Clinical Trial Protocol: C1701-201-P-06

Final Version, 03 October 2017

Study Title:	A Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group, Single-dose, Phase 2a Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IW-1701 in Patients with Achalasia
Study Number:	C1701-201
Study Phase:	2a
Product Name:	IW-1701 Tablets, 1 mg
Indication:	Achalasia
Sponsor:	Ironwood Pharmaceuticals, Inc.
Sponsor Contact:	[redacted]
Medical Monitor:	[redacted]

	Date
Original Protocol:	13 September 2016
Amendment #1	18 October 2016
Amendment #2	28 November 2016
Amendment #3	01 May 2017
Amendment #4	30 June 2017
Amendment #5	03 October 2017

STUDY IDENTIFICATION

A summary of key study participants is provided in Table 1. All study contact details will be provided prior to the Site Initiation Visit.

Table 1. Key Study Participants

Role	Contact Information
Medical Monitor:	[redacted]
Clinical Laboratory:	[redacted]
Contract Research Organization (CRO) Contact:	[redacted]
CRO Medical Monitor	[redacted]

Table 1. Key Study Participants

Role	Contact Information
Ironwood Contact:	[redacted]
Safety Officer:	
Dedicated SAE Facsimile and Email:	

SYNOPSIS

Sponsor

Ironwood Pharmaceuticals, Inc.

Name of Finished Product

IW-1701 Tablets, 1 mg

Name of Active Ingredient

IW-1701

Study Title

A Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group, Single-dose, Phase 2a Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IW-1701 in Patients with Achalasia

Study Number

C1701-201

Study Phase: 2a

Objectives

In patients with primary Type I or II achalasia, following a single 5-mg oral dose of IW-1701,

- To assess safety and tolerability
- To determine the effects on measures of esophageal function by high-resolution impedance manometry (HRIM)
- To determine the pharmacokinetic (PK) parameters, C_{max}, T_{max}, AUC_{last}.

Study Design

This multicenter, randomized, double-blind, placebo-controlled, parallel-group, single-dose study will randomize up to 20 patients to receive study drug (15 patients to IW-1701 and 5 to matching placebo).

Study Population

The study will randomize patients diagnosed with primary Type I or II achalasia with an integrated relaxation pressure (IRP) > 15 mm Hg by baseline HRIM. Up to 20 patients are planned.

See Eligibility Criteria for full inclusion and exclusion criteria.

Test Product, Dose, and Mode of Administration

Test product (IW-1701 Tablets, 1 mg) will be 1 mg oral tablets; the dose will be 5 mg IW-1701 (5 tablets) administered orally.

Reference Therapy, Dosage, and Mode of Administration

Placebo will match the IW-1701 oral tablets. The number of placebo tablets administered will match the number of tablets used for the IW-1701 treatment arm.

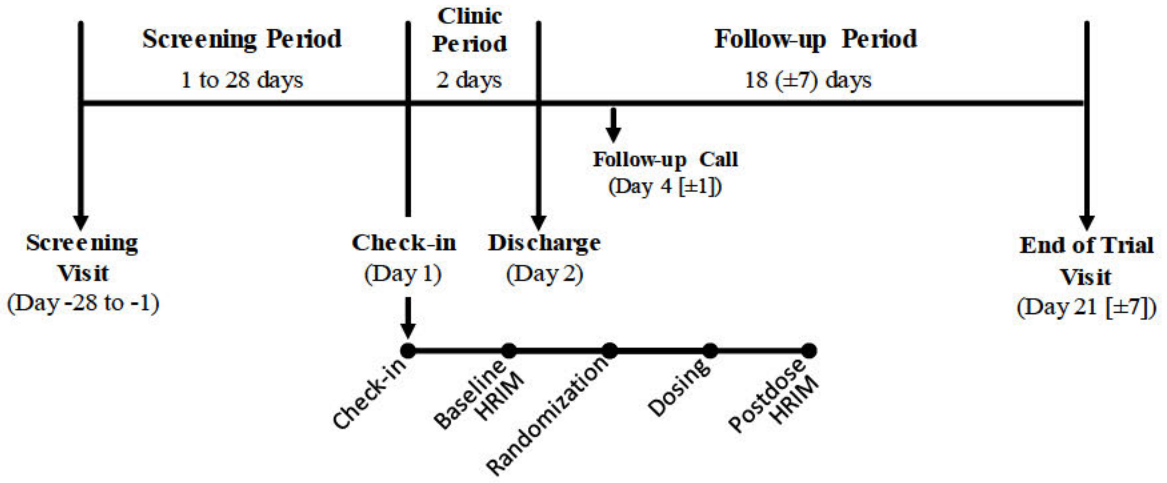
Study Periods

***Screening Period*:** The Screening Period will begin with the signature of the informed consent form (ICF) at the Screening Visit (which may occur from Day -28 to Day -1) and will last 1 to 28 days (see Study Schematic). At the Screening Visit, the patient will undergo screening procedures (see Schedule of Events) to determine their preliminary eligibility for the study (see Inclusion/Exclusion Criteria). Patients will begin a liquid diet on Day -1 and then will fast overnight. The Screening Period will end at Check-in on the morning of Day 1.

Clinic Period: The Clinic Period will begin at Check-in on the morning of Day 1 and will end at Discharge from the clinic on Day 2. To confirm eligibility (see Eligibility Criteria), patients will undergo a baseline protocol-specific HRIM procedure (see HRIM Methods). After the procedure, the HRIM catheter will be removed and patients will complete a baseline symptom assessment. Patients who meet all eligibility criteria in addition to having confirmed Type I or II achalasia and IRP > 15 mm Hg will be randomized to receive a single 5-mg dose of IW-1701 or matching placebo (see Study Drug Administration). Following study drug administration, the HRIM catheter will be reinserted for the postdose HRIM procedure following the protocol-specific swallowing protocol. The HRIM catheter will be removed after the postdose recording, and patients will complete a postdose symptom assessment. Safety, PK, and pharmacodynamic (PD) assessments, including blood collections, will be performed at prespecified times (see Schedule of Events). Following all study assessments, and at the Investigator's discretion, patients may be discharged on Day 2 at least 30 hours after study drug administration if they meet discharge criteria.

Follow-up Period: The Follow-up Period will begin immediately after the patient is discharged from the clinic. Patients will have a Follow-up Call on Day 4 (± 1 day) to review adverse events (AEs). The Follow-up Period will end after the End of Trial Visit on Day 21 (± 7 days).

Study Schematic



Study Drug Administration

On the morning of Day 1, after an overnight fast of ≥ 8 hours and after the baseline HRIM procedure, randomized patients will receive 5 mg IW-1701 or placebo in a tablet formulation. Study drug will be administered with ~ 240 mL (8 fluid ounces) of water; patients should be encouraged to consume all ~ 240 mL of water. (*Note*: Patients are allowed to take multiple tablets together, and additional water is allowed to complete dosing.) Fluids will not be allowed for at least 1 hour before study drug administration, and except for the water given during HRIM study procedures, fluids will not be allowed from at least 1 hour before HRIM catheter insertion until after catheter removal. Solid food will not be allowed from Day -1 until at least 4 hours postdose. See Exclusion Criteria for prohibited concomitant medications. Oral medications not prohibited by the protocol may be taken on Day 1; administration should be timed to avoid interfering with study procedures.

Stopping Criteria

Dosing of additional patients will be suspended if any of the following occur:

- Drug-related SAEs in 2 or more patients (per causality and SAE definitions in this protocol)
- Drug-related AE in 1 or more patients that is life threatening or requires urgent intervention ‖
- An overall pattern of clinically significant treatment-emergent AEs that may appear minor in terms of an individual event but collectively represents a safety concern
- Treatment-emergent AEs judged to be drug-related and reported per the description and in the number of patients indicated in the table below. These events are based on clinical experience with IW-1701 and the prescribing information for riociguat.

Treatment-emergent, Drug-related Event	Event Description	# of patients that trigger stopping
Symptomatic hypotension-related events (eg, syncope, presyncope)	Medical intervention or hospitalization indicated*	2
Vomiting	≥ 6 episodes (separated by at least 5 minutes) in 24 hours†	2
Spontaneous bleeding events ‡ (including hemoptysis, vaginal hemorrhage, ovarian hemorrhage, subdural hemorrhage, hematemesis, hematochezia)	Moderate symptoms, medical intervention indicated §	2
	Life-threatening consequences; urgent intervention indicated ‖	1

* Consistent with National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, Grade 3
† CTCAEv4 Grade 3
‡ Based on riociguat prescribing information
§ Consistent with CTCAEv4 Grade 2
‖ Consistent with CTCAEv4, Grade 4

If stopping criteria are met, the study may continue only after the study protocol has been amended, if appropriate, and approved by the IRB.

(*Note*: This is a single-dose study; therefore, there are no individual subject stopping rules.)

Discharge Criteria

Patients may be discharged on Day 2 at the Investigator's discretion and at least 30 hours after study drug administration if the following criteria are met:

- Systolic BP ≥ 90 mm Hg and diastolic BP ≥ 60 mm Hg
- No unresolved AEs that the Investigator judges to be drug related

Duration of Treatment

Patients will receive a single oral dose of IW-1701 and will be followed in the clinic for at least 30 hours after dosing. Total patient participation will be 15 to 56 days, including the Screening, Clinic, and Follow-up Periods.

Pharmacodynamic Assessments

HRIM

HRIM Methods: After calibration, the HRIM catheter will be inserted transnasally to record manometric data from the hypopharynx to the stomach. After insertion and ~ 2-minute baseline (relaxed) recordings to assess basal esophagogastric junction (EGJ) pressure, the following set of swallowing protocols will be recorded:

- 10 supine (at ~ 30-degree incline) ~ 5-mL saline swallows
- 5 upright ~ 5-mL saline swallows (after upright and relaxed for ~ 1 minute)
- (Optional) Standing ~ 200-mL saline bolus challenge (at both baseline and postdose)

This procedure will be performed 2 times, once at baseline and once after dosing. The baseline and postdose procedures should be identical. For the postdose recording, the swallowing protocol should begin at 3 hours (+15 minutes) postdose.

HRIM Measurements

HRIM measurements for PD analyses will be made by central, blinded reader(s).

- **Bolus Flow Time (BFT**) – time in seconds of bolus (~ 5 mL swallow) transit through the EGJ from the 10-swallow supine and 5-swallow upright protocols; median measurement from the 10-swallow supine and 5-swallow upright protocols
- **Integrated Relaxation Pressure (IRP)** – mean of the 4s of maximal deglutitive relaxation in the 10-s window beginning at upper esophageal sphincter relaxation. Contributing times can be contiguous or non-contiguous (eg, interrupted by diaphragmatic contraction). Referenced to gastric pressure; median measurement from the 10-swallow supine and 5-swallow upright protocols
- **Impedance bolus height (IBH)** – height in esophagus of ~ 200 mL saline bolus at ~ 1, ~ 2, and ~ 5 minutes postbolus from the standing ~ 200-mL bolus challenge *(if performed)*

Patient-reported Symptom Questions

The patient will complete a symptom assessment just after removal of the catheter at both the baseline and the postdose HRIM procedures.

Pharmacokinetic Assessments

If systemic levels of IW-1701 are detectable, the following PK parameters will be calculated, when appropriate:

- AUC_{last}: Area under the plasma concentration time curve from time zero to T_{last}, the time at which the last measurable plasma concentration (C_{last}) is observed
- C_{max}: Maximum observed plasma concentration, occurring at T_{max}
- T_{max}: Time of maximum observed plasma concentration

Additional Questionnaires

- Health status as assessed employing the EQ-5D-3L
- Health-related quality of life as assessed employing the SF-12v2
- Screening Patient-reported Symptom Questions

Safety Assessments

Adverse event recording, physical examination, clinical laboratory tests, vital sign parameters, and ECGs

Statistical Methods:

Sample Size Determination

For this Phase 2a study, a sample size of up to 20 patients is planned. The sample size of 20 was determined outside of statistical considerations and is considered reasonable based on precedent set by prior studies of similar nature and design.

Analysis Populations

All patients who receive study drug will be included in the Safety Population.

All patients who receive study drug and have at least 1 postdose PK parameter assessment will be included in the PK Population.

All patients who receive study drug and have at least 1 postdose PD assessment will be included in the PD Population.

Statistical Analysis

Descriptive statistics (n, mean, standard deviation, minimum, median, and maximum) will be calculated to summarize continuous variables. Frequency and percent of patients in each category will be calculated to summarize categorical variables.

Pharmacodynamic Analyses

Change from baseline will be calculated for continuous variables and summarized by treatment group.

Safety Analyses

Adverse events will be summarized by system organ class (SOC), preferred term (PT), and treatment group. Listings will be provided for pretreatment AEs, treatment-related AEs, severe AEs, drug-related AEs, serious AEs (SAEs), and AEs leading to study discontinuation. Descriptive statistics will be provided for the safety parameters.

ECGs, vital signs, and clinical laboratory tests will be summarized at each time point and listings will be provided for patients with abnormal values.

Pharmacokinetic Analyses

If plasma concentrations of IW-1701 are detected, the PK parameters will be determined and tabulated, and summary statistics will be reported.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

Patients must meet all of the following criteria to be eligible for enrollment in this study:

1. Patient has signed an ICF before any study-specific procedures are performed.
2. Patient is a male or female between 18 and 75 years old at the Screening Visit.
3. Female patient is not pregnant or breastfeeding at the time of the Screening Visit and must have a negative serum pregnancy test at the Screening Visit and a negative urine pregnancy test on Day 1 confirmed negative before Randomization.
4. Patient has a diagnosis of primary Type I or II achalasia per Investigator judgement, and median IRP > 15 mm Hg from supine 10-swallow protocol on baseline HRIM procedure as read by the Investigator.
5. Patient must have an endoscopy documented in their medical history that was performed within the 90 days before Day 1.
6. Patient is in generally good health, is in medically stable condition, and has no clinically significant findings on a physical examination, 12-lead ECG, and clinical laboratory tests (serum chemistry, hematology, coagulation, urine drug screen, and urinalysis) after signing the ICF but before receiving the dose of study drug. (*Note*: The Investigator will determine if a particular finding is clinically significant. In making this determination, the Investigator will consider whether the particular finding could prevent the patient from performing any of the protocol-specified assessments, could represent a condition that would exclude the patient from the study, could represent a safety concern if the patient participates in the study, or could confound the protocol-specified assessments).
7. Patient has seated systolic BP from 100 to 160 mm Hg and seated diastolic BP from 60 to 100 mm Hg at the Screening Visit. (*Note*: BPs are the averages of 3 measurements obtained with an appropriately sized cuff at 2-minute intervals after the patient has been sitting quietly for ≥ 5 minutes.)
8. Patient has platelet count, prothrombin time, International Normalized Ratio (INR), and activated partial thromboplastin time (aPTT) within laboratory normal ranges at the Screening Visit; if values are out of normal ranges, test may be repeated to determine eligibility.
9. Patient has no contraindications to the performance of the baseline and postdose HRIM procedures per Investigator discretion.
10. Female patient must be postmenopausal or surgically sterile (ie, bilateral oophorectomy, hysterectomy, or tubal ligation); must agree to completely abstain from heterosexual

intercourse; or, if heterosexually active, must agree to use 1 of the following methods of birth control from the date she signs the ICF until 90 days after the dose of study drug:

a. Combination of 2 highly effective birth control methods (eg, condom with spermicide plus intrauterine device, condom with spermicide plus a diaphragm or cervical cap, hormonal contraceptive [including progesterone implant] combined with a barrier method)
b. Maintenance of a monogamous relationship with a male partner who has been surgically sterilized by vasectomy (vasectomy procedure must have been conducted ≥ 60 days before the Screening Visit or confirmed via sperm analysis) plus a hormone or barrier method

11. Male patient must be surgically sterile by vasectomy (vasectomy procedure conducted ≥ 60 days before the Screening Visit or confirmed via sperm analysis), must agree to completely abstain from heterosexual intercourse, or, if heterosexually active, must agree to use a combination of 2 highly effective birth control methods (eg, condom with spermicide plus intrauterine device, condom with spermicide plus a diaphragm or cervical cap, hormonal contraceptive [including progesterone implant] combined with a barrier method) from date they sign the ICF until 90 days after the dose of study drug.
12. Patient is fluent and literate in English.

EXCLUSION CRITERIA

Patients who meet any of the following criteria will not be eligible to participate in the study:

1. Patient has any autoimmune disease, peptic esophagitis, or evidence of esophageal rings, per Investigator judgement.
2. Patient has malignant or premalignant esophageal lesions.
3. Patient has history of migraine requiring treatment in the last 6 months.
4. Patient has had any of the following:

- Any prior esophageal, periesophageal, or gastric surgery, or treatment with sclerosing agent
- More than 1 pneumatic dilation procedure to a diameter of > 2 cm
- Pneumatic dilation procedure to a diameter of > 2 cm within 1 year prior to randomization
- Prior esophageal injection of botulinum toxin (Botox) within 6 months prior to randomization

- More than 2 esophageal Botox injection procedures in his/her lifetime

Note: Prior bougie dilation(s) or pneumatic dilation(s) of ≤ 2 cm are allowed.

5. Patient has known megaesophagus (esophageal diameter > 5 cm), sigmoid esophagus, or esophageal diverticulum, per Investigator judgement.
6. Patient has history of platelet dysfunction, hemophilia, von Willebrand disease, coagulation disorder, or other bleeding diathesis condition(s), even if patient has normal complete blood count, prothrombin time, and aPTT.
7. Patient has used PDE5 inhibitors (including sildenafil and tadalafil), mono- or di-nitroglycerine products, riociguat, calcium-channel blockers, opiates, or aspirin in the 7 days before Check-in on Day 1. Patient has used bisphosphonates in the 3 months before Check-in on Day 1. These medications are not allowed until after the Follow-up Call. Patient has used CYP3A4 inhibitors or inducers in the 7 days before Check-in on Day 1. CYP3A4 inhibitors or inducers are not allowed until after the End of Treatment Visit.
8. Patient has taken any supplements for the treatment of erectile dysfunction during the 7 days before Check-in/Day 1. Supplements for the treatment of erectile dysfunction are not allowed until after the Follow-up Call.
9. Patient has a history of active alcoholism or drug addiction during the year before the Screening Visit or has a positive result for illicit drugs at the Screening Visit. Alcoholic beverages are not permitted from the Screening Visit until after the Follow-up Call (Day 4 ± 1 day).
10. Patient has taken any drug that can affect GI motility in the 72 hours before Check-in through Discharge from the clinic.
11. Patient has a 12-lead ECG demonstrating severe bradycardia (heart rate < 40 beats per minute) or average QTcF ≥ 450 msec for male patients or ≥ 470 msec for female patients at the Screening Visit. (*Note*: If on initial ECG, QTcF exceeds criteria, the ECG will be repeated 2 more times, and the average of the 3 QTcF values will be used to determine the patient's eligibility).
12. Patient has elevated (> 1.5 times the upper limit of normal as defined by the laboratory) levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), or creatinine at the Screening Visit.
13. Patient has history of cancer within the past 5 years, other than basal cell carcinoma.
14. Patient has active hepatitis B, hepatitis C, or history of human immunodeficiency virus infection.
15. Patient has hepatic impairment defined as Child-Pugh A, B, C.
16. Patient has known eating disorder (eg, anorexia nervosa, binge eating, bulimia).
17. Patient is chronic, heavy smoker (> 25 cigarettes/day).

18. Patient has a history of clinically significant hypersensitivity or allergies to any of the inactive ingredients contained in the active or placebo drug products.
19. Patient has previously received IW-1701 in a study, or received an investigational drug during the 30 days or 5 half-lives of that investigational drug (whichever is longer) before the Screening Visit, or is planning to receive another investigational drug at any time during the study.
20. Patient has undergone a surgery during the 30 days before Check-in except minor dental and dermatological procedures.
21. Female patient who may wish to become pregnant and/or plan to undergo egg donation or egg harvesting for current or future in vitro fertilization during the course of the study and for at least 30 days after the dose of study drug.
22. Male patient unwilling to refrain from sperm donation during the study and for at least 90 days after the dose of study drug.
23. Patient has an acute or chronic condition that, in the Investigator's opinion, would limit the patient's ability to complete or participate in this clinical study.
24. Patient is involved in the conduct and administration of this study as an Investigator, sub-Investigator, study coordinator, other study staff, or Sponsor member.

Note: Screening Visit assessments may take place over more than 1 day. Patients may be rescreened.

At the Investigator's discretion and after consultation with the Medical Monitor, during the Screening Period, laboratory values that are outside specified ranges may each be repeated once to confirm eligibility

SCHEDULE OF EVENTS

Study Period	**Screening Period**	**Clinic Period**		**Follow-up Period**	
Visit Days → Study Procedure ↓	**Screening Visit Day -28 to -1**	**Dosing Day 1**	**Discharge Day 2**	**Follow-up Call Day 4 (±1)**	**End of Trial Visit Day 21 (±7)**
Informed consent signed	X				
Inclusion/exclusion evaluation	X	X			
Demographics	X				
Medical history	X				
Drug screen (a)	X				
HBsAg, HCV, & HIV Screen	X				
Pregnancy test (b)	X	X			X
Weight (W) & height (H)	W, H	W			W
Physical exam	X				X
12-lead ECG (c)	X	predose pd: 4h (±30m)	pd: 24h (±30m)		X
Vital signs (oral temperature, respiratory rate, seated BP & pulse) (d)	X	pre: 0 (≤ 15m) *BP & pulse only:* pd: 0.5h (±10m), 1, 2, 3, 4, 6, 8, 12h (±15m)	pd: 24h (±30m) At discharge (30 h or later): *BP only*		X
Clinical chemistry, coagulation, hematology, urinalysis (e)	X		pd: 24h (±30m)		X
Adverse event evaluations	X	X	X	X	X
Prior & concomitant medications	X	X	X	X	X
EQ-5D-3L	X				X
SF-12v2	X				X
Screening patient-reported symptom questions	X				
Optional genotyping sample (f)			pd: 24h (±30m)		
PK/PD blood samples (g)		pre: 0 (≤ 15m) pd: 0.5h (±2m),1,2,3,4,5 6,8h (±5m),12h (±15m)	pd: 17h (±15m), 24h (±30m)		X

Study Period	Screening Period	Clinic Period		Follow-up Period	
Visit Days → Study Procedure ↓	**Screening Visit Day -28 to -1**	**Dosing Day 1**	**Discharge Day 2**	**Follow-up Call Day 4 (±1)**	**End of Trial Visit Day 21 (±7)**
Randomization		X			
Study drug administration (h)		X			
High resolution impedance manometry (HRIM) procedure (i)		predose pd: 3h (+15m)			
Patient-reported symptom questions		after pre HRIM (+15m) after pd HRIM (+15m)			
Discharge (j)			X		
Study completion					X

0=time of dose (ie, swallowing of initial tablet[s]); BP = blood pressure; ECG = electrocardiogram; h = hour; H = height; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; m = minute; msec = millisecond; pd = postdose; PK = pharmacokinetic; PD = pharmacodynamic; pre = predose; W = weight

a. Urine drug screen for selected illicit drugs
b. For female patients, a negative serum pregnancy test must be documented at the Screening and Follow-up Visits; a negative urine pregnancy test must be documented on Day 1 with results available before Randomization.
c. Patients must be supine for ≥ 5 m before the ECG recording (*Note*: If on initial ECG, QTcF is ≥ 450 msec for male patients or is ≥ 470 msec for female patients, the ECG will be repeated 2 more times, and the average of the 3 QTcF values will be used to determine the patient's eligibility). Before blood draws and vital signs where applicable. The predose ECG may be completed any time on Day 1 prior to dose administration.
d. Oral temperature (°C), respiratory rate, systolic and diastolic BP, and pulse after the patient has been sitting for ≥ 5 m. At the Screening Visit, BPs will be the average of 3 measurements obtained at 2-m intervals after the patient has been sitting quietly for ≥ 5 m; vital sign measurements will be taken before HRIM procedures and before blood draws where applicable.
e. Patients must fast for ≥ 8 h before sample collection in the morning. If the Screening Visit is Day -2 or -1, local labs results may be used to determine eligibility.
f. Optional: one 4-mL blood sample for genotyping
g. One ~ 6 mL blood sample at each timepoint for PK and exploratory PD (plasma renin activity) assessment, each sample divided into 2 equal plasma aliquots; samples taken before HRIM procedures when applicable; record exact time of blood draw
h. In the morning, after overnight fast of ≥ 8 h, after baseline HRIM procedure and randomization, study drug will be administered with ~ 240 mL (~ 8 fluid ounces) of water; patients should be encouraged to consume all ~ 240 mL of water. (*Note*: Patients are allowed to take multiple tablets together, and additional water is allowed to complete dosing.) Fluids will not be allowed for at least 1 h before dosing. Solid food will not be allowed from the morning of Day -1 until at least 4 hours postdose.
i. All recordings will include supine 10-swallow and upright 5-swallow protocols, and may include an optional 200 mL bolus challenge. Baseline and postdose procedures should be identical. The postdose swallowing protocol should begin at 3 h (+15 m) postdose. Fluids are not allowed for at least 1 h before catheter insertion until after catheter removal.
j. Patients may be discharged at the Investigator's discretion a minimum of 30 h postdose if discharge criteria are met.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
ADP	adenosine diphosphate
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC_{last}	area under the plasma concentration time curve from time zero to the last observation
BFT	bolus flow time
BP	blood pressure
BUN	blood urea nitrogen
CBC	complete blood count
cGMP	cyclic guanosine 3', 5'-monophosphate
C_{max}	maximum observed plasma concentration
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EGJ	esophagogastric junction
FDA	Food and Drug Administration
GCP	good clinical practice
GGT	gamma glutamyl transferase
GLP	good laboratory practice
HDPE	high-density polyethylene
HPF	high power field
HRIM	high-resolution impedance manometry
IBH	impedance bolus height
IC_{50}	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IRB	Institutional Review Board
IRP	integrated relaxation pressure

Abbreviation	Term
IWRS	interactive web response system
kg	kilogram
kg/m^2	kilograms/meters squared (body mass index)
LDH	lactate dehydrogenase
LES	lower esophageal sphincter
MAD	multiple ascending dose
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeters of mercury
MPV	mean platelet volume
msec	millisecond
nNOS	neuronal NO synthase
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
pd	postdose
PD	pharmacodynamic(s)
PDE5	phosphodiesterase 5
PEG	polyethylene glycol
PK	pharmacokinetic(s)
pre	predose
PT	preferred term
QTcF	QT interval corrected using Fridericia's formula
RBC	red blood cell
RDW	red blood cell distribution width
SAD	single ascending dose

Abbreviation	Term
SAE	serious adverse event
SAS®	Statistical Analysis System
sGC	soluble guanylate cyclase
SNP	single nucleotide polymorphisms
SOC	system organ class
T_{max}	time of maximum observed plasma concentration
TRAP	thrombin receptor activating peptide
UGT	uridine diphosphate–glucuronosyl transferase
WBC	white blood cell

1. INTRODUCTION

1.1 ACHALASIA

Achalasia is a primary motility disorder characterized by impaired esophageal peristalsis and insufficient relaxation of the lower esophageal sphincter (LES) during swallowing. Symptoms of the disorder include dysphagia, heartburn, chest pain, and regurgitation.(1) Patients may also aspirate undigested food leading to respiratory complications and symptoms including pneumonia, cough, hoarseness, and wheezing.(2) Patients with more severe symptoms may also have weight loss, esophageal dilation, or retention of food in the esophagus. The disease is chronic, and symptoms worsen over time. The condition substantially affects patients' quality-of-life, interfering with their attendance and productivity at work and limiting their lifestyle.(3)

1.2 THE NO-SGC-CGMP PATHWAY

Soluble guanylate cyclase (sGC) is an enzyme that catalyzes the formation of cyclic guanosine 3', 5'-monophosphate (cGMP) from guanosine 5'-triphosphate (GTP) in response to binding and activation by nitric oxide (NO).(4) The NO-sGC-cGMP signaling pathway plays an important role in several physiological processes including smooth muscle relaxation.(4) Reduced NO bioavailability and reduced responsiveness to endogenous NO have been implicated in the pathogenesis of many disease processes.(5,6) sGC stimulators are compounds that directly stimulate sGC and synergize with endogenous NO to increase cGMP production.(7)

1.3 RATIONALE FOR USE OF AN SGC STIMULATOR IN ACHALASIA

Esophageal peristalsis and LES tone are regulated by excitatory and inhibitory neurons, which release smooth muscle constrictors (such as acetylcholine) resulting in contraction and release neurotransmitters (such as NO) resulting in relaxation. In achalasia, reductions in NO signaling are believed to result in an imbalance between excitatory and inhibitory neurons during swallowing, leading to impaired LES relaxation.

Evidence from animal models as well as from achalasia patients supports the role of NO in this disorder. Mice lacking neuronal NO synthase (nNOS) show achalasia-like symptoms including LES hypertension with impaired relaxation.(8) Consistent with this animal model, some

achalasia patients have polymorphisms of genes encoding NO synthase (NOS).(9) Low nNOS activity has also been observed in biopsies of the muscularis externa of the esophagus from achalasia patients.(10) The reported benefit of treatment (off-label) with nitrate donors and phosophodiesterase 5 (PDE5) inhibitors provides further evidence supporting the potential of the NO-sGC-cGMP pathway in achalasia. Both nitrates, which increase NO concentration, and the PDE5 inhibitor sildenafil, which blocks the degradation of cGMP, have been shown to reduce LES pressure in achalasia patients.(11-13) Recent genetic evidence also supports the role of sGC in achalasia: 9 individuals with early onset achalasia from 3 unrelated consanguineous families were found to have loss-of-function mutations in a gene that encodes sGC.(14)

In total, these data strongly suggest that achalasia can be caused by a failure of the NO-sGC-cGMP signaling pathway to relax the LES. Thus, in achalasia patients, the augmentation of cGMP production by sGC stimulators in response to impaired NO signaling may ameliorate excessive pressure in the LES and potentially elsewhere in the esophageal body, and consequently may improve the symptoms of achalasia.

1.4 IW-1701

Ironwood Pharmaceuticals is developing IW-1701, an orally administered sGC stimulator, for the treatment of conditions associated with deficient NO-sGC-cGMP signaling, including achalasia.

1.4.1 Nonclinical

[redacted]

[redacted]

1.4.2 Clinical Studies

The safety, tolerability, pharmacokinetic (PK) profile, and pharmacodynamic (PD) effects of IW-1701 were evaluated in a placebo-controlled, Phase 1a, single-ascending-dose (SAD) study (ICP-1701-101)

1.4.3 IW-1701 Tablet Formulation

[redacted]

2. STUDY OBJECTIVES

In patients with primary Type I or II achalasia, following a single 5-mg oral dose of IW-1701,

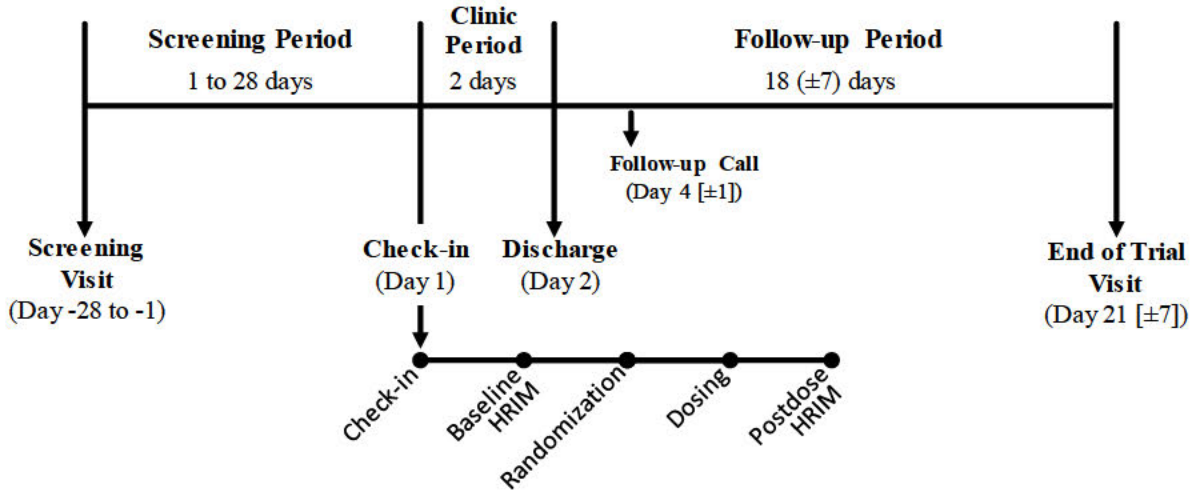
- To assess safety and tolerability
- To determine the effects on measures of esophageal function by high-resolution impedance manometry (HRIM)
- To determine the PK parameters, C_{max}, T_{max}, AUC_{last}

3. INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN

This is a multicenter, randomized, double-blind, placebo-controlled, parallel-group, single-dose study with a Screening Period, a Clinic Day, and a Follow-up Period (Figure 1). The study will enroll up to 20 patients diagnosed with primary Type I or II achalasia, with an integrated relaxation pressure (IRP) >15 mm Hg by baseline HRIM.

Figure 1. Study Schematic



Patients who are suspected or known to have primary Type I or II achalasia by the Investigator and who are interested in participating in a clinical trial will be scheduled for a Screening Visit.

Screening Period: The Screening Period will begin with the signature of the informed consent form (ICF) at the Screening Visit (which may occur from Day -28 to Day -1) and will last 1 to 28 days (Figure 1). At the Screening Visit, the patient will undergo screening procedures (see Schedule of Events) to determine their preliminary eligibility for the study (see Eligibility Criteria). Patients will begin a liquid diet on Day -1 and then will fast overnight. The Screening Period will end at Check-in on the morning of Day 1.

Clinic Day: The Clinic Day will begin at Check-in on the morning of Day 1 and will end at Discharge from the clinic. To confirm eligibility (see Eligibility Criteria), patients will undergo a baseline protocol-specific HRIM procedure (see Section 3.8.4.1). After the procedure, the HRIM

catheter will be removed, and patients will complete a baseline symptom assessment. Patients with confirmed Type I or II achalasia including an IRP > 15 mm Hg will be randomized to receive a single 5-mg dose of IW-1701 or matching placebo (see Section 3.5.1.1). Dosing of study drug should occur such that the patient may be observed in the clinic for at least 30 hours after dosing (see Section 3.5.4, Selection and Timing of Dose for Each Patient). Following study drug administration, the HRIM catheter will be reinserted for the postdose HRIM procedure following the protocol-specific swallowing protocol. The HRIM catheter will be removed after the postdose recording, and patients will complete a postdose symptom assessment. Safety, PK, and PD assessments, including blood collections, will be performed at prespecified times (see Schedule of Events). At the Investigator's discretion, patients may be discharged 30 hours after dosing if they meet discharge criteria (Section 3.7).

Follow-up Period: The Follow-up Period will begin immediately after the patient is discharged from the clinic. Patients will have a Follow-up Call on Day 4 (±1 day) to review AEs. The Follow-up Period will end after the End of Trial Visit on Day 21 (± 7 days).

3.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

A double-blind, randomized, placebo-controlled, parallel-group study design was chosen in order to provide comparable treatment groups. Patients, Investigators, Sponsor personnel involved in study conduct, and central reader(s) of the HRIM recordings are blinded to individual treatment assignment to minimize the chance of bias during selection and treatment of patients as well as during data collection and assessment. Randomization, use of placebo control, and blinding in this study are consistent with the concepts in International Conference on Harmonisation (ICH) E10, Choice of Control Groups and Related Issues in Clinical Trials (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2001). (See Section 3.5.5, Blinding.)

A 3:1 drug:placebo randomization ratio will be used in order to maximize exposure to study medication while allowing for a sufficient placebo control.

The parameters used to assess the efficacy of study medication will be the HRIM measurements. HRIM is the gold standard for the diagnosis and assessment of achalasia. Centralized analysis of

HRIM recordings will be performed in a blinded manner to maintain objectivity and provide a uniform standard for interpretation of results.

3.3 STUDY DURATION

Patients will receive a single oral dose of IW-1701 and will be followed in the clinic for at least 30 hours after dosing. Total patient participation will be 15 to 56 days, including the Screening, Clinic, and Follow-up Periods.

3.4 STUDY POPULATION SELECTION

3.4.1 Study Population

This study will be conducted in patients diagnosed with primary Type I or II achalasia with an IRP >15 mm Hg by baseline HRIM procedure. Up to 20 patients will be randomized in a 3:1 ratio, IW-1701 to placebo. The study will be conducted at up to 10 study centers in the United States.

Refer to Eligibility Criteria for full inclusion and exclusion criteria.

3.4.2 Removal of Patients from Therapy or Assessment

A premature discontinuation will occur when a patient who has signed the ICF and has been randomized ceases participation in the study, regardless of circumstances, before completion of the End of Trial Visit. A patient will be considered to have completed the study after completing the End of Trial Visit.

Patients will be informed that they are free to withdraw from the study at any time and for any reason. The Investigator may remove a patient from the study if, in the Investigator's opinion, it is not in the best interest of the patient to continue the study. Patients may also be discontinued from the study by the Investigator or the Sponsor at any time for any reason, including the following:

- Adverse event(s)
- Protocol violation, including lack of compliance

- Lost to follow-up (every effort must be made to contact the patient; a certified letter must be sent)
- Withdrawal of consent (attempts should be made to determine the reason for the patient withdrawing consent if possible)
- Study termination by the Sponsor
- Other reasons (eg, administrative reasons or pregnancy)

The Sponsor will be notified of any patient discontinuation after randomization. The date the patient is withdrawn from the study and the reason for discontinuation will be recorded on the study termination form of the electronic case report form (eCRF). Patients who discontinue from the study will be followed until resolution of all of their AEs or until the unresolved AEs are judged by the Investigator to have stabilized.

If a patient does not return for a scheduled visit, the study center should contact the patient. An effort must be made to contact the patient, including sending a certified letter. In every case, the patient outcome, including lost to follow-up information, will be documented.

3.5 STUDY TREATMENT(S)

3.5.1 Description of Treatment

3.5.1.1 Investigational Product

The investigational product, IW-1701 Tablets, consists of 1 mg of IW-1701 in a 200-mg oral tablet. The dose will be 5 mg IW-1701 (5 tablets) administered orally.

3.5.1.2 Placebo

Placebo will match IW-1701 Tablets in appearance. The number of placebo tablets administered will match the number of tablets used for the IW-1701 treatment arm.

3.5.1.3 Packaging and Labeling

IW-1701 Tablets and placebo to match will be supplied to the site in 60cc high-density polyethylene (HDPE) induction-sealed bottles, 35 tablets per bottle. Bottles will be supplied open label to the clinical site.

3.5.1.4 Storage and Accountability

IW-1701 Tablets and placebo to match will be shipped at ambient temperature.

IW-1701 Tablets and placebo to match must be stored under controlled room temperature conditions, 15 °C to 30 °C (59 °F to 86 °F) per the instructions in the study Pharmacy Manual. Any deviation from these storage conditions must be reported to Ironwood and use of the study drug suspended until authorization for its continued use has been provided by Ironwood.

The Investigator's duly trained designee must ensure that the receipt and use of all study drug supplied is recorded and must supervise the storage and allocation of these supplies. All study drug supplies must be retained in a locked room that may only be accessed by the pharmacist or other duly designated persons. Study drug must not be used outside the context of this protocol, and under no circumstances should the Investigator or study center personnel allow the supplies to be used other than as directed by this protocol without prior authorization from Ironwood.

3.5.2 Method of Assigning Patients to Treatment Groups

Patients who meet all of the inclusion criteria and none of the exclusion criteria will be randomized to treatment on Clinic Day (Day 1). Patients will be randomized through central randomization in a 3:1 ratio to receive either 5 mg IW-1701 or placebo.

The computer-generated randomization schedule will be prepared by an independent statistician not otherwise associated with the study.

Patients, Investigators, and central reader(s) of the HRIM recordings will be blinded to individual treatment assignments in this study.

3.5.3 Selection of Dosage in the Study

In this Phase 2a study, C1701-201, a single, oral dose of 5 mg IW-1701 Tablet will be studied. This dose was chosen based on final PD and safety data from ICP-1701-101, the Phase 1 SAD study in healthy subjects, and based on preliminary, blinded AE and Day 1 PK data from ICP-1701-102, the Phase 1 MAD study in healthy subjects.

[redacted]

3.5.4 Selection and Timing of Dose for Each Patient

Patients will be randomized to receive either 5 mg IW-1701 or placebo. On the morning of Day 1, after a liquid diet on Day -1 and an overnight fast of ≥ 8 hours, and after the baseline HRIM procedure, randomized patients will receive 5 mg IW-1701 or placebo in a tablet formulation. Study drug will be administered with approximately 240 mL (~ 8 fluid ounces) of water; patients should be encouraged to consume all ~ 240 mL of water. (*Note*: Patients are allowed to take multiple tablets together, and additional water is allowed to complete dosing.) Fluids will not be allowed for at least 1 hour before study drug administration, and except for the

water given during HRIM study procedures, fluids will not be allowed from at least 1 hour before HRIM catheter insertion until after catheter removal. Solid food will not be allowed from Day -1 until at least 4 hours postdose. Oral medications not prohibited by the protocol may be taken on Day 1; administration should be timed to avoid interfering with study procedures.

3.5.5 Blinding

This study is double-blind and placebo-controlled. The patients, Investigators, and site study staff, with the exception of specifically designated unblinded Study Center pharmacy staff, will be blinded to individual treatment assignments. Sponsor personnel involved in the conduct of the study (eg, study medical monitor, study statistician, study data manager, and clinical operations study personnel) will be blinded to individual patient treatment assignments; however, Sponsor personnel may be unblinded to data at the treatment-group level. In addition, the HRIM recordings will be interpreted by central, blinded reader(s) for PD analyses. Further, the investigational product and placebo will consist of identical oral tablets.

Unblinding of an individual patient's treatment assignment to an Investigator is restricted to emergency situations where knowledge of the treatment is necessary for the safety of the patient. Except in a medical emergency, the Investigator and blinded study center staff will remain blinded to patient treatment assignments during the conduct of the study and until, at a minimum, all discrepancies in the clinical database are resolved (ie, at the time of the database lock). Individual patient treatment assignment unblinding is available to the Investigator through the interactive web response system (IWRS) in the event of an emergency. The Investigator should make all reasonable efforts to notify and discuss the circumstances requiring unblinding with the Medical Monitor or designee in advance of breaking the blind. If the treatment blind is broken, the reason and the date should be recorded and signed by the Investigator and information regarding the unblinding should be submitted as soon as possible to the Sponsor. If the Investigator is unblinded to the treatment assignment of a patient, the patient will be immediately withdrawn from study drug dosing.

3.5.6 Concomitant Therapy

At the Screening Visit, the following information will be recorded for each patient:

- All medications the patient is taking (ongoing)
- All prior medications taken during the 30 days before the Screening Visit

Beginning at the Screening Visit, any medication taken by a patient during the course of the study (including medication for sedation/anesthesia, new medications added, or changes in medication previously reported) and the reason for use will be documented in the source documents and the eCRF.

3.5.7 Restrictions

3.5.7.1 Prohibited Medicines

- CYP3A4 inhibitors (including but not limited to itraconazole, ketoconazole, fluconazole, clarithromycin, telithromycin, erythromycin, nefazodone, suboxone, and protease inhibitors) are prohibited from 7 days before Check-in on Day 1 until after the End of Treatment Visit on Day 21 (± 7 days).
- CYP3A4 inducers (including but not limited to carbamazepine, enzalutamide, mitotane, phenytoin, phenobarbital, rifampin/rifampicin, rifabutin, rifapentine, and St. John's wort) are prohibited from 7 days before Check-in on Day 1 until after the End of Treatment Visit on Day 21 (± 7 days).
- PDE5 inhibitors (including sildenafil and tadalafil), mono- or di-nitroglycerine products, riociguat, calcium-channel blockers, opiates, and aspirin are prohibited from 7 days before Check-in on Day 1 through the Follow-up Call on Day 4 (± 1 day). Bisphosphonates are prohibited from 3 months before Check-in on Day 1 through the Follow-up Call on Day 4 (± 1 day).

3.5.7.2 Prohibited Supplements

Any supplements for the treatment of erectile dysfunction are prohibited from 7 days before Check-in on Day 1 through the Follow-up Call on Day 4 (± 1 day).

3.5.7.3 Fluid and Food Restrictions

Patients must fast overnight before sample collections for clinical chemistry, coagulation, hematology, and urinalysis assessments at the Screening and End of Trial Visits.

Patients must adhere to a liquid diet on Day -1 and then fast overnight until at least 4 hours after study drug administration. Fluids will not be allowed for at least 1 hour before study drug administration, and except for the water given during HRIM procedures, fluids will not be allowed from at least 1 hour before HRIM catheter insertion until after catheter removal.

Alcoholic beverages are prohibited from the Screening Visit until after the Follow-up Call (± 1 day).

3.5.7.4 Patient Activity Restrictions

Female patients of childbearing potential (ie, women who are not postmenopausal or who have not had a bilateral oophorectomy, hysterectomy, or tubal ligation) must agree to completely abstain from heterosexual intercourse or, if heterosexually active, must agree to use 1 of the following methods of birth control from the date they sign the ICF until 90 days after the dose of study drug:

a. Combination of 2 highly effective birth control methods (eg, condom with spermicide plus intrauterine device, condom with spermicide plus a diaphragm or cervical cap, hormonal contraceptive [including progesterone implant] combined with a barrier method)
b. Maintenance of a monogamous relationship with a male partner who has been surgically sterilized by vasectomy (vasectomy procedure must have been conducted ≥ 60 days before the Screening Visit or confirmed via sperm analysis) plus a hormone or barrier method

In addition, female patients may not donate or harvest eggs during the course of the study through at least 30 days after the dose of study drug.

Male patients who are not surgically sterile by vasectomy (vasectomy procedure conducted ≥ 60 days before the Screening Visit or confirmed via sperm analysis) must agree to completely abstain from heterosexual intercourse, or, if heterosexually active, must agree to use a combination of 2 highly effective birth control methods (eg, condom with spermicide plus intrauterine device, condom with spermicide plus a diaphragm or cervical cap, hormonal

contraceptive [including progesterone implant] combined with a barrier method) from date they sign the ICF until 90 days after the dose of study drug.

In addition, male patients may not donate sperm during the study through at least 90 days after the dose of study drug.

3.6 STOPPING CRITERIA

Dosing of additional patients will be suspended if any of the following occur:

- Drug-related SAEs in 2 or more patients (per causality and SAE definitions in this protocol)
- Drug-related AEs in 1 or more patients that are life threatening or require urgent intervention‖
- An overall pattern of clinically significant treatment-emergent AEs that may appear minor in terms of an individual event but collectively represents a safety concern
- Treatment-emergent AEs judged to be drug-related and reported per the description and in the number of patients indicated in Table 2. These events are based on clinical experience with IW-1701 and the prescribing information for riociguat.

Table 2. Adverse Event Stopping Criteria

Treatment-emergent, Drug-related Event	Event Description	# of patients that trigger stopping
Symptomatic hypotension-related events (eg, syncope, presyncope)	Medical intervention or hospitalization indicated*	2
Vomiting	≥ 6 episodes (separated by at least 5 minutes) in 24 hours†	2
Spontaneous bleeding events ‡ (including hemoptysis, vaginal hemorrhage, ovarian hemorrhage, subdural hemorrhage, hematemesis, hematochezia)	Moderate symptoms, medical intervention indicated §	2
	Life-threatening consequences; urgent intervention indicated ‖	1

* Consistent with National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, Grade 2 (15)
† CTCAEv4 Grade 3
‡ Based on riociguat prescribing information
§ Consistent with CTCAEv4 Grade 2
‖ Consistent with CTCAEv4, Grade 4

If stopping criteria are met, the study may continue only after the study protocol has been amended, if appropriate, and approved by the IRB.

(Note: This is a single-dose study; therefore, there are no individual subject stopping rules.)

3.7 DISCHARGE CRITERIA

Patients may be discharged at the Investigator's discretion a minimum of 30 hours after study drug administration if the following criteria are met:

- Systolic BP ≥ 90 mm Hg and diastolic BP ≥ 60 mm Hg
- No unresolved AEs that the Investigator judges to be drug related

3.8 STUDY PROCEDURES

3.8.1 Informed Consent

Informed consent procedures will comply with the Code of Federal Regulations (CFR) 21 CFR, Parts 50 and 312.

The written ICF must be approved by the Institutional Review Board (IRB) for the purposes of obtaining and documenting consent.

Before entry into the study, each patient will be provided with a written explanation of the study. It is the responsibility of the Investigator or appropriately trained health professional to give each patient full and adequate information regarding the objectives and procedures of the study and the possible risks involved. Patients will then be given the opportunity to ask questions and the Investigator will be available to answer questions as needed. Patients will be informed of their right to withdraw from the study at any time without prejudice. After this explanation and before entering the study, the patient will voluntarily sign an ICF. The patient should receive a copy of the signed and dated ICF. The Investigator must retain each patient's original signed ICF.

If new information becomes available that may be relevant to the patient's consent and willingness to participate in the study, the ICF will be revised. The revised ICF must be submitted to the IRB for review and approval prior to its use.

3.8.2 Medical History

A complete medical history will be recorded at the Screening Visit.

3.8.3 Questionnaires

3.8.3.1 EQ-5D-3L

The EuroQol (EQ)-5D-3L (Appendix 1) is a generic measure of health status widely used in Europe.(16) The first component consists of 5 questions assessing the following dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Responses to the 5 questions define a health state for which a utility index can be derived from published algorithms.(17) The second component of the EQ-5D is a visual analogue scale asking patients to rate their health from 0 to 100 (0 represents worst imaginable health state and 100 represents best imaginable health state). Patients will complete the EQ-5D-3L at the Screening and End of Trial Visits.

3.8.3.2 SF-12v2 Health Survey

The SF-12v2 (Appendix 2) is a widely used generic measure of health-related quality of life and measures 8 concepts of health: physical functioning, role limitations due to physical health problems, bodily pain, general health, vitality (energy/fatigue), social functioning, role limitations due to emotional problems, and mental health (psychological distress and psychological well-being). These 8 scales are aggregated into 2 summary measures: the physical component and mental component summary scores.(18) Patients will complete the SF-12v2 at the Screening and End of Trial Visits.

3.8.3.3 Screening Patient-reported Symptom Questions

Patients will answer the following Screening Patient-reported Symptom Questions at the Screening Visit; responses will be entered into the respective patient's eCRF.

[redacted]

ere

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

[redacted]

3.8.4 Pharmacodynamic Assessments

The assessments used to determine the pharmacodynamic parameters will include baseline and postdose HRIM recordings and patient-reported symptom questions.

3.8.4.1 High Resolution Impedance Manometry

On Clinic Day (Day 1), the HRIM procedure will be performed 2 times, once at baseline and once after study drug administration. Catheter insertion should be timed to minimize the total duration of catheter placement during the procedure; baseline and postdose procedures, including the timing of prerecording placement of the catheter and use of sedation/anesthesia, should be identical (± 10 minutes). For the postdose recording, the swallowing protocol should begin at 3 hours (+ 15 minutes) postdose. HRIM measurements for PD analyses will be made by central, blinded reader(s).

During HRIM procedures, patients may experience gagging, watery eyes, slight nosebleed, discomfort in nose and throat. Afterwards, patients may have mild side effects including sore throat, stuffy nose, and minor nosebleed. Rare severe complications may include irregular heartbeat, aspiration, and perforation of the esophagus.

HRIM procedure

- Calibrate the HRIM catheter per manufacturer's instructions.
- Administer sedation (optional per Investigator standard protocol).
- Anesthetize transnasally with lidocaine (2% jelly) and/or oropharyngeally with Ketocaine® per Investigator standard protocol.
- Insert catheter transnasally to record manometric data from the hypopharynx to the stomach.
- Recline patient into a supine position (at ~ 30-degree incline).
- Begin recording with ~ 2-minute resting measurement to assess basal esophagogastric junction (EGJ) pressure without swallowing.
- With patient supine, have patient swallow ~ 5 mL saline in a single swallow; repeat 9 times allowing ~ 30 seconds for each swallow. Mark each swallow and encourage patient not to swallow between sips; repeat any technically inadequate swallows.
- With patient upright, repeat the ~ 2-minute resting measurement without swallowing.
- With patient upright, have patient swallow ~5 mL saline in a single swallow; repeat 4 times allowing ~ 30 seconds for each swallow. Mark each swallow and encourage patient not to swallow between sips.
- (Optional) With patient standing, have patient drink a ~ 200-mL saline bolus as quickly as possible. Patient should be encouraged to drink the full bolus.
- Remove catheter and save the data.

3.8.4.2 Predose (Baseline) and Postdose Patient-reported Symptom Questions

Following removal of the HRIM catheter after both the baseline and postdose procedures, the patient will answer a set of questions related to their most recent swallowing protocols; the answers will be entered into the patient's eCRF.

[redacted]

[redacted]

3.8.4.3 Exploratory Biomarker

Blood samples for determination of plasma renin activity will be collected according to the Schedule of Events.

3.8.5 Safety Assessments

3.8.5.1 Physical Examination

A complete physical examination will be performed according to the Schedule of Events. The physical examination of each patient should include examination and assessment of the following:

General appearance	Head, eyes, ears, nose, and throat
Cardiovascular system	Neck
Respiratory system	Musculoskeletal system
Abdomen/liver/spleen	Nervous system
Lymph nodes	Skin
Neurologic status	Mental status

Breast, genitourinary, and rectal examinations are optional and may be performed at the discretion of the Investigator. Any new, clinically significant abnormal findings from the physical examination will be reported as an AE.

Each patient's weight will be recorded at each study visit; height will only be recorded at the Screening Visit.

3.8.5.2 Vital Signs

Vital signs will be measured according to the Schedule of Events and documented on the eCRF. Vital sign measurements include oral temperature (°C), respiratory rate, BP, and pulse. Respiratory rate, BP, and pulse measurements will be taken after the patient has been seated for at least 5 minutes. Where applicable, vital signs should be measured before blood draws.

3.8.5.3 Electrocardiograms

A 12-lead ECG will be performed according to the Schedule of Events and documented on the eCRF. Electrocardiograms should be obtained after the patient has been supine for at least 5 minutes. (*Note*: If on initial ECG, QTcF is ≥ 450 msec for male patients or is ≥ 470 msec for female patients, the ECG will be repeated 2 more times, and the average of the 3 QTcF values will be used to determine the patient's eligibility.)

3.8.5.4 Clinical Laboratory Tests

3.8.5.4.1 Laboratory Parameters

Blood and urine samples for clinical laboratory tests will be collected according to the laboratory study procedures at the days and times defined in the Schedule of Events. Patients must have fasted for at least 8 hours before sample collections.

The clinical laboratory evaluations will include the serum chemistry, hematology, coagulation, urinalysis and urine drug tests presented in Table 3.

Table 3. Clinical Laboratory Tests

Serum Chemistry	Hematology (CBC)	Complete Urinalysis
Albumin	Hematocrit	Color and appearance
Alkaline Phosphatase	Hemoglobin	pH and Specific Gravity
ALT	Platelet count	Bilirubin
AST	MPV	Glucose
Bicarbonate	RBC count	Ketones
BUN	WBC count	Leukocytes
Calcium	WBC differential	Nitrites
Chloride	(% & absolute):	Occult blood
Cholesterol	Basophils	Protein
Creatinine	Eosinophils	Urobilinogen
GGT	Lymphocytes	Microscopic
Glucose	Monocytes	Including bacteria, RBCs, WBCs
HDL-c	Neutrophils	per HPF if dipstick is abnormal
LDH	RBC indices	
LDL-c (calculated)	MCH	
Magnesium	MCHC	**Additional Urine Tests**
Phosphorus	MCV	Urine Cotinine
Potassium	RDW	
Sodium		
Total Bilirubin	**Coagulation**	
Total Protein	aPTT	
Triglycerides	Prothrombin time	
Uric acid	INR	

ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CBC = complete blood count; GGT = gamma glutamyl transferase; HPF = high power field; INR = International Normalized Ratio; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MPV = mean platelet volume; RBC = red blood cell; RDW = red blood cell distribution width; WBC = white blood cell.

For female patients, a negative serum pregnancy test must be documented at the Screening and Follow-up Visits; a negative urine pregnancy test must be documented on Day 1 with results available before Randomization. In the event of a positive pregnancy test, the test will be repeated. If pregnancy is confirmed, see Section 3.8.5.7.

Screens for a hepatitis panel (including HBsAg and anti-HCV) and HIV antibody will be performed at the Screening Visit.

A urine drug screen for the following illicit drugs will be performed at the Screening Visit:

Amphetamines	Cocaine	Opiates
Barbiturates		Phencyclidine (PCP)
Benzodiazepines	Marijuana	Propoxyphene

3.8.5.5 Adverse Events

All patients will be monitored for AEs throughout the study. All AEs will be recorded in accordance with the procedures outlined in this section.

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE, therefore, can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes, but is not limited to, the following:

- Any unfavorable changes in general condition
- Any clinically significant worsening of a preexisting condition
- Any intercurrent diseases and accidents

Note: A procedure is not an AE, but the reason for a procedure may be an AE.

3.8.5.5.1 Causality Assessment

For all AEs, the Investigator must provide an assessment of causal relationship to study drug. The causality assessment must be recorded in the patient's source documentation and on the AE page of the subject's eCRF. Causal relationship must be assessed according to the following:

Related: An event where there is a reasonable possibility of a causal relationship between the event and the study drug

Unrelated: Any other event

3.8.5.5.2 Severity Assessment

The Investigator will provide an assessment of the severity of each AE by recording a severity rating in the patient's source documentation and on the AE page of the patient's eCRF. *Severity,* which is a description of the intensity of manifestation of the AE, is distinct from *seriousness,* which implies a patient outcome or AE-required treatment measure associated with a threat to life or functionality. Severity will be assessed according to the following scale:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

3.8.5.6 Serious Adverse Events

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening: the patient was at immediate risk of death from the reaction as it occurred (ie, it does not include a reaction that hypothetically might have caused death if it had occurred in a more severe form)
- Hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity: a substantial disruption of a person's ability to conduct normal daily functions
- Congenital anomaly/birth defect
- Important medical events: events that may not result in death, be life threatening, or require hospitalization. Such an event may be considered serious when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency department or at home,

blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Emergency room visits that do not result in admission to the hospital should be evaluated for 1 of the other serious outcomes (eg, life-threatening, other serious [medically important] event).

3.8.5.7 Recording Adverse Events

Adverse events will be collected and recorded from the time the patient signs the ICF at the Screening Visit through the End of Trial Visit. All AEs, regardless of the assumption of a causal relationship with study procedures or study medication, must be recorded in the patient's source documentation and subsequently on the appropriate AE page of the patient's eCRF. This record includes AEs the patient reports spontaneously, those observed by the Investigator, and those elicited by the Investigator in response to open-ended questions during the study, such as "Have you had any health problems since your last visit?"

For every AE, the Investigator must:

- Provide an assessment of the severity, causal relationship to the study medication, and seriousness of the event
- Document all actions taken with regard to the study medication (ie, no action taken, treatment temporarily interrupted, or treatment discontinued)
- Detail any other treatment measures taken for the AE, including concomitant medications and/or procedures

Pretreatment AEs will be collected from the time the patient signs the ICF until the patient receives study drug. Pretreatment AEs will be captured in the patient's source documentation but will only be entered for randomized patients on the AE page of the patient's eCRF.

Laboratory abnormalities and changes in vital signs, physical examination findings, and 12-lead ECG parameters should be considered AEs and reported on the AE page of the patient's eCRF if the Investigator considers them clinically significant and/or they necessitate intervention.

Any medical condition that is present when a patient is screened and does not worsen in severity and/or frequency should be reported as Medical History and not as an AE. However, if the

condition does deteriorate in severity and/or frequency at any time during the study, it should be reported as an AE.

3.8.5.8 Reporting Serious Adverse Events

An AE that meets any of the serious criteria must be reported to Ironwood within 24 hours from the time that site personnel first learn of the event, using the SAE Report form provided for the study. Regardless of causality, all SAEs must be reported and will be collected and recorded from the time the subject signs ICF at the Screening Visit until the End of Trial Visit. All SAEs must also be recorded in the subject's source documentation and on the AE page of the subject's eCRF.

The initial report should include at least the following information:

- Patient identification number
- Description and onset of the event
- Serious criteria
- Causality assessment to study drug

Special Situation: Exposure to Study Drug during Pregnancy

In the event that a pregnancy occurs in a patient, study personnel must report the pregnancy as soon as possible (within 24 hours after notification) on the pregnancy notification form provided for this study. The study personnel must follow the pregnancy until the end and report the pregnancy outcome on the pregnancy outcome form provided for this study. If the pregnancy is associated with an SAE (eg, if the mother is hospitalized), a separate SAE form must be completed.

All relevant SAE or pregnancy information should be emailed to Ironwood Drug Safety and Pharmacovigilance.

All SAE Report Forms should be emailed to:

Clinical Drug Safety & Pharmacovigilance
Ironwood Pharmaceuticals, Inc.
[redacted]

If follow-up is obtained, or requested by Ironwood, the additional information should be emailed on an SAE Report Form to Ironwood, in a timely manner according to the procedures outlined above. Copies of discharge summaries, consultant reports, autopsy reports, and any other relevant documents may also be requested.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the subject's response to these measures should be recorded. All SAEs regardless of relationship to study drug will be followed by the Investigator until satisfactory resolution, until the Investigator deems the SAE to be chronic or stable, or until the subject is lost to follow-up. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

The Investigator will be responsible for reporting all SAEs to the IRB. Ironwood will be responsible for reporting to the regulatory authorities.

3.8.6 Pharmacokinetic Assessments

Blood samples for PK and exploratory PD assessments (Section 3.8.4.3) will be collected according to the Schedule of Events.

3.8.7 Genotyping

An optional single blood sample for genotyping of single nucleotide polymorphisms (SNPs) that may be related to interindividual differences in drug disposition or PD response (eg, uridine

diphosphate–glucuronosyl transferase [UGT] drug metabolizing enzymes) may be collected according to the Schedule of Events.

3.9 STUDY ACTIVITIES

3.9.1 Screening Period (Day -28 to Day -1)

3.9.1.1 Screening Visit (Days -28 to -1)

- Signing of ICF
- Review of inclusion and exclusion criteria
- Demographics
- Medical history
- Weight and height
- Physical examination
- 12-lead ECG
- Seated vital signs (oral temperature, respiratory rate, blood pressure and pulse)
- Prior medication recording (all medicines taken during the 30 days before Screening Visit)
- Collection of blood and urine samples for:
  - Clinical chemistry
  - Hematology
  - Coagulation
  - Urinalysis
  - Urine cotinine test
  - Hepatitis and HIV screen
  - Drug screen
  - Serum pregnancy test for all females (must be confirmed negative)
- AE evaluation
- EQ-5D-3L
- SF-12v2
- Screening patient-reported symptom questions

3.9.2 Clinic Period (Day 1 to Day 2)

3.9.2.1 Dosing (Day 1)

- Review of inclusion and exclusion criteria
- Prior and concomitant medication recording
- Weight
- Urine pregnancy test for all females (must be confirmed negative before Randomization)
- 12-lead ECG prior to dosing and at 4 hours (± 30 minutes) postdose
- Oral temperature and seated respiratory rate predose (≤ 15 minutes)
- Seated blood pressure and pulse predose (≤ 15 minutes) and at 0.5 hours (± 10 minutes), 1, 2, 3, 4, 6, 8, and 12 hours (± 15 minutes) postdose
- Collection of blood samples for:
  - PK and exploratory PD assessments predose (≤ 15 minutes) and at 0.5 hours (± 2 minutes), 1, 2, 3, 4, 5, 6, 8 hours (± 5 minutes) and 12 hours (± 15 minutes) postdose
- AE evaluations throughout day
- Baseline HRIM procedure
- Baseline patient-reported symptom questions
- Review of IRP eligibility criterion
- Randomization
- Study drug administration
- Postdose HRIM procedure (the swallowing protocol should begin at 3 hours [+ 15 minutes] postdose)
- Postdose patient-reported symptom questions

3.9.2.2 Discharge (Day 2)

- 12-lead ECG at 24 hours (± 30 minutes) postdose
- Seated vital signs (oral temperature, respiratory rate, blood pressure, and pulse) at 24 hours (± 15 minutes) postdose
- Collection of blood samples for:
  - Clinical chemistry at 24 hours (± 30 minutes) postdose

- Hematology at 24 hours (± 30 minutes) postdose
- Coagulation at 24 hours (± 30 minutes) postdose
- Urinalysis at 24 hours (± 30 minutes) postdose
- Genotyping at 24 hours (± 30 minutes) postdose (optional)
- PK and exploratory PD assessments at 17 (± 15 minutes) and 24 hours (± 30 minutes) postdose

- AE evaluations throughout day
- Concomitant medication recording
- Discharge at least 30 hours postdose, per Investigator discretion and discharge criteria (Section 3.7)
- BP prior to discharge (Section 3.7)

3.9.3 Follow-up Period (Day 2 at Discharge to Day 21 ± 7)

3.9.3.1 Follow-up Phone Call (Day 4 ± 1)

- Concomitant medication recording
- AE evaluation

3.9.3.2 End of Trial Visit (Day 21 ± 7)

- Weight
- EQ-5D-3L
- SF-12v2
- Physical examination
- 12-lead ECG
- Seated vital signs (oral temperature, respiratory rate, blood pressure, and pulse)

- Collection of blood and urine samples for:
  - Clinical chemistry
  - Hematology
  - Coagulation
  - Urinalysis
  - PK and exploratory PD assessments
  - Serum pregnancy test for all females
- Concomitant medication recording
- AE evaluation

3.10 STATISTICAL METHODS

Detailed statistical methods will be provided in the Statistical Analysis Plan (SAP), to be finalized prior to the clinical database lock.

3.10.1 Determination of Sample Size

For this Phase 2a study, a total sample size of up to 20 patients is planned (subjects will be randomized to either IW-1701 or placebo in a 3:1 ratio, resulting in approximately 15 patients receiving IW-1701 and approximately 5 patients receiving placebo). For this exploratory Phase 2a study, the sample size of up to 20 patients was determined outside of statistical considerations and is based on precedent set by prior studies of similar nature and design.(11,20-24)

3.10.2 Analysis Populations

Safety Population

All patients who receive study drug will be included in the Safety Population. Patients in the Safety Population will be evaluated for safety according to the treatment they actually received.

Pharmacokinetic Population

All patients who receive study drug and have at least 1 postdose PK parameter assessment will be included in the PK Population.

Pharmacodynamic Population

All patients who receive study drug and have at least 1 postdose PD assessment will be included in the PD Population.

3.10.3 Statistical Methods

3.10.3.1 General Considerations

Descriptive statistics (n, mean, standard deviation, minimum, maximum, median, and interquartile range [IQR]) will be calculated to summarize continuous variables. Frequency and percentage of patients in each category will be calculated to summarize categorical variables. Due to the exploratory nature of the trial, no adjustments will be made for multiplicity. Details of the data handling methods will be specified in the SAP. Inferential statistics will be used for descriptive purposes only. If not otherwise specified, the baseline value is defined as the last nonmissing value measured before administration of study drug on Day 1. All statistical analyses will be performed using SAS® Version 9.3 (or later) for Windows.

3.10.3.2 Patient Disposition, Demographics, and Baseline Characteristics

The overall number of patients screened and the number of patients randomized to each treatment group will be presented. The number and percentage of randomized patients included in the Safety Population as well as the PK and PD Populations will be presented by treatment group and overall. The distribution of randomized patients by completion status, as well as reason for discontinuation, will also be presented by treatment group and overall.

Demographic parameters (eg, age, race, ethnicity, sex, weight, and height) and other baseline characteristics will be summarized by treatment group for the PD Population.

This table will be repeated for the Safety Population if it is different from the PD Population.

3.10.3.3 Pharmacodynamic Analyses

All PD analyses will be conducted using the PD Population.

3.10.3.3.1 HRIM Measurements

HRIM measurements for PD analyses will be made by central, blinded reader(s).

- Bolus Flow Time (BFT) – time in seconds of bolus (5 mL swallow) transit through the EGJ from the 10-swallow supine and 5-swallow upright protocols; median measurement from the 10-swallow supine and 5-swallow upright protocols
- Integrated Relaxation Pressure (IRP) – mean of the 4s of maximal deglutitive relaxation in the 10-s window beginning at upper esophageal sphincter relaxation. Contributing times can be contiguous or non-contiguous (eg, interrupted by diaphragmatic contraction). Referenced to gastric pressure; median measurement from the 10-swallow supine and 5-swallow upright protocols
- Impedance bolus height (IBH) – height in esophagus of 200 mL saline bolus at 1, 2, and 5 minutes postbolus from the standing 200-mL bolus challenge

A single HRIM procedure consists of a set of swallowing protocols. For postdose recordings, the swallowing protocols begin at 3 hours (+ 15 minutes) postdose. The procedures at baseline and postdose should be identical.

The following PD parameters will be derived for each subject from the HRIM measurements:

- **Supine BFT_{3h}**: median of the measurements from the 10 supine swallows at baseline and at 3 hours postdose
- **Upright BFT_{3h}**: median of the measurements from the 5 upright swallows at baseline and at 3 hours postdose
- **Supine IRP_{3h}**: median of the measurements from the 10 supine swallows at baseline and at 3 hours postdose
- **Upright IRP_{3h}**: median of the measurements from the 5 upright swallows at baseline and at 3 hours postdose
- **IBH_{1m}**: height in esophagus of standing 200 mL saline bolus at 1-minute postbolus (if performed for the subject)
- **IBH_{2m}**: height in esophagus of standing 200 mL saline bolus at 2 minutes postbolus (if performed for the subject)
- **IBH_{5m}**: height in esophagus of standing 200 mL saline bolus at 5 minutes postbolus (if performed for the subject)

For both baseline and postdose HRIM procedures, the central reader will assess the HRIM recording and determine the presence (or absence) of achalasia and, if present, will define the achalasia type (I, II, III).(25)

Descriptive statistics will be presented by treatment group for each parameter at baseline and postdose. Mean and median change from baseline for each parameter will be presented by treatment group. Percent reduction (from baseline to postdose) in IRP parameters will also be calculated and summarized by treatment group.

3.10.3.3.2 Patient-reported Symptom Questions

Descriptive statistics will be presented by treatment group for each question at baseline and postdose.

3.10.3.4 Safety Analyses

All safety analyses will be conducted using the Safety Population.

3.10.3.4.1 Adverse Events

AEs will be coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA) available at the start of the study. Treatment-emergent AEs (TEAEs) are AEs that first occurred or worsened in severity after study drug administration. AEs occurring more than 72 hours after administration of study drug will not be considered treatment emergent. Incidence (number and percentage) of TEAEs will be presented by system organ class (SOC), preferred term (PT), and treatment group. Listings, including verbatim terms, will be provided for TEAEs, drug-related AEs, SAEs, and AEs leading to study discontinuation.

3.10.3.4.2 Vital signs, ECGs, and Clinical Laboratory Tests

ECGs, vital signs, and clinical laboratory tests at each assessment time point and the change from baseline at each postbaseline time point will be summarized by treatment group. Listings will be provided for patients with abnormal values.

3.10.3.5 Pharmacokinetic Analyses

If systemic levels of IW-1701 are detectable, the following PK parameters will be calculated, when appropriate:

- AUC_{last}: Area under the plasma concentration time curve from time zero to T_{last}, the time at which the last measurable plasma concentration (C_{last}) is observed
- C_{max}: Maximum observed plasma concentration, occurring at T_{max}
- T_{max}: Time of maximum observed plasma concentration

3.10.3.6 Interim Analysis

No interim analysis is planned for the study.

3.10.3.7 Computer Methods

Statistical analyses will be performed using version 9.3 (or newer) of SAS® for Windows.

3.11 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Any amendment to this protocol will be provided to the Investigator in writing by Ironwood or its designee. Prior to implementation, any protocol amendment regarding reportable deviations (as defined by the IRB) must be approved by the IRB and the signature page must be signed by the Investigator and received by Ironwood or its designee, with the following exception: If the protocol is amended to eliminate or reduce the risk to patients, the amendment may be implemented before IRB review and approval. However, the IRB must be informed in writing of such an amendment, and approval must be obtained within reasonable time limits.

Deviating from the protocol is permitted only if absolutely necessary for the safety of the patients and must immediately be reported to Ironwood or its designee.

4. ETHICAL CONSIDERATIONS

4.1 INSTITUTIONAL REVIEW BOARD

Prior to the study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for patient recruitment, and any other written information regarding this study to be provided to a patient or patient's legal guardian must be approved by the IRB.

All IRB approvals must be dated and signed by the IRB Chairman or his or her designee and must identify the IRB by name and address, the clinical protocol by title and/or protocol number, and the date upon which approval or favorable opinion was granted for the clinical research. Copies of IRB approvals should be forwarded to Ironwood. All correspondence with the IRB should be maintained in the Investigator File.

No drug will be released to the site to dose a patient until written IRB approval has been received by Ironwood.

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IRB. The Investigator must supply Ironwood with written documentation of the approval of the continued clinical research.

The IRB must be constituted in accordance with Federal and ICH Good Clinical Practice (GCP) guidelines and any relevant and applicable local regulations.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by Ironwood and by the IRB that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB for approval prior to patients being enrolled into the amended protocol.

4.2 PATIENT INFORMATION AND INFORMED CONSENT

Informed consent procedures will comply with the Code of Federal Regulations (CFR) 21 CFR, Parts 50 and 312.

The written ICF must be approved by the IRB for the purposes of obtaining and documenting consent.

Before entry into the study, each patient will be provided with a written explanation of the study. It is the responsibility of the Investigator or appropriately trained health professional to give each patient full and adequate information regarding the objectives and procedures of the study and the possible risks involved. Patients will then be given the opportunity to ask questions and the Investigator will be available to answer questions as needed. Patients will be informed of their right to withdraw from the study at any time without prejudice. After this explanation and before entering the study, the patient will voluntarily sign an ICF. The patient should receive a copy of the signed and dated ICF. The Investigator must retain each patient's original signed ICF.

If new information becomes available that may be relevant to the patient's consent and willingness to participate in the study, the ICF will be revised. The revised ICF must be submitted to the IRB for review and approval prior to its use.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

This study will be performed at approximately 10 study centers in the US. The Investigator at each study center will be responsible for ensuring that the study is conducted according to the signed Clinical Trial Agreement, the protocol, IRB requirements, and GCP guidelines.

The Investigator will be responsible for the oversight of the site's conduct of the study, which will consist of completing all protocol assessments, maintaining the study file and the patient records, drug accountability, corresponding with the IRB, and completing the eCRF.

5.1 GENERATION OF STUDY RECORDS

Ironwood or its designated representative will conduct a study center visit to verify the qualifications of each Investigator, inspect study center facilities, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study patient. All information recorded in the eCRFs for this study must be consistent with the patient's source documentation.

During the course of the study, the Clinical Site Monitor will make study center visits to review protocol compliance, compare eCRFs and individual patient's medical records, assess drug accountability (in a blinded manner), and ensure that the study is being conducted according to pertinent regulatory requirements. All eCRFs will be verified with source documentation. The review of medical records will be performed in a manner that ensures patient confidentiality is maintained.

The Clinical Site Monitor will discuss instances of missing or uninterpretable data with the Investigator for resolution. Any changes to the study data will be made to the eCRF and documented via an electronic audit trail associated with the affected eCRF.

5.2 DATA QUALITY ASSURANCE

Ironwood performs quality control and assurance checks on all of its clinical studies. Section 5.4 provides details regarding study monitoring procedures.

The study may be subject to audit by Ironwood, its representatives, or regulatory authorities. In the event of an audit, the Investigator must agree to allow Ironwood, representatives of Ironwood, or the FDA or other regulatory agencies access to all study records.

5.3 ELECTRONIC CASE REPORT FORMS AND DATA MANAGEMENT

All data relating to the study will be recorded in the patient's source documentation and eCRF to be provided by Ironwood or designee via the electronic data capture (EDC) system. Source documentation supporting the eCRF data should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs, all observations, and patient status. The Investigator is responsible for verifying that all data entries on the eCRFs are accurate and correct and ensuring that all data are entered in a timely manner, as soon as possible after the information is collected. An explanation should be provided for any missing data. The Investigator as well as the central reader(s) must provide his or her formal approval through the EDC system of all the information in the eCRFs and changes to the eCRFs to endorse the final submitted data for each patient.

Ironwood will retain the final eCRF data and corresponding audit trails. A copy of the final archival eCRF in the form of a compact disc or other electronic media will be placed in the Investigator's study file.

A record of screen failures and pretreatment failures will be maintained for patients who do not qualify for randomization, including the reason for the failure.

5.4 STUDY MONITORING

Ironwood performs quality control and assurance checks on all of its clinical studies. Before any patients are enrolled in the study, a representative of Ironwood or its authorized designee will meet with the Investigator and his/her staff to review relevant and important study-related information including, but not limited to, the protocol, the Investigator's Brochure, the eCRFs

and instructions for their completion using the EDC system, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs.

An Ironwood representative, the Clinical Site Monitor, will monitor the progress and conduct of the study by periodically conducting monitoring visits and by frequent communications (telephone, e-mail, letter, and fax) with the study centers. The site monitor will ensure that the study is conducted according to the protocol and regulatory requirements. During monitoring visits, the information recorded on the eCRFs will be verified against source documents. Upon request of the monitor, auditor, IRB, or regulatory authority, the Investigator should make all requested study-related records available for direct access.

All aspects of the study will be carefully monitored by Ironwood or its designee for compliance with applicable government regulations with respect to GCP and current standard operating procedures.

6. STUDY SPONSORSHIP

6.1 INVESTIGATOR AND STUDY TERMINATION

Ironwood may terminate Investigator participation at any institution for any reason. If participation is ended at the site by either Ironwood or the Investigator, the Investigator must

- Return all study medications and any study materials to Ironwood;
- In cases where the Investigator opts to self-terminate, provide a written statement describing why the study was terminated prematurely.

Ironwood may terminate the study in its entirety or at a specific center at any time for any reason, including but not limited to the following:

- Failure to enroll patients
- Protocol violations
- Inaccurate or incomplete data
- Unsafe or unethical practice
- Questionable safety of the study medication
- Administrative decision

6.2 REPORTING AND PUBLICATION

All data generated in this study will be the property of Ironwood. An integrated clinical and statistical report will be prepared at the completion of the study.

Publication of the results by the Investigator will be subject to mutual agreement between the Investigator and Ironwood.

7. INVESTIGATOR OBLIGATIONS

7.1 DOCUMENTATION

The Investigator must provide the Sponsor with the following documents BEFORE the enrollment of any subjects, in accordance with ICH E6 (*Note*: Ironwood must be notified if there are any changes to these documents):

1. Completed and signed Form FDA 1572 (Statement of Investigator) including all sub-investigators involved in the study
2. Financial disclosure form(s) for the Investigator and all sub-investigators listed on Form FDA 1572
3. Current, signed curricula vitae of the Investigator and all sub-investigators
4. Copy of current medical license of the Investigator and all sub-investigators (as applicable)
5. Copy of the IRB approval letter for the protocol and ICF
6. Copy of the IRB-approved ICF to be used
7. Copy of the IRB approval of recruitment advertising (if applicable)
8. A list of IRB members and their qualifications, and a description of the committee's working procedures
9. Investigator's Statement page, signed by the Investigator
10. Fully executed Clinical Trial Agreement
11. Written document containing the name, location, certification number, and date of certification of the local laboratory to be used for laboratory assays and those of other facilities conducting tests
12. List of normal laboratory values and units of measurements for all laboratory tests required by the protocol. This list is required for each local laboratory to be used during the study.

During the study, the Investigator must maintain the following essential/administrative documents related to the study:

1. Copy of the signed Investigator's Statement Page
2. Copy of financial disclosure form(s) for the Investigator and all sub-investigators (as applicable) if updated
3. Curricula vitae of any new Investigator(s) and/or sub-investigators involved in the study
4. Copy of current medical license of the Investigator and all sub-investigators (as applicable) if updated

5. Copy of the signed Form FDA 1572
6. IRB Approval Notification for the following:
    a. Protocol
    b. Informed consent document
    c. Recruitment advertising (if applicable)
    d. Amendment(s) (if applicable)
    e. Annual review of the protocol and the informed consent document
    f. SAEs
    g. Study closure
7. SAE Reports
8. Drug Inventory Forms (drug receipts, drug dispensing, and inventory forms)
9. Name and address of local or central laboratory, list of normal laboratory values and units of measurement, as well as laboratory certification or hospital accreditation
10. Updates of medical/laboratory/technical procedures/tests:
    a. Normal value(s)/ranges(s)
    b. Certification
    c. Accreditation
    d. Established quality control and/or external quality assessment
    e. Other validation (where required)
11. Record of retained body fluids/tissue samples (if any)
12. Correspondence with Sponsor
13. Written assurance of continuing approval (at least annually) as well as a copy of the annual progress report submitted to the IRB must also be provided to the Sponsor. Any changes in this study or unanticipated problems involving risks to the patients must be reported promptly to the IRB. An Investigator must not make any changes in a study without IRB and Sponsor approval, except when necessary to eliminate apparent immediate hazards to the subjects. All protocol amendments must be submitted to the IRB and approved.
14. Responsibility Log
15. Other logs (eg, prescreening/screening, enrollment)
16. Signed ICFs
17. Patient source documentation
18. eCRFs
19. Audit certificate(s), if applicable

7.2 PERFORMANCE

The Investigator must demonstrate reasonable efforts to obtain qualified patients for the study. The Sponsor may terminate the study with any Investigator for any reason, including, but not limited to, Investigator nonperformance or Investigator noncompliance.

7.3 USE OF INVESTIGATIONAL MATERIALS

The Investigator will acknowledge that the drug supplies are investigational and as such must be used strictly in accordance with the protocol and only under the supervision of the Investigator or sub-investigators. Study medication must be stored in a safe and secure temperature-monitored location. The Investigator's duly trained designee must maintain adequate records documenting the receipt and disposition of all study supplies. The study center must record the date the study medication was received and maintain a dispensing record in which to record each patient's use. A complete reconciliation of study medication will be performed at the site close-out visit, with a final accountability report provided to Ironwood as part of the site close-out report. Written instructions for return of all unused and reconciled study medication to an appropriate waste handler will be provided prior to the end of the study. No study medication may be destroyed by study centers without prior written permission of Ironwood.

7.4 RETENTION AND REVIEW OF RECORDS

Records and documents pertaining to the conduct of this study, including eCRFs, source documents, ICFs, laboratory test results, and medication inventory records, must be retained by the Investigator in accordance with locally applicable regulatory requirements; and, in any event, for a minimum period of 5 years.

No study records shall be destroyed without notifying the Sponsor and giving the Sponsor the opportunity to take such study records or authorizing in writing the destruction of records after the required retention period.

If the Investigator retires, relocates, or otherwise withdraws from the responsibility of keeping the study records, custody must be transferred to another person (Ironwood, IRB, or other Investigator) who will accept the responsibility. Ironwood must be notified of and agree to the change.

7.5 PATIENT CONFIDENTIALITY

All data collected in the context of this study will be stored and evaluated in such a way as to guarantee patient confidentiality in accordance with the legal stipulations applying to confidentiality of data. All patient records will be identified only by initials and patient identification (PID) number. Patient names are not to be transmitted to Ironwood or its authorized designee. The Investigator will keep a Master Patient List on which the PID number and the full name, address, and telephone number of each patient is listed.

8. REFERENCE LIST

1. Patel DA, Kim HP, Zifodya JS, Vaezi MF. Idiopathic (primary) achalasia: a review. Orphanet J Rare Dis 2015;10:89.

2. Allaix ME, Patti MG. What is the best primary therapy for achalasia: medical or surgical treatment? Who owns achalasia? J Gastrointest Surg 2013 Sep;17(9):1547-9.

3. Nenshi R, Takata J, Stegienko S, Jacob B, Kortan P, Deitel W, et al. The cost of achalasia: quantifying the effect of symptomatic disease on patient cost burden, treatment time, and work productivity. Surg Innov 2010 Dec;17(4):291-4.

4. Derbyshire ER, Marletta MA. Structure and Regulation of Soluble Guanylate Cyclase. Annu Rev Biochem 2012;81:533-59.

5. Nossaman BD, Kadowitz PJ. Stimulators of soluble guanylyl cyclase: future clinical indications. The Ochsner journal 2013;13(1):147-56.

6. Stasch JP, Pacher P, Evgenov OV. Soluble guanylate cyclase as an emerging therapeutic target in cardiopulmonary disease. Circulation 2011;123(20):2263-73.

7. Follmann M, Griebenow N, Hahn MG, Hartung I, Mais FJ, Mittendorf J, et al. The Chemistry and Biology of Soluble Guanylate Cyclase Stimulators and Activators. Angew Chem Int Ed Engl 2013;52(36):9442-62.

8. Sivarao DV, Mashimo HL, Thatte HS, Goyal RK. Lower esophageal sphincter is achalasic in nNOS(-/-) and hypotensive in W/W(v) mutant mice. Gastroenterology 2001 Jul;121(1):34-42.

9. Gyawali CP. Achalasia: new perspectives on an old disease. Neurogastroenterol Motil 2016 Jan;28(1):4-11.

10. Hoshino M, Omura N, Yano F, Tsuboi K, Kashiwagi H, Yanaga K. Immunohistochemical study of the muscularis externa of the esophagus in achalasia patients. Dis Esophagus 2013 Jan;26(1):14-21.

11. Bortolotti M, Mari C, Lopilato C, Porrazzo G, Miglioli M. Effects of sildenafil on esophageal motility of patients with idiopathic achalasia. Gastroenterology 2000 Feb;118(2):253-7.

12. Nassri A, Ramzan Z. Pharmacotherapy for the management of achalasia: Current status, challenges and future directions. World J Gastrointest Pharmacol Ther 2015 Nov 6;6(4):145-55.

13. Eherer AJ, Schwetz I, Hammer HF, Petnehazy T, Scheidl SJ, Weber K, et al. Effect of sildenafil on oesophageal motor function in healthy subjects and patients with oesophageal motor disorders. Gut 2002 Jun;50(6):758-64.

14. Herve D, Philippi A, Belbouab R, Zerah M, Chabrier S, Collardeau-Frachon S, et al. Loss of alpha1beta1 soluble guanylate cyclase, the major nitric oxide receptor, leads to moyamoya and achalasia. Am J Hum Genet 2014 Mar 6;94(3):385-94.

15. National Cancer Institute. Common Terminology Criteria for Adverse Events v4.0.. May 29, 2009. NCI, NIH, DHHS; NIH Publications #09-7473.

16. EuroQol Group. EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group. Health Policy 1990;16(3):199-208.

17. Shaw JW, Johnson JA, Coons SJ. US valuation of the EQ-5D health states: development and testing of the D1 valuation model. Med Care 2005;43(3):203-20.

18. Ware J, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 1996;34(3):220-33.

19. Urbach DR, Tomlinson GA, Harnish JL, Martino R, Diamant NE. A measure of disease-specific health-related quality of life for achalasia. Am J Gastroenterol 2005 Aug;100(8):1668-76.

20. Robertson CS, Hardy JG, Atkinson M. Quantitative assessment of the response to therapy in achalasia of the cardia. Gut 1989;30(6):768-73.

21. Gelfond M, Rozen P, Gilat T. Isosorbide dinitrate and nifedipine treatment of achalasia: a clinical, manometric and radionuclide evaluation. Gastroenterology 1982;83(5):963-9.

22. Traube M, Dubovik S, Lange RC, McCallum RW. The role of nifedipine therapy in achalasia: results of a randomized, double-blind, placebo-controlled study. The American journal of gastroenterology 1989;84(10):1259-62.

23. Traube M, Hongo M, Magyar L, McCallum RW. Effects of nifedipine in achalasia and in patients with high-amplitude peristaltic esophageal contractions. JAMA 1984;252(13):1733-6.

24. Bortolotti M, Labò G. Clinical and manometric effects of nifedipine in patients with esophageal achalasia. Gastroenterology 1981;80(1):39-44.

25. Kahrilas PJ, Bredenoord AJ, Fox M, Gyawali CP, Roman S, Smout AJ, et al. The Chicago Classification of esophageal motility disorders, v3.0. Neurogastroenterol Motil 2015 Feb;27(2):160-74.

9. SPONSOR SIGNATURE

Study Title:	A Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group, Single-dose, Phase 2a Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IW-1701 in Patients with Achalasia
Study Number:	C1701-201
Final Date:	03 October 2017

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

Signed: [redacted] Date: 05 OCT 2017

Ironwood Pharmaceuticals, Inc.

10. INVESTIGATOR'S STATEMENT

Study Title:	A Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group, Single-dose, Phase 2a Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IW-1701 in Patients with Achalasia
Study Number:	C1701-201
Final Date:	03 October 2017

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.

Signed:_____ Date:_____

Investigator Name:_____

11. APPENDICES

APPENDIX 1 [redacted]

[redacted]

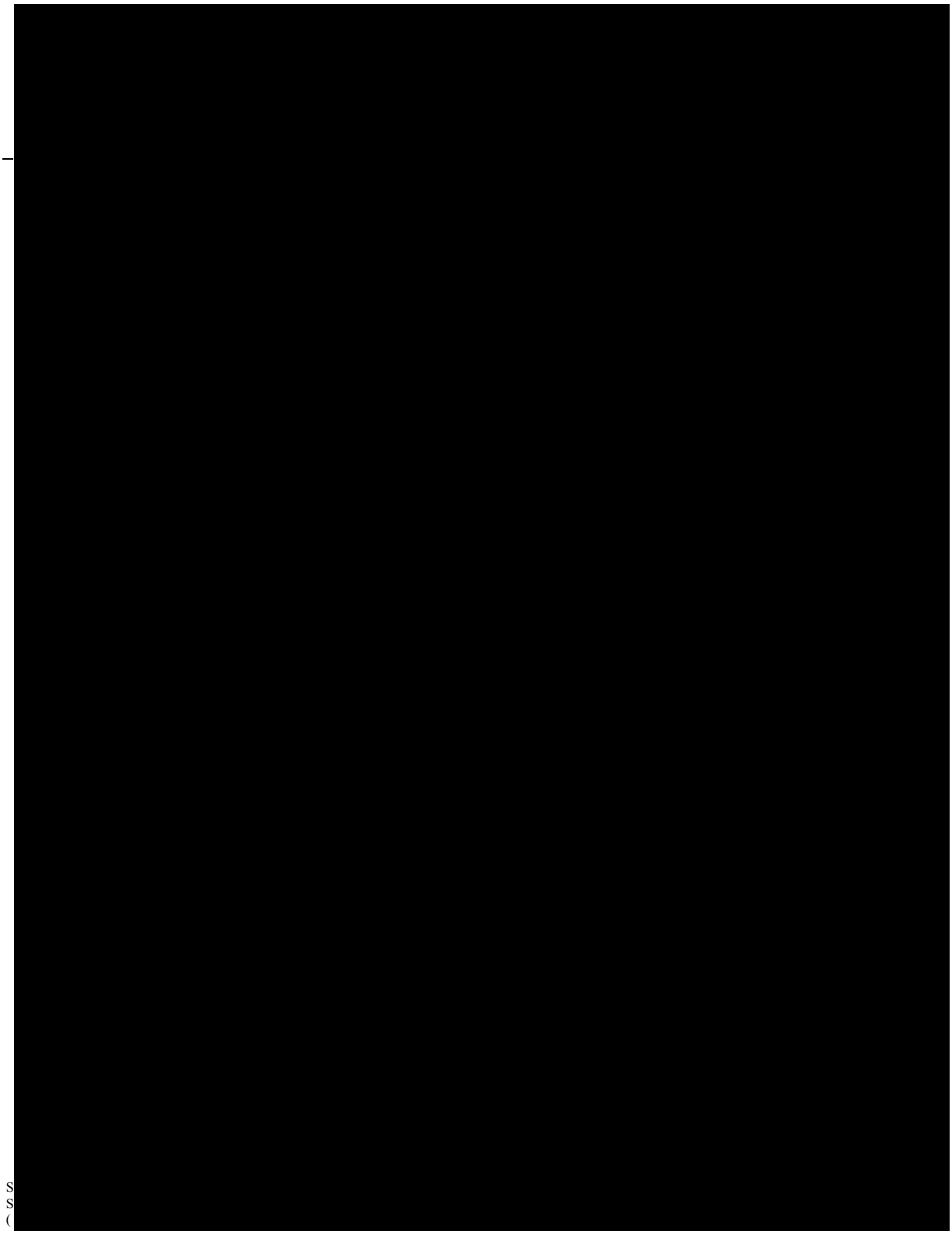
[redacted]

[redacted]

[redacted]

APPENDIX 2 SF-12V2

Protocol Number: C1701-201 Study Period: Subject ID:
Visit Date:



Protocol Number: C1701-201 Study Period: Subject ID:
Visit Date:

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Protocol Number: C1701-201 Study Period: Subject ID:
Visit Date:

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