# Statistical Analysis Plan: C1701-201-SAP

## Final Version, July 23, 2018

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|---|---|
| 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 |
| Protocol Number: | C1701-201 |
| Product Name: | IW-1701 Tablets, 1 mg |
| Sponsor: | Ironwood Pharmaceuticals, Inc.<br>[redacted]<br>Cambridge, MA 02142 |
| Final Date: | July 23, 2018 |



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# 2. LIST OF ABBREVIATIONS

| Abbreviation | Full Term |
|---|---|
| ADO | adverse event leading to drop out |
| AE | adverse event |
| ATC | Anatomical Therapeutic Chemical |
| $AUC_{last}$ | area under the plasma concentration time curve from time zero to the last observation |
| BFT | bolus flow time |
| BLQ | below the limit of quantitation |
| BP | blood pressure |
| bpm | beats per minute |
| $C_{last}$ | last quantifiable plasma concentration |
| $C_{max}$ | maximum observed plasma concentration |
| CS | clinically significant |
| CSR | clinical study report |
| ECG | electrocardiogram |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| EGJ | esophagogastric junction |
| EOT | end of treatment |
| HDL-c | high density lipoprotein cholesterol |
| HRIM | high-resolution impedance manometry |
| IBH | impedance bolus height |
| ICH | International Conference on Harmonisation |
| IPD | important protocol deviation |
| IQR | interquartile range |
| IRP | integrated relaxation pressure |
| IWRS | interactive web response system |
| kg | kilogram |
| $kg/m^2$ | kilograms per meters squared (body mass index) |
| LDH | lactate dehydrogenase |

| Abbreviation | Full Term |
|---|---|
| LDL-c | low density lipoprotein cholesterol |
| LES | lower esophageal sphincter |
| LLN | lower limit of normal |
| LLQ | lower level of quantification |
| MCV | mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mg | milligram |
| mL | milliliter |
| mmHg | millimeters of mercury |
| MPV | mean platelet volume |
| msec | millisecond |
| NCS | not clinically significant |
| PCS | potentially clinically significant |
| pd | postdose |
| PD | pharmacodynamic(s) |
| 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 |
| SAE | serious adverse event |
| SAS® | Statistical Analysis System |
| SE | standard error |
| SOC | system organ class |
| $T_{max}$ | time of maximum observed plasma concentration |
| TEAE | treatment-emergent adverse event |
| WBC | white blood cell |
| WHO | World Health Organization |

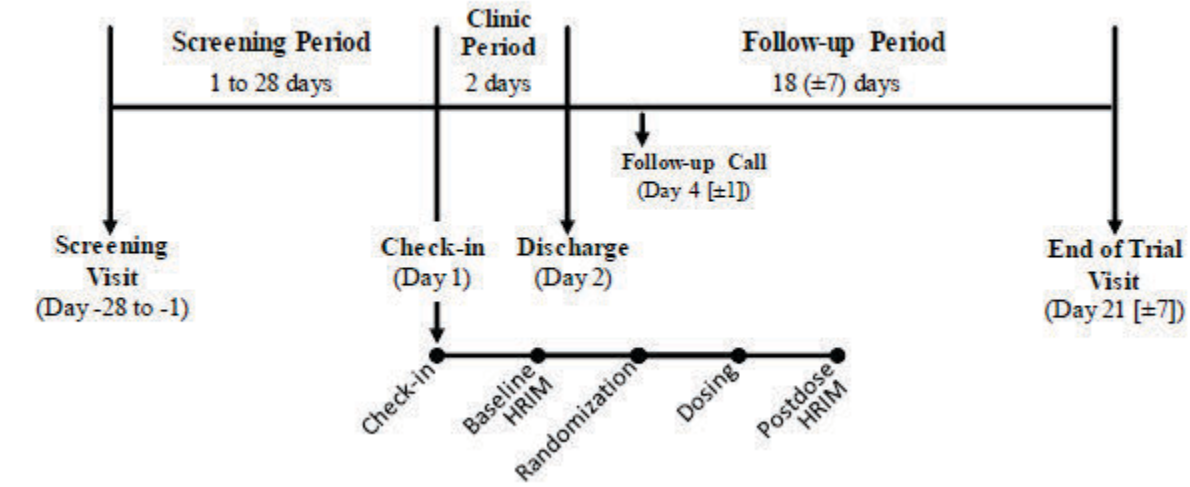
# 3. INTRODUCTION

This statistical analysis plan (SAP) provides a more technical and detailed elaboration of the statistical analyses of the pharmacokinetics (PK), pharmacodynamics (PD), and safety data as outlined and/or specified in the final protocol of Study C1701-201 (Amendment 5, dated October 3, 2017). Specifications for the tables, figures, and data listings are contained in a separate document.

## 3.1 STUDY DESIGN SUMMARY

Study C1701-201 is a Phase 2a, multicenter, randomized, double-blind, placebo-controlled, parallel-group, single-dose study consisting of 3 distinct periods (Screening, Clinic, and Follow-up); see Figure 1. The study will enroll subjects aged 18-75 who have been diagnosed with Type I or Type II achalasia, with an integrated relaxation pressure (IRP) >15 mmHg as assessed by baseline high-resolution impedance manometry (HRIM). As originally designed, approximately 20 patients will be randomized to either IW-1701 or placebo in a 3:1 ratio.

**Figure 1. Study Schematic**




On the morning of Day 1, after an overnight fast of ≥8 hours and after a baseline HRIM procedure, randomized patients will receive 5 mg IW-1701 or placebo in a tablet formulation. Safety, PK, and PD assessments, including blood collections, will be performed at prespecified times (see Table 1). Following all study assessments and at the Investigator's discretion, patients will be discharged on Day 2, at least 30 hours after study drug administration. 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).

## 3.2 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, $C_{max}$, $T_{max}$, $AUC_{last}$

## 3.3 SCHEDULE OF EVENTS

The schedule of events for Study C1701-201 is presented in Table 1.

## Table 1. Schedule of Events

| Study Period | Screening Period | Clinic Period | | Follow-up Period | |
|---|---|---|---|---|---|
| Visit Days →<br>Study Procedure ↓ | Screening Visit<br>Day -28 to -1 | Dosing<br>Day 1 | Discharge<br>Day 2 | Follow-up Call<br>Day 4 (±1) | End of Trial Visit<br>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<br>pd: 4h (±30m) | pd: 24h (±30m) | | X |
| Vital signs (oral temperature, respiratory rate, seated BP & pulse) (d) | X | pre: 0 (≤ 15m)<br>*BP & pulse only:*<br>pd: 0.5h (±10m), 1, 2, 3, 4, 6, 8, 12h (±15m) | pd: 24h (±30m)<br>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)<br>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 →<br>Study Procedure ↓ | Screening Visit<br>Day -28 to -1 | Dosing<br>Day 1 | Discharge<br>Day 2 | Follow-up Call<br>Day 4 (±1) | End of Trial Visit<br>Day 21 (±7) |
| Randomization | | X | | | |
| Study drug administration (h) | | X | | | |
| High resolution impedance manometry (HRIM) procedure (i) | | predose<br>pd: 3h (+15m) | | | |
| Patient-reported symptom questions | | after pre HRIM (+15m)<br>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.

# 4. STATISTICAL METHODS, STUDY ASSESSMENTS AND ENDPOINTS, AND DETERMINATION OF SAMPLE SIZE

## 4.1 STATISTICAL AND ANALYTICAL PLAN

Statistical analyses defined in this SAP will be performed using SAS Version 9.4 or newer.

### 4.1.1 General Methods

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. Additionally, the geometric mean and geometric CV% will be calculated for PK parameters when data allows (i.e., concentration >0). Due to the exploratory nature of the trial, no adjustments will be made for multiplicity. 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.

#### 4.1.1.1 Analysis Populations

Screened Population

All screened patients who have signed the informed consent form for the study and received a patient identification number will be included in the Screened Population.

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 IW-1701 and have at least 1 evaluable postdose PK parameter assessment will be included in the PK Population.

Protocol deviations or events that may affect the evaluability of the PK results are described under Section 4.1.5.2 .

Pharmacodynamic Population

All patients who receive study drug and have at least 1 postdose PD assessment will be included in the PD Population. Protocol deviations or events that may affect IW-1701 systemic exposure (described in Section 4.1.5.2) will be evaluated prior to database lock to determine if PD results should be excluded.

#### 4.1.1.2 Study Medication

During the clinic period, patients will receive a single dose of either 5-mg of IW-1701 or matching placebo.

#### 4.1.1.3 Statistical Methods

For descriptive summaries, N will represent the total number of patients within the summarized population and n will represent the number of non-missing patients for the summarized endpoint. All summary statistics will be presented by treatment and based on non-missing endpoint values, unless otherwise specified.

**Table 2. Statistical Methods**

| Method | Definition |
|---|---|
| Categorical Summaries | • Number and percent of patients in each category (Percent based on number of non-missing values)<br>• Number of missing patients in each category |
| Continuous Summaries | • Number of non-missing patients, mean, median, standard deviation, minimum, maximum, interquartile range<br>• Geometric mean and geometric CV% (PK parameters only) |

#### 4.1.1.4 Missing Data

No imputation will be performed for missing PD observations. All safety and tolerability data will be summarized and analyzed when data are available for a patient. Data handling for missing dates and other key safety data are described in Section 5. Refer to Section 5.7 for details regarding handling of missing PK data.

#### 4.1.1.5 Study Periods

The study periods are outlined in Table 3.

**Table 3. Study Periods**

| Period | Start Date | End Date |
|---|---|---|
| Screening | Date of Signed ICF | Day -1 |
| Clinic | Check-in (morning of Day 1) | Discharge (Day 2) |
| Follow-up Period* | Immediately after Discharge | End of Treatment Visit Date (Day 21±7) |

*Includes Follow-up Call on Day 4 ±1.

### 4.1.2 Interim Analyses

At least one administrative interim analysis will be conducted. For details, please refer to the full administrative interim analysis plan in the appendices of this document.

### 4.1.3 Demographics

#### 4.1.3.1 Disposition of Subjects

Subject disposition will be presented as outlined in Table 4.

**Table 4. Disposition Analyses**

| Analysis Population | Description | Summary Method |
|---|---|---|
| Screening, PK, PD, Safety | Disposition by investigational site, overall | Counts |
| Screening | Reason for screening and pretreatment failure | Categorical summary |
| PD, Safety | Premature discontinuation<br>• Completed study<br>• Prematurely discontinued<br>- Reason for discontinuation | Categorical summary |

#### 4.1.3.2 Protocol Deviations

Protocol deviations and important protocol deviations (IPD) will be identified and documented for all randomized patients prior to unblinding through select individual data reviews and possible programmatic checks of the study data. IPDs will be determined based on review of all protocol deviations and protocol deviation categories (i.e., Use of Prohibited Medication) by members of the Ironwood Clinical Trials team, including but not limited to the Sponsor Medical Monitor and study Biostatistician.

**Table 5. Important Protocol Deviations**

| Analysis Population | Description | Summary Method |
|---|---|---|
| Safety | Important Protocol Deviations<br>• those who entered the study even though they did not satisfy the entry criteria<br>• those who developed discontinuation criteria during the study but were not withdrawn<br>• those who received the wrong treatment or incorrect dose<br>• those who received a prohibited concomitant treatment | Listing |

#### 4.1.3.3 Demographics and Baseline Characteristics

Patient demographics will be summarized by treatment arm and overall for the PD and, if different, the Safety Population as shown in Table 6. Baseline characteristics will be summarized for the PD population as shown in Table 7.

**Table 6. Demographics**

| Endpoint | Description | Summary Method |
|---|---|---|
| Age | Age at informed consent date | Continuous summary |
| Weight | Weight (kg) | |
| Height | Height (m) | |
| BMI | Weight (kg)/ [Height (m)]$^2$ | |
| Age category | <40 years<br>40 to <65 years<br>≥65 years | Categorical summary |
| Sex | Male<br>Female | |
| Race | • White<br>• Non-white<br>- Black or African American<br>- Asian<br>- American Indian or Alaska Native<br>- Native Hawaiian or Other Pacific Islander<br>- Other | |
| Ethnicity | • Hispanic or Latino<br>• Not Hispanic or Latino | |

**Table 7. Baseline Characteristics (PD Population)**

| Endpoint | Description | Summary Method |
|---|---|---|
| Baseline Pharmacodynamic Parameters | • Bolus Flow Time (BFT)<br>  ○ Upright<br>  ○ Supine<br>• Integrated Relaxation Pressure (IRP)<br>  ○ Upright<br>  ○ Supine<br>• Impedance Bolus Height (IBH)<br>  ○ ~1 minute<br>  ○ ~2 minutes<br>  ○ ~5 minutes | Continuous summary |

#### 4.1.3.4 Medical History

Medical History (abnormalities and surgeries) reported at screening will be coded using MedDRA version 20.0. A complete listing by subject of medical and surgical history will be presented.

#### 4.1.3.5 Prior and Concomitant Medication

Reported medication will be coded using the World Health Organization (WHO) Drug Dictionary (version December 2016 LT) to the respective Anatomical Therapeutical Chemical (ATC) class and PT (Table 8). A complete listing by subject of prior and concomitant medications will be presented.

**Table 8. Prior and Concomitant Medication**

| Parameter | Description | Summary |
|---|---|---|
| Prior medications | Medication taken prior to first dose of study medication date | Listing |
| Concomitant medication | Medication taken on or after first dose of study medication date | |

#### 4.1.3.6 Achalasia Disease History

A complete listing by subject of Achalasia Disease History reported at Screening will be presented.

#### 4.1.3.7 Study Drug Compliance

This is an in-clinic dosing study and, therefore, an assessment of compliance is not necessary. A data listing containing the study drug dosing information will be provided and will include the number of tablets each patient swallowed.

#### 4.1.3.8 Achalasia Diagnosis

Per Protocol, a diagnosis of achalasia is determined from supine 10-swallow protocol on baseline HRIM procedure as read by the Investigator at the site. Subjects enrolled under C1701-201-P-05 or earlier will have two sets of predose swallows; diagnosis will be determined from the first set of predose swallows. In the event that the first set of predose swallows is missing, the diagnosis will be determined from the second set of predose swallows. The EDC allows the Investigator at the site to enter a diagnosis only after the first predose set of swallows. A patient with a missing first set of predose swallows, but an applicable diagnosis on the second set of predose swallows will be randomized and included in analysis. Because a diagnosis from a second set of swallows cannot be entered into the EDC, correspondence with the site and documentation of the Type I or Type II achalasia diagnosis will be included as an Appendix in this SAP (see Appendix 7.3).

A complete by subject listing of Achalasia diagnosis will be presented.

### 4.1.4 Pharmacodynamic Assessments and Analyses

#### 4.1.4.1 High Resolution Impedance Manometry (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.

Timing

- 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)

Measurement

- **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
- **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 (e.g., interrupted by diaphragmatic contraction).
- **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)*

HRIM measurements will be recorded at each site and then sent to a central reader for analysis. For all PD analyses, the values determined by the central reader will be used.

Subjects randomized under protocol version C1701-201-P-05 or earlier will have 2 sets of swallowing protocols both pre- and postdose. For subjects with 2 sets of predose swallowing protocols, the first predose recording will be used to obtain the baseline BFT and IRP values. For subjects with 2 sets of postdose swallowing protocols, the second postdose recording will be used to define the postdose BFT and IRP values. If a subject randomized under C1701-201-P-05 or earlier is missing the first predose recording, the second predose recording will be used to obtain the baseline BFT and IRP values. Similarly, if a subject randomized under C1701-201-P-05 or earlier is missing the second postdose recording, the first postdose recording will be used to define the postdose BFT and IRP values.

Descriptive statistics will be calculated at baseline and each postdose assessment. Mean and median change from baseline for each parameter will be presented by treatment group. Percent change (from baseline to postdose) in IRP parameters will also be calculated and summarized by treatment group. A shift table from baseline to postdose will also be created for BFT to summarize improvement in flow (BFT=0 and BFT>0). Refer to Table 9 for a full description of endpoints. All pre- and postdose values (not just the median) will be plotted for each HRIM measure (BFT and IRP) by patient and timepoint. A complete listing of all HRIM measurements provided from both the site (only available for predose assessments) and central read will be provided for each patient.

**Table 9. Pharmacodynamic Endpoints and Analyses**

| Endpoint | Description | Timing | Summary Method |
|---|---|---|---|
| Change from Baseline in *Supine* Bolus Flow Time (BFT) | Supine BFT defined as the median measurement from the 10 available swallows in supine position as determined by the central read (seconds; longer times=more severe achalasia). Change=(postdose supine BFT – predose supine BFT). | Predose, postdose (3 hours +15 minutes after dosing), and change. | Continuous summary |
| Change from Baseline in *Upright* BFT) | Upright BFT defined as the median measurement from the 5 available swallows in the upright position as determined by the central read (seconds; longer times=more severe achalasia). Change = (postdose upright BFT – predose upright BFT). | | |
| Change from Baseline in *Supine* Integrated Relaxation Pressure (IRP) | Supine IRP defined as the median measurement from the 10 available swallows in supine position as determined by the central read (mmHg; higher pressure=more severe achalasia). Change = (postdose supine IRP – predose supine IRP). Percent change = ((postdose supine IRP – predose supine IRP) ÷ (predose supine IRP)) × 100 | | |
| Change from Baseline in *Upright* IRP | Upright IRP defined as the median measurement from the 5 available swallows in the supine position as determined by the central read (mmHg; higher pressure=more severe achalasia). Change = (postdose upright IRP – predose upright IRP). Percent change = ((postdose upright IRP – predose upright IRP) ÷ (predose upright IRP)) × 100 | | |
| Change from Baseline in 1-minute Impedance Bolus Height (IBH) (if performed) | 1-minute IBH defined by the height in esophagus of 200 mL saline bolus 1-minute post-bolus as determined by the central read (cm; greater height=more severe achalasia). Change = (postdose height 1 min IBH – predose height 1 min IBH) | | |
| Change from Baseline in 2-minute IBH (if performed) | 2-minute IBH defined by the height in esophagus of 200 mL saline bolus 2 minutes post-bolus as determined by the central read (cm; greater height=more severe achalasia). Change = (postdose height 2 min IBH – predose height 2 min IBH). | | |
| Change from Baseline in 5-minute IBH (if performed) | 5-minute IBH defined by the height in esophagus of 200 mL saline bolus 5 minutes post-bolus as determined by the central read (cm; greater height=more severe achalasia). Change = (postdose height 5-min IBH – predose height 5-min IBH) | | |

#### 4.1.4.2 Esophagogastric Junction (EGJ) Pressure

Basal EGJ pressure will be recorded to assess the resting pressure without swallowing. For subjects randomized under C1701-201-P-05 and earlier resting EGJ pressure will be recorded

after insertion of HRIM catheter and every 15 minutes for 1 hour (baseline, 15 min, 30 min, 45 min, and 60 min EGJ pressure) both predose and postdose. Subjects randomized under C1701-201-P-06 only have a single measure of EGJ pressure assessed following insertion of the catheter. These values will be presented by subject and timepoint in a data listing.

#### 4.1.4.3 Exploratory Biomarker Analyses

Blood samples for determination of plasma renin activity will be collected according to the schedule of events. As data permit, summaries for each postdose timepoint and change from baseline may be calculated. Results may be presented outside of the clinical study report (CSR).

#### 4.1.4.4 Multiple Comparisons/Multiplicity

Due to the exploratory nature of the trial, no adjustments will be made for multiplicity.

### 4.1.5 Pharmacokinetic Assessments and Analyses

#### 4.1.5.1 PK Parameters

The PK parameters listed in Table 10 will be calculated for each patient, whenever possible, if systemic levels of IW-1701 are quantifiable. PK parameter calculations will be performed using noncompartmental methods with Phoenix® WinNonlin® Version 6.4, or higher, (Certara LP, Princeton, NJ).

Plasma concentrations that are below the lower level of quantification (LLQ) or missing will be treated as described in Section 4.1.5.2 for the calculation of PK parameters. Actual sampling times relative to IW-1701 dosing times will be used in calculations of the PK parameters. Area under the concentration-time curve will be calculated by linear up/log down trapezoidal summation.

**Table 10. PK Parameters**

| Parameter* | Description |
|---|---|
| $AUC_{last}$ | Area under the plasma concentration time curve from time zero to $T_{last}$, the time at which the last quantifiable plasma concentration ($C_{last}$) is observed. |
| $T_{max}$ | Time of maximum observed plasma concentration, obtained directly from the concentration-time profile. |
| $C_{max}$ | Maximum observed plasma concentration, occurring at $T_{max}$ |

*To be calculated relative to the morning dose, unless otherwise noted.

#### 4.1.5.2 PK Analysis

Plasma concentration of IW-1701 will be summarized using the PK Population for each assessment timepoint for the active treatment group. Concentrations that are below LLQ (BLQ) will be treated as zero for the computation of descriptive statistics. A by-subject listing of all concentration-time data and scheduled sample collection time will be presented. A listing of PK blood sample collection times, and elapsed times relative to dose, and time deviations will be provided.

Derived PK parameters for IW-1701 will be summarized using the PK Population. A by-subject listing of all calculated PK parameters will be presented.

Changes to protocol procedures or events which many impact the quality of the PK data or alter the evaluation of the PK will be reviewed on a case-by-case basis to determine if PK data may be excluded from relevant PK summaries. Not all the events may be considered a protocol deviation; as an event may be secondary to an AE or to a circumstance outside of the control of the study site or the patient. Examples include, but may not be limited to, vomiting following oral dosing occurring within the time frame of 2 times the median time to $T_{max}$, sample processing errors that lead to inaccurate bioanalytical results, inaccurate dosing or administration of a prohibited medication expected to impact IW-1701 exposure. Deviations to procedures or events which do not impact the quality of the PK data such as a missed blood sample or deviations from blood collection times will not be considered for exclusion from PK summaries.

### 4.1.6 Graphical Presentation of PK Data

Geometric mean plasma concentrations will be plotted over time on both a linear and a semi-logarithmic scale. Individual drug plasma concentrations will also be plotted over time.

### 4.1.7 Patient-reported Symptom Assessments and Analyses

Patients will answer a number of Screening Patient-reported Symptom Questions at the Screening Visit. Refer to Table 11 for full description.

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

Descriptive statistics will be calculated at baseline and each postdose assessment for the patient-reported symptoms data. Change from baseline for each symptom will be calculated and presented by treatment group. Refer to Table 11 for full endpoint descriptions.

## Table 11. Patient Symptom Endpoints and Analyses

| Endpoint | Description | Timing | Summary Method | Analysis Population |
|---|---|---|---|---|
| Severity of difficulty swallowing *food during/between* meals[a] | During the last 7 days, how would you rate the severity of your difficulty swallowing *food during/between* meals? 0=Did not have, 1=Very mild, 2=Mild, 3=Moderate, 4=Moderatively severe, 5=Severe | Screening | Continuous summary | Screened population; PD population |
| Severity of pain while swallowing *food during/between* meals[a] | During the last 7 days, how would you rate the severity of your pain while swallowing *food during/between* meals? 0=Did not have, 1=Very mild, 2=Mild, 3=Moderate, 4=Moderatively severe, 5=Severe | | | |
| Frequency of cough while swallowing *food during/between* meals[a] | During the last 7 days, how often did you experience cough while swallowing *food during/between* meals? 0=Never, 1=Rarely, 2=Sometimes, 3=Often, 4=Very often | | | |
| Impact of achalasia/ swallowing disorder symptoms on *what/when* you ate[b] | During the last 7 days, how much did your achalasia/swallowing disorder symptoms impact *what/when* you ate? 0=Not at all, 1=A little bit, 2=Somewhat, 3=Quite a bit, 4=A lot | | | |
| Overall severity of achalasia/ swallowing disorder symptoms | How would you rate the overall severity of achalasia/swallowing disorder symptoms over the past 7 days? 0=Did not have, 1=Very mild, 2=Mild, 3=Moderate, 4=Moderately severe, 5=severe | | | |
| Ability to swallow *raw hard fruits and vegetables/rice/ clear fluids (water, juice, coffee, tea)*[c] | Please indicate which of the following types of foods you are able to swallow without experiencing any problems such as pain or food "sticking" as it goes down. Check one box for each type of food. Options: Can swallow without problem; can swallow, but with some difficulty; Can swallow with great difficulty or not at all | | Categorical summary | |
| Change from baseline in severity of difficulty swallowing during swallowing tests | Severity of difficulty swallowing during swallowing tests (0=Did not have, 1=Very mild, 2=Mild, 3=Moderate, 4=Moderately severe, 5=Severe); Change=(postdose severity – predose severity). | Within 15 minutes of predose HRIM; within 15 minutes of postdose HRIM; Change | Continuous summary | PD population |
| Change from baseline in severity of pain while swallowing during swallowing tests | Severity of pain while swallowing during swallowing tests (0=Did not have, 1=Very mild, 2=Mild, 3=Moderate, 4=Moderately severe, 5=Severe); Change=(postdose severity – predose severity). | | | |
| Change from baseline in frequency of coughing while swallowing during swallowing tests | Frequency of cough while swallowing during swallowing tests (0=Never, 1=Rarely, 2=Sometimes, 3=Often, 4=Very often); Change=(postdose frequency – predose frequency). | | | |
| Relief of achalasia symptoms | Overall achalasia symptoms since treatment with study medication (1=Significantly relieved, 2=Moderately relieved, 3=Somewhat relieved, | Within 15 minutes of | Continuous summary | |

| Endpoint | Description | Timing | Summary Method | Analysis Population |
|---|---|---|---|---|
| | 4=Unchanged, 5=Somewhat worse, 6=Moderately worse, 7=Significantly worse) | postdose HRIM | | |

[a]Two separate endpoints; one for during meals and one for between meals.
[b]Two separate endpoints; one for what you ate and one for when you ate.
[c]Three separate endpoints; one for fruits and vegetables, one for rice, and a third for clear fluids.

### 4.1.8 Health Outcomes Assessments and Analyses

#### 4.1.8.1 EuroQol-5 Dimension (EQ-5D-3L)

Patients will complete the EQ-5D-3L at Baseline (Screening Visit) and the EOT visit. Patient responses to the descriptive system (e.g., health state) will be converted to the corresponding utility score and the descriptive statistics (n, mean, SD, median, minimum and maximum) will be presented for utility score by treatment group at each timepoint for the PD Population. The same statistics will be calculated for the analog scale reflecting the patient's preference for their health state.

**Table 12. EQ-5D-3L Endpoints and Analyses**

| Endpoint | Description | Timing | Summary Method | Analysis Population |
|---|---|---|---|---|
| Utility Score | Multi-component, single measure derived from 5 general health items: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each item measured on 0 to1 scale. | Screening, EOT Visit | Continuous descriptive | PD Population |
| Visual Analog Scale | Rating of Current Health as of today (0-100): Higher score indicates better health | | | |

The 5-item responses of the EQ-5D-3L define a health state that can be converted into a single summary utility index that assigns weights (i.e., value) to each level. Different weights are available. For this study, the US TTO (time trade-off) weights will be used.(1)

The EQ-5D-3L index is computed according to the following equation as:

[redacted]

**Table 13. EuroQol Quality-of-Life Questionnaire Utility Index**

If any dimension is missing in the EQ-5D-3L, the EQ-5D-3L index will not be calculated and will be set to missing.

#### 4.1.8.2 Short Form 12 Health Survey Version 2 (SF-12v2)

Patients will complete the SF-12v2 at Baseline (Screening Visit) and the EOT visit. SF-12v2 scores will be calculated using QualityMetric Health Outcomes(™) Scoring Software 5.0. Summaries will be provided by treatment for the PD population at each timepoint as well as the change from baseline as described in Table 14.

**Table 14. SF-12v2 Endpoints and Analyses**

| Endpoint | Description | Timing | Summary Method | Analysis Population |
|---|---|---|---|---|
| SF-12v2 Physical Component Summary Score | 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); aggregated into 2 summary measures: the physical component and the mental component summary scores. | Screening, EOT Visit, change from baseline | Continuous descriptive | PD population |
| SF-12v2 Mental Component Summary Score | | | | |

The SF-12 is a multipurpose, short-form health survey consisting of 12 questions designed for use in clinical practice and research, health policy evaluations, and general population surveys. The SF-12, Version 2.0, contains 12 items that, when scored, yield an 8-scale profile of functional health and well-being, as well as physical and mental summary measures.

Two distinct, higher-order summary norm-based scores: Physical Component Summary (PCS-12) and Mental Component Summary (MCS-12) scores are standardized to have a mean of 50 and an SD of 10 in the general US Population. These two scores will be summarized as described in Table 14.

The steps involved in scoring SF-12 can be found at Ware et al. (2,3)

If any item is missing in SF-12, then the PCS-12 and MCS-12 scores will not be calculated and will be set to missing.

## 4.2 SAMPLE SIZE JUSTIFICATION

For this exploratory 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). The sample size was determined outside of statistical considerations and is based on precedent set by prior studies of similar nature and design.(3, 4)

## 4.3 SAFETY ANALYSIS

### 4.3.1 Adverse Events

All adverse events reported during the study will be coded using MedDRA version 20.0 and will be classified by MedDRA system organ class (SOC) and preferred term (PT) as outlined in Table 15.

**Table 15. AE Classifications and Definitions**

| AE classification | Definition |
|---|---|
| Treatment-emergent Adverse Event (TEAE) | TEAE if:<br>• Treatment Start date/time ≤ AE Start Date/Time ≤ (Date of Last Treatment +72 hours)<br>And<br>• AEs that first occurred or worsened in severity after study drug administration |
| Serious Adverse Events | SAEs will be recorded from the time the subject signs the ICF at the Screening Visit through the End of Trial Visit (Day 21 ±7).<br><br>An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:<br>• Death<br>• Life-threatening: the patient was at immediate risk of death from the reaction as it occurred<br>• Hospitalization or prolongation of existing hospitalization<br>• Persistent or significant disability/incapacity: a substantial disruption of a person's ability to conduct normal daily functions<br>• Congenital anomaly/birth defect<br>• 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.<br><br>These criteria will be identified by AE Serious criteria = Yes |

Patients reporting multiple incidence of an adverse event mapping to the same PT will be counted only once overall, at the highest severity level, and at the closest relationship to treatment. AEs will be summarized as described in Table 16.

**Table 16. AE Summary Tables Specifications**

| Summaries | Description | Summary Method | Analysis Population |
|---|---|---|---|
| Overall Summary | • TEAEs<br>• Deaths<br>• SAEs<br>• Withdrawals Due to TEAEs (ADOs)<br>• Premature Discontinuations<br>• | Categorical summary | Safety population |
| TEAEs | • At least 1 TEAE<br>• By SOC<br>• By PT within SOC | | |
| TEAEs by Severity | | | |
| TEAEs by Relationship to Study Drug | | | |
| Severity | Overall Summary by Severity Level<br>• Mild<br>• Moderate<br>• Severe | | |
| Relationship to Study Drug | Overall Summary by Relationship to Study Drug<br>• Related<br>• Unrelated | | |
| Listings of All Adverse Events (including pretreatment) for Patients with:<br>1. SAEs<br>2. ADOs<br>3. Fatal | For Patients meeting the defined criteria, list all of the patient's Adverse Events | Data listing – See table shells for variables to include | |
| Listing of Screening Period Adverse Events | AEs occurring prior to first dose of study drug | Data listing | |

### 4.3.2 Clinical Laboratory Data

Clinical laboratory results recorded over the course of the study will be summarized as defined in Table 17. Baseline is defined as the last non-missing laboratory assessment reported prior to dosing with study medication. Complete clinical laboratory listings will be provided for each patient. Central laboratory results are preferred. If patients have both central and local labs for a given assessment, the central laboratory results will be used.

**Table 17. Clinical Laboratory Summary Specifications**

| Endpoint | Description | Timing | Summary Method | Analysis Population |
|---|---|---|---|---|
| Shift from baseline | Summary by laboratory category and parameter<br>• Normal<br>• Abnormal NCS<br>• Abnormal CS | Postdose Day 1, Day 2 (Discharge), Day21 (End of Trial Visit) | Categorical summary (denominator based on patients with both baseline and follow-up measure) | Safety population |

### 4.3.3 Vital Signs Parameters

Vital signs endpoints will be defined and summarized as described in Table 18. Criteria for potentially clinically significant post-baseline vital signs is summarized in Table 19. Baseline is defined as the last nonmissing vital sign value prior to dosing with study medication. Complete vital sign listings will be provided for each patient.

**Table 18. Vital Signs Summaries**

| Endpoint | Description | Timing | Summary Method |
|---|---|---|---|
| PCS Vital Signs | Summarized by vital signs category (**Table 19**) and parameter | Baseline (predose values Day 1), Day 1 (postdose: 0.5h ±10m, 1h, 2h, 3h, 4h, 6h, 8h, 12h±15m), Day 2 (postdose: 24h ±30m; at discharge (*BP only*): 30h or later), EOT visit (Day 21±7) | Categorical summary (denominator based on patients with both baseline and follow-up measure) |
| Descriptive | • Summary BP and Pulse at each timepoint measured<br>• Change from baseline | Baseline (predose values Day 1), Day 1 (postdose: 0.5h ±10m, 1h, 2h, 3h, 4h, 6h, 8h, 12h±15m), Day 2 (postdose: 24h ±30m; at discharge (*BP only*): 30h or later), EOT visit (Day 21±7) | Continuous summary |
| | • Summary oral temperature and respiratory rate | Baseline (predose values Day 1), Day 2 (24h ±30m), EOT visit (Day 21 ±7) | Continuous summary |

**Table 19. Potentially Clinically Significant Post-Baseline Vital Signs**

| Vital Sign Parameter | Flag | Criteria* | |
|---|---|---|---|
| | | Observed Value | Change from Study Baseline |
| Seated systolic blood pressure (mmHg) | High | ≥ 180 | Increase of ≥ 20 |
| | Low | ≤ 90 | Decrease of ≥ 20 |
| Seated diastolic blood pressure (mmHg) | High | ≥ 105 | Increase of ≥ 15 |
| | Low | ≤ 50 | Decrease of ≥ 15 |
| Seated pulse rate (bpm) | High | ≥ 110 | Increase of ≥ 15 |
| | Low | ≤ 50 | Decrease of ≥ 15 |

*A postbaseline value is considered as a notable value if it meets both criteria for observed value and change from baseline.

### 4.3.4 ECG Parameters

Baseline ECG is defined as the last nonmissing value prior to dosing with study medication. Shift tables will be provided to show the categorical shift from baseline at each postdose assessment (Normal, Abnormal NCS, Abnormal CS). Complete ECG listings will be provided for each patient.

### 4.3.5 Physical Examination

Physical examination results for all subjects will be presented in a data listing for the Safety Population.

# 5. CHANGES FROM ANALYSES PLANNED IN THE PROTOCOL

The following analyses are added to those specified in the study protocol:

1. The addition of a Screened Population to the Analysis Populations.

2. Descriptive statistics for chemistry, vital signs, and ECG parameters have been removed and shift tables only will be presented due to the small number of subjects enrolled (N=9).

# 6. DATA HANDLING CONVENTIONS

## 6.1 REPEATED OR UNSCHEDULED ASSESSMENTS OF SAFETY PARAMETERS

If a patient has repeated safety assessments (e.g., for a lab parameter) prior to dosing of study drug, then the results from the final non-missing assessment made prior to the start of study drug will be used as baseline. If there is more than one safety measurement (e.g., for a lab parameter) at a postbaseline timepoint, only the last measurement will be used. All postbaseline assessments including unscheduled assessments, if any, will be used for notable value determination, and all assessments will be presented in data listings.

## 6.2 CONVENTIONS FOR SUMMARIZING ADVERSE EVENTS

The following conventions will be followed in summarizing TEAEs within a treatment group:

- For patient incidence summaries, each patient will be counted only once within each SOC, PT, or the overall AE summary
- If a patient reported more than one AE within an SOC or PT, then the TEAE with the highest severity or strongest study drug relationship within each SOC and each PT will be included in the summaries by severity or relationship, respectively.

## 6.3 MISSING DATE INFORMATION FOR ADVERSE EVENTS

If it is not possible to determine when an AE started due to incomplete start date/times, it will be assumed to be treatment emergent.

## 6.4 MISSING SEVERITY ASSESSMENTS FOR ADVERSE EVENTS

If severity is missing for an AE that started prior to the dosing of study drug, all efforts should be made to obtain the severity from the Investigator. If it is still missing after all efforts, then a severity of “Mild” will be assigned. If the severity is missing for an AE that started on or after the date of dosing of study drug, then a severity of “Severe” will be assigned. The imputed values for the missing severity assessment will be used for the incidence summary, while the actual missing values will be presented in data listings.

## 6.5 MISSING RELATIONSHIP TO STUDY DRUG FOR ADVERSE EVENTS

If the relationship to study drug is missing for an AE that started on or after the date of dosing of study drug, all efforts should be made to obtain the relationship from the Investigator. If it is still missing after all efforts, a causality of "Related" will be assigned in the corresponding analysis-derived data set. The imputed values for the missing relationship to study drug will be used only for incidence summary, while the actual missing values will be presented in data listings.

## 6.6 MISSING DATE INFORMATION FOR PRIOR OR CONCOMITANT MEDICATIONS

If the start date of a medication is missing or incomplete (i.e., partially missing), then the medication will be assumed to be concomitant.

## 6.7 HANDLING OF MISSING PK DATA

- Handling of predose samples prior to the IW-1701 administration: Concentrations that are BLQ or missing will be assigned a numerical value of zero for the calculation of AUC. Any anomalous concentration values observed at predose will be included in the computation of AUC. If the predose concentration is greater than 5% of $C_{max}$, the data will be evaluated on a case-by-case basis to determine if exclusion of the affected profile is warranted.

- Handling of all other BLQ concentrations: Any other BLQ concentrations will be assigned a value of zero if at predose or if they precede quantifiable samples in the initial portion of the profile. A BLQ value that occurs between quantifiable data points, especially prior to $C_{max}$, will be evaluated to determine if an assigned concentration of zero makes sense, or if exclusion of the data is warranted. Following $C_{max}$, BLQ values embedded between 2 quantifiable data points will be treated as missing when calculating AUC. If BLQ values occur at the end of the collection interval (after the last quantifiable concentration), these will be set to zero. If consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal portion of the concentration curve, these quantified values will be excluded from the PK analysis by setting them to missing, unless otherwise warranted by the concentration-time profile.

- Handling of missing data: In general, missing data in a given profile will not be imputed. The affected profile(s) will be evaluated whether sufficient IW-1701 concentrations are available to calculate any of the planned PK parameters.

# 7. REFERENCES

1. 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.

2. Ware J, Jr., 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.

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

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

5. 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.

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

7. 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.

8. 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.

# 8. APPENDICES

## 8.1 ADMINISTRATIVE INTERIM STATISTICAL ANALYSIS PLAN

**Ironwood Pharmaceuticals, Inc.**
[redacted]
**Cambridge, MA 02142**

**Administrative Interim Analysis**
**Statistical Analysis Plan**

**Study C1701-201**

**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**

**Final: December 19, 2017**

# 1. INTRODUCTION

This document describes in detail the analysis plan for the unblinded administrative interim analyses being performed for corporate planning, for patients randomized in C1701-201**, *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 details are presented in the full protocol, C1701-201-P-06, finalized on October 3, 2017.

The analyses documented in this plan will be executed by an unblinded statistician (employee of Ironwood) not otherwise associated with the day-to-day study execution. As defined in the protocol blinding section, *Sponsor personnel involved in the conduct of the study (e.g., study medical monitor, study statistician, study data manager, and clinical operations study personnel) will be blinded to individual patient treatment assignments; however, descriptive and inferential statistics will be presented with a treatment identifier*. In contrast, patient-level data such as minimum, maximum, median, and graphical analysis will not be presented with patient identifiers so that study conduct decisions regarding individual patients can be addressed without explicit knowledge of the patient's treatment assignment. A list of study personnel and their blinded/unblinded status is presented below.

Safety is monitored blinded in an ongoing basis throughout the course of the study. Safety assessments and endpoints will not be part of the administrative interim analysis.

# 2. 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).

## 2.1 Study Population

This study will randomize up to 20 patients diagnosed with primary Type I or II achalasia with an integrated relaxation pressure (IRP) >15 mmHg by baseline High Resolution Impedance Manometry (HRIM).

## 2.2 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 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 mmHg will be randomized to receive a single 5-mg dose of IW-1701 or matching placebo. 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. 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).

**2.3 Method of Assigning Patients to Treatment Groups**

Patients who meet all 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.

**2.4 Blinding**

This study is double-blind and placebo-controlled. The patients, Investigators, and site study staff, except for specifically designated unblinded Study Center pharmacy staff, will be blinded to individual treatment assignments. Sponsor personnel involved in the conduct of the study (e.g., 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.

**2.5 Administrative Unblinded Interim Analysis Objective**

The objective of this unblinded interim analysis is to determine if a single 5 mg dose of IW-1701 has an effect on a patient's IRP measures (supine or upright).

## 3. STATISTICAL METHODS

For any unblinded interim analysis, descriptive statistics and graphical analyses (e.g., number of subjects, mean, standard deviation, median, minimum, and maximum) will be calculated for IRP in the supine and upright position at both the predose (baseline) and postdose (HRIM) procedures, as well as change from baseline in IRP measured in both the supine and upright position. However, for the first interim analysis, number of subjects, standard deviation, minimum, and maximum will not be calculated in order to maintain blinding to individual

treatment assignments. Summaries will be presented by treatment group and overall. Where descriptive statistics or graphical analyses might unblind individual patients due to the sparsity of data, select descriptive statistics of graphical analyses may be omitted at the discretion of the unblinded statistician. Inferential statistics will not be calculated except if specifically requested by the Study Medical Monitor. All statistical analyses will be performed using SAS Version 9.4 (or later). Analysis will be conducted using the Pharmacodynamic Population.

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

### 3.1 Pharmacodynamic methods for unblinded interim analysis

Integrated Relaxation Pressure (IRP): mean of the 4s of maximal deglutitive relaxation in the 10-s window beginning at the upper esophageal sphincter relaxation measured in mmHg. Contributing times can be contiguous or non-contiguous (e.g., interrupted by diaphragmatic contraction). Referenced to gastric pressure; median measurement from the 10-swallow supine and 5-swallow upright protocols. IRP was measured in both the supine position (a subject's value is the median of their 10 swallows) and the upright position (a subject's value is the median of their 5 swallows). Both the median supine and upright IRP values will be used in the primary IRP analysis.

Predose IRP: For subjects with 2 sets of swallowing protocols (subjects randomized under version C1701-201-P-05 or earlier), the first recording, or the IRP from the first set of predose swallowing protocol, will be used as the predose IRP values.

Postdose IRP: For subjects with 2 sets of swallowing protocols (subjects randomized under C1701-201-P-05 or earlier), the second recording, or the median supine and upright IRP values from the second set of postdose swallowing protocol will be used for the postdose IRP values.

Mean change from predose (postdose supine IRP - predose supine IRP) will be calculated for supine IRP (which is recorded as the median of the 10 supine swallows from each swallowing protocol).

Mean change from predose (postdose upright IRP – predose upright IRP) will be calculated for upright IRP (which is recorded as the median of the 5 upright swallows from each swallowing protocol).

See the appendix 4.1 for a more detailed list of variables used for this administrative interim analysis.

### 3.2 Sensitivity Analysis

We will perform a sensitivity analysis where the average of a subject's two supine IRP values is calculated for subjects with 2 sets of swallowing protocols (subjects randomized under version C1701-201-P-05 or earlier). The mean change from predose for supine will be recalculated for all patients using the mean values for patients with 2 sets of swallowing protocols. This analysis will also be repeated for upright IRP.

### 3.3 Timing of Administrative Interim Analyses

Administrative unblinded interim analyses will be performed after the first 5 patients have completed both pre- and postdose HRIMs, and then subsequently when approximately 10, and 15 patients have completed both pre- and postdose HRIMs.

### 3.4 Roles and Responsibilities

The unblinded statistician will execute the statistical analyses defined in this document in a secure location, accessible to him/her only, to ensure the limited access to the unblinded results. The unblinded statistician will create a summary report of all interim finding and present the report to the Study Medical Monitor.

In turn, the Study Medical Monitor and unblinded statistician will present the results to a Senior Management Group consisting of: President of R & D, Chief Development Officer, and SVP of Clinical Affairs with assistance from the Head of Biostatistics of VP of Data Science, as needed.

The Study Execution Team (SET), with the exception of the Study Medical Monitor, will remain blinded at both the group and individual level.

**List of 1701-201 Study Personnel**

| Study Personnel | Role | Title | Blinding Status |
|---|---|---|---|
| [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] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |
| [redacted] | [redacted] | [redacted] | [redacted] |

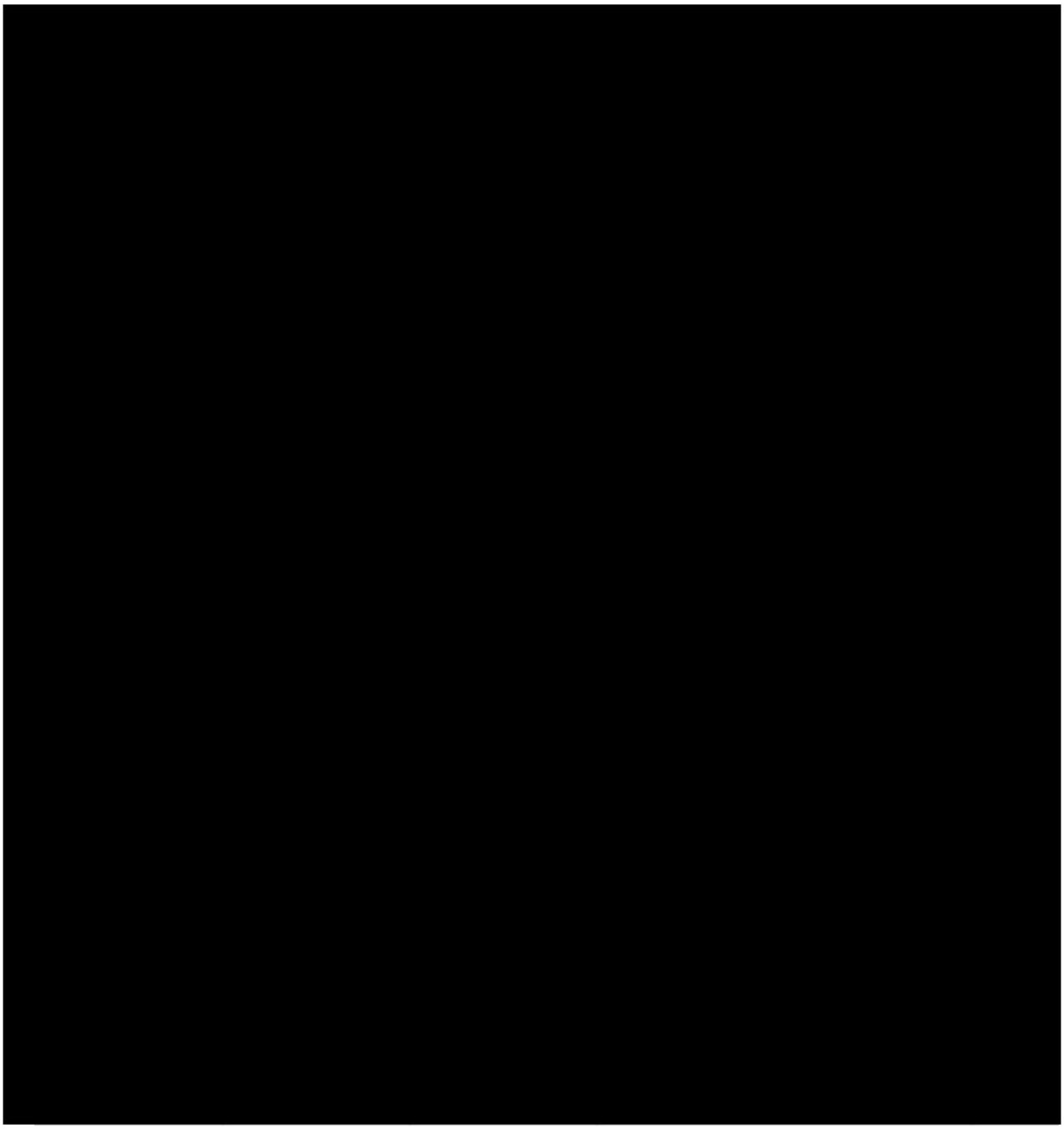
# 4. APPENDIX

## 4.1 Key variables for administrative interim analysis

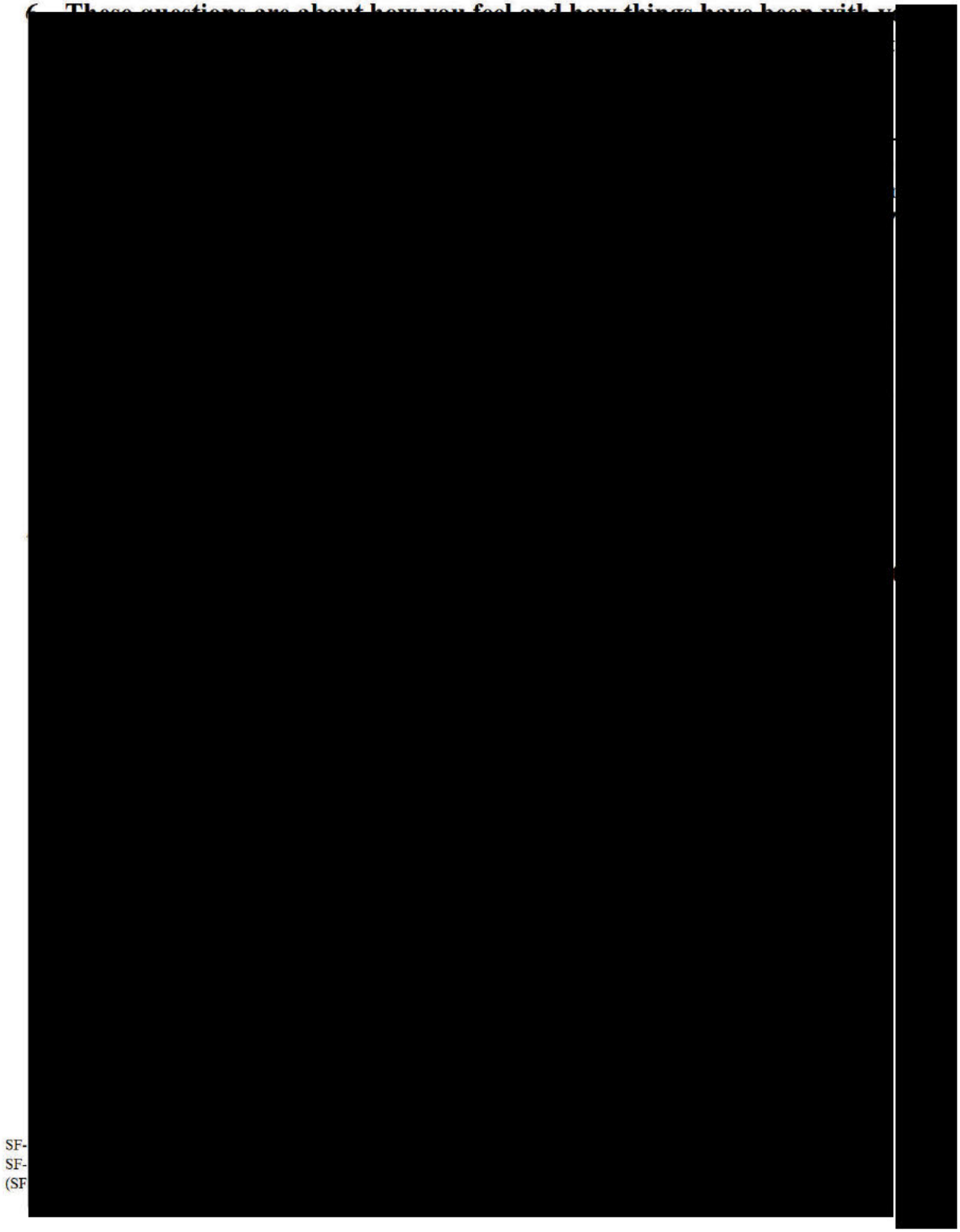
| Variable | Definition |
|---|---|
| CR1SIRPM | IRP in supine position from first recording (this value is the median from the first set of 10 supine swallows) |
| CR2SIRPM | IRP in supine position from second recording; not all subjects will have this (this value is the median from the second set of 10 supine swallows) |
| CR1UIRPM | IRP in upright position from first recording (this value is the median from the first set of 5 upright swallows) |
| CR2UIRPM | IRP in upright position from second recording; not all subjects will have this (this value is the median from the second set of 5 upright swallows) |

## 8.2 HEALTH OUTCOMES SURVEYS

### 8.2.1 Short Form 12 Health Survey Version 2



[redacted]



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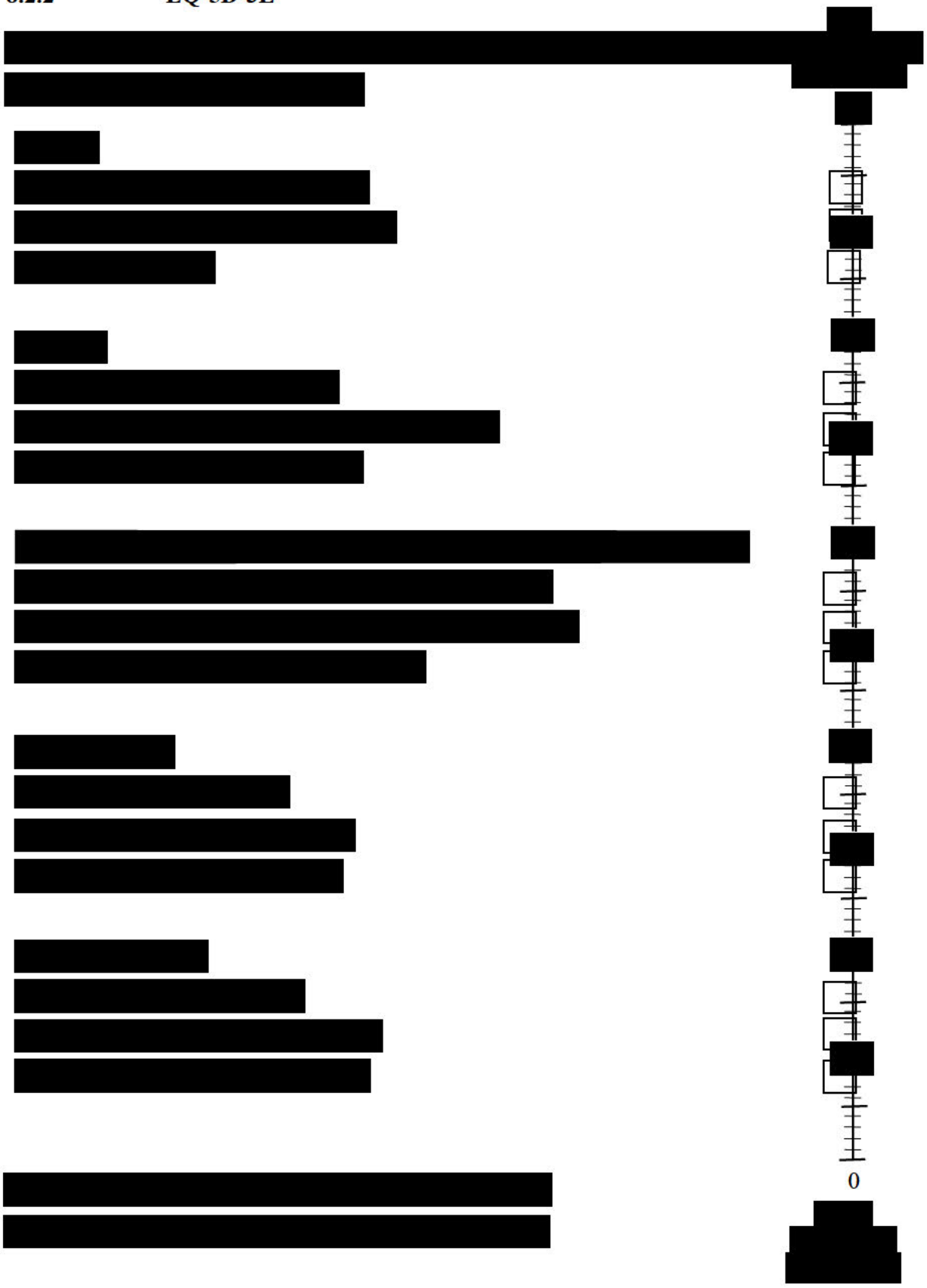
### 8.2.2 EQ-5D-3L



[redacted]

[redacted]

[redacted]

## 8.3 SITE DOCUMENTATION

**Vanderbilt University Medical Center**

Division of Gastroenterology, Hepatology and Nutrition

Vanderbilt Digestive Disease Center
Division of Gastroenterology and Hepatology
[redacted]

**NOTE-TO-FILE**

**Date:** June 28, 2018

**Protocol No.:** IW C1701-201

**Principal Investigator:** [redacted]

**Subject:** Protocol Deviation – Subject Eligibility - HRIM Recording) – Subject [redacted]

The purpose of this communication is to serve as documentation of summary of events leading to protocol deviation specific to the above-referenced subject.

Subject [redacted] for dosing visit. Per protocol, pre-dose HRIM was performed and all guidelines were followed per manual of procedures provided by Sponsor. During two-hour wait period between dosing and second HRIM procedures, manometry nurse realized that software did not save full recording of initial HRIM due to programming of maximum record time running total of 60 minutes. Record start time was 08:03 and record end time was 09:09. Therefore, the first six (6) minutes of recording was not saved and initial IRP measurements could not be calculated.

Lead CRA, [redacted] was on site for this study visit and was informed immediately of the issue. She contacted Sponsor representative, [redacted] and informed her of the issue and [redacted] conferred with Sponsor colleagues regarding next steps. Manometry nurse [redacted], communicated via telephone with [redacted] to inquire as to whether or not the data could be retrieved. Although it was determined that data could not be retrieved, representative was able to walk [redacted] through the necessary steps to extend recording time for subsequent recordings.

After deliberation, Site was informed by Sponsor that personnel should proceed with study visit per protocol, noting that failure to save initial recording in full would result in a protocol deviation as eligibility was based on pre-dose recording #2 only. Although Site secured data supporting upright IRP measurements from recording #1, criteria for confirmation of eligibility stated 'recording 1 median supine IRP > 15mmHg.

Site PI, [redacted] were both heavily involved in the communications that took place concerning this event and neither felt that the deviation posed increased risk to the research patient.

Post-dose HRIM was performed on time and all data were successfully recorded. Study visit proceeded without further complication.

[redacted]

6/21/18
Date