Clinical Trial Protocol: C1973-201-P-03

Final Version, 09 January 2017

Study Title:	An Open-label, Phase 2a Trial to Evaluate the Effect of Escalating Doses of IW-1973 on Tolerability, Endothelial Function, and Hemodynamics in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-201
Study Phase:	2a
Product Name:	IW-1973 Tablet
Indication:	Type 2 diabetes with hypertension
Investigator:	[redacted]
Sponsor:	Ironwood Pharmaceuticals, Inc.
Sponsor Contact:	[redacted]
Medical Monitor:	[redacted]

	Date
Original Protocol:	15 August 2016
Amendment #1:	25 October 2016
Amendment #2:	09 January 2017

STUDY IDENTIFICATION

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

Table 1. Key Study Participants

Role	Contact Information
Principal Investigator and Study Center:	[redacted] ICON Early Phase Services, LLC 8307 Gault Lane San Antonio, TX 78209 [redacted]
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]
Dedicated SAE Facsimile Number:	[redacted]

SYNOPSIS

Sponsor

Ironwood Pharmaceuticals, Inc.

Name of Finished Product

IW-1973 Tablet

Name of Active Ingredient

IW-1973

Study Title

An Open-label, Phase 2a Trial to Evaluate the Effect of Escalating Doses of IW-1973 on Tolerability, Endothelial Function, and Hemodynamics in Patients with Stable Type 2 Diabetes and Hypertension

Study Number

C1973-201

Study Phase: 2a

Objectives

In adult patients (N = 12) with stable type 2 diabetes mellitus and controlled hypertension:

- To evaluate the safety and tolerability of escalating doses of IW-1973
- To evaluate the impact of escalating doses of IW-1973 on
  - endothelial function using EndoPAT™ (Itamar Medical; Caesarea, Israel) to measure fingertip small vessel pulse volume
  - blood pressure (BP) and heart rate

Study Design

This open-label, single-center trial will enroll 12 patients (at least 4 men and 4 women) to receive once-daily study drug for 6, sequential, 3-day dosing cycles (18 total days) starting with placebo (baseline) for 3 days and then progressing to 5 escalating dose levels of IW-1973 (10, 20, 30, 40, and 50 mg) for 3 days each (see Study Schematic).

Study Population

The study will enroll 12 patients with

- Type 2 diabetes with a hemoglobin A1c (HbA1c) level of ≤ 10.5% and a fasting (≥ 8 hours) blood glucose level of ≤ 240 mg/dl on a regimen of ≥ 1 medication for glycemic control with no change in medication for at least 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 for at least 30 days before the Screening Visit

that includes an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocking agent (ARB)

See Eligibility Criteria for full inclusion and exclusion criteria.

Test Product, Planned Doses, and Mode of Administration

Test product (IW-1973 Tablet, 5 mg) will be a 5-mg oral tablet. Doses will comprise multiple tablets. Planned doses are 10, 20, 30, 40, and 50 mg.

Reference Therapy, Dosage, and Mode of Administration

Reference therapy will be placebo to match IW-1973 Tablet, 5 mg. During the placebo cycle, doses will comprise 2 placebo tablets.

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

The 12 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 and will last 1 to 26 days. Patients will undergo preliminary screening procedures to determine their eligibility for the study at the Screening Visit. 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 -1 and will end at Discharge on Day 19. Patients who meet eligibility criteria based on Screening Visit assessments will be admitted to the Study Center on Day -1 for procedures to confirm eligibility and will be confined to the Study Center until Discharge on Day 19. Patients will receive a standard diet for diabetics. On Days 1 to 18, following an overnight fast of ≥ 8 hours, 12 eligible patients will receive once-daily study drug (see Study Drug Administration). Safety (adverse event [AE] collection, vital signs, fasting glucose, serum insulin, electrocardiograms [ECGs]) and pharmacokinetic (PK) assessments, including blood collections, will be performed at prespecified times daily (see Schedule of Events). Pharmacodynamic assessments will be performed on a 3-day cycle that will repeat for each of the 6 dosing cycles (see Study Schematic and Schedule of Events): the first day will include supine and standing cuff BP and pulse measurements; the second day will include ambulatory BP monitoring (ABPM), and the third day will include EndoPAT assessments of endothelial function. [redacted] On Day 19, after assessments have been completed, patients will be discharged from the Study Center at the Investigator's discretion.

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.

Follow-up Period: The Follow-up Period will begin immediately after Discharge of the patient from the Study Center and will last for 27 (± 3) days. Patients will return to the Study Center 14 (± 2) days after the final dose of study drug (Day 32) for the Follow-up Visit. At this visit, in addition to safety and PK assessments, patients will have EndoPAT assessments

before and after a 0.4 mg dose of sublingual nitroglycerin. Patients will remain in the Study Center for ≥ 2 hours until BP and pulse return to predose or acceptable, safe levels. Patients will return to the Study Center 28 (± 3) days after the final dose of study drug (Day 46) for the End of Trial Visit for final safety and PK assessments (see Schedule of Events).

Patients who prematurely discontinue dosing will remain in the clinic for at least 24 hours after their final dose of study drug and will complete all Discharge day assessments. In addition, these patients will return to the Study Center for their Follow-up and End of Trial Visits 14 (± 2) and 28 (± 3) days, respectively, after their final dose of study drug.

Study Drug Administration

Patients will receive orally administered study drug at approximately the same time (± 15 minutes) every day in the morning (8 to 10AM), after an overnight fast of at least 8 hours. Patients may take multiple tablets together. Permitted concomitant medications that the patient may be taking in the morning for diabetes and hypertension, or any other allowed concomitant conditions, should be taken at the same time as study drug. Breakfast should begin within 30 minutes after dosing.

Stopping Criteria

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

- Drug-related SAEs in 2 or more patients (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

Note: Safety and tolerability will be assessed daily and dosing may be discontinued or dose escalation may be cancelled on an individual patient basis. Patients who discontinue dosing or do not escalate will remain in the clinic for at least 24 hours after their final dose of study drug and will complete all Discharge day assessments. In addition, these patients will return to the Study Center for their Follow up and End of Trial Visits 14 (± 2) and 28 (± 3) days, respectively, after their final dose of study drug**.**

Planned Number of Patients:

Twelve patients may receive study drug in this trial. Additional patients (approximately 2 to 6) may be checked into the Study Center on Day -1 as back-ups. If the back-up patients are not needed, they will be discharged from the Study Center on Day -1 or Day 1. Patients withdrawing from the study after dosing will not be replaced.

Duration of Treatment

Patients will receive once-daily study drug for 18 days: placebo for 3 days and escalating doses of IW-1973 for 15 days. Patients will be in clinic for 20 days, from Check in on Day -1 to Discharge on Day 19. Total patient participation will be 45 to 77 days including Screening, Clinic, and Follow-up Periods.

Criteria for Evaluation

Pharmacodynamics

Hemodynamics

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

- From supine measurements – change from baseline (placebo cycle) in systolic and diastolic BP and pulse rate measurements
- From orthostatic (standing minus supine) measurements – orthostatic change in systolic and diastolic BP and pulse rate
- From ambulatory monitoring – change from baseline (placebo cycle) in 4-hour, 30-minute, and daytime (awake) averages of systolic and diastolic BP, mean arterial pressure, and pulse rate

Endothelial Function Assessments

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 (22–24°C) room to reduce vascular tone fluctuation. The recording 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 RHI parameter.

Platelet Function Testing (PFA-100®)

Blood samples for platelet function assessment (2.7 mL at each timepoint) will be collected according to the Schedule of Events.

[redacted]

Pharmacokinetics

Blood samples will be collected according the Schedule of Events and the following PK parameters will be calculated, when possible:

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

Safety

Physical examination findings, vital sign parameters, 12-lead ECG results, clinical laboratory results (ie, clinical chemistry, hematology, coagulation, urinalysis, HbA1c, fasting glucose, and serum insulin), and patient- and Investigator-reported AEs will be evaluated.

Statistical Methods:

Sample Size Determination

The sample size in this trial was determined outside of statistical considerations. A sample size of 12 patients is considered sufficient and is based on precedent set by prior studies of similar nature and design, as well as a review of published literature regarding the assessment of endothelial functioning by the EndoPAT technology.

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 RHI (from EndoPAT); supine, standing, and ambulatory BP and pulse rate values; and platelet function testing (from PFA-100). Changes from baseline (placebo cycle) in these variables will be compared across dose levels.

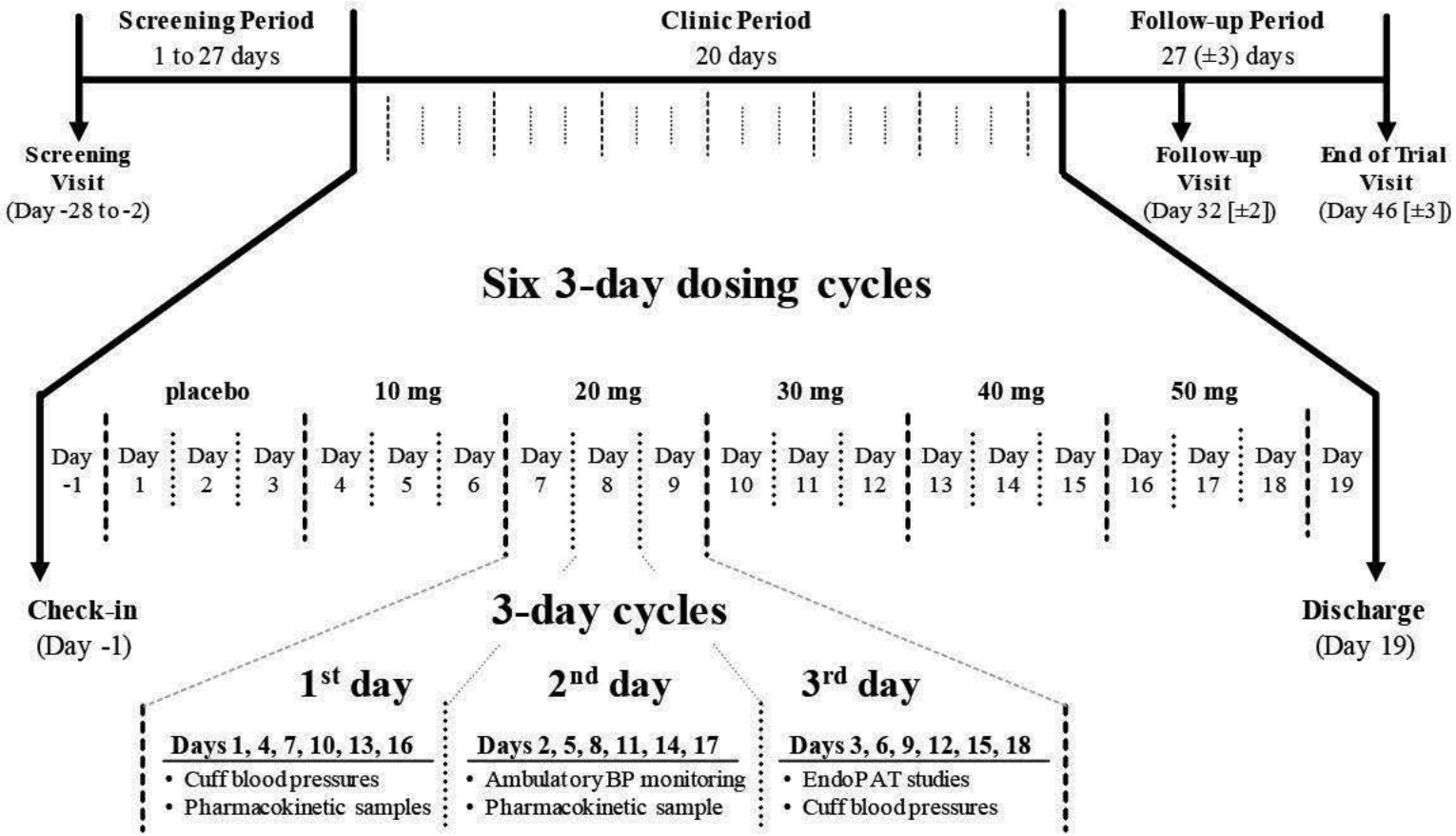
Pharmacokinetic Analyses

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

Safety Analyses

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

STUDY SCHEMATIC



SCHEDULE OF EVENTS

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

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

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

a. Urine drug screen for selected drugs of abuse and alcohol breathalyzer screen
b. For female patients, a negative serum pregnancy test must be documented at the Screening Visit, at Check-in with results available before dosing, and at the End of Trial Visit; a negative urine pregnancy test must be documented at the Follow-up Visit before nitroglycerin dosing.
c. On in-clinic days, in the morning after voiding, before any water or food intake
d. Patients must be supine for ≥ 5 m before the ECG recording (Note: if on initial ECG, QTcF is ≥ 450 msec for men or is ≥ 470 msec for women, the ECG will be repeated 2 more times, and the average of the 3 QTcF values will be used to determine the patient's eligibility); before blood draws where applicable
e. Respiratory rate after the patient has been seated for ≥ 5 m
f. After ≥ 8-h fast, after EndoPAT and BP/pulse measurements and before dosing, except at Follow-up Visit; left arm preferred. Urinalysis may be performed prior to EndoPAT and blood pressure assessments.
g. Must fast ≥ 8 hours; left arm preferred

h. Always precedes PD BP/pulse measurements and blood collections, except at Follow-up Visit; ≥ 3 h after any caffeine-containing foods/beverages, ≥ 1 h after allowed concomitant medications, after opportunity to void and then sitting quietly in a temperature-controlled room for ≥ 15 m; occlusion/finger measurement right arm/hand

i. At Screening Visit & Check-in, supine BP is average of 3 measurements obtained at 2-m intervals after the patient has been lying quietly for ≥ 5 m; thereafter, 1 measurement after the patient has been lying quietly for ≥ 5 m.
For supine to standing: patient must lie quietly for ≥ 5 m before supine measurements are taken, then assume sitting position for 1 m, and finally assume standing position for 2 m before standing measurements are taken.
All measurements before blood draws where applicable; always left arm

[redacted]

k. ≈ 2.7-mL blood sample in citrate tube at each timepoint; left arm preferred

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

[redacted]

o. In the morning, after overnight fast of ≥ 8 h, may take multiple tablets together; permitted concomitant morning medications should be taken at the same time. Breakfast begins ≤ 30 m after dosing.

p. Sublingual nitroglycerin 0.4 mg, administered according to label, food not allowed from 1 h before dosing to 30 m after dosing; patients should stay in the clinic for ≥ 2 h postdose.

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 65 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 patient must be postmenopausal, 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 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 stable 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. Per Investigator discretion, doses of supplemental short-acting insulin can be varied as needed to achieve adequate glycemic control.

b. Has HbA1c level ≤ 10.5% and fasting (≥ 8 hours) blood glucose level ≤ 240 mg/dl at the Screening Visit and at Check-in; at Investigator discretion, if fasting blood 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 ≥ 30 days before the Screening Visit with no indication that the regimen will need to be changed for the duration of the study. The medication(s) must include an ACEi or ARB and may include diuretics and/or calcium channel blockers. Other antihypertensive agents may be acceptable per the Investigator's discretion. (See Section 3.5.6.1 for prohibited medications.)

   b. Has supine systolic BP of 120 to 160 mm Hg and supine diastolic BP of 70 to 100 mm Hg at the Screening Visit

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

9. Patient has a valid reactive hyperemia index (RHI) measured by EndoPAT at the Screening Visit. (Note: If initial EndoPAT assessment cannot be completed successfully at the Screening Visit, [ie, the RHI value cannot be confirmed], the test may be repeated after at least 3 hours have passed. All other instructions regarding EndoPAT testing should be followed.)
10. Patient agrees to adhere to the study requirements.
11. 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.
12. 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), or 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 at 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 at Check-in, or 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 at the Screening Visit.
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) levels > 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.
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 assessments.
17. Patient has donated blood products 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.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

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
AUC_{tau}	area under the plasma concentration time curve during a dosing interval (tau)
BP	blood pressure
BUN	blood urea nitrogen
CBC	complete blood count
cGMP	cyclic guanosine 3', 5'-monophosphate
C_{max}	maximum observed plasma concentration
C_{trough}	trough plasma concentration observed at the end of a dosing interval
DNA	deoxyribonucleic acid
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FDA	Food and Drug Administration
GCP	good clinical practice
GGT	gamma glutamyl transferase
GLP	good laboratory practice
GTP	guanosine triphosphate
HbA1c	hemoglobin A1c (glycated hemoglobin)
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDPE	high-density polyethylene
hERG	ether-a-go-go related gene
HIV	human immunodeficiency virus

Abbreviation	Term
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
NG	nitroglycerin
NO	nitric oxide
pd	postdose
PD	pharmacodynamic(s)
PDE	phosphodiesterase
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

Abbreviation	Term
SAE	serious adverse event
SAS®	Statistical Analysis System
sGC	soluble guanylate cyclase
SNP	single nucleotide polymorphisms
SOC	system organ class
tau	dosing interval
$t_{1/2}$	apparent terminal phase half-life
T_{max}	time of maximum observed plasma concentration
UGT	uridine diphosphate–glucuronosyl transferase
WBC	white blood cell

1. INTRODUCTION

1.1 STUDY RATIONALE

1.1.1 Background

The vascular endothelium is a single continuous layer of endothelial cells that lines the blood vessels. It serves as a barrier between the circulating blood in the vessel lumen and the vascular smooth muscle in the vessel wall. Through the production of vasoactive molecules, the endothelium regulates vascular tone, permeability, inflammation, cellular adhesion, and thromboresistance, thus maintaining vascular homeostasis.(reviews in 1-3) Modulation of vascular tone balances vasoconstriction and vasodilation in response to chemical and mechanical stimuli. (review in 4) In particular, vasodilation is critical for reducing arterial blood pressure (BP) and adapting organ perfusion in response to flow-mediated shear stress associated with changes in cardiac output or postocclusion restoration of blood flow (reactive hyperemia).(review in 5,6)

Nitric oxide (NO) is the primary mediator of vasodilation. The release of NO by endothelial cells activates the heterodimeric enzyme, soluble guanylate cyclase (sGC), to convert guanosine triphosphate (GTP) to cyclic guanosine-3', -5'-monophosphate (cGMP). In turn, cGMP stimulates protein kinases, which decrease intracellular calcium levels and relax the vascular smooth muscle. The cGMP is then degraded by phosphodiesterase (PDE) enzymes. (review in 7) The NO-sGC-cGMP pathway is the main mechanism by which NO regulates vasodilation.

Impaired vasodilation is the hallmark of endothelial dysfunction and reflects reduced NO production or activity. Endothelial dysfunction underlies the earliest development of atherosclerosis; is associated with multiple cardiovascular risk factors including type 1 and 2 diabetes, obesity, hypertension, hypercholesterolemia, metabolic syndrome, and chronic smoking; and is a predictor of cardiovascular disease.(1,2,4,5)

Endothelial dysfunction may be improved, both acutely and chronically, by treatment with therapeutic agents that work by inhibiting vasoconstriction or by promoting vasodilation.(5) Agents that promote vasodilation act on various components of the NO-sGC-cGMP pathway. For example, organic nitrates exploit the beginning of the pathway by providing exogenous NO, and PDE5 inhibitors exploit the end by preventing degradation of cGMP thereby prolonging its

activity.(2,8,9) sGC, a central enzyme in this pathway, may also be targeted. Agonists of sGC may compensate for NO deficiency by acting directly on sGC to increase cGMP production.(5,10) By restoring endothelial function, sGC stimulators may be useful for treating and/or preventing a broad range of diseases that may ensue from dysfunction in this fundamental process.(reviews in 11,12)

1.1.2 IW-1973

Ironwood Pharmaceuticals is developing IW-1973, an orally administered stimulator of soluble guanylate cyclase (sGC), for the treatment of cardiovascular disorders modulated by the NO-cGMP-sGC signaling pathway.

1.1.3 Study Population

This exploratory, proof-of-mechanism study aims to evaluate the effect of the sGC stimulator, IW-1973, on impaired endothelial function by assessing reactive hyperemia, an endothelium-mediated vascular response, both before and after treatment. Endothelial function will be evaluated by measuring pulsatile volume changes in the small vessels of the fingertip after brachial artery occlusion using an EndoPAT™ instrument (Itamar Medical; Caesarea, Israel).

To investigate the hypothesis that IW-1973 will improve impaired endothelial function, the study requires a sample of patients who may have impaired endothelial function. Therefore, this study will enroll patients with type 2 diabetes and hypertension, a population that commonly has some degree of endothelial dysfunction. 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.(13) 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.(14) Hypertension is the most common comorbid condition and may affect over 80% of patients with type 2 diabetes.(14) 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.(reviews in 15,16)

Endothelial dysfunction has a complex relationship to diabetes, hypertension, and their comorbidities because it is both a cause and an effect. Additionally, the combination of disorders often present in diabetic patients leads to acceleration and exacerbation of endothelial dysfunction, which in turn leads to worsened disease states. Improving endothelial function in these patients may help avert this cascade of effects.

1.2 IW-1973: NONCLINICAL AND CLINICAL BACKGROUND

1.2.1 Nonclinical

IW-1973 is a potent and selective stimulator of sGC. In vitro, IW-1973 was shown to sensitize sGC to endogenous NO, and to directly stimulate sGC independently of NO. IW-1973 also potently relaxed precontracted human subcutaneous resistance arteries ex vivo. Oral administration of IW-1973 to normotensive and spontaneously hypertensive rats and to normotensive dogs was shown to elicit a dose-dependent decrease in systolic, diastolic, and mean arterial BP, as well as a concomitant rise in heart rate.

[redacted]

In Good Laboratory Practices (GLP) safety pharmacology studies, IW-1973-related effects observed in neurobehavioral (decreased rearing, locomotor activity) and cardiovascular (decreased BP and associated increased heart rate) functional studies were consistent with the expected pharmacology of IW-1973.

[redacted]

[redacted]

[redacted]

2. STUDY OBJECTIVES

In adult patients (N = 12) with stable type 2 diabetes mellitus and controlled hypertension:

- To evaluate the safety and tolerability of escalating doses of IW-1973
- To evaluate the impact of escalating doses of IW-1973 on
  - endothelial function using EndoPAT to measure fingertip small vessel pulse volume
  - BP and heart rate

3. INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN

This is an open-label, single-center study with a Screening Period, Clinic Period, and a Follow-up Period (Study Schematic). The study will enroll 12 patients (at least 4 men and 4 women) with type 2 diabetes, hypertension, and impaired endothelial function (see Eligibility Criteria); the 12 patients may be enrolled separately. Additional patients (approximately 2 to 6) may be checked into the Study Center on Day -1 as back-ups. If the back-up patients are not needed, they will be discharged from the Study Center on Day -1 or Day 1. Patients will receive once-daily study drug for 6, sequential, 3-day dosing cycles (18 total days) starting with placebo (2 tablets) for 3 days (baseline) and then progressing to 5 escalating dose levels of IW-1973 (10, 20, 30, 40, and 50 mg) for 3 days each (see Study Schematic).

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 and will last 1 to 27 days. Patients will undergo preliminary screening procedures to determine their eligibility for the study at the Screening Visit. 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 -1 and will end at Discharge on Day 19. Patients who meet eligibility criteria based on Screening Visit assessments will be admitted to the Study Center on Day -1 for procedures to confirm eligibility. Patients will be confined to the Study Center and will receive a standard diet for diabetics until Discharge. On Days 1 to 18, following an overnight fast of at least 8 hours, 12 eligible patients will receive once-daily study drug (see Section 3.5.3). Safety (AE collection, vital signs, fasting glucose, serum insulin, electrocardiograms [ECGs],) and PK assessments, including blood collections, will be performed at prespecified times (see Schedule of Events). Pharmacodynamic assessments will be performed on a 3-day cycle that will repeat for each of the 6 dosing cycles (see Study Schematic and Schedule of Events): the first day will include supine and standing cuff BP and pulse measurements; the second day will include ambulatory BP monitoring (ABPM), and the third day will include EndoPAT assessments of endothelial function. In addition, blood samples will be collected for platelet function testing (PFA-100®) [redacted]

[redacted] (see Schedule of Events). On Day 19, 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 of the patient from the Study Center and will last for 27 (± 3) days. Patients will return to the Study Center 14 (± 2) days after the final dose of study drug (Day 32) for the Follow-up Visit. At this visit, in addition to safety and PK assessments, patients will have EndoPAT assessments before and after a 0.4 mg dose of sublingual nitroglycerin to test endothelium-independent vasodilation. Patients will remain in the Study Center for at least 2 hours until BP and pulse return to predose or acceptable, safe levels. Patients will return to the Study Center 28 (± 3) days after the final dose of study drug (Day 46) for the End of Trial Visit for final safety and PK assessments (see Schedule of Events).

3.2 DISCUSSION OF STUDY DESIGN INCLUDING THE CHOICE OF CONTROL GROUPS

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

3.3 STUDY DURATION

Patients will receive once-daily study drug for 18 days: placebo for 3 days and escalating doses of IW-1973 for 15 days. Patients will be in clinic for 20 days, from Check in on Day -1 to Discharge on Day 19. Total patient participation will be 45 to 77 days including Screening, Clinic, and Follow-up Periods.

3.4 STUDY POPULATION SELECTION

3.4.1 Study Population

This study will enroll 12 patients with type 2 diabetes and hypertension as defined below; in addition, patients must have impaired endothelial function at baseline. 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 less than 6 months before the Screening Visit and must have been on a stable regimen of at least 1 medication specifically for control of glycemia, either oral or injectable, for at least 12 weeks before the Screening Visit. To be eligible, patients must have a hemoglobin A1c (HbA1c) level of ≤ 10.5% and a fasting (≥ 8 hours) blood glucose level of ≤ 240 mg/dl.

All screened patients with HbA1c or blood 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 less than 6 months before the Screening Visit and must have been on a stable regimen of at least one medication for BP control for at least 30 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.

3.4.1.3 Endothelial Function Assessment Requirements

The trial will investigate the hypothesis that escalating doses of the sGC stimulator, IW-1973, will improve endothelial vascular function. Endothelial function will be defined by the reactive hyperemic index (RHI) determined using the EndoPAT device, which assesses changes in fingertip small vessel pulse volume by tonometry.

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 received at least 1 dose of study drug ceases participation in the study, regardless of circumstances, before completion of Clinic Period.

A patient will be considered to have completed the study after completing the Clinic Period.

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 first dose of study drug. 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 first dose will not be replaced.

3.5 STUDY TREATMENT(S)

3.5.1 Description of Treatment(s)

3.5.1.1 Study Drug

3.5.1.1.1 Investigational Product

The investigational product, IW-1973 Tablet, is a 5 mg oral tablet. Doses will comprise multiple tablets (eg, a 30 mg dose will be six 5 mg tablets).

3.5.1.1.2 Placebo

Placebo will match the IW-1973 Tablet in appearance. During the placebo cycle, doses will comprise 2 placebo tablets.

3.5.1.1.3 Packaging and Labeling

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

3.5.1.1.4 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.

3.5.1.2 Nitroglycerin

Nitroglycerin, 0.4 mg, sublingual tablet, which will be administered at the Follow-up Visit to test endothelium-independent vasodilation, will be supplied by the Study Center and will be stored in accordance with manufacturer's instructions.

3.5.2 Selection of Dosage in the Study

In this Phase 2a exploratory study, 5 escalating doses of IW-1973 (10, 20, 30, 40, and 50 mg) will be studied in sequential 3-day cycles. In the Phase 1a and Phase 1b studies in healthy volunteers, dose levels ranging from 10 to 40 mg were tolerated. In the Phase 1a SAD study (ICP-1973-101), 35 mg was considered the maximum single tolerated dose and in the Phase 1b MAD study (ICP-1973-102), 40 mg in the tablet formulation was considered the maximum tolerated repeat dose. (In the Phase 1b study, 1 subject in Cohort 4 [Low Dose (≤ 0.5 mg/kg [in 5-mg increments])/High Dose (40 mg or ≤ 0.5 mg/kg for body weight ≥ 90 kg, whichever greater)] received 50 mg IW-1973 for 7 days as the High (up-titration) Dose. This subject reported no AEs.) In this Phase 2a trial, gradual dose escalation starting with a low dose may satisfy 2 secondary objectives: 1) to identify the minimum dose at which an improvement in endothelial function is detected, and 2) to determine if gradual escalation improves tolerability. The highest planned dose, 50 mg, exceeds the maximum tolerated dose level in healthy volunteers but is planned for evaluation in this Phase 2 study because gradual escalation may allow a 50 mg dose to be tolerated and, in addition, the maximum tolerated dose in patients may

be higher than in healthy volunteers. This study will be conducted in clinic and will employ stopping criteria (Section 3.6) to ensure the safety of patients.

3.5.3 Selection and Timing of Dose for Each Patient

Patients will receive orally administered study drug at approximately the same time (± 15 minutes) every day in the morning (8 to 10AM), after an overnight fast of at least 8 hours. (Note: for each patient, the first dose on Day 1 may be administered between 8 and 10AM; thereafter, doses on Days 2 to 18 must be administered within 15 minutes of the time of dosing on Day 1.) Patients are allowed to take multiple tablets together. Permitted concomitant medications that the patient may be taking in the morning should be taken at the same time as study drug. Breakfast should begin within 30 minutes after dosing.

On the 3rd day of each cycle the predose EndoPAT assessment (from the time the EndoPAT recording starts) should occur no more than 1 hour before study drug administration; predose BP and pulse measurements should be measured no more than 30 minutes before study drug administration.

In summary, the order of procedures around dosing on the 1st and 2nd day of each cycle should be:

- Overnight fast of at least 8 hours
- Predose safety, PK, and PD assessments
- Study drug and allowed concomitant medication administration between 8 and 10AM at the same time (± 15 minutes) every day
- Breakfast begins within 30 minutes after study drug administration

The order of procedures around dosing on the 3rd day of each cycle should be:

- Overnight fast of at least 8 hours
- Predose EndoPAT no more than 1 hour before study drug administration (from time EndoPAT recording starts)
- Predose BP and pulse measurements no more than 30 minutes before study drug administration

- Study drug and allowed concomitant medication administration between 8 and 10AM at the same time (± 15 minutes) every day
- Breakfast begins within 30 minutes after study drug administration

3.5.4 Blinding

This is an open-label study.

3.5.5 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 medications that the patient may be taking in the morning should be taken at the same time as study drug. Permitted concomitant medications that the patients may be taking at other times of day should not be taken 1 hour before or during EndoPAT assessments.

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

3.5.6 Restrictions

3.5.6.1 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), or phosphodiesterase (PDE) 5 inhibitors (including sildenafil, tadalafil, and vardenafil), alpha adrenergic blockers, riociguat, and sodium-glucose co-transporter 2 (SGLT2) inhibitors.

3.5.6.2 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.

3.5.6.3 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.
- 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 study drug administration. Breakfast should begin within 30 minutes of study drug administration.
- 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.

3.5.6.4 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 SAEs in 2 or more patients (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

Note: Safety and tolerability will be assessed daily and dosing may be discontinued or dose escalation may be cancelled on an individual patient basis. Patients who discontinue dosing or do not escalate will remain in the clinic for at least 24 hours after their final dose of study drug and will complete all Discharge day assessments. In addition, these patients will return to the clinic for their Follow-up and End of Trial Visits 14 (±2) and 28 (±3) days, respectively, after their final dose of study drug.

3.7 STUDY PROCEDURES

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

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

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 and at Check-in, the blood pressure assessments should be completed at medication trough level, i.e. prior to subjects taking their hypertensive medications. In addition, at the Screening Visit and at Check-in 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. 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 in the arm not used for EndoPAT occlusion and before blood draws where applicable.

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, preferably from the left arm, 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 2.

Table 2. 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	MPV	Glucose
Bicarbonate	RBC count	Ketones
BUN	WBC count	Leukocytes
Calcium	WBC differential	Nitrates
Chloride	(% & absolute):	Occult blood
Cholesterol	Basophils	Protein
Creatinine	Eosinophils	Urobilinogen
GGT	Lymphocytes	Microscopic
Glucose	Monocytes	Including bacteria, RBCs, WBCs per HPF if dipstick is abnormal
HDL-c	Neutrophils	
LDH	RBC indices	
LDL-c (calculated)	MCH	**Additional tests**
Magnesium	MCHC	Hemoglobin A1c
Phosphorus	MCV	Fasting blood glucose
Potassium	RDW	Serum insulin
Sodium		
Total Bilirubin	**Coagulation Panel**	
Total Protein	aPTT	
Triglycerides	Prothrombin time	
Uric acid	INR	

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

For female patients, a negative serum pregnancy test must be documented at the Screening Visit, at Check-in with results available before dosing, and at the End of Trial Visit; a negative urine pregnancy test must be documented at the Follow-up Visit before nitroglycerin dosing. In the event of a positive pregnancy test, the test will be repeated. If pregnancy is confirmed, see Section 3.7.3.8.

At the Screening Visit, blood will be collected for a hepatitis panel (including hepatitis B surface antigen [HBsAg] and anti- hepatitis 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 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.

3.7.3.5.1 Causality Assessment

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

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

Unrelated: Any other event

3.7.3.5.2 Severity Assessment

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

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

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

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

3.7.3.6 Serious Adverse Events

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

- Death
- Life-threatening: the patient was at immediate risk of death from the reaction as it occurred (ie, it does not include a reaction that hypothetically might have caused death if it had occurred in a more severe form)

- Hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity: a substantial disruption of a person's ability to conduct normal daily functions
- Congenital anomaly/birth defect
- Important medical events: events that may not result in death, be life threatening, or require hospitalization. Such an event may be considered serious when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency department or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

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

3.7.3.7 Recording Adverse Events

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

For every AE, the Investigator must

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

Pretreatment AEs will be collected from the time the patient signs the ICF until the patient receives study drug. Pretreatment AEs will be captured in the patient's source documentation but will only be entered for 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.

3.7.3.8 Reporting Serious Adverse Events

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

The initial report should include at least the following information:

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

Special Situation: Exposure to Study Drug during Pregnancy

In the event that a pregnancy occurs in a patient, 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
Ironwood Pharmaceuticals, Inc.
[redacted]

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

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

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

3.7.4 Pharmacodynamic Assessments

Hemodynamic and endothelial 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 collected according to the Schedule of Events. The left arm will be used for BP measurements. Hemodynamic effects will be assessed using the following parameters:

- From supine and standing measurements – change from baseline (placebo cycle) in systolic and diastolic BP and pulse rate measurements
- From orthostatic (standing minus supine) measurements – orthostatic change in systolic and diastolic BP and pulse rate
- From ambulatory monitoring – change from baseline (placebo cycle) in 4-hour, 30-minute, and daytime (awake) averages of systolic and diastolic BP, mean arterial pressure, and pulse rate

3.7.4.2 Endothelial Function

Endothelial function in the finger will be measured according to the Schedule of Events using the noninvasive EndoPAT device. Assessments will occur at least 3 hours after any caffeine-containing foods or beverages and at least 1 hour after administration of any allow concomitant medications. All measurements will be performed in a quiet, dimly lit, temperature-controlled (22–24°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. The right arm will be used for occlusion and finger measurements. Endothelial function will be assessed using the RHI parameter.

The EndoPAT procedure requires data entry of height, weight, and blood pressure assessments in the EndoPAT software as follows. The Screening height will be used for all EndoPAT assessments. The weight assessments obtained at Screening, Check-in and Follow-up will be used for the Screening, Check-in, and Follow-up EndoPAT assessments respectively. The weight assessment from Day 1 of each cycle will be used for the EndoPAT completed on Day 3 of each dosing cycle. 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. If multiple EndoPAT procedures are completed on the same day, just one

blood pressure assessment will be taken prior to the first EndoPAT on that day (e.g. for Day 3 of the dosing cycle, blood pressure will be measured prior to the first EndoPAT assessment only).

3.7.4.3 Platelet Function Assessment

Blood samples for platelet function assessment using the PFA-100 instrument will be collected according to the Schedule of Events. Blood samples should preferably be taken from the left arm.

[redacted]

[redacted]

3.7.5 Pharmacokinetic Assessments

Blood samples for PK assessments will be collected according to the Schedule of Events. Samples should preferably be taken from the left arm. The following PK parameters will be calculated:

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

3.8 STUDY ACTIVITIES

3.8.1 Screening Period (Days –28 to Day -2)

3.8.1.1 Screening Visit (Days –28 to Day -2)

- 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
- EndoPAT assessment (before BP measurements and blood sample collection)
- Supine BP (average of 3 measurements) and pulse
- Supine-to-standing (orthostatic) pulse and BP
- Collection of blood and urine samples for clinical laboratory test, including:
  - Clinical chemistry
  - Hematology (CBC)
  - Coagulation
  - Urinalysis
  - Serum pregnancy test for all females (must be confirmed negative)
  - Hemoglobin A1c
  - Fasting blood glucose
  - Serum 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.

3.8.2 Clinic Period (Days -1 to Day 19)

Patients will be confined to the clinic from Check in on Day -1 until Discharge on Day 19.

3.8.2.1 Check-in (Day -1)

- Review of inclusion and exclusion criteria
- Weight
- Physical examination
- Respiratory rate and oral temperature
- Prior (since the Screening Visit) and concomitant medications *
- EndoPAT assessment *
- Supine BP* (average of 3 measurements) and pulse
- 12-lead ECG
- Collection of blood and urine samples for clinical laboratory test, including:
  - Clinical chemistry
  - Hematology (CBC)
  - Coagulation
  - Urinalysis
  - Serum pregnancy test for all females (must be confirmed negative) *
  - Hemoglobin A1c *
  - Fasting blood glucose *
  - Serum insulin
  - Drug screen *
  - [redacted]
- Alcohol breathalyzer *
- AE evaluation

* must meet Eligibility Criteria at Check-in

3.8.2.2 First Day of Each 3-day Dosing Cycle (Days 1, 4, 7, 10, 13, 16)

- Weight
- 12-lead ECG predose (≤ 15 minutes) and at 1 and 4 hours (± 15 minutes) postdose
- Supine-to-standing (orthostatic) pulse and BP predose (≤ 30 minutes) and at 1, 2, 4, 8, and 12 hours (± 10 minutes) postdose
- PFA-100 test predose (≤ 15 minutes) and at 4 hours (± 15 minutes) postdose
- PK blood samples predose (≤ 15 minutes) and at 1, 3, 6, and 12 hours (± 5 minutes) postdose

[redacted]

- Administration of study drug and allowed concomitant medications
- Breakfast (≤ 30 minutes) after study drug administration
- AE evaluation
- Concomitant medication recording

3.8.2.3 Second Day of Each 3-day Dosing Cycle (Days 2, 5, 8, 11, 14, 17)

- Fasting blood glucose & serum insulin samples predose (≤ 15 minutes)
- PK blood sample predose (≤ 15 minutes)
- Ambulatory BP monitoring: start predose (≤ 30 minutes) and end 12 hours (+ 30 minutes) postdose
- Administration of study drug and allowed concomitant medications
- Breakfast (≤ 30 minutes) after study drug administration
- AE evaluation
- Concomitant medication recording

3.8.2.4 Third Day of Each 3-day Dosing Cycle (Days 3, 6, 9, 12, 15, 18)

- EndoPAT assessment predose (≤ 1 hour) and at 4 and 12 hours (± 15 minutes) postdose
- Supine pulse and BP predose (≤ 30 minutes) and at 1, 2, and 8 hours (± 10 minutes) postdose
- Administration of study drug and allowed concomitant medications
- Breakfast (≤ 30 minutes) after study drug administration
- AE evaluation
- Concomitant medication recording

3.8.2.5 Discharge (Day 19)

- Weight
- Supine-to-standing (orthostatic) pulse and BP at 24 hours (± 15 minutes) postdose
- Physical examination
- 12-lead ECG
- Respiratory rate and oral temperature
- Collection of blood and urine samples for clinical laboratory test, including:
  - PK blood sample at 24 hours (± 15 minutes) postdose
  - [redacted]
  - Clinical chemistry
  - Hematology (CBC)
  - Coagulation
  - Urinalysis
  - Hemoglobin A1c
  - Fasting blood glucose
  - Serum insulin
- AE evaluation
- Concomitant medications
- Discharge from the clinic

3.8.3 Follow-up Period (Day 20 to Day 46 ±3)

3.8.3.1 Follow-up Visit (Day 32 ±2)

- Collection of blood samples for the following assessments ≥ 1 hour before predose EndoPAT assessment:
  - PK
  - [redacted]
  - Clinical chemistry
  - Hematology (CBC)
  - Coagulation
  - Hemoglobin A1c
  - Fasting blood glucose
  - Serum insulin
- Collection of urine samples for the following assessments predose (≥ 1 hour)
  - Urine pregnancy test for all females (must be confirmed negative before nitroglycerin dose)
  - Urinalysis
  - Drug screen
- Weight
- Alcohol breathalyzer
- EndoPAT assessment predose (> 1 hour after blood draws and ≤ 1 hour before nitroglycerin dose)
- Supine BP and pulse predose (≤ 30 minutes)
- Respiratory rate and oral temperature predose (≤ 15 minutes)
- Sublingual nitroglycerin administration according to label
- EndoPAT assessment postdose (45 minutes [± 15 minutes] after nitroglycerin dose)
- Supine BP and pulse 2 hours (+ 30 minutes) postdose
- AE evaluation
- Concomitant medications

3.8.3.2 End of Trial Visit (Day 46 ± 3)

- Weight
- Respiratory rate and oral temperature
- Supine BP and pulse
- Physical examination
- Collection of blood samples for:
  - Serum pregnancy test for all females
  - PK
  - [redacted]
- AE evaluation
- Concomitant medications

3.9 STATISTICAL METHODS

3.9.1 Determination of Sample Size

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

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 received at least 1 dose of study drug and had at least 1 postdose PK parameter assessment.

3.9.2.3 PD Population

The PD Population will consist of all patients who received at least 1 dose of study drug and had at least 1 postdose PD assessment.

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 number and percentage of subjects 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 in each of the 3 analysis populations (Safety, PK, and PD) who completed the study or discontinued early, as well as the reasons for discontinuation, will be presented.

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

3.9.3.3 Pharmacodynamic Analyses

3.9.3.3.1 Hemodynamics

Descriptive statistics including 95% confidence intervals will be presented for the following hemodynamic parameters by treatment group:

- Change from study baseline (Day -1 assessment) and time-matched baseline (placebo cycle assessments) in supine pulse, systolic BP, and diastolic BP
- 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 time-matched baseline (placebo cycle assessments) in 4-hour, 30-minute, and daytime averages of systolic BP, diastolic BP, mean arterial pressure and pulse measurements from ambulatory BP monitoring
- Change from pre-nitroglycerin dose assessment to post-nitroglycerin dose assessment in supine pulse, systolic BP, and diastolic BP

3.9.3.3.2 Endothelial Function

Descriptive statistics, including 95% confidence intervals, will be presented for the following endothelial function parameters by treatment group:

- Change from study baseline (Day -1 assessment) in RHI
- Change from time-matched baseline (placebo cycle assessments) in RHI
- Change from pre-nitroglycerin dose assessment of RHI to the post-nitroglycerin dose assessment on the Follow-up Visit

3.9.3.3.3 Platelet Function Assessment

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

- Change from baseline in platelet function assessments (based on PFA-100)

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. TEAEs 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, Clinical Laboratory Tests

Vital signs, 12-lead ECGs, clinical laboratory evaluations at each assessment timepoint and the change from study baseline (Day -1) 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 and mean plasma concentrations will be plotted over time for each dose level.

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

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

The PK parameters will be summarized for each dose level.

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 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 1 study center in the US. The Investigator at the 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 ELECTRONIC CASE REPORT FORMS AND DATA MANAGEMENT

All data relating to the study will be recorded in the patient's source documentation and eCRF to be provided by Ironwood or designee via the electronic data capture (EDC) system. Source documentation supporting the eCRF data should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs, all observations, and patient status. The Investigator is responsible for verifying that all data entries on the eCRFs are accurate and correct and ensuring that all data are entered in a timely manner, as soon as possible after the information is collected. An explanation should be provided for any missing data. The Investigator 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. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007;115(10):1285-95.

2. Munzel T. [Endothelial dysfunction: pathophysiology, diagnosis and prognosis]. Dtsch Med Wochenschr 2008 Nov;133(47):2465-70.

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9. SPONSOR SIGNATURE

Study Title:	An Open-label, Phase 2a Trial to Evaluate the Effect of Escalating Doses of IW-1973 on Tolerability, Endothelial Function, and Hemodynamics in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-201

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

Signed: _____ Date: _____

[redacted]
Senior Director, Clinical Research
Ironwood Pharmaceuticals, Inc.

10. INVESTIGATOR SIGNATURE

Study Title:	An Open-label, Phase 2a Trial to Evaluate the Effect of Escalating Doses of IW-1973 on Tolerability, Endothelial Function, and Hemodynamics in Patients with Stable Type 2 Diabetes and Hypertension
Study Number:	C1973-201

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: _____

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