Statistical Analysis Plan: C1973-201-02

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Study Title:	An Open-label, Phase 2a Trial to Evaluate the Effect of Escalating Doses of IW-1973 on Tolerability, Endothelial Function, and Hemodynamics in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-201
Product Name:	IW-1973 Tablet
Sponsor:	Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142

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LIST OF ABBREVIATIONS

Abbreviation	Full Term
AE	adverse event
ALT	alanine aminotransferase
aPTT	activated partial prothrombin time
AST	aspartate aminotransferase
AUC	area under the curve
AUC_{tau}	Area under the plasma concentration time curve during a dosing interval
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
cGMP	cyclic guanosine 3', 5'-monophosphate
C_{max}	maximum observed plasma concentration
C_{trough}	trough plasma concentration observed at the end of a dosing interval (collected before the next administration)
CV	coefficient of variation
ECG	Electrocardiogram
eCRF	electronic case report form
GGT	gamma glutamyl transferase
HbA1c	Hemoglobin A1c (glycated hemoglobin)
HIV	human immunodeficiency virus
HPF	high power field
Kg	Kilogram
LDH	lactate dehydrogenase
MAP	mean arterial pressure
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
mL	Milliliter

Abbreviation	Full Term
mmHg	millimeters of mercury
MPV	mean platelet volume
PCS	potentially clinically significant
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	preferred term
RBC	red blood cell
RDW	red blood cell distribution width
RHI	reactive hyperemia index
SAE	serious adverse event
SAP	statistical analysis plan
SAS®	Statistical Analysis System
SOC	system organ class
$t_{1/2}$	apparent terminal phase half-life
tau	dosing interval
TEAE	treatment emergent adverse event
T_{max}	sampling time of maximum observed plasma concentration
WBC	white blood cell

1. INTRODUCTION

Study C1973-201 is a Phase 2a, open-label, single-center study in patients with Type 2 diabetes, hypertension and impaired endothelial function to evaluate the effect of escalating doses of IW-1973 on tolerability, endothelial function, and hemodynamics.

This statistical analysis plan (SAP) provides a more technical and detailed description of the data presentations and statistical analyses of the data as outlined and/or specified in the final protocol for Study C1973-201 (C1973-201-P-03, dated 09 January 2017). Specifications of tables, figures, and data listings are contained in a separate document.

2. STUDY OBJECTIVES

The objectives of the study are to evaluate the following in adult patients with stable type 2 diabetes mellitus and controlled hypertension:

- Safety and tolerability of escalating doses of IW-1973.
- Impact of escalating doses of IW-1973 on
    - endothelial function using EndoPAT™ to measure fingertip small vessel pulse volume
    - blood pressure (BP) and heart rate

3. STUDY DESIGN

3.1 GENERAL DESCRIPTION

This open-label, single-center trial will enroll 12 patients (at least 4 men and 4 women) to receive once-daily study drug for 6, sequential, 3-day dosing cycles (18 total days) starting with placebo (baseline) for 3 days and then progressing to 5 escalating dose levels of IW-1973 (10, 20, 30, 40, and 50 mg) for 3 days each. Patients will be in clinic for 20 days, from Check-in on Day -1 to Discharge on Day 19. Total patient participation will be 45 to 77 days.

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

In this open-label study, patients will undergo 3 days of placebo dosing before beginning dosing with IW-1973, thereby serving as their own control. This design is sufficient for the stated objectives of this study.

3.3 TREATMENTS ADMINISTERED

Patients will receive once-daily (QD) study drug in the morning (8 to 10 AM) for 6 sequential, 3-day dosing cycles starting with placebo (2 tablets) for 3 days and then progressing to 5 escalating dose levels of IW-1973 (10, 20, 30, 40, and 50 mg) administered as multiple 5 mg tablets for 3 days each.

Nitroglycerin, 0.4 mg, sublingual tablet, will be administered to all patients at the Follow-up Visit to test endothelium-independent vasodilation.

3.4 METHODS OF ASSIGNING PATIENTS TO TREATMENT GROUPS

All patients will receive placebo and 5 escalating dose levels of IW-1973.

3.5 BLINDING

This is an open-label study.

4. DETERMINATION OF SAMPLE SIZE

The sample size in this trial was determined outside of statistical considerations. A sample size of 12 patients is considered sufficient and is based on precedent set by prior studies of similar nature.

5. PHARMACOKINETIC, PHARMACODYNAMIC, AND SAFETY ASSESSMENTS

5.1 STUDY SCHEMATIC AND SCHEDULE OF ASSESSMENTS

The schedule of evaluations for Study C1973-201 is presented in the following table.

Table 1 Schedule of Events

Visit Days → Study Procedure ↓	Screening Period	Clinic Period					Follow-up Period	
	Screening Visit (Day -28 to -2)	Check-in Day -1	1st day of cycle Days 1, 4, 7, 10, 13, 16	2nd day of cycle Days 2, 5, 8, 11, 14, 17	3rd day of cycle Days 3, 6, 9, 12, 15, 18	Discharge Day 19	Follow-up Visit Day 32 (± 2 days)	End of Trial Visit Day 46 (± 3 days)
Informed consent signed	X							
Inclusion/exclusion evaluation	X	X						
Demographics	X							
Medical history	X							
Physical exam	X	X				X		X
HBsAg, HCV & HIV screen	X							
Drug & alcohol screen (a)	X	X					X	
Pregnancy test (b)	X	X					X	X
Weight (W) & height (H) (c)	W, H	W	W			W	W	W
12-lead ECG (d)	X	X	pre: 0 (≤ 15m) pd: 1, 4h (± 15m)			X		
Respiratory rate & oral temp (e)	X	X				X	preNG: 0 (≤ 15m)	X
Clinical chemistry, coagulation, hematology, urinalysis (f)	X	X				X	preEndoPAT (≥1h)	
Hemoglobin A1c	X	X				X	preEndoPAT (≥1h)	
Fasting blood glucose & serum insulin (g)	X	X		pre: 0 (≤15m)		X	preEndoPAT (≥1h)	
Adverse event evaluations	X	X	X	X	X	X	X	X
Prior & concomitant meds	X	X	X	X	X	X	X	X
EndoPAT (h)	X	X			pre: 0 (≤ 1h) pd: 4, 12h (±15m)		preNG: 0 (≤ 1h) pdNG: 45m(±15m)	
Supine/standing BP & pulse supine only (i)	X	X	pre: 0 (≤ 30m) pd: 1,2,4,8,12h (±10m)		Supine only pre: 0 (≤ 30m) pd: 1,2,8h (±10m)	24h (± 15m)	Supine only preNG: 0 (≤ 30m) pdNG: 2h (+30m)	X

Visit Days → Study Procedure ↓	Screening Period	Clinic Period					Follow-up Period	
	Screening Visit (Day -28 to -2)	Check-in Day -1	1st day of cycle Days 1, 4, 7, 10, 13, 16	2nd day of cycle Days 2, 5, 8, 11, 14, 17	3rd day of cycle Days 3, 6, 9, 12, 15, 18	Discharge Day 19	Follow-up Visit Day 32 (± 2 days)	End of Trial Visit Day 46 (± 3 days)
[redacted]		[redacted]						
Platelet function (k)			pre: 0 (≤ 15m) pd: 4h (± 15m)					
Pharmacokinetic samples (l)			pre: 0 (≤ 15m) pd: 1,3,6,12h (± 5m)	pre: 0 (≤15m)		24h (± 15m)	preEndoPAT (≥1h)	X
[redacted]			[redacted]			[redacted]	[redacted]	[redacted]
[redacted]			[redacted]			[redacted]	[redacted]	[redacted]
Study drug administration (o)			X	X	X			
Ambulatory BP monitoring				Start: pre 0(≤30m) End: pd12h(+30m)				
Confined to clinic		X	X	X	X			
Discharge from clinic						X		
Nitroglycerin administration (p)							X	
Study completion								X

BP = blood pressure; ECG = electrocardiogram; EDTA = ethylenediaminetetraacetic acid; h = hour; H = height; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; ICF = informed consent form; m = minute; NG = nitroglycerin; pd = postdose; PD = pharmacodynamic; pre = predose; W = weight

a. Urine drug screen for selected drugs of abuse and alcohol breathalyzer screen
b. For female patients, a negative serum pregnancy test must be documented at the Screening Visit, at Check-in with results available before dosing, and at the End of Trial Visit; a negative urine pregnancy test must be documented at the Follow-up Visit before nitroglycerin dosing.
c. On in-clinic days, in the morning after voiding, before any water or food intake
d. Patients must be supine for ≥ 5 m before the ECG recording (Note: if on initial ECG, QTcF is ≥ 450 msec for men or is ≥ 470 msec for women, 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 where applicable
e. Respiratory rate after the patient has been seated for ≥ 5 m
f. After ≥ 8-h fast, after EndoPAT and BP/pulse measurements and before dosing, except at Follow-up Visit; left arm preferred. Urinalysis may be performed prior to EndoPAT and blood pressure assessments.
g. Must fast ≥ 8 hours; left arm preferred

h. Always precedes PD BP/pulse measurements and blood collections, except at Follow-up Visit; ≥ 3 h after any caffeine-containing foods/beverages, ≥ 1 h after allowed concomitant medications, after opportunity to void and then sitting quietly in a temperature-controlled room for ≥ 15 m; occlusion/finger measurement right arm/hand
i. At Screening Visit & Check-in, supine BP is average of 3 measurements obtained at 2-m intervals after the patient has been lying quietly for ≥ 5 m; thereafter, 1 measurement after the patient has been lying quietly for ≥ 5 m.
For supine to standing: patient must lie quietly for ≥ 5 m before supine measurements are taken, then assume sitting position for 1 m, and finally assume standing position for 2 m before standing measurements are taken.
All measurements before blood draws where applicable; always left arm
[redacted]
k. ≈ 2.7-mL blood sample in citrate tube at each timepoint; left arm preferred
l. ≈ 2-mL blood sample in one K_2EDTA tube at each timepoint; each sample divided into 2 equal plasma aliquots; left arm preferred
[redacted]
o. In the morning, after overnight fast of ≥ 8 h, may take multiple tablets together; permitted concomitant morning medications should be taken at the same time. Breakfast begins ≤ 30 m after dosing.
p. Sublingual nitroglycerin 0.4 mg, administered according to label, food not allowed from 1 h before dosing to 30 m after dosing; patients should stay in the clinic for ≥ 2 h postdose.

5.2 PHARMACODYNAMIC ASSESSMENTS

The following hemodynamic, endothelial function and platelet function assessments will be performed throughout the study, as outlined in the Schedule of Assessments.

Ambulatory BP Monitoring:

Patients will undergo 12-hour ambulatory BP monitoring on the second day of each cycle, starting at predose (≤ 30 minutes) and ending at 12 hours postdose. 30-minute, 4-hour, and daytime averages of mean arterial pressure (MAP), systolic and diastolic BP, and pulse measurements will be calculated.

Supine and Orthostatic Pulse and BP Assessments:

Supine and standing BP and pulse measurements will be obtained on the first day of each cycle at 0, 1, 2, 4, 8, and 12 hours postdose. Supine measurements will also be obtained on the third day of each cycle at 0, 1, 2, 8, and 24 hours postdose, and at the follow-up visit prior to and 2 hours after the nitroglycerine dose.

For an individual patient, the supine BP and pulse determination at a particular timepoint will be based on a single measurement after the patient has been lying quietly for at least 5 minutes, and the standing assessment will be obtained 2 minutes after the patient has assumed the standing position after assuming sitting position for 1 minute after the supine assessment. The left arm will be used for BP measurements.

Orthostatic assessments, defined as the change in systolic and diastolic BP and pulse measurements after the patient has gone from the supine to the standing position, will be calculated.

Endothelial Function Assessments

Endothelial function in the finger will be measured using the noninvasive EndoPAT™ (Itamar Medical; Caesarea, Israel) device on the third day of each cycle at 0, 4, and 12 hours postdose, and at the follow-up visit prior to and 45 minutes after the nitroglycerine dose. All measurements will be performed in a quiet, dimly lit, temperature controlled room after the patient has been

resting quietly for 15 minutes. The right arm will be used for occlusion and finger measurements. Endothelial function will be assessed using the Reactive Hyperemia Index (RHI) parameter.

Platelet Function Assessments:

Blood samples for platelet function assessment using the PFA-100® instrument will be collected on the first day of each cycle. Platelet function will be assessed using time to adhesion /aggregation.

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[redacted]

5.3 PHARMACOKINETIC ASSESSMENTS

Blood samples for PK assessments will be collected as outlined in the Schedule of Events. If systemic levels of IW-1973 are detectable, and data support the calculations, the following PK parameters may be calculated:

- AUC_{tau}: Area under the plasma concentration time curve during a dosing interval (tau);
- C_{max}: Maximum observed plasma concentration, occurring at T_{max};
- C_{trough}: Trough plasma concentration observed at the end of a dosing interval (collected before the next administration)
- $t_{1/2}$: Apparent terminal phase half-life (whenever possible);
- T_{max}: Time of maximum observed plasma concentration;

5.4 SAFETY ASSESSMENTS

The following safety assessments are performed throughout the study, as indicated in the Schedule of Events.

- Adverse Events
- Physical Examination
- Height and Weight
- Vital Signs including oral temperature, respiratory rate, pulse, and cuff BP measurements
- Laboratory Parameters
    - Serum Chemistry: albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bicarbonate, blood urea nitrogen (BUN), calcium, chloride, cholesterol, creatinine, gamma glutamyl transferase (GGT), glucose, HDL-c, lactate dehydrogenase (LDH), LDL-c (calculated), magnesium, phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, uric acid.
    - Hematology: hematocrit, hemoglobin, platelet count, mean platelet volume (MPV), RBC count, WBC count, WBC differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils [% and absolute]), RBC indices (mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and mean corpuscular volume [MCV], red blood cell distribution width [RDW]).
    - Coagulation: activated partial thromboplastin time (aPTT), Prothrombin time, INR.
    - Urinalysis: pH and specific gravity, bilirubin, glucose, ketones, leukocytes, nitrates, occult blood, protein, urobilinogen, miscroscopic, including bacteria, RBCs, WBCs per high power field (HPF) only if dipstick is abnormal.
    - Additional tests: Hemoglobin A1c, fasting blood glucose, serum insulin
- 12-lead Electrocardiogram

6. STATISTICAL METHODS

6.1 GENERAL METHODOLOGY

Descriptive statistics (number of patients, mean, standard deviation, median, minimum and maximum) will be calculated for continuous variables. For summaries of continuous PK parameters, the geometric mean and the coefficient of variation (CV) will also be presented. For summaries of continuous PD parameters, the CV, 95% confidence intervals (CI), as well as the 25^{th} and 75^{th} percentiles will be presented. For categorical variables, frequencies and percentages for each category will be presented. Percentages will be based on the total number of patients with non-missing values. If there are missing values, the number missing will be presented, but without a percentage.

Inferential statistics, if calculated, will only be used for descriptive purposes.

All statistical analyses will be performed using SAS Version 9.1 or later [1]. Additional analyses using alternative software may be performed as part of the pharmacokinetic analysis.

Summary data will be presented by cycle group:

- Placebo
- IW-1973 10 mg
- IW-1973 20 mg
- IW-1973 30 mg
- IW-1973 40 mg
- IW-1973 50 mg

Definition of Baseline:

The following definitions of baseline will be used to calculate change from baseline parameters, unless specified otherwise-

- Study baseline is defined as the last non-missing assessment before first administration of study drug, that is, the Day -1 assessment.

- Time-matched baseline for each day/timepoint in the IW-1973 cycles is defined as the corresponding assessment during the placebo cycle.

- Cycle baseline is defined as the assessment taken prior to drug administration at the beginning of each cycle.

- Trough baseline is defined as the last assessment prior to initiation of IW-1973 administration, that is, the Study Day 4 predose assessment.

6.2 ADJUSTMENTS FOR COVARIATES

There are no adjustments for covariates planned for this study.

6.3 HANDLING OF DROPOUTS OR MISSING DATA

Missing observations will be treated as missing at random, and no imputation will be performed. All safety and tolerability data will be summarized and analyzed when data values are available for a patient. With respect to PK data, parameters will be derived as defined in Section 5.3 and although these methods may employ some inherent estimation of missing values (i.e., calculation of AUC), no formal imputation methods will be performed for the study.

6.4 INTERIM ANALYSIS AND DATA MONITORING

No formal interim analyses are planned to compare treatment arms (cycle groups) with respect to efficacy or safety prior to formal completion of the trial.

6.5 MULTICENTER STUDIES

This study is conducted at a single center.

6.6 MULTIPLE COMPARISONS/MULTIPLICITY

No multiple comparison adjustments are planned for the safety and tolerability analyses, which are qualitative in nature. No multiple comparison adjustments will be employed for the PK or PD analyses, as the analyses of these endpoints focus more on estimation rather than inferential testing.

6.7 USE OF AN EFFICACY SUBSET OF PATIENTS

Not applicable.

6.8 ACTIVE-CONTROL STUDIES INTENDED TO SHOW EQUIVALENCE

Not applicable.

6.9 EXAMINATION OF SUBGROUPS

Subgroup analyses of daytime ABPM assessments and adverse events will be performed by race (blacks versus non-blacks).

7. ANALYSIS METHODS

7.1 ANALYSIS POPULATIONS

The following analysis populations will be defined for the study:

Safety Population: The Safety Population will consist of all patients who received at least 1 dose of study drug and will be grouped according to actual study drug taken. All safety and tolerability assessments will be performed using the Safety Population.

PK Population: The PK Population will consist of all patients who received at least 1 dose of study drug and had at least 1 postdose PK parameter assessment. All analyses of PK data will be performed using the PK Population.

PD Population: The PD Population will consist of all patients who received at least 1 dose of study drug and had at least 1 postdose PD assessment. All analyses of PD data will be performed using the PD Population.

7.2 PROTOCOL DEVIATIONS

Protocol deviations will be listed by patient.

7.3 DISPOSITION OF PATIENTS

The number and percentage of patients who were included in each of the 3 analysis populations (Safety, PK, and PD), who completed each cycle, completed the study or discontinued early (along with the reasons for discontinuation) will be presented.

In addition, a data listing of patient disposition, including all patients who terminated early, will be presented.

7.4 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Patient demographics (age, sex, race, ethnicity, weight, height, and body mass index [BMI, defined as weight in kg divided by height in meters squared]) and other baseline characteristics will be summarized for the Safety Population.

If either the PD or PK Population differs from the Safety Population, the presentation will be repeated for the respective populations.

7.5 MEASUREMENTS OF TREATMENT COMPLIANCE

This is an in-clinic dosing study and, therefore no assessment of compliance is necessary. A data listing containing the study drug dosing information will be provided.

7.6 EXTENT OF EXPOSURE

This is an in-clinic dosing study and therefore no assessment of exposure is necessary, other than the data listing of study drug dosing information.

7.7 PHARMACODYNAMIC (PD) ANALYSIS

7.7.1 PD Parameters

The pharmacodynamic parameters are described below:

Ambulatory BP Monitoring

- Change from time-matched baseline in average daytime systolic BP, MAP, diastolic BP, and pulse. The daytime average for any IW-1973 cycle is defined as the average of the 30-minute average ambulatory assessments from the time of dosing on the ABPM assessment day to the end of the 12-hour ABPM recording for that cycle. Time-matched baseline is defined as the average of ambulatory assessments from the time of dosing on the ABPM assessment day of the Placebo cycle (Study Day 2) to the end of the 12-hour ABPM recording.

- Change from time-matched baseline in average daytime systolic BP, MAP, diastolic BP, and pulse by race (blacks vs. non-blacks)

- Change from time-matched baseline in 4-hour averages of MAP, systolic BP, diastolic BP, and pulse. The 4-hour averages are calculated from the time of dosing on the ABPM assessment day, using the 30-minute average values. Baseline is defined as the corresponding 4-hour average on Day 2 of the placebo cycle.

- Change from time-matched baseline in systolic BP, diastolic BP, MAP, and pulse at 30-minute postdose intervals. See Section 9.7 for details on calculation of assessment time intervals. Baseline is defined as the corresponding 30-minute average on Day 2 of the placebo cycle.

Supine Pulse and BP Measurements

- Change from study baseline in supine systolic BP, diastolic BP, and pulse
- Change from time-matched baseline (placebo cycle) in supine systolic BP, diastolic BP, and pulse
- Change from cycle baseline in supine systolic BP, diastolic BP, and pulse
- Change from trough baseline in trough systolic BP, diastolic BP, and pulse. The trough value for each cycle is defined as the 24 hour postdose assessment after the last dose of that cycle. Trough baseline is the last assessment prior to initiation of IW-1973 administration, that is, the Study Day 4 predose assessment
- Change from pre- to post-nitroglycerine dose assessments of supine systolic BP, diastolic BP, and pulse at the follow-up visit

Orthostatic BP Measurements

- Orthostatic changes in systolic BP, diastolic BP, and pulse. An orthostatic measurement is obtained by subtracting the supine measurement from the standing measurement.

Endothelial Function Assessments

- Change from study baseline in RHI
- Change from time-matched baseline (placebo cycle) in RHI
- Change from cycle baseline in RHI
- Change from pre- to post-nitroglycerine dose assessments of RHI at the follow-up visit

Platelet Function Assessments (PFA-100)

- Change from time-matched baseline (placebo cycle) in time to adhesion/aggregation
- Change from cycle baseline in time to adhesion/aggregation

[redacted]

[redacted]

7.7.2 PD Analysis

Descriptive statistics will be presented for the observed and change from baseline results for the PD parameters at each scheduled timepoint by cycle group. 95% confidence intervals of the mean difference will be included for summaries of change from baseline results.

For orthostatic BP parameters, descriptive statistics will be presented by cycle group for the change from supine to standing measurements at each scheduled timepoint.

7.7.3 Graphical Presentation of PD Data

Boxplots of change from time-matched baseline in daytime, and 4-hour averages of ambulatory mean arterial pressure, systolic BP, diastolic BP, and pulse will be presented by cycle group. Mean plots of change from time-matched baseline in 30-minute averages of ABPM assessments over time along with the corresponding 95% confidence intervals will be plotted by cycle group.

Mean plots of change from time-matched baseline in supine cuff blood pressure and pulse assessments over time, along with the corresponding 95% confidence intervals will be presented by cycle group. Individual patient plots of change from time-matched baseline in supine cuff blood pressure and pulse assessments as well as change from trough baseline in trough assessments of supine cuff blood pressure and pulse assessments will be plotted over time.

Individual patient plots of RHI values as well as trough RHI values will be plotted over time as patients escalate from placebo through the IW-1973 doses. Furthermore, boxplots of change from time-matched baseline in RHI values and platelet function assessments will also be presented by cycle group.

7.8 PHARMACOKINETIC (PK) ANALYSIS

7.8.1 PK Parameters

The PK parameters listed in Table 2 will be calculated for each patient, whenever possible, if systemic levels of IW-1973 are detectable. PK parameter calculations will be performed using standard methods in widely available software. Plasma concentrations that are below the LLQ will be treated as described in Section 6.3. Actual sampling times will be used in calculations of the PK parameters.

Table 2. Pharmacokinetic Parameters

Parameter	Description
AUC_{tau}	Area under the plasma concentration-time curve during a dosing interval (tau)
C_{max}	Maximum observed plasma concentration, occurring at T_{max}
C_{trough}	Trough plasma concentration observed at the end of a dosing interval (collected before the next administration)
$t_{1/2}$	Apparent terminal phase half-life
T_{max}	Time of maximum observed plasma concentration

7.8.2 PK Analysis

Plasma concentration of IW-1973 will be summarized using the PK Population for each assessment timepoint by cycle group. PK parameters will be summarized by cycle group using the PK Population.

7.8.3 Graphical Presentation of PK Data

Geometric mean plasma concentrations will be plotted over time by cycle group on a semi-logarithmic scale. Individual drug plasma concentrations will also be plotted over time. Scatterplots of individual C_{max}, AUC_{tau}, C_{trough}, and T_{max} values will be presented by cycle group for the PK population, with means and medians also presented on these graphs when possible.

7.8.4 PK-PD Analysis

In order to assess potential relationships between the PK assessments and the time course of the PD assessments, scatterplots of change from time-matched baseline in daytime averages of ambulatory assessments and time to adhesion (PFA-100) against C_{max}, C_{trough}, and AUC_{tau} will be included for each cycle group using the PK population.

Furthermore, to assess potential relationships between IW-1973 plasma concentrations and PD assessments, scatterplots of change from time-matched baseline in 30-minute averages of ambulatory blood pressure and pulse assessments and change from time-matched baseline in RHI versus nominal time-matched IW-1973 plasma concentration will be presented by cycle group for the PK population.

7.9 SAFETY ANALYSIS

All safety analyses will be performed using the Safety Population.

7.9.1 Adverse Events

All adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.1 and will be classified by MedDRA system organ class (SOC), and preferred term (PT).

Treatment-emergent adverse events (TEAEs) are those AEs that started or worsened in severity after the administration of study drug. If it is not possible to determine when the event started due to incomplete start dates/times, it will be assumed to be treatment emergent.

TEAEs will be assigned to the cycle in which they originated. That is, TEAEs that started on or after the first dose of study drug in a cycle but before dosing in the next cycle will be assigned to the treatment received in that cycle. Adverse events that occurred after the Nitroglycerin administration during the follow-up period will be summarized separately.

The number and percentage of patients who experience at least one TEAE as well as those who experience each specific SOC and PT will be presented by cycle group as well as overall using the Safety Population. Subgroup analyses of TEAEs will also be presented by race (blacks vs. non-blacks). Summaries of study drug related TEAEs will also be presented by cycle group.

Prevalent TEAEs in a cycle are defined as TEAEs that either started in or continued into a cycle, and will be presented by SOC and PT for each cycle group using the Safety Population.

For presentation of AE incidence, AEs will be sorted alphabetically by SOC, and within each SOC, by decreasing incidence of PT in the overall group.

In addition, TEAEs, severe TEAEs, drug-related AEs (those that are determined by the Investigator to be related to study drug), serious AEs, AEs that resulted in study discontinuation, and AEs that resulted in death (if any) will be presented in separate listings.

7.9.2 Clinical Laboratory Parameters

For each quantitative clinical laboratory parameter (including Hemoglobin A1c, fasting blood glucose, and serum insulin) descriptive statistics of the observed values as well as change from study baseline will be presented overall for each assessment timepoint. Change from time-matched baseline will also be presented for fasting blood glucose, and serum insulin by cycle group.

Parameter values will also be categorized as low, normal, or high based on reference ranges provided by the lab, and shifts from baseline to each later timepoint will be tabulated. If there is more than one measurement for a lab parameter at a postbaseline timepoint, only the last measurement will be used.

Individual patient plots of fasting blood glucose and serum insulin values will be plotted over time as patients escalate from placebo through the IW-1973 doses.

7.9.3 Vital Signs Parameters

Vital signs evaluations at each assessment timepoint not included in the PD analyses and the corresponding change from study baseline will be summarized for each cycle group.

The number and percentage of patients who had a notable change from baseline in BP and pulse (based on the criteria in Table 3), will be presented by cycle group.

Table 3. Criteria for Notable Changes in Post-Baseline Vital Signs

Vital Sign Parameter	***Flag***	***Criteria****	
		Observed Value	*Change from Study Baseline*
Supine/Standing Systolic Blood Pressure (mmHg)	High	≥ 180	Increase of ≥ 30
	Low	≤ 90	Decrease of ≥ 30
Supine/Standing Diastolic Blood Pressure (mmHg)	High	≥ 105	Increase of ≥ 20
	Low	≤ 50	Decrease of ≥ 20
Supine Pulse Rate (bpm)	High	≥ 110	Increase of ≥ 20
	Low	≤ 50	Decrease of ≥ 20
Standing Pulse Rate (bpm)	High	≥ 120	Increase of ≥ 30
	Low	≤ 50	Decrease of ≥ 20

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

The number and percentage of patients who meet the following criteria at any postdose timepoint will also be summarized by cycle group:

- Orthostatic decrease in systolic BP of > 20 mmHg from supine to standing
- Orthostatic decrease in diastolic BP of > 10 mmHg from supine to standing
- Orthostatic increase in pulse of > 20 beats per minute from supine to standing

7.9.4 ECG Parameters

For each ECG parameter, descriptive statistics will be presented by cycle group for each assessment timepoint. Change from study baseline (Day -1) statistics will also be presented for the predose assessment of each cycle. Shift tables from study baseline (Day -1) to the discharge visit (Day 19) for the overall ECG interpretation (normal, abnormal not clinically significant, or abnormal clinically significant) will be summarized for all subjects in the safety population. Change from time-matched baseline (placebo cycle) will be presented for QTc-F for each scheduled postdose assessment.

7.9.5 Other Parameters

7.9.5.1 Concomitant Medications

Any medication taken by patients after administration of study drug will be considered concomitant and presented in a data listing for the Safety Population.

7.9.5.2 Physical Examination

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

8. CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

The following analyses will be performed in addition to those described in the study protocol.

- Change from cycle baseline (defined as the assessment taken prior to drug administration at the beginning of each cycle) will be presented for cuff BP assessments (supine systolic BP, diastolic BP, and pulse), RHI assessments, and platelet function assessments.

- Change from trough baseline (defined as the last assessment prior to initiation of IW-1973 administration, that is, the Study Day 4 predose assessment) will be presented for trough supine systolic BP, diastolic BP, and pulse.

- Subgroup analyses by race (blacks vs. non-blacks) will be performed for daytime ABPM assessments and adverse events.

- Prevalent TEAEs in a cycle, defined as TEAEs that either started in or continued into a cycle, will be presented by SOC and PT for each cycle group using the Safety Population.

- Change from time-matched baseline (placebo cycle) will be presented for blood glucose, serum insulin and QTc-F for each scheduled postdose assessment.

- Change from study baseline in ECG assessments will be summarized only for the predose assessments in each cycle.

9. DATA HANDLING CONVENTIONS

9.1 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.

9.2 CONVENTIONS FOR SUMMARIZING ADVERSE EVENTS

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

- For patient incidence summaries by cycle group, each patient will be counted only once within each SOC, PT, or the overall AE summary within each cycle
- If a patient reported more than one AE within an SOC or PT within a particular cycle, 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.

9.3 MISSING DATE INFORMATION FOR ADVERSE EVENTS

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

9.4 MISSING SEVERITY ASSESSMENT 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.

9.5 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 date 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.

9.6 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.

9.7 ASSESSMENT TIME WINDOWS FOR AMBULATORY BP ANALYSIS

Table 4 presents the timepoints used for the ambulatory BP analysis and the corresponding assessment windows during which the actual assessment may have occurred.

Table 4 . Timepoint Windows for Ambulatory BP Analysis

Postdose Timepoints	*Assessment Window [1]*
30 min postdose	dosing to 0.5h + 15min on Cycle Day 2
1 h postdose	1h ± 15min on Cycle Day 2
1.5 h postdose	1.5h ± 15min on Cycle Day 2
…	…
12 h postdose	12h - 15mins to 12h postdose on Cycle Day 2

[1] Postdose timepoints are relative to dosing time on Cycle Day 2.

10. REFERENCES

1. SAS Institute Inc. 2015. SAS Version 9.4 Language Reference: Concepts, Fifth Edition. SAS Publishing, SAS Institute Inc., Cary, NC, USA.