included for the analysis.

The analysis of the microbiome at the genus level will be based on the Microbiome Analysis Set.

The relative abundance of different phyla, class, order, family, and genera will be constructed and summarized by treatment (or cohort) at baseline and following 7 days of study drug administration using descriptive statistics. Changes from baseline will be calculated.

9.4.5. Safety Analyses

All safety analyses, including the BBMD analyses, will be based 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. 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, inflammatory marker, and urinalysis parameter
- Vital signs (ie, pulse rate, systolic and diastolic blood pressure, oral temperature, and body weight)

- ECG parameters (ie, heart rate and measures of PR, QRS, QT, and QTcF intervals)
  - Additionally, the overall 12-lead ECG interpretation (normal, abnormal not clinically significant, or abnormal clinically significant) will be summarized.

For the BBMD parameters, descriptive statistics at each assessment timepoint will be presented by treatment (or cohort) for each study period (Screening Period and Clinic Period): BM frequency, stool consistency, fecal incontinence, urinary frequency, urinary urgency, nocturia, and bladder/pelvic pain.

BM-related baseline 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. Bladder-related baseline values will be derived from the BBMD data collected predose on Day 1 (with a 24-hour recall). The Clinic Period BM- and bladder-related 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 8 (Discharge).

9.4.6. Other Analysis/Analyses

Not applicable to this study

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 SoA (Table 1).

- The laboratory tests are detailed in Table 2.
- 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 the Discharge (Day 8)/ET Visit. The decision to repeat a laboratory test would be based on the investigator's clinical judgment.

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		
Inflammatory Markers	• ESR • CRP			
Hemoccult	Point-of-care hemoccult test			
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)			
Other Screening Tests	• Drug and alcohol screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids, and benzodiazepines) • FSH • HCG (blood-based test at Screening and urine-based test at Check-in/Day -1) • SARS-CoV-2 testing (Screening and Check-in/Day -1) • 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 is measured, which may indicate severe liver injury (possible Hy's Law case), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).

Investigators must document their review of each laboratory safety report.

Laboratory/analyte results that could unblind the study will not be reported to 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 one of the following categories, according to the 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.

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

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

10.3.3.3. Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult IB and/or Product Information, for marketed products, in 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 21.

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 ICF from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

10.4.2.1.2. Female Subjects Who Become Pregnant

- The investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. The 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
CRO	contract research organization
CRP	c-reactive protein
CRU	clinical research unit
CV%	coefficient of variation
DMC	data monitoring committee
DR	delayed release
eCRF	electronic case report form
EDC	electronic data capture
ESR	erythrocyte sedimentation rate
ET	early termination
FSH	follicle-stimulating hormone
GC-C	guanylate cyclase C

Abbreviation	Definition
GI	gastrointestinal
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HCG	human chorionic gonadotropin
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
IBS-C	irritable bowel syndrome with constipation
IBS-D	irritable bowel syndrome with diarrhea
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
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MRP	multidrug resistance protein
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIMP	noninvestigational medical product
NOAEL	no-observed-adverse-effect level
PK	pharmacokinetic(s)
PKGII	protein kinase G type II
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan

Abbreviation	Definition
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SID	subject identification
SoA	schedule of activities
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	terminal elimination half-life
TBD	to be determined
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
T_{max}	time to maximum plasma concentration
UA	urinalysis
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, Multiple-ascending Doses of IW-3300 in Healthy Subjects
Short Title:	A Phase 1 Study of Multiple-ascending Doses of IW-3300 in Healthy Subjects
Protocol Number:	C3300-102
Version Number:	Version 1
Final Date:	14 February 2022

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

{Electronic signature page appended}

[redacted]
Head of Clinical Development and Medical Scientific Affairs
Ironwood Pharmaceuticals, Inc.

Date

10.6.2. Investigator Signature

Protocol Title:	A Phase 1 Placebo-controlled Study of the Safety and Tolerability of Rectally Administered, Multiple-ascending Doses of IW-3300 in Healthy Subjects
Short Title:	A Phase 1 Study of Multiple-ascending Doses of IW-3300 in Healthy Subjects
Protocol Number:	C3300-102
Version Number:	Version 1
Final Date:	14 February 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:

_____ _____

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

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