Clinical Trial Protocol: C1973-202-P-03

Final Version, 16 March 2017

Study Title:	A Phase 2 Study to Compare the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of 2 Dose Regimens of IW-1973 in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-202
Study Phase:	2a
Product Name:	IW-1973 Tablet
Indication:	Type 2 diabetes with hypertension
Investigator(s):	up to 3 study centers
Sponsor:	Ironwood Pharmaceuticals, Inc.
Sponsor Contact:	[redacted]
Medical Monitor:	[redacted]

	Date
Original Protocol:	21 September 2016
Amendment #1	06 January 2017
Amendment #2	16 March 2017

Confidentiality Statement

The contents of this document are confidential and belong to Ironwood Pharmaceuticals, Inc. Except as may be otherwise agreed to in writing, by accepting or reviewing these materials, you (including any colleagues or subordinates) agree to hold such information in confidence and not to disclose it to others (except where required by applicable law), nor to use it for unauthorized purposes. In the event of actual or suspected breach of this obligation, Ironwood should be promptly notified.

STUDY IDENTIFICATION

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

Table 1. Key Study Participants

Role	Contact Information
Ironwood Contact:	[redacted] Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142 [redacted]
Medical Monitor:	[redacted] Ironwood Pharmaceuticals, Inc 301 Binney Street Cambridge, MA 02142 [redacted]
Safety Officer:	[redacted] Ironwood Pharmaceuticals, Inc 301 Binney Street Cambridge, MA 02142 [redacted]
Serious Adverse Event (SAE) E-mail:	[redacted]
[redacted]	[redacted]

SYNOPSIS

Sponsor

Ironwood Pharmaceuticals, Inc.

Name of Finished Product

IW-1973 Tablet

Name of Active Ingredient

IW-1973

Study Title

A Phase 2 Study to Compare the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of 2 Dose Regimens of IW-1973 in Patients with Stable Type 2 Diabetes and Hypertension

Study Number

C1973-202

Study Phase: 2a

Objectives

To compare the safety, tolerability, pharmacokinetic (PK) profile, and pharmacodynamic (PD) effects of 2 treatment regimens of IW-1973 Tablet (40 mg per day) administered orally for 2 weeks to patients with stable type 2 diabetes mellitus and hypertension

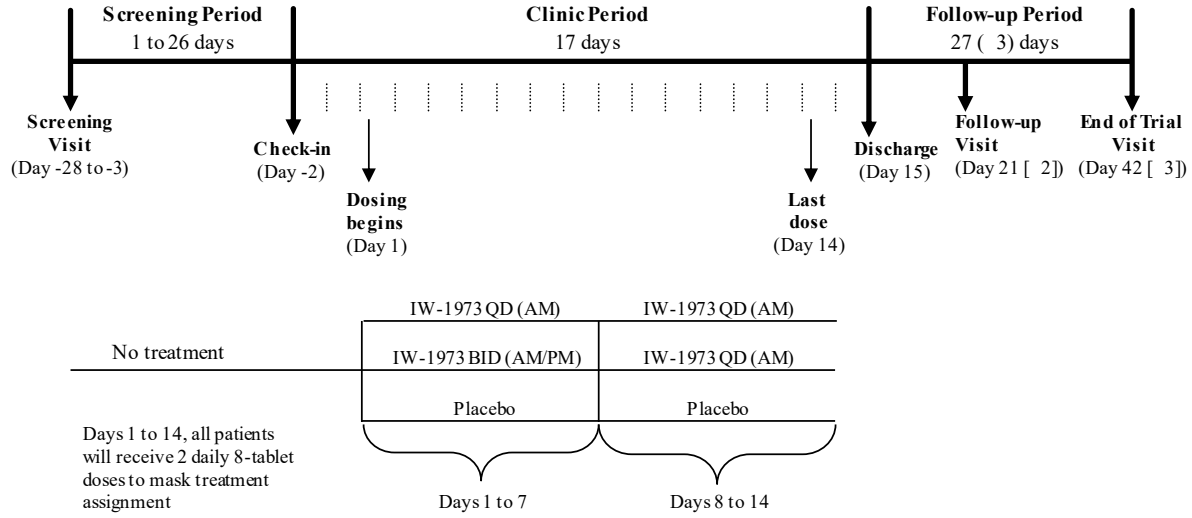
Study Design

This is a randomized, double-blind, placebo-controlled study consisting of 3 distinct periods (see Study Schematic). The study will enroll 26 patients (at least 11 male and 11 female) with stable type 2 diabetes and hypertension. Patients will be randomized in a 5:5:3 ratio to receive 1 of 2 IW-1973 treatment regimens or placebo (all regimens will be masked) as outlined in the table.

Treatment Arm	Dose time	Days 1 to 7	Days 8 to 14
QD/QD	AM	40 mg IW-1973	40 mg IW-1973
	PM	Placebo	Placebo
BID/QD	AM	20 mg IW-1973	40 mg IW-1973
	PM	20 mg IW-1973	Placebo
PBO/PBO	AM	Placebo	Placebo
	PM	Placebo	Placebo

Refer to Test Product, Dosage, and Mode of Administration for details on the number of IW-1973 and/or placebo tablets that will make up each dose.

Study Schematic



Study Population

The study will enroll 26 patients with

- Type 2 diabetes with a hemoglobin A1c (HbA1c) level of ≤ 10.5% and a fasting (≥ 8 hours) serum glucose level of ≤ 240 mg/dL on a regimen of ≥ 1 medication for glycemic control with no change in medication for ≥ 12 weeks before Check-in and on a stable regimen (ie, same drug and same dose) for ≥ 28 days before Check-in
- Hypertension with systolic BP of 120 to 160 mm Hg and diastolic BP of 70 to 100 mm Hg while on a stable regimen of ≥ 1 medication, which includes an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocking agent (ARB), for ≥ 28 days before Check-in

See Eligibility Criteria for full inclusion and exclusion criteria.

Test Product, Dosage, and Mode of Administration

Test product (IW-1973 Tablet, 5 mg) will be a 5-mg oral tablet

On Days 1 to 14, all patients will receive 2 daily 8-tablet doses (AM and PM), 12 hours (± 30 minutes) apart. Matching placebo tablets will be administered with IW-1973 Tablets when required to mask treatment assignments. The table outlines study drug dosage by week and time for each regimen/treatment arm.

Regimen/ Treatment arm	Dose time	Dosage Days 1 to 7	Dosage Days 8 to 14
QD/QD	AM	8 x IW-1973 Tablet	8 x IW-1973 Tablet
	PM	8 x placebo tablet	8 x placebo tablet
BID/QD	AM	4 x IW-1973 Tablet 4 x placebo tablet	8 x IW-1973 Tablet
	PM	4 x IW-1973 Tablet 4 x placebo tablet	8 x placebo tablet
PBO/PBO	AM	8 x placebo tablet	8 x placebo tablet
	PM	8 x placebo tablet	8 x placebo tablet

Reference Therapy, Dosage, and Mode of Administration

Reference therapy will be placebo to match IW-1973 Tablet, 5 mg.

Study Periods

The 26 patients may be enrolled separately. Each patient will progress through 3 study periods.

***Screening Period*:** The Screening Period will begin with the signature of the informed consent form (ICF) at the Screening Visit (which can occur from Day -28 to Day -3) and will last 1 to 26 days. At the Screening Visit, patients will undergo preliminary screening procedures to determine their eligibility for the study. The end of the Screening Period will coincide with the beginning of the Clinic Period at Check-in.

***Clinic Period*:** The Clinic Period will begin at Check-in on Day -2 (2 days before dosing) and will end at the time of Discharge on Day 15. During the 17-day Clinic Period, patients will be confined to the Study Center and will receive a standard diet for diabetics. Patients who meet eligibility criteria based on Screening Visit assessments will be admitted to the Study Center on Day -2 for baseline procedures. On the morning of Day 1 (there is no Day 0), eligible patients (at least 11 male and 11 female) will be randomized in a 5:5:3 ratio to 1 of 3 masked treatment regimens: QD/QD, BID/QD, or PBO/PBO. On Days 1 to 14, patients will receive a morning (AM) dose and an evening (PM) dose.

Safety, PK, and PD assessments, including blood collections, will be performed at specified times throughout the Clinic Period (see Criteria for Evaluation and Schedule of Events). On Day 15, after assessments have been completed, patients will be discharged from the Study Center at the Investigator's discretion.

***Follow-up Period*:** The Follow-up Period will begin immediately after Discharge from the Study Center on Day 15 and will last for 27 (± 3) days. On Day 21 (± 2 days), 7 (± 2) days after the last dose of study drug, patients will return to the Study Center for the Follow-up Visit (see Schedule of Events). On Day 42 (± 3 days), 28 (± 3) days after the last dose of study drug, patients will return to the Study Center for the End of Trial Visit (see Schedule of Events).

Study Drug Administration

All patients will receive 2 orally administered doses per day: an AM dose and a PM dose. Except for Day 13, patients will receive the AM dose at approximately the same time (± 15 minutes) every day in the morning (8 to 10AM) following an overnight fast of ≥ 8 hours. (Note: For each patient, the first dose on Day 1 may be administered between 8 and 10AM; thereafter, AM doses on Days 2 to 12 and on Day 14 must be administered within 15 minutes of the time of dosing on Day 1.) On Day 13, if EndoPAT is planned, the first dose should be administered in the morning between 7 and 10:30AM, after the EndoPAT assessment. Breakfast should begin within 30 minutes after dosing. Each patient will receive their PM dose 12 hours (± 30 minutes) after their AM dose and at least 30 minutes after completing a normal dinner.

Patients may take multiple tablets together. Permitted concomitant medications may be taken at the same time as study drug.

Stopping Criteria

All dosing will be stopped if the Sponsor and Investigator determine that any of the following have occurred:

- Drug-related serious adverse events (SAEs) in 2 or more patients on a given dosing regimen (per causality and SAE definitions in the protocol)
- An overall pattern of clinically significant AEs or an overall pattern of patient tolerability issues, which may appear minor in terms of an individual event but, in the opinion of the Sponsor or Investigator, collectively represents a safety concern.

Planned Number of Patients:

Up to 26 patients may receive study drug in this trial. Additional patients may be checked into the Study Center on Day -2 as backups. If the backup patients are not needed, they will be discharged from the Study Center before Randomization. Patients withdrawing from the study after Randomization will not be replaced.

Duration of Treatment

Patients will be in the Study Center for 17 days, from Check-in on Day -2 to Discharge on Day 15. Patients will receive oral doses of study drug for 14 consecutive days and will be followed in the Study Center for 24 hours after the last dose. Total patient participation will be 35 to 73 days, including the Screening, Clinic, and Follow-up Periods.

Criteria for Evaluation

Pharmacodynamics

Hemodynamics

Cuff BP and pulse measurements and ambulatory BP and pulse monitoring will occur according to the Schedule of Events. BP effects will be assessed using the following parameters:

- From supine measurements: change from baseline in pulse and systolic and diastolic BP measurements; proportion of patients with supine BP < 120 mm Hg and diastolic BP < 70 mm Hg
- From orthostatic measurements: change in systolic and diastolic BP and pulse measurements after patient has gone from the supine to the standing position
- From ambulatory monitoring: change from baseline in 24-hour, 4-hour, daytime, and nighttime averages of systolic and diastolic BP, mean arterial pressure, and pulse measurements

Endothelial Function Assessments

If available at the Study Center, endothelial function in the finger will be measured according to the Schedule of Events using the noninvasive EndoPAT device. All measurements will be performed in a quiet, dimly lit, temperature-controlled (21–24°C) room to reduce vascular tone fluctuation. The procedure will begin after the patient has had the opportunity to void and has been resting quietly for ≥ 15 minutes. Endothelial function will be assessed using the Reactive Hyperemia Index (RHI) parameter.

Platelet Function Testing

Blood samples for platelet function assessments will be collected according to the Schedule of Events.

Fasting plasma glucose and insulin

Blood samples for determination of fasting plasma glucose and insulin levels will be collected according to the Schedule of Events. Values will be used in the Homeostatic Model Assessment to estimate insulin resistance (HOMA-IR).

[redacted]

Pharmacokinetics

Blood samples for determination of plasma concentrations of IW-1973 will be collected according the Schedule of Events and the following PK parameters will be calculated, when possible:

- AUC_{last}: Area under the plasma concentration time curve from time zero to T_{last}, the time at which the last measurable plasma concentration (C_{last}) is observed
- AUC_{tau}: Area under the plasma concentration time curve during a dosing interval (tau)
- AUC_{inf}: Area under the plasma concentration time curve extrapolated to infinity
- 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)
- CL/F: Apparent total body clearance after oral administration
- $t_{1/2}$: Apparent terminal phase half-life
- T_{max}: Time of maximum observed plasma concentration
- V_z/F: Apparent volume of distribution during the terminal phase after oral administration

Safety

Physical examination, vital sign parameters, 12-lead electrocardiograms (ECGs), clinical laboratory evaluations (including clinical chemistry, hematology, coagulation, urinalysis), and patient- and Investigator-reported AEs will be evaluated. Estimated glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation and urine albumin creatinine ratio (UACR; urine albumin [mg/dL] / urine creatinine [g/dL]) will be calculated.

Statistical Methods:

Sample Size Determination

Sample size for this trial was determined outside statistical considerations.

Analysis Populations:

All patients who receive at least 1 dose of study drug will be included in the Safety Population.

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

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

Statistical Analysis

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

Pharmacodynamic Analyses

Summary statistics will be provided by treatment for supine and ambulatory BP and pulse rate values, endothelial function testing (RHI value), platelet function assessment results, and HOMA-IR values; changes from baseline in these variables will be compared across treatment groups. Summary statistics will be provided by treatment for the proportion with postdose supine BP less than 130/80 mm Hg.

Pharmacokinetic Analyses

If plasma concentrations of IW-1973 are detected, the PK parameters will be determined and tabulated, and summary statistics will be reported by treatment group.

Safety Analyses

Adverse events (AEs) will be summarized by system organ class (SOC), preferred term (PT), and treatment. Listings will be provided for severe AEs, drug-related AEs, SAEs, and AEs leading to study discontinuation. Descriptive statistics will be calculated on the safety parameters (ECG, vital signs, and clinical laboratory tests, eGFR, UACR) by treatment.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

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

1. Patient has signed an ICF before any study-specific procedures are performed.
2. Patient is an ambulatory male or female aged 30 to 75 years at the Screening Visit.
3. Female patient is not pregnant or breastfeeding at the time of the Screening Visit and Check-in. Negative serum pregnancy tests must be documented at the Screening Visit and at Check-in before dosing.
4. Female patients must be postmenopausal (no menses for 12 consecutive months), surgically sterile (ie, bilateral oophorectomy, hysterectomy, or tubal ligation), or, if of childbearing potential, agree to use 1 of the following methods of birth control from the date they sign the ICF until after the End of Trial Visit:
   a. Combination of 2 highly effective birth control methods (eg, condom with spermicide plus intrauterine device, condom with spermicide plus a diaphragm or cervical cap, hormonal contraceptive [including progesterone implant] combined with a barrier method)
   b. Maintenance of a monogamous relationship with a male partner who has been surgically sterilized by vasectomy (vasectomy procedure must have been conducted ≥ 60 days before the Screening Visit or confirmed via sperm analysis) plus a hormone or barrier method
5. Patient's body mass index (BMI) score is > 20 and < 40 kg/m^2 at the Screening Visit.
6. Patient's health is stable with no clinically significant findings on a physical examination, 12-lead ECG, alcohol breathalyzer, and clinical laboratory tests (serum chemistry, hematology, coagulation, urine drug screen, and urinalysis) that would prevent participation in the trial. (Note: The Investigator will determine if a particular finding is clinically significant. In making this determination, the Investigator will consider whether the particular finding could prevent the patient from performing any of the protocol-specified assessments, could represent a condition that would exclude the patient from the study, could represent a safety concern if the patient participates in the study, or could confound the study-specified assessments.)
7. Patient has type 2 (ie, adult onset) diabetes mellitus diagnosed by a physician or nurse practitioner ≥ 6 months before the Screening Visit and meets all of the following:
   a. Has been on a regimen of ≥ 1 medication for glycemic control, which may include long-acting insulin, with no change in medication for ≥ 12 weeks before Check-in and on a stable regimen (ie, same drug and same dose) for ≥ 28 days before Check-in with no

indication that the regimen will need to be changed for the duration of the study. Modification of short-acting insulin throughout the Screening Period will not affect eligibility. During the Clinic Period, per Investigator discretion, doses of supplemental short-acting insulin may be varied as needed to achieve adequate glycemic control.

b. Has HbA1c level ≤ 10.5% and fasting (≥ 8 hours) serum glucose level ≤ 240 mg/dL at the Screening Visit and at Check-in. Glucose value from serum chemistry panel; at Investigator discretion, if fasting serum glucose is > 240 mg/dL, the test may be repeated for determination of eligibility

c. Has, in the clinical judgement of the Investigator, sufficient diabetes stability to participate in the trial

8. Patient has hypertension diagnosed by a physician or nurse practitioner ≥ 6 months before the Screening Visit and meets all of the following:

   a. Has been on a stable regimen of ≥ 1 medication to control hypertension for ≥ 28 days before Check-in with no indication that the regimen will need to be changed for the duration of the study. The medication(s) must include an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocking agent (ARB) and may include diuretics and/or calcium channel blockers. Other antihypertensive agents may be acceptable per the Investigator's discretion. (See Exclusion Criteria for prohibited medications.)

   b. Has supine systolic blood pressure (BP) of 120 to 160 mm Hg and supine diastolic BP of 70 to 100 mm Hg at the Screening Visit. Eligibility will be based on the average of 3 measurements.

   c. Has, in the clinical judgement of the Investigator, sufficient hypertension stability to participate in the trial.

9. Patient agrees to adhere to the study requirements.
10. Patient has a negative hepatitis panel (hepatitis B surface antigen [HBsAg] and antihepatitis C virus [HCV]) and human immunodeficiency virus (HIV) antibody at the Screening Visit.
11. Patient agrees to refrain from making any major lifestyle changes (eg, changing his or her exercise pattern) from the time of signature of the ICF to the End of Trial Visit.

EXCLUSION CRITERIA

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

1. Patient has a clinically significant active or unstable medical condition that, in the opinion of the Investigator, would preclude trial participation, including active or unstable metabolic; hepatic; renal; hematological; pulmonary; cardiovascular; gastrointestinal; musculoskeletal; dermatological; urogenital; eye, ear, nose, and throat; psychiatric; or neurological conditions.
2. Patient is on medication(s) that when co-administered with a soluble guanylate cyclase (sGC) stimulator, could increase the risk of hypotension. These include (but may not be limited to) nitrates, nitroglycerin, direct vasodilators (including hydralazine or systemic minoxidil), phosphodiesterase (PDE) 5 inhibitors (including sildenafil, tadalafil, and vardenafil), alpha adrenergic blockers, riociguat, and sodium-glucose co-transporter 2 (SGLT2) inhibitors. Patients should not take these medications from 6 days before Check-in to the End of Trial Visit.
3. Patient has evidence of severe or active end-organ damage attributable to diabetes (eg, active diabetic nephropathy, retinopathy, or neuropathy) at the Screening Visit or Check-in.
4. Patient has evidence of active end-organ morbidity associated with uncontrolled hypertension (eg, progressive kidney insufficiency, myocardial infarction, or stroke) at the Screening Visit or Check-in. Patient has had an in-patient hospitalization for a cardiovascular, renal, or metabolic cause in the 6 months before the Screening Visit.
5. Patient has orthostatic decrease in systolic BP of > 20 mm Hg or orthostatic decrease in diastolic BP of > 15 mm Hg.
6. Patient has severe renal insufficiency (eg, current or past need for dialysis) has undergone renal transplantation, or has planned renal transplantation.
7. Patient has a history of malignancy, diagnosed or known to be active or actively treated within the past 5 years, other than resected lesions of low malignant potential, such as basal cell skin cancers.
8. Patient has bleeding diathesis or history of clinically significant bleeding episodes (eg, gastrointestinal bleed) in the 12 months before the Screening Visit.
9. Patient has a 12-lead ECG demonstrating severe bradycardia (heart rate < 40 beats per minute) or QTcF is ≥ 450 msec for male patients or is ≥ 470 msec for female patients at the Screening Visit. (Note: If on initial ECG, QTcF exceeds the limit, 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).
10. Patient has alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level > 2 times the upper limit of normal as defined by the laboratory or creatinine level > 1.5 times the normal as defined by the laboratory at the Screening Visit.

11. Patient has a history of clinically significant hypersensitivity or allergies to any of the inactive ingredients contained in the active or placebo drug products.
12. Patient has a history of active alcoholism or drug addiction during the year before the Screening Visit, or has a positive drug screen at the Screening Visit or at Check-in.
13. Patient has previously received IW-1973 in a study or has received an investigational drug during the 30 days or 5 half-lives of that investigational drug (whichever is longer) before the Screening Visit or is planning to receive another investigational drug at any time during the study.
14. Patient is an active smoker or has used any nicotine-containing products (cigarettes, e-cigarettes, vape pens, cigars, chewing tobacco, gum, patches) during the 6 months before Check-in. Use of nicotine is excluded during the study until after the End of Trial Visit. All positive nicotine tests will result in screen failure.
15. Patient has consumed grapefruit or grapefruit juice during the 72 hours before Check-in, taken vitamins or herbal supplements during the 7 days before Check-in, or taken any supplements for the treatment of erectile dysfunction during the 14 days before Check-in. Grapefruit, grapefruit juice, vitamins, herbal supplements, or any supplements for the treatment of erectile dysfunction are excluded during the study until after the End of Trial Visit.
16. Patient has consumed any alcohol-containing foods or beverages during the 7 days before Check-in. Use of alcohol-containing foods or beverages is prohibited from 7 days before Check-in through Discharge. In the clinic, patient may consume up to 2 cups of coffee or tea per day but not within 1 hour of study drug administration or within 3 hours before EndoPAT assessment.
17. Patient has donated blood products (including plasma and platelet donation) during the 6 weeks before Check-in.
18. Patient has received blood products during the 2 months before Check-in.
19. Patient has undergone a surgical procedure during the 30 days before Check-in, other than minor dermatologic procedures.
20. Patient has an acute or chronic condition that, in the Investigator's opinion, would limit the patient's ability to complete or participate in this clinical study.
21. Patient is involved in the conduct and administration of this study as an Investigator, sub-Investigator, study coordinator, other study staff, or Sponsor member.
22. Patient will not be able to adhere to the trial assessment schedule, or, in the clinical judgment of the Investigator, the patient is otherwise not suitable for trial participation.

NOTE: Patients may be rescreened should they discontinue in the Screening Period due to visit window deviations or other administrative reasons. In addition, laboratory values, ECG values, or BPs that are outside the range specified in the protocol may be repeated to confirm eligibility during the Screening period at the Investigator's discretion after consultation with the Medical Monitor.

SCHEDULE OF EVENTS

Study Period→		**Screening Period**	**Clinic Period**				
Visit Days → **Study Procedure ↓**		**Screening Visit** **Day -28 to -3**	**Check-in** **Day -2**	**Day -1**	**Day 1**	**Day 2**	**Days** **3-6**
ICF Signed		X					
Demographics		X					
Medical History		X					
Inclusion/Exclusion Evaluation		X	X				
Physical Exam		X	X				
Hepatitis (HBsAg, HCV) & HIV Screen		X					
Drug & Alcohol Screen (a)		X	X				
Pregnancy Test (b)		X	X				
Weight (W) & Height (H) (c)		W, H		W			
12-lead electrocardiogram (d)		X	X		preAM: 0 (≤30m) pdAM: 4 (±15m)		
Respiratory rate (R) & oral temperature (T)		X	X	X	T,R preAM: 0 (≤30m) T pdAM: 1, 4 h (±15m)	X	X
Supine/standing BP & pulse (e) [3] indicates triplicate supine BPs supine only	AM	X [3]	X	preD1: 0 (≤30m) [3] pdD1: 1, 4h (±5m)	pre: 0 (≤30m) pd: 1, 4, 8h (±5m)	pre: 0 (≤15m) pd: 1, 4h (±5m)	pre: 0 (≤15m)
	PM			preD1: 0 (≤15m) pdD1: 1, 4h (±5m)	pre: 0 (≤15m) pd: 1, 4h (±5m)	pre: 0 (≤15m) pd: 1, 4h (±5m)	
Hemoglobin A1c		X	X		preAM: 0 (≤15m)		
Fasting plasma glucose & serum insulin (f)		X	X		preAM: 0 (≤15m)		
Clinical chemistry, coagulation, hematology, urinalysis (f)		X	X		serum creatinine only preAM: 0 (≤15m)		
Urine albumin & creatinine (g)		X		first void			
AE Evaluations		X	X	X	X	X	X

Study Period→		**Screening Period**	**Clinic Period**				
Visit Days → **Study Procedure ↓**		**Screening Visit** **Day -28 to -3**	**Check-in** **Day -2**	**Day -1**	**Day 1**	**Day 2**	**Days 3-6**
Prior & concomitant medications		X	X	X	X	X	X
[redacted]			[redacted]				
Ambulatory BP monitoring (i)				Start: preD1: 0 (≤ 15m)	Continue	End: pd 12h [= preAM: 0(≤15m)]	
EndoPAT (j)				pre D13: 0 (end ≥30 m)			
[redacted]					[redacted]		
Randomization					X		
Study drug administration (l)	AM				X	X	X
	PM				X	X	X
PK blood samples (m)	AM				pre: 0 (≤ 15m) pd: 1, 3, 6h (± 5m)	…pd 6h (5m) pre: 0 (≤ 5m)	
	PM				pre: 0 (≤ 5m) pd: 1, 3h (± 5m)…	pre: 0 (≤ 5m)	
Platelet function (n)					preAM: 0 (≤15m)		
Confined to clinic			X	X	X	X	X
Discharge from clinic							
Study completion							

AM = morning; BP = blood pressure; h = hour; H = height; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; ICF = informed consent form; m = minute; msec = millisecond; pd = postdose; pdD1 = corresponding to postdose Day 1; PM = evening; pre = predose; preD1 = corresponding to predose Day 1; R = respiratory rate; T = oral temperature; 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 randomization, and at the Follow-up Visit. A urine pregnancy test must be documented at the End of Trial Visit.
c. On in-clinic days, in the morning after voiding, before any water or food intake
d. Patients must be supine for ≥ 5 minutes 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).
e. Triplicate supine to standing assessment (Screening Visit, predose on Day -1, and on Day 15) after the subject has been lying quietly for ≥ 5 m supine BP and pulse is the average of 3 measurements obtained at 2-m intervals, after final supine measurement subject assume sitting position for 1 m, and finally assume standing position for 2 m before standing measurements are taken Note: The change in blood pressure from supine to standing will be determined

using the third supine blood pressure and pulse measurements in comparison to the standing blood pressure and pulse measurements. Single supine to standing measurement assessed after the patient has been lying 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. Single supine measurements are assessed after the subject has been laying quietly for ≥ 5 m. Predose measurements should precede all oral and any antihypertensive concomitant medications. All measurements before blood draws where applicable. Note: Supine-only (without standing) pulse and BP may be taken at the 4-h post PM dose timepoints (approximately midnight) if, in the Investigator's clinical opinion, earlier postdose orthostatic (supine and standing) pulse and BPs for the given dosing and for previous dosings at the 4-hour postdose timepoint indicate it is safe to eliminate the standing (orthostatic) pulse and BP measurement.

f. After ≥ 8-h fast, after EndoPAT (when applicable) and BP/pulse measurements and before dosing, where applicable. Does not apply to urine collection.

g. At the Screening and Follow-up Visit, single urine sample, which may not be first void; during Clinic Period, first-void urine sample

[redacted]

i. ABPM assessments should be performed on the same arm of the subject throughout the study; non-dominant arm is preferred.

j. If EndoPAT is available at the Study Center, for each patient, the Day -1 and Day 13 assessments should be at approximately the same time of day (± 15 m). Before all oral and any antihypertensive concomitant medications and study drug, BP/pulse measurements (for PD/Safety), and blood collections; after opportunity to void and then resting quietly in a temperature-controlled room for ≥ 15 m; occlusion/finger measurement right arm/hand. See Section 3.7.4.2

[redacted]

l. 2 orally administered 8-tablet doses: an AM 8-tablet dose at ~ same time (± 15 m) every day after overnight fast of ≥ 8 hours on Days 1 to 12 and on Day 14. On Day 13, first dose may be administered in the morning between 7 and 10:30AM, after the EndoPAT assessment (if applicable). Breakfast should begin within 30 m after dosing. PM 8-tablet dose, 12 hours (± 30 minutes) after AM dose and ≥ 30 minutes after completing dinner. May take multiple tablets together. Permitted concomitant medications may be taken at the same time as study drug.

m. ≈ 2-mL blood sample in one K_2EDTA tube at each timepoint; each sample divided into 2 equal plasma aliquots

n. CPRS assessment ≈ 1.8-mL blood sample in one 3.2% citrate tube at each timepoint as well as ≈ 4.5-mL blood sample in one 3.2% citrate tube at each timepoint; VerifyNow Aspirin Test and VerifyNow PRU Test ≈ 2-mL blood sample in 3.2% citrate tube at each timepoint for each test.

SCHEDULE OF EVENTS (…CONTINUED)

Study Period→		**Clinic Period**				**Follow-up Period**	
Visit Days → **Study Procedure ↓**		**Day 7**	**Days 8-13**	**Day 14**	**Discharge Day 15**	**Follow-up Visit Day 21 (± 2)**	**End of Trial Visit Day 42 (± 3)**
ICF Signed							
Demographics							
Medical History							
Inclusion/Exclusion Evaluation							
Physical Exam					X		X
Hepatitis (HBsAg, HCV) & HIV Screen							
Drug & Alcohol Screen (a)						X	
Pregnancy Test (b)						X	X
Weight (W) & Height (H) (c)			Day 13 W		W	W	W
12-lead electrocardiogram (d)					X		
Respiratory rate (R) & oral temperature (T)		X	X	X	X	X	X
Supine/standing BP & pulse (e) [3] indicates triplicate supine BPs supine only	AM	pre: 0 (≤15m) pd: 1, 4h (±5m)	Days 8 & 13 pre: 0 (≤ 15m) pd: 1, 4 (± 5m) Days 9-12 pre: 0 (≤15m)	pre: 0 (≤15m)	pd: 12h (±15m) [3]	X	X
	PM		Day 8 & 13 pre: 0 (≤15m)				
Hemoglobin A1c			Day 8 preAM: 0 (≤15m)	preAM:0 (≤15m)	X		X
Fasting plasma glucose & serum insulin (f)			Day 8 preAM: 0 (≤15m)		X		
Clinical chemistry, coagulation, hematology, urinalysis; (f)					X		X
Urine albumin & creatinine (g)					first void		X
AE Evaluations		X	X	X	X	X	X
Prior & concomitant medications		X	X	X	X	X	X

Study Period→		**Clinic Period**				**Follow-up Period**	
Visit Days → **Study Procedure ↓**		**Day 7**	**Days 8-13**	**Day 14**	**Discharge Day 15**	**Follow-up Visit Day 21 (± 2)**	**End of Trial Visit Day 42 (± 3)**
[redacted]							
Ambulatory BP monitoring		Start: pre: 0 (≤15m)	End: Day 8 preAM: 0 (≤15m)	Start: preAM: 0 (≤15m)	End: pd: 12h (±15m)		
EndoPAT (j)			Day 13 preAM 0 (end ≥30m)				
[redacted]					[redacted]	[redacted]	[redacted]
Randomization							
Study drug administration (l)	AM	X	X	X			
	PM	X	X	X			
PK blood samples (m)	AM	pre: 0 (≤5m) pd: 1, 3, 6h (±5m)	Day 8 pre: 0 (≤5m) pd: 1, 3, 6h (±5m)	pre: 0 (≤5m) pd: 1,3,6h (±5m)	pd: 12h (±15m)	X	X
	PM	pre: 0 (≤5m)	Day 8 pre: 0 (≤5m)	pre: 0 (≤5m)			
Platelet function (n)			Day 8 preAM: 0 (≤15m)	preAM: 0 (≤15m)			
Confined to clinic		X	X	X			
Discharge from clinic					X		
Study completion							X

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
ABPM	ambulatory blood pressure monitoring
ACEi	angiotensin-converting enzyme inhibitor
AE	adverse event
ALT	alanine aminotransferase
ARB	angiotensin receptor blocking agent
AST	aspartate aminotransferase
AUC_{inf}	area under the plasma concentration time curve extrapolated to infinity (whenever possible)
AUC_{last}	area under the plasma concentration time curve from time zero to the last observation
AUC_{tau}	area under the plasma concentration time curve during a dosing interval (tau)
BMI	body mass index (kg/m^2)
BP	blood pressure
BUN	blood urea nitrogen
CBC	complete blood count
[redacted]	[redacted]
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)
CL/F	apparent clearance (clearance relative to bioavailability)
C_{max}	maximum observed plasma concentration
C_{trough}	trough plasma concentration observed at the end of a dosing interval
DNA	deoxyribonucleic acid
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate ($mg/mL/1.73\ m^2$)
eNOS	endothelial nitric oxide synthase
FDA	Food and Drug Administration
FPG	fasting plasma glucose
FPI	fasting plasma insulin

Abbreviation	Term
GCP	good clinical practice
GGT	gamma glutamyl transferase
GLP	good laboratory practice
HbA1c	hemoglobin A1c (glycated hemoglobin)
HBsAG	hepatitis B surface antigen
HCV	hepatitis C virus
HDPE	high-density polyethylene
HEK	human embryonic kidney
hERG	ether-a-go-go related gene
HIV	human immunodeficiency virus
HOMA-IR	homeostatic model assessment to quantify insulin resistance
HPF	high power field
IC_{50}	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IRB	Institutional Review Board
kg	kilogram
kg/m^2	kilograms/meters squared (body mass index)
LDH	lactate dehydrogenase
MAD	multiple ascending dose
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm Hg	millimeters of mercury
MPV	mean platelet volume
msec	millisecond
NO	nitric oxide

Abbreviation	Term
pd	postdose
PD	pharmacodynamic(s)
PDE	phosphodiesterase
PID	patient identification
PK	pharmacokinetic(s)
PT	preferred term
QTcF	QT interval corrected using Fridericia's formula
RBC	red blood cell
RDW	red blood cell distribution width
RHI	reactive hyperemia index
SAD	single ascending dose
SAE	serious adverse event
SAS®	Statistical Analysis System
Scr	serum creatinine
sGC	soluble guanylate cyclase
SGLT2	sodium-glucose co-transporter 2
[redacted]	[redacted]
SOC	system organ class
tau	dosing interval
$t_{1/2}$	apparent terminal phase half-life
T_{max}	time of maximum observed plasma concentration
UACR	urine albumin creatinine ratio
UGT	uridine diphosphate–glucuronosyl transferase
V_z/F	apparent volume of distribution (volume of distribution relative to bioavailability)
WBC	white blood cell

1. INTRODUCTION

1.1 BACKGROUND AND STUDY RATIONALE

The NO-sGC-cGMP (nitric oxide – soluble guanylate cyclase – cyclic guanosine 3',5'-monophosphate) pathway plays a critical role in the cardiovascular system. In the vascular endothelium, sGC is stimulated by NO to produce cGMP, which acts on downstream targets including cGMP-dependent protein kinases, phosphodiesterases, and ion channels. This leads to relaxation of the vascular smooth muscle and vasodilation. In addition, intracellular cGMP is involved in processes that affect vascular proliferation, fibrosis, and inflammation. Impaired NO-sGC-cGMP signaling, has been implicated in the pathogenesis of cardiovascular diseases such as systemic and pulmonary hypertension, coronary artery disease, congestive heart failure, peripheral vascular disease, atherosclerosis, and kidney disease. Agents that stimulate sGC to increase cGMP production (1,2) may be useful for treating and/or preventing a broad range of diseases that may ensue from dysfunction in the fundamental NO-sGC-cGMP pathway.(3,4)

[redacted]

This exploratory, proof-of-concept study will evaluate the safety, tolerability, PK, and PD of 2 dosing regimens of daily 40 mg IW-1973 administered orally for 2 weeks to patients with type 2 diabetes mellitus and hypertension. This study is designed to explore the tolerability profile of the MAD MTD, 40 mg IW-1973, in a patient population, and to explore whether tolerability can be improved with an initial week of BID dosing.

In addition to safety, tolerability, and PK, this study will evaluate the PD effects (hemodynamics and endothelial function) of daily 40 mg IW-1973 in patients with type 2 diabetes and hypertension, a population with likely dysfunction in the NO-cGMP-sGC pathway. Type 2 diabetes is a chronic metabolic disorder in which the body does not use insulin effectively, leading to hyperglycemia and glucose intolerance. The prevalence of diabetes in the United States (US) is 9.3%, or about 29.1 million people, and 95% of diagnosed cases are type 2.(5) This multifactorial disease is associated with numerous comorbidities, including hypertension, obesity, hyperlipidemia, chronic kidney disease, and cardiovascular disease. Nearly all diabetic patients have at least 1 comorbidity and almost 90% have at least 2.(6) Hypertension is the most common comorbid condition and may affect over 80% of patients with type 2 diabetes.(6) Hypertension in diabetic patients increases the risk of cardiovascular disease and associated complications such as stroke, coronary artery disease, and peripheral vascular disease. In addition, hypertension in diabetic patients also increases the risk of microvascular diseases including retinopathy, nephropathy, and neuropathy.(7,8) Many of the common comorbid disorders in diabetic patients are related to impairment in the NO-sGC-cGMP pathway; therefore, improving dysfunction in the NO-cGMP-sGC pathway could be useful in treating multiple disorders in these patients.

1.2 IW-1973 BACKGROUND

For a detailed description of the properties of IW-1973 and the results of the nonclinical and clinical studies conducted thus far, please refer to the most recent Investigator's Brochure.

.

2. STUDY OBJECTIVES

To compare the safety, tolerability, PK profile, and PD effects of 2 treatment regimens of IW-1973 Tablet (40 mg per day) administered orally for 2 weeks to patients with stable type 2 diabetes mellitus and hypertension.

3. INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN

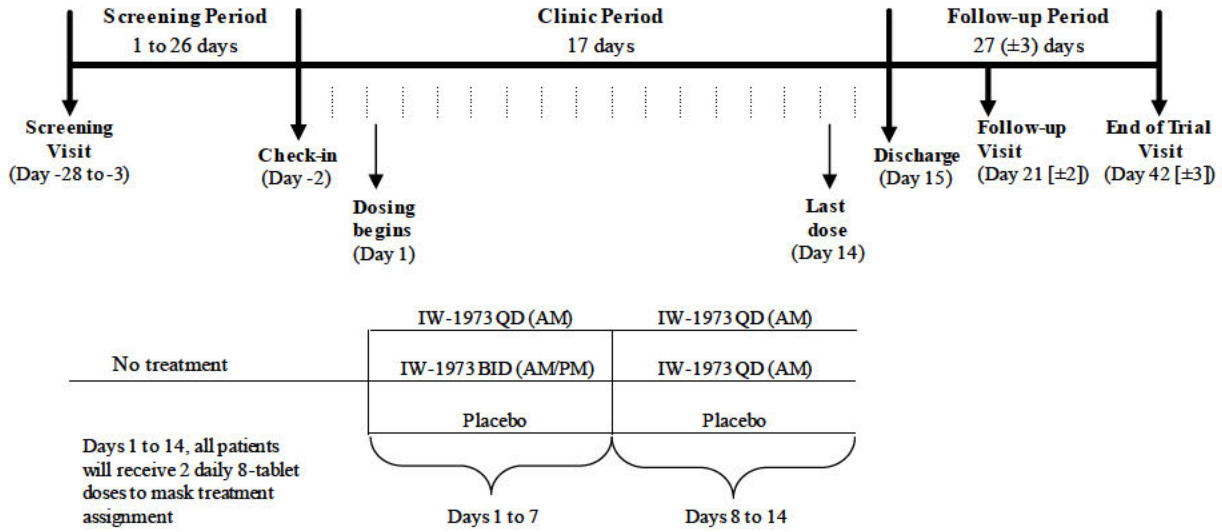
This is a randomized, double-blind, placebo-controlled study consisting of 3 distinct periods (see Figure 1). The study will enroll 26 patients (at least 11 male and 11 female) with stable type 2 diabetes and hypertension (see Eligibility Criteria). Additional patients may be checked into the Study Center on Day -2 as backups. If the backup patients are not needed, they will be discharged from the Study Center before Randomization. Patients withdrawing from the study after Randomization will not be replaced. Patients will be randomized in a 5:5:3 ratio to 1 of 2 IW-1973 treatment regimens or placebo (all regimens will be masked) as outlined in Table 2.

Table 2. Treatment Arm Dosing Regimens

Treatment Arm	Dose time	Days 1 to 7	Days 8 to 14
QD/QD	AM	40 mg IW-1973	40 mg IW-1973
	PM	Placebo	Placebo
BID/QD	AM	20 mg IW-1973	40 mg IW-1973
	PM	20 mg IW-1973	Placebo
PBO/PBO	AM	Placebo	Placebo
	PM	Placebo	Placebo

Refer to Section 3.5.5 for details on the number of IW-1973 and/or placebo tablets that will make up each dose.

Figure 1. Study Schematic



The 26 patients may be enrolled separately. Each patient will progress through 3 study periods.

Screening Period: The Screening Period will begin with the signature of the informed consent form (ICF) at the Screening Visit (which can occur from Day -28 to Day -3) and will last 1 to 26 days. At the Screening Visit, patients will undergo preliminary screening procedures to determine their eligibility for the study. The end of the Screening Period will coincide with the beginning of the Clinic Period at Check-in.

Clinic Period: The Clinic Period will begin at Check-in on Day -2 (2 days before dosing) and will end at the time of Discharge on Day 15. During the 17-day Clinic Period, patients will be confined to the Study Center and will receive a standard diet for diabetics. Patients who meet eligibility criteria based on Screening Visit assessments will be admitted to the Study Center on Day -2 for baseline procedures. On the morning of Day 1 (there is no Day 0), eligible patients will be randomized 5:5:3 to 1 of 3 masked treatment regimens: QD/QD, BID/QD, or PBO/PBO. On Days 1 to 14, patients will receive a morning (AM) dose and an evening (PM) dose. See Section 3.5.4 for details on dosing.

Safety, PK, and PD assessments, including blood collections, will be performed at specified times throughout the Clinic Period (see Schedule of Events). On Day 15, after assessments have been completed, patients will be discharged from the Study Center at the Investigator's discretion.

Follow-up Period: The Follow-up Period will begin immediately after Discharge from the Study Center on Day 15 and will last for 27 (± 3) days. On Day 21 (± 2 day), 7 (± 2) days after the last dose of study drug, patients will return to the Study Center for the Follow up Visit (see Schedule of Events). On Day 42 (± 3 days), 28 days after the last dose of study drug, patients will return to the Study Center for the End of Trial Visit.

3.2 DISCUSSION OF STUDY DESIGN INCLUDING THE CHOICE OF CONTROL GROUPS

A double-blind, placebo-controlled, randomized study design was chosen to investigate the safety PK, PD, and tolerability of 2 daily 40-mg IW-1973 dosing regimens. Patients will be randomized to ensure that the treatment groups are comparable and to minimize the potential for selection bias. The study will be double blind and treatments will be masked to ensure that the subjects and clinic staff are unaware of the dosing assignment and to minimize potential for bias in study assessments or in reporting of AEs. Placebo was chosen as the control so that the rate of spontaneously occurring AEs can be determined and to reduce the potential for bias in the reporting of AEs. Randomization will occur so that, within a particular cohort, an optimal number of subjects can be exposed to a particular dose of IW-1973, while at the same time ensuring that a reasonable number of subjects across the cohorts receive placebo for AE and PD comparisons. This design is in accordance with the concepts in International Conference on Harmonisation (ICH) E10, Choice of Control Groups and Related Issues in Clinical Trials (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2001).

Patients will be confined to the Study Center for the full duration of dosing through 24 hours after the final dose to allow monitoring of safety parameters and assessment of any PD effects of IW-1973. In addition, Stopping Criteria (Section 3.6) have been established to ensure that dosing will stop should a safety signal be detected. Patients will have a Follow-up Visit 7 (± 2) days after the final dose of study drug and an End of Trial Visit 28 (± 3) days after the final dose to determine if any AEs have developed and if any AEs that were ongoing at the time of Discharge have resolved.

3.3 STUDY DURATION

Patients will be in the Study Center for 17 days, from Check-in on Day -2 to Discharge on Day 15. Patients will receive oral doses of study drug for 14 consecutive days and will be followed in the Study Center for 24 hours after the last dose. Total patient participation will be 35 to 73 days, including the Screening, Clinic, and Follow-up Periods.

3.4 STUDY POPULATION SELECTION

3.4.1 Study Population

This study will enroll 26 patients (at least 11 male and 11 female) with type 2 diabetes and hypertension as defined below. Refer to Eligibility Criteria for full inclusion and exclusion requirements.

3.4.1.1 Type 2 Diabetes Requirement

Patients must have type 2 diabetes diagnosed by a physician or nurse practitioner no fewer than 6 months before the Screening Visit and must have been on a regimen of at least 1 medication specifically for control of glycemia, either oral or injectable, with no change in medication for at least 12 weeks before Check-in and have been on a stable regimen (ie, same drug and same dose) for at least 28 days before Check-in. To be eligible, patients must have a hemoglobin A1c (HbA1c) level of ≤ 10.5% and a fasting (≥ 8 hours) serum glucose level of ≤ 240 mg/dL.

All screened patients with HbA1c or serum glucose levels in the abnormal range (as defined by the laboratory) at any point during the trial will be informed of these abnormal lab values, and with the patient's permission, this information will be shared with a designated healthcare provider at the conclusion of their participation in the trial.

3.4.1.2 Hypertension Requirement

In addition to type 2 diabetes, patients must have hypertension diagnosed by a physician or nurse practitioner no fewer than 6 months before the Screening Visit and must have been on a stable medical regimen of at least 1 medication for BP control for at least 28 days before the Screening Visit; the medication(s) must include an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocking agent (ARB). Patients must have systolic BP of 120 to 160 mm Hg and diastolic BP of 70 to 100 mm Hg.

All patients with systolic pressures ≥ 140 mm Hg or diastolic pressures ≥ 90 mm Hg at the end of trial visit will be referred to their healthcare provider. All patients with systolic pressure ≥ 160 mm Hg or diastolic pressure ≥ 100 mm Hg at any point during the study, who, in the clinical judgement of the Investigator, are experiencing or at immediate risk for end-organ events will be immediately referred to an appropriate healthcare provider.

3.4.2 Removal of Patients from Therapy or Assessment

A premature discontinuation will occur when a patient who has signed the ICF and has been randomized ceases participation in the study, regardless of circumstances, before completion of Clinic Period. Patients who prematurely discontinue dosing should remain in the clinic for at least 24 hours after their final dose of study drug and should complete all Discharge day assessments. In addition, these patients should return to the Study Center for their Follow-up and End of Trial Visits, 7 (± 2) and 28 (± 3) days, respectively, after their final dose of study drug.

A patient will be considered to have completed the study after completing the End of Trial Visit.

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

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

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

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

3.4.3 Replacement Procedures

Patients withdrawing from the study after Randomization will not be replaced.

3.5 STUDY TREATMENT(S)

3.5.1 Study Drug

3.5.1.1 Investigational Product

The investigational product, IW-1973 Tablet, is a 5 mg oral tablet.

3.5.1.2 Placebo

Placebo will match the IW-1973 Tablet in appearance.

3.5.1.3 Packaging and Labeling

IW-1973 Tablet and placebo to match will be supplied to the site(s) in 60cc high-density polyethylene (HDPE) induction sealed bottles, 35 tablets per bottle. Bottles will be supplied as open label to the clinical site(s).

3.5.1.4 Storage and Accountability

IW-1973 Tablet and placebo to match will be shipped under refrigerated conditions, 2°C - 8°C (36 - 46°F).

IW-1973 Tablet and placebo to match must be stored under refrigerated conditions, 2°C - 8°C (36°F - 46°F) per the instructions in the study's Pharmacy Manual. Once opened the bottles will

be kept at room temperature storage (15 -30°C/59-86°F) for up to 30 days. Any deviation from these storage conditions must be reported to Ironwood and use of the study drug suspended until authorization for its continued use has been provided by Ironwood.

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

3.5.2 Method of Assigning Patients to Treatment Groups

Patients who meet all of the inclusion criteria and none of the exclusion criteria will be randomized to treatment on Day 1. Patients will be randomized in a 5:5:3 ratio to receive QD/QD IW-1973, BID/QD IW-1973, or placebo.

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

3.5.3 Selection of Dosage in the Study

This Phase 2a exploratory study will evaluate 40 mg IW-1973 administered daily for 2 weeks according to 2 different regimens: QD for both weeks or BID for the 1st week and QD the 2nd week. In the Phase 1b MAD study (ICP-1973-102), 40 mg IW-1973 was considered the maximum tolerated repeat dose, therefore, 40 mg was chosen for evaluation in this patient population.

3.5.4 Selection and Timing of Dose for Each Patient

All patients will receive 2 orally administered doses per day: an AM dose and a PM dose. Except for Day 13, patients will receive the AM dose at approximately the same time (± 15 minutes) every day in the morning (8 to 10AM) following an overnight fast of ≥ 8 hours. (Note: For each patient, the first dose on Day 1 may be administered between 8 and 10AM; thereafter, AM doses on Days 2 to 12 and on Day 14 must be administered within 15 minutes of the time of dosing on Day 1.) On Day 13, first dose should be administered in the morning between 7 and 10:30AM,

after the EndoPAT assessment (when applicable). Breakfast should begin within 30 minutes after dosing. Each patient will receive their PM dose 12 hours (± 30 minutes) after their AM dose and at least 30 minutes after completing a normal dinner.

Patients may take multiple tablets together. Permitted concomitant may be taken at the same time as study drug.

3.5.5 Dosage

On Days 1 to 14, all patients will receive 2 daily 8-tablet doses (AM and PM), 12 hours (± 30 minutes) apart.

Matching placebo tablets will be administered with IW-1973 Tablets when required to mask treatment assignments. Table 3 outlines study drug dosage by week and time, for each regimen/treatment arm.

Table 3. Dosage by Week and Time for Each Treatment Arm

Regimen/ Treatment arm	Dose time	Dosage Days 1 to 7	Dosage Days 8 to 14
QD/QD	AM	8 x IW-1973 Tablet	8 x IW-1973 Tablet
	PM	8 x placebo tablet	8 x placebo tablet
BID/QD	AM	4 x IW-1973 Tablet 4 x placebo tablet	8 x IW-1973 Tablet
	PM	4 x IW-1973 Tablet 4 x placebo tablet	8 x placebo tablet
PBO/PBO	AM	8 x placebo tablet	8 x placebo tablet
	PM	8 x placebo tablet	8 x placebo tablet

3.5.6 Blinding

This study is double blind and placebo controlled. The patients and Sponsor will be blinded to treatment assignments. Except for specifically designated unblinded Study Center pharmacy staff, the Investigator and remaining site study staff will be blinded as to treatment. The investigational product and placebo will be matching oral tablets.

Site unblinding of a subject's treatment assignment is restricted to emergency situations. In the scenario that the Investigator must unblind a subject, the Investigator, or person designated by the Investigator, should contact Ironwood's Medical Monitor directly to discuss the need for emergency unblinding. Individual sealed unblinding envelopes, which can be opened to identify the treatment assignment for an individual subject in an emergency, will be provided to the Study Center pharmacist. The reason for breaking the blind must be documented in the subject's source documentation and eCRF.

3.5.7 Concomitant Medications

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

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

Permitted concomitant medications should be taken at the same time (± 15 minutes) each day and may be taken at the same time as study drug. All oral and any antihypertensive concomitant medications should be taken after EndoPAT (when applicable) and after predose BP assessments.

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

3.5.8 Restrictions

3.5.8.1 Prior Therapy and Prohibited Medications

Medication(s) that when co-administered with an sGC stimulator could increase the risk of hypotension are prohibited from 6 days before Check-in through the End of Trial Visit. These medications include (but may not be limited to) nitrates, nitroglycerin, direct vasodilators (including hydralazine or systemic minoxidil), phosphodiesterase (PDE) 5 inhibitors (including sildenafil, tadalafil, and vardenafil), alpha adrenergic blockers, riociguat, and sodium-glucose co-transporter 2 (SGLT2) inhibitors.

3.5.8.2 Vitamins and Supplements

All vitamins and herbal supplements are prohibited within 7 days before Check-in through the End of Trial Visit. Supplements for the treatment of erectile dysfunction are prohibited within 14 days before Check-in through the End of Trial Visit.

3.5.8.3 Fluid and Food Intake and Nicotine-containing Product Use

The following food, fluid, and tobacco restrictions apply:

- All nicotine-containing products (eg, cigarettes, e-cigarettes, vape pens, cigars, chewing tobacco, gum, patches) are prohibited within 6 months before Check-in through the End of Trial Visit.
- All alcohol-containing foods or beverages are prohibited within the 7 days before Check-in through Discharge.
- Patients may consume up to 2 cups of coffee or tea per day but not within 1 hour of study drug administration or within 3 hours before EndoPAT assessments (when applicable).
- Grapefruit and grapefruit juice are prohibited within 72 hours before Check-in through the End of Trial Visit.
- Food is not allowed for 8 hours before AM study drug administration. Breakfast should begin within 30 minutes of AM study drug administration. Dinner should be completed at least 30 minutes before PM dose.
- Food is not allowed 8 hours before clinical laboratory sample collections.
- While in clinic, patients will follow a standard diet for diabetics.

Because poppy seeds can sometimes cause a positive result on the drugs of abuse test, subjects are advised to avoid eating poppy seeds or foods containing poppy seeds for at least 48 hours before the drug screen at the Screening Visit, Check-in, and Follow-up Visit.

The subject's food consumption will be recorded in the subject's source documentation.

3.5.8.4 Patient Activity Restrictions

The following subject activity restrictions apply:

- Subjects are not to donate blood within the 6 weeks before Check-in.
- Subjects are not to have received blood products within the 2 months before Check-in.
- Subjects are not to have undergone a surgical procedure (other than minor dermatologic procedures) during the 30 days before Check-in.
- Subjects should refrain from making any major lifestyle changes (eg, changing his or her exercise pattern) from the time of signature of the ICF to the End of Trial Visit.
- Female subjects are not breastfeeding.

3.6 STOPPING CRITERIA

All dosing will be stopped if the Sponsor and Investigator determine that any of the following have occurred:

- Drug-related serious adverse events (SAEs) in 2 or more patients on a given dosing regimen (per causality and SAE definitions in the protocol)
- An overall pattern of clinically significant AEs or an overall pattern of patient tolerability issues, which may appear minor in terms of an individual event but, in the opinion of the Sponsor or Investigator, collectively represents a safety concern.

3.7 STUDY PROCEDURES

3.7.1 Informed Consent

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

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

Before entry into the study, each patient will be provided with a written explanation of the study. It is the responsibility of the Investigator or appropriately trained health professional to give each patient full and adequate information regarding the objectives and procedures of the study and

the possible risks involved. Patients will then be given the opportunity to ask questions and the Investigator will be available to answer questions as needed. Patients will be informed of their right to withdraw from the study at any time without prejudice. After this explanation and before entering the study, the patient will voluntarily sign an ICF. The patient should receive a copy of the signed and dated ICF. The Investigator must retain each patient's original signed ICF.

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

3.7.2 Medical History

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

3.7.3 Safety Assessments

3.7.3.1 Physical Examination

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

General appearance	Lymph nodes	Skin
Cardiovascular system	Head, eyes, ears, nose, and throat	Central nervous system
Respiratory system	Neck	Peripheral nervous system
Abdomen/liver/spleen	Musculoskeletal system	

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

Height will only be recorded at the Screening Visit; weight will be recorded according to the Schedule of Events.

3.7.3.2 Vital Signs

Vital signs will be measured according to the Schedule of Events and documented on the eCRF. Vital sign measurements include oral temperature (°C), respiratory rate, and supine and standing

BP and pulse. Respiratory rate will be taken after the patient has been seated for at least 5 minutes. At the Screening Visit, on Day -1, and on Day 15, the supine BP measurements will be the average of 3 measurements obtained at 2-minutes intervals after the patient has been lying quietly for at least 5 minutes; for supine-only BP and pulse measurements thereafter, patient must lie quietly for at least 5 minutes before measurements are taken. In addition, at the Screening Visit, these blood pressure assessments should be completed at medication trough level, ie, prior to subjects taking their hypertensive medications, for determination of eligibility. For supine and standing BP and pulse measurements, patient must lie quietly for at least 5 minutes before supine measurements are taken, then assume a sitting position for 1 minute, and finally assume a standing position for 2 minutes before standing measurements are taken. All BP and pulse measurements should be taken before blood draws where applicable. All predose BP and pulse measurements should be taken before all oral and any antihypertensive concomitant medications.

3.7.3.3 Electrocardiograms

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

3.7.3.4 Clinical Laboratory Tests

Blood and urine samples for clinical laboratory tests will be collected according to the laboratory study procedures at the days and times defined in the Schedule of Events. Patients must have fasted for at least 8 hours before sample collections. The clinical laboratory evaluations will include the serum chemistry, hematology, coagulation, and urinalysis panels and the additional tests presented in Table 4.

Table 4. Clinical Laboratory Tests

Serum Chemistry Panel	Hematology Panel (CBC)	Complete Urinalysis
Albumin	Hematocrit	Color and appearance
Alkaline Phosphatase	Hemoglobin	pH and Specific Gravity
ALT	Platelet count	Bilirubin
AST	RBC count	Glucose
Bicarbonate	WBC count	Ketones
BUN	WBC differential	Leukocytes
Calcium	(% & absolute):	Nitrates
Chloride	Basophils	Occult blood
Cholesterol	Eosinophils	Protein
Creatinine	Lymphocytes	Urobilinogen
GGT	Monocytes	Microscopic
Glucose	Neutrophils	Including bacteria, RBCs, WBCs
HDL-c	RBC indices	per HPF if dipstick is abnormal
LDH	MCH	
LDL-c (calculated)	MCHC	
Magnesium	MCV	
Phosphorus	RDW	**Additional tests**
Potassium		Hemoglobin A1c
Sodium		Fasting plasma glucose
Total Bilirubin	**Coagulation Panel**	Fasting plasma insulin
Total Protein	aPTT	Urine albumin
Triglycerides	Prothrombin time	Urine creatinine
Uric acid	INR	

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

For female patients, a negative serum pregnancy test must be documented at the Screening Visit, at Check-in with results available before dosing, and at the Follow-up Visit. A urine pregnancy

test must be documented at the End of Trial Visit. In the event of a positive pregnancy test, the test will be repeated. If pregnancy is confirmed, see Section 3.7.3.10.

At the Screening Visit, blood will be collected for a hepatitis panel (including hepatitis B surface antigen [HBsAg] and antihepatitis C virus [HCV]) and human immunodeficiency virus (HIV) antibody screen.

A urine drug screen for selected drugs of abuse and an alcohol breathalyzer screen will be performed at the Screening Visit, Check-in, and the Follow-up Visit. Patients will be screened for the following drugs of abuse:

Amphetamines	Cocaine	Opiates
Barbiturates	Cotinine	Phencyclidine (PCP)
Benzodiazepines	Marijuana	Propoxyphene

3.7.3.5 Urine Creatinine Ratio (UACR)

Urine creatinine ratio (UACR) will be calculated as urine albumin (mg/dL) / urine creatinine (g/dL), At the Screening and Follow-up Visits, single urine samples, which may not be first void, will be collected for determination of albumin and creatinine levels. During the Clinic Period, first-void urine samples will be collected according to the Schedule of Events for determination of albumin and creatinine levels.

3.7.3.6 Estimated Glomerular Filtration Rate (eGFR)

Estimated glomerular filtration rate will be based on the serum creatinine level determined as part of the clinical laboratory tests (Table 4) and will be calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation.(9)

The CKD-EPI equation, expressed as a single equation, is

$$GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1\,209} \times 0.993^{Age} \times 1.018\ [\text{if female}]\ \_\ 1.159\ [\text{if black}]$$

where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κor 1, and max indicates the maximum of Scr/κ or 1.

3.7.3.7 Adverse Events

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

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

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

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

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

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

3.7.3.7.1 Causality Assessment

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

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

Unrelated: Any other event

3.7.3.7.2 Severity Assessment

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

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

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

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

3.7.3.8 Serious Adverse Events

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

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

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

3.7.3.9 Recording Adverse Events

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

For every AE, the Investigator must

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

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

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

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

3.7.3.10 Reporting Serious Adverse Events

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

The initial report should include at least the following information:

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

Special Situation: Exposure to Study Drug during Pregnancy

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

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

All SAE Report Forms should be emailed to:

Clinical Drug Safety & Pharmacovigilance

[redacted]

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

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

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

3.7.4 Pharmacodynamic Assessments

Hemodynamic, endothelial function, and platelet function assessments will be used to determine the PD parameters for PD analyses.

3.7.4.1 Hemodynamics

Blood pressure and pulse measurements for PD analyses will include supine and standing pulse and BP (for calculation of orthostatic pulse rate and BP) and ABPM assessed according to the Schedule of Events; refer to Section 3.7.3.2 for detailed instructions.

3.7.4.2 Endothelial Function

If available at the Study Center, endothelial function in the finger will be measured according to the Schedule of Events using the noninvasive EndoPAT device. (Note: For each patient, the Day -1 and Day 13 assessments should occur at approximately the same time of day [±15 minutes].) All measurements will precede all antihypertensive concomitant medications and will be performed in a quiet, dimly lit, temperature-controlled (21–24°C) room to reduce vascular tone fluctuation. The procedure will begin after the patient has had the opportunity to void and has been resting quietly for at least 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.

The EndoPAT procedure requires data entry of height, weight, and BP assessments in the EndoPAT software as follows. The Screening Visit height will be used for all EndoPAT assessments. The weight assessments obtained on Day -1 and on Day 13 will be used for the Day -1 and Day 13 assessments, respectively. Blood pressure is required as part of the EndoPAT procedure to determine the range of occlusion pressure necessary to complete the procedure. Therefore, as part of the EndoPAT procedure, BP assessments will be taken at least 15 minutes prior to starting the EndoPAT recording.

3.7.4.3 Platelet Function Assessment

Blood samples for platelet function assessment (VerifyNow®, Accriva Diagnostics, San Diego, CA) and Evaluation of Platelet Function Markers (Center for Platelet Research Studies, Boston, MA) will be collected according to the Schedule of Events. Additional platelet function tests may be performed.

3.7.4.4 HOMA-IR

Blood samples for determination of fasting plasma glucose and insulin levels (Table 4) will be collected according to the Schedule of Events. Values will be used in the Homeostatic Model Assessment to estimate insulin resistance (HOMA-IR).(10,11)

The HOMA-IR equation is

$$\text{HOMA-IR} = (\text{FSI} \times \text{FPG})/22.5,$$

where FSI is fasting serum insulin concentration (μU/l) and FPG is fasting plasma glucose (mmol/l)

3.7.4.5 [redacted]

[redacted]

3.7.4.6 [redacted]

[redacted]

[redacted]

[redacted]

3.7.5 Pharmacokinetic Assessments

Blood samples for determination of plasma concentrations of IW-1973 will be collected according to the Schedule of Events.

3.8 STUDY ACTIVITIES

3.8.1 Screening Period (Days –28 to Day -3)

3.8.1.1 Screening Visit (Days –28 to Day -3)

- Signing of ICF
- Review of inclusion and exclusion criteria
- Demographics and medical history
- Prior medications (all medicines taken during the 30 days before the Screening Visit)
- Weight and height
- Physical examination
- Respiratory rate and oral temperature
- 12-lead ECG
- Supine BP (average of 3 measurements) and pulse
- Supine-to-standing (orthostatic) pulse and BP
- Collection of blood and urine samples for:
  - Clinical chemistry, hematology (complete blood count [CBC]), coagulation
  - Urinalysis
  - Urine albumin and creatinine
  - Serum pregnancy test for all females (must be confirmed negative)
  - Hemoglobin A1c
  - Fasting plasma glucose
  - Fasting plasma insulin

- Drug screen
- HBsAg, HCV, and HIV screen

- Alcohol breathalyzer
- AE evaluation

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

Patients may be rescreened should they discontinue in the Screening Period due to visit window deviations or other administrative reasons. In addition, laboratory values, ECG values, or BPs that are outside the range specified in the protocol may be repeated to confirm eligibility during the Screening period at the Investigator's discretion after consultation with the Medical Monitor.

3.8.2 Clinic Period (Day -2 to Day 15)

Patients will be confined to the clinic from Check in on Day -2 until Discharge on Day 15.

3.8.2.1 Check-in (Day -2)

- Review of inclusion and exclusion criteria
- Physical examination
- Respiratory rate and oral temperature
- Prior (since the Screening Visit) and concomitant medications *
- Supine-to-standing (orthostatic) pulse and BP *
- 12-lead ECG
- Collection of blood and urine samples for:
  - Clinical chemistry, hematology (CBC), coagulation (including fasting serum glucose *)
  - Urinalysis
  - Serum pregnancy test for all females (must be confirmed negative) *
  - Hemoglobin A1c *
  - Fasting plasma glucose
  - Fasting plasma insulin
  - Drug screen *
  - [redacted]
- Alcohol breathalyzer *

- AE evaluation

* must meet Eligibility Criteria at Check-in

3.8.2.2 Day -1

- Weight
- If planned, EndoPAT assessment completed > 30 minutes before the time corresponding to AM dose on Day 13 and before all oral and any antihypertensive concomitant medications (Note: For each patient, the Day -1 and Day 13 assessments should occur at approximately the same time of day [±15 minutes])
- Respiratory rate and oral temperature
- Collection of urine samples:
  - Urine albumin and creatinine (first void)
- Supine BP (average of 3 measurements) at the time corresponding to AM dose (≤ 15 minutes) on Day 1
- Supine-to-standing (orthostatic) pulse and BP
  - at the time corresponding to AM dose (≤ 30 minutes) on Day 1 and at 1 and 4 hours (± 5 minutes) after that time
  - at the time corresponding to PM dose (≤ 15 minutes) on Day 1 and at 1 and 4 hours (± 5 minutes) after that time (Note: Supine-only pulse and BP may be taken at the 4-hour post PM dose timepoint if, in the Investigator's clinical opinion, earlier orthostatic pulse and BPs indicate it is safe to eliminate the standing [orthostatic] pulse and BP measurement.)
- ABPM starts at the time corresponding to AM dose (≤ 15 minutes)
- AE evaluation
- Concomitant medication recording

3.8.2.3 Day 1

- Respiratory rate and oral temperature AM predose (≤ 30 minutes)
- Oral temperature at 1 and 4 hours (± 30 minutes) AM postdose
- 12-lead ECG predose (≤ 30 minutes) and at 4 hours (± 15 minutes) postdose
- Supine-to-standing (orthostatic) pulse and BP
  - AM predose (≤ 30 minutes) and at 1, 4 and 8 hours (± 5 minutes) postdose

- PM predose (≤ 15 minutes) and at 1 and 4 hours (± 5 minutes) postdose
  (Note: Supine-only pulse and BP may be taken at the 4-hour post PM dose timepoint if, in the Investigator's clinical opinion, earlier postdose orthostatic pulse and BPs for the given dosing and for previous measurements at the 4-hour postdose timepoint indicate it is safe to eliminate the standing (orthostatic) pulse and BP measurement.)

- Collection of blood samples for:
  - PK
    - AM predose (≤ 15 minutes) and at 1, 3, and 6 hours (± 5 minutes) postdose
    - PM predose (≤ 5 minutes) and at 1 and 3 hours (± 5 minutes) postdose
  - Serum creatinine AM predose (≤ 15 minutes)
  - Hemoglobin A1c AM predose (≤ 15 minutes)
  - Fasting plasma glucose AM predose (≤ 15 minutes)
  - Fasting plasma insulin AM predose (≤ 15 minutes)
  - Platelet function assessments AM predose (≤ 15 minutes)
  - [redacted]
- Randomization
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- ABPM continues
- AE evaluation
- Concomitant medication recording

3.8.2.4 Day 2

- Respiratory rate and oral temperature
- ABPM ends AM predose (≤ 15 minutes)
- Supine-to-standing (orthostatic) pulse and BP
  - AM predose (≤ 15 minutes) and at 1 and 4 hours (± 5 minutes) postdose
  - PM predose (≤ 15 minutes) and at 1 and 4 hours (± 5 minutes) postdose
    (Note: Supine-only pulse and BP may be taken at the 4-hour post PM dose timepoint if, in the Investigator's clinical opinion, earlier postdose orthostatic pulse and BPs for the given dosing and for previous dosings at the 4-hour postdose timepoint indicate it is safe to eliminate the standing (orthostatic) pulse and BP measurement.)

- Collection of blood samples for PK:
  - 6 hours (± 5 minutes) post Day 1 PM dose
  - AM predose (≤ 5 minutes)
  - PM predose (≤ 5 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.5 Day 3-6

- Respiratory rate and oral temperature
- Supine pulse and BP AM predose (≤ 15 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.6 Day 7

- Respiratory rate and oral temperature
- Supine-to-standing (orthostatic) pulse and BP
  - AM predose (≤ 15 minutes) and at 1 and 4 (± 5 minutes) postdose
- ABPM starts AM predose (≤ 15 minutes)
- Collection of blood samples for PK:
  - AM predose (≤ 5 minutes) and at 1, 3, and 6 hours (± 5 minutes) postdose
  - PM predose (≤ 5 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.7 Day 8

- Respiratory rate and oral temperature
- ABPM ends AM predose (≤ 15 minutes)
- Supine-to-standing (orthostatic) pulse and BP
  - AM predose (≤ 15 minutes) and at 1 and 4 hours (± 5 minutes) postdose
  - PM predose (≤ 15 minutes)
- Collection of blood samples for:
  - PK
    - AM predose (≤ 5 minutes) and at 1, 3, and 6 hours (± 5 minutes) postdose
    - PM predose (≤ 5 minutes)
  - Hemoglobin A1c AM predose (≤ 15 minutes)
  - Fasting plasma glucose AM predose (≤ 15 minutes)
  - Fasting plasma insulin AM predose (≤ 15 minutes)
  - Platelet function assessments AM predose (≤ 15 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.8 Days 9 to 12

- Respiratory rate and oral temperature
- Supine pulse and BP AM predose (≤ 15 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.9 Day 13

- Weight
- If planned, EndoPAT assessment completed > 30 minutes before AM dose and before all oral and any antihypertensive concomitant medications; for each patient, assessments should be at time (±15 minutes) of EndoPAT on Day -1
- Respiratory rate and oral temperature
- Supine-to-standing (orthostatic) pulse and BP
  - AM predose (≤ 15 minutes) and at 1 and 4 hours (± 5 minutes) postdose
  - PM predose (≤ 15 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.10 Day 14

- Respiratory rate and oral temperature
- Supine pulse and BP AM predose (≤ 15 minutes)
- ABPM starts AM predose (≤ 15 minutes)
- Collection of blood samples for:
  - PK
    - AM predose (≤ 5 minutes) and at 1, 3, and 6 hours (± 5 minutes) postdose
    - PM predose (≤ 5 minutes)
  - Hemoglobin A1c AM predose (≤ 15 minutes)
  - Platelet function assessments AM predose (≤ 15 minutes)
- Study drug administration AM and PM, 12 hours (± 30 minutes) apart
- AE evaluation
- Concomitant medication recording

3.8.2.11 Discharge (Day 15)

- Weight

- Respiratory rate and oral temperature
- ABPM ends 12 hours (± 15 minutes) postdose
- 12-lead ECG
- Supine BP (average of 3 measurements) and pulse at 12 hours (± 15 minutes) postdose
- Supine-to-standing (orthostatic) pulse and BP at 12 hours (± 15 minutes) postdose
- Physical examination
- Collection of blood at 12 hours (± 15 minutes) postdose:
  - PK
  - Clinical chemistry, hematology (CBC), coagulation
  - Hemoglobin A1c
  - Fasting plasma glucose
  - Fasting plasma insulin
  - [redacted]
- Collection of urine samples, first void
  - Urinalysis
  - Urine albumin and creatinine
- AE evaluation
- Concomitant medication recording
- Discharge from the clinic

3.8.3 Follow-up Period (Day 16 to Day 42 ±3)

3.8.3.1 Follow-up Visit (Day 21 ±2)

- Weight
- Respiratory rate and oral temperature
- Supine BP and pulse
- Collection of blood and urine samples for:
  - PK
  - Serum pregnancy test for all females
  - Drug screen
  - [redacted]
- Alcohol breathalyzer
- AE evaluation
- Concomitant medication recording

3.8.3.2 End of Trial Visit (Day 42 ± 3)

- Weight
- Respiratory rate and oral temperature
- Supine BP and pulse
- Physical exam
- Collection of blood and urine samples for:
  - PK
  - Urine pregnancy test for all females
  - Clinical chemistry, hematology (CBC), coagulation
  - Urinalysis
  - Urine albumin and creatinine
  - Hemoglobin A1c
  - [redacted]
- AE evaluation
- Concomitant medication recording

3.9 STATISTICAL METHODS

Details regarding the statistical methods will be provided in the Statistical Analysis Plan (SAP), to be finalized before unblinding of the study.

3.9.1 Determination of Sample Size

Twenty-six patients (at least 11 males and 11 females) will be randomized in a 5:5:3 ratio to 1 of 2 IW-1973 treatment regimens or placebo. The sample size for this study was determined outside of statistical considerations.

3.9.2 Analysis Populations

3.9.2.1 Safety Population

The Safety Population will consist of all patients who receive at least 1 dose of study drug.

3.9.2.2 PK Population

The PK Population will consist of all patients who receive at least 1 dose of study drug and have at least 1 postdose PK parameter assessment.

3.9.2.3 PD Population

The PD population will consist of all patients who receive at least 1 dose of study drug and have at least 1 postdose PD parameter assessment.

3.9.3 Statistical Methods

3.9.3.1 General Considerations

Continuous variables will be summarized using the mean, standard deviation, minimum, median, interquartile range, and maximum. Categorical variables will be summarized using the frequency and percentage of patients in each category. Data summaries will be presented by treatment group.

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

3.9.3.2 Patient Disposition, Demographics, and Baseline Characteristics

The number and percentage of patients who were randomized, included in each of the 3 analysis populations (Safety, PK, and PD), completed the study or discontinued early, as well as the reasons for discontinuation will be presented by treatment group.

Patient demographics (age, sex, race, ethnicity, weight, height, BMI), and other baseline characteristics will be summarized for the Safety Population by treatment group.

3.9.3.3 Pharmacodynamic Analyses

3.9.3.3.1 Hemodynamics

Descriptive statistics will be presented for the following hemodynamic PD parameters by treatment group for each postdose assessment:

- Change from baseline in supine and standing pulse, systolic BP, and diastolic BP measurements
- Proportion of patients with postdose supine BP less than 130/80 mm Hg
- Orthostatic change in pulse, systolic BP, and diastolic BP. An orthostatic measurement is obtained by subtracting the supine measurement from the standing measurement
- Change from baseline in 24-hour, 4-hour, 30-minute, daytime, and nighttime averages of systolic BP, diastolic BP, mean arterial pressure and pulse measurements from ambulatory BP monitoring

3.9.3.3.2 Endothelial Function

Endothelial function will be assessed using the RHI parameter. Descriptive statistics will be presented by treatment group for the change from baseline in RHI on Day 14.

3.9.3.3.3 Other PD Parameters

Descriptive statistics will be presented for the following PD parameters by treatment group for each postdose assessment:

- Proportion of patients with decreased platelet reactivity
- Change from baseline in platelet function assessments

- Change from baseline in insulin resistance (HOMA-IR)

3.9.3.4 Safety Analyses

3.9.3.4.1 Adverse Events

Adverse events will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) available at the start of the study. Treatment-emergent adverse events (TEAEs) are those AEs that started or worsened in severity after the administration of study drug. All TEAEs as well as those that occurred during the first 7 days of treatment will be summarized by system organ class (SOC) and preferred term (PT) for each treatment group. In addition, listings of severe TEAEs, drug-related TEAEs, SAEs, AEs leading to study discontinuation, and AEs leading to death (if any) will be provided.

3.9.3.4.2 Vital signs, ECGs, and Clinical Laboratory Tests

Vital signs, 12-lead ECGs, and clinical laboratory evaluations (including clinical chemistry, hematology, coagulation, urinalysis, and UACR) at each assessment timepoint and the change from study baseline at each postdose timepoint will be summarized by treatment group.

3.9.3.4.3 Estimated glomerular filtration rate (eGFR)

The eGFR will be calculated according to the CKD-EPI creatinine equation (9); each assessment timepoint and the change from study baseline at each postdose timepoint will be summarized by treatment group.

3.9.3.5 Pharmacokinetic Analyses

Plasma concentration values of IW-1973 will be summarized for each assessment timepoint by treatment group. Mean plasma concentrations will be plotted over time for each active treatment group.

If systemic levels of IW-1973 are detectable, the following PK parameters will be calculated, when appropriate:

- AUC_{last}: Area under the plasma concentration time curve from time zero to T_{last}, the time at which the last measurable plasma concentration (C_{last}) is observed
- AUC_{tau}: Area under the plasma concentration time curve during a dosing interval (tau)
- AUC_{inf}: Area under the plasma concentration time curve extrapolated to infinity
- 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)
- CL/F: Apparent total body clearance after oral administration
- $t_{1/2}$: Apparent terminal phase half-life
- T_{max}: Time of maximum observed plasma concentration
- V_z/F: Apparent volume of distribution during the terminal phase after oral administration

3.9.3.6 Interim Analysis

There are no interim analyses planned for this study.

3.9.3.7 Computer Methods

Statistical analyses will be performed using Statistical Analysis System (SAS®), version 9.3 (or newer).

3.10 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Any amendment to this protocol will be provided to the Investigator in writing by Ironwood or its designee. Prior to implementation, any protocol amendment regarding reportable deviations (as defined by the IRB) must be approved by the IRB and the signature page must be signed by

the Investigator and received by Ironwood or its designee, with the following exception: If the protocol is amended to eliminate or reduce the risk to patients, the amendment may be implemented before IRB review and approval. However, the IRB must be informed in writing of such an amendment, and approval must be obtained within reasonable time limits.

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

4. ETHICAL CONSIDERATIONS

4.1 INSTITUTIONAL REVIEW BOARD

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

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

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

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

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

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

4.2 PATIENT INFORMATION AND INFORMED CONSENT

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

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

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

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

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

This study will be performed at up to 3 study centers in the US. The Investigator at each study center will be responsible for ensuring that the study is conducted according to the signed Clinical Trial Agreement, the protocol, IRB requirements, and ICH GCP guidelines.

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

5.1 GENERATION OF STUDY RECORDS

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

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

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

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

5.2 DATA QUALITY ASSURANCE

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

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

5.3 ELEC TRONIC CASE REPORT FORMS AND DATA MANAGEMENT

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

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

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

5.4 STUDY MONITORING

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

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

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

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

6. STUDY SPONSORSHIP

6.1 INVESTIGATOR AND STUDY TERMINATION

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

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

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

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

6.2 REPORTING AND PUBLICATION

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

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

7. INVESTIGATOR OBLIGATIONS

7.1 DOCUMENTATION

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

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

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

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

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

7.2 PERFORMANCE

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

7.3 ACCOUNTABILITY

Ironwood requires accountability of all study drug received and administered by the Study Center. Required records of drug disposition are to include logs or dispensing-records capturing, but not limited to, the date study drug was received, date(s) individual dosing units were prepared and labeled, date administered, quantity administered, and the subject to whom study drug was administered. At the end of the study, a complete reconciliation of the study drug supplies will be performed. All unused and reconciled drug supplies will be destroyed according to standard institutional policy or per written instruction from Ironwood should an alternate disposition be requested. No study drug is to be destroyed without prior written permission of Ironwood. A copy of the Certificate of Destruction or equivalent shall be provided to Ironwood once available.

7.4 RETENTION AND REVIEW OF RECORDS

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

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

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

7.5 PATIENT CONFIDENTIALITY

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

8. REFERENCE LIST

1. Vanhoutte PM, Vanhoutte PM, Shimokawa H, Feletou M, Tang EH, Shimokawa H, et al. Endothelial Dysfunction and Vascular Disease - A Thirthieth Anniversary Update. Acta Physiologica 2015;n/a.

2. Corson MA, James NL, Latta SE, Nerem RM, Berk BC, Harrison DG. Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. Circulation research 1996;79(5):984-91.

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

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

5. Centers for Disease Control and Prevention. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services; 2014.

6. Iglay K, Hannachi H, Joseph HP, Xu J, Li X, Engel SS, et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. Current medical research and opinion 2016;32(7):1243-52.

7. Arauz-Pacheco C, Parrott MA, Raskin P. Hypertension management in adults with diabetes. Diabetes care 2004;27 Suppl 1:S65-S67.

8. Rahman S, Rahman T, Ismail AA, Rashid AR. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. Diabetes, obesity & metabolism 2007;9(6):767-80.

9. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine 2009;150(9):604-12.

10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.

11. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care 2004;27(6):1487-95.

9. SPONSOR SIGNATURE

Study Title:	A Phase 2 Study to Compare the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of 2 Dose Regimens of IW-1973 in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-202
Final Date:	16 March 2017

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

Signed:_____ Date:_____

[redacted]
Ironwood Pharmaceuticals, Inc.

Print Name:_____

10. INVESTIGATOR'S SIGNATURE

Study Title:	A Phase 2 Study to Compare the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of 2 Dose Regimens of IW-1973 in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-202
Final Date:	16 March 2017

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____

Print Name:_____