A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-LRx, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients With Uncontrolled Blood Pressure

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Ionis.com

2855 Gazelle Court
Carlsbad, CA 92010

(760) 931-9200

IONIS PHARMACEUTICALS, INC.

ISIS 757456-CS4

A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure

Amendment 4 – 4 November 2021

ASO Targeting **RAAS**

Trial Sponsor

Ionis Pharmaceuticals
2855 Gazelle Court
Carlsbad, CA 92010
Phone: 760-931-9200
Fax: 760-603-2588

[redacted]

ISIS 757456-CS4

Amendment 4

Clinical Phase: 2

A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure

ASTRAAS

ASO Targeting **RAAS**

Protocol History

Original Protocol:	8 September 2020
Amendment 1:	5 November 2020
Amendment 2:	12 April 2021
Amendment 3:	15 June 2021

Sponsor
Ionis Pharmaceuticals, Inc.
Carlsbad, CA 92010

[redacted]

Confidentiality Statement

Protocol Signature Page

Protocol Number:	ISIS 757456-CS4
Protocol Title:	A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure
Amendment:	4
Date:	4 November 2021

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled "A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure," dated 4 November 2021, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice and the Declaration of Helsinki.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

Investigator's Signature

Investigator's Name *(please print)*

Date (DD Month YYYY)

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Protocol Number:	ISIS 757456-CS4
Protocol Title:	A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure
Amendment:	4
Date:	4 November 2021

The main purpose of this amendment is to update some of the inclusion and exclusion criteria related to baseline requirements for ECG abnormalities, renal function, diuretic use and the use of medications that may cause hyperkalemia. Based on the collective safety and laboratory data currently available across ISIS 757456 clinical studies and the lack of safety signals related to hyperkalemia, ECG, and renal function, the criteria below have been revised to allow patients with these baseline abnormalities: Other changes are outlined below as well as the rationale for the updates:

i) update the eGFR exclusion criterion to < 30 mL/min/1.73 m^2

ii) update the UPCR exclusion criterion to ≥ 0.5 mg/mg and remove the UACR exclusion criterion

iii) revise the renal stopping rules to align with the modified eligibility criteria

iv) allow patients with ECG abnormalities, aside from those who have clinically relevant prolonged QTc per Investigator judgement

v) allow patients on potassium sparing diuretics while excluding patients with a history of hyperkalemia per Investigator judgement

vi) revise the definition of baseline for renal stopping rules in order to more accurately determine if additional monitoring or stopping of Study Drug is necessary

Further, to clarify that in the event Day 1 lab results are uninterpretable (e.g., due to hemolysis or missing sample), Day 8 dosing is allowed but the investigator is instructed to obtain an interpretable result before study drug dosing on Day 15. And finally, to clarify the testing needed for HBV at screening

The table below provides a list of modifications to the protocol; added text in bold, deleted text in strikethrough.

Protocol Section	Description of Change	Rationale
Protocol Synopsis Section 5.1 Inclusion Criteria	6. At Screening, the patient must have been on a stable, maximally tolerated regimen (per Investigator judgement) of 3 or more antihypertensive medications for at least 1 month prior to screening and will be required to maintain this regimen throughout the Study. The combination of antihypertensive medications must be in the following categories: a. angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) b. beta blocker c. calcium channel blocker d. ~~**non-potassium sparing**~~ diuretic e. alpha-1 blocker f. centrally acting sympatholytic agent g. direct acting vasodilators (e.g., hydralazine)	Based on the collective safety and laboratory data currently available across ISIS 757456 clinical studies and lack of safety signals related to hyperkalemia, the inclusion criterion has been updated to also allow patients on potassium sparing diuretics. This modification is further supported by the updated eligibility criterion that excludes patients with a known history of hyperkalemia per Investigator judgement.
Protocol Synopsis Section 5.2 Exclusion Criteria	5. The use of the following at time of Screening and during the course of the study: a. Other medications for the treatment of HTN (e.g., minoxidil, diazoxide, renin inhibitors) b. Medications that may cause hyperkalemia **unless on a stable dose at least 1 month prior to the Screening visit and no known history of hyperkalemia per Investigator judgement** ~~(e.g., cyclosporine or tacrolimus, pentamidine, trimethoprim-sulfamethoxazole, all heparins)~~ c. Use of oral anticoagulants, unless stable for 4 weeks prior to the first dose of Study Drug and regular monitoring must be performed per clinical practice during the study unless the patient is receiving vitamin K agonists. If the patient is receiving vitamin K antagonists (e.g., warfarin) international normalized ratio (INR) should be in therapeutic range, as established by the Investigator, for 4 weeks prior to the first dose d. Chronic administration (defined as > 3 days per week for the duration of the trial) of NSAIDs or Cox-2 inhibitors (except aspirin for cardiovascular provided the total daily dose does not exceed 325 mg) e. Use of phosphodiesterase 5 inhibitors (e.g., sildenafil, tadalafil, vardenafil, avanafil) within 7 hours prior to any scheduled visit f. ~~Potassium sparing diuretics (e.g., eplerenone, spironolactone, amiloride, triamterene)~~ **Intentionally left blank**	The exclusion criterion 5b has been updated to allow patients who are on medications that may cause hyperkalemia, but do not have hyperkalemia at Screening or a history of hyperkalemia per Investigator judgement. This will allow patients who have stable, within normal range potassium levels. Exclusion criterion 5f has been removed and added to inclusion criterion 6.

Protocol Section	Description of Change	Rationale
Protocol Synopsis Section 5.2 Exclusion Criteria	8. Active infection with human immunodeficiency virus (HIV)~~, hepatitis C (HCV)~~ hepatitis B (HBV) **(as evidenced by a positive test for hepatitis B surface antigen) or hepatitis C (HCV) (**diagnosed by initial serological testing and confirmed with RNA testing) or prior treatment for hepatitis C. ~~Patients at Screening who test positive by serology, but negative by RNA may be allowed in consultation with the Sponsor Medical Monitor or designee~~	Correction. HBV diagnosis is based on a positive test for hepatitis B surface antigen.
	12. Unstable/underlying known cardiovascular disease defined as: a. Any history of congestive heart failure (New York Heart Association [NYHA] Class III-IV) b. Any history of previous myocardial infarction, coronary revascularization, unstable or stable angina pectoris < 1 year prior to screening c. ~~12-lead ECG demonstrating a QT interval (corrected using Fridericia's formula [QTcF]) > 450 msec in males and > 470 msec in females at Screening, or a history or evidence of long QT syndrome~~ **Clinically relevant prolonged QTc (using Fridericia's formula [QTcF]) per Investigator judgement** d. Any hemodynamically unstable atrial or ventricular arrhythmias e. Significant uncorrected valvular heart disease f. Any history of stroke or transient ischemic attack < 1 year prior to screening	The exclusion criterion was updated to allow patients with ECG abnormalities, aside from those who have clinically relevant prolonged QTc per Investigator judgement and are otherwise eligible.

Protocol Section	Description of Change	Rationale
Protocol Synopsis Section 5.2 Exclusion Criteria	16. Screening laboratory results as follows, or any other clinically significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion a. Urine protein-creatinine ratio (UPCR) ≥ 0.**5**~~3~~ mg/mg ~~In the event of a UPCR above this threshold, eligibility may be confirmed by a quantitative total urine protein measurement of < 300 mg/24 hr~~ b. ~~Urine albumin creatinine ratio (UACR) > 300 mg/g~~ **Intentionally left blank** c. A positive test for blood (including trace) on urinalysis that is subsequently confirmed with urine microscopy showing > 5 red blood cells per high power field and is related to glomerulopathies. In women, this exclusion criterion must be assessed outside of menstrual period. If, in the opinion of the Investigator, the haematuria is not considered related to glomerulopathies the patient may be considered eligible, pending proper follow-up and a discussion with the Sponsor Medical Monitor or designee. Patients with history of bladder cancer must have been treated with curative intent and have not presented recurrence within the prior 5 years d. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) > 1.5 × upper limit of normal (ULN) e. Total bilirubin ≥ 1.5 × ULN (patients with total bilirubin ≥ 1.5 × ULN may be allowed on study if only indirect bilirubin is elevated, ALT/AST is not greater than the ULN, and known to have Gilbert's disease) f. Platelet count < 125,000/mm^3 g. Serum potassium > 5.1 mmol/L h. Estimated glomerular filtration rate (eGFR) of < **30**~~45~~ mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration formula i. Hemoglobin A1c (HbA1c) of > 9% or diabetes mellitus type 2 not well-controlled based on the Investigator's judgement	Based on the collective renal safety and laboratory data currently available across ISIS 757456 clinical studies, the UPCR and eGFR criteria were modified to allow the inclusion of patients who have a higher UPCR and lower eGFR. The UACR criterion has been removed as patients with macroalbuminuria will still be excluded per the UPCR threshold for eligibility.

Protocol Section	Description of Change	Rationale
Section 2.5.2 Risk Assessment, Table 1	ISIS 757456 is expected to have less effect on the renal RAAS system (less elevation of serum potassium) than ACEi, ARB or direct renin antagonists Most likely to occur in patients with high serum potassium. Eligibility criteria excludes K+ > 5.1 mmol/L and patients on medications that may cause hyperkalemia **unless on a stable dose at least 1 month prior to the Screening visit and no known history of hyperkalemia per Investigator judgement.** Eligibility criteria exclude patients at enhanced risk Monitoring and stopping rules in place for hyperkalemia	The language is updated to be consistent with the revised eligibility criteria.
Section 3.2 Number of Study Centers	This study will be conducted at multiple centers in the United States **and Canada**.	This study will now also be conducted at sites in Canada.
Section 6.2 Laboratory Assessments	Laboratory analyte samples will be collected throughout the study. A list of these analytes is contained in Appendix B. If the platelet value, ~~serum creatinine~~ **eGFR, UPCR,** potassium, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing, a repeat blood specimen should be re drawn as soon as possible (ideally within 7 days, either in clinic, by Home Healthcare or local laboratory). Any uninterpretable platelet count, ~~serum creatinine~~ **eGFR, UPCR,** liver enzyme, or potassium result must be rechecked and determined not to have met a stopping rule before dosing can continue. **If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15.** If there is suspicion of EDTA mediated platelet clumping, a repeat platelet count should be collected in a sodium citrate tube as soon as possible. Treatment should be held if there is no evaluable platelet count within 18 days prior to the scheduled dose (Section 8.5.2).	Correction. Added eGFR and UPCR tests to align with safety monitoring rules and clarified how to manage uninterpretable values after Day 1.

Protocol Section	Description of Change	Rationale
Section 8.5.2 Safety Monitoring Rules for Platelet Count Results	Treatment should be held if there is no evaluable platelet count within 18 days prior to the scheduled dose. Any unreportable platelet count result must be rechecked and determined not to have met a stopping rule before dosing can continue. **If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15.**	Provide further clarification on how to manage uninterpretable values after Day 1.
Section 8.6 Stopping Rules	For the purposes of the stopping rules, Baseline is defined as: • Temporary stopping rules for renal function tests - Baseline is defined as the **average of the pre-dose test closest to Day 1 and Day 1** ~~last non missing measurement prior to the first dose~~ • Stopping rules for platelets – Baseline is defined as the last non missing measurement prior to the first dose	The definition of baseline for renal stopping rules was modified in order to provide a more accurate pre-dose clinical status while taking physiological variability into consideration. This will better indicate if additional monitoring or the stopping of Study Drug is necessary.
Section 8.6.2 Stopping Rules for Renal Function Test Results	1.Confirmed 30% decline in eGFR from Baseline eGFR values **[eGFR should be calculated using the CKD-EPI creatinine equation (Levey et al. 2009)]** 2. Confirmed proteinuria ~~(UPCR ≥ 0.7 mg/mg)~~**: Absolute UPCR value ≥ 1000 mg/g** ~~The follow-up schedule for any events meeting either of these stopping criteria will be determined by the Investigator in consultation with the Sponsor Medical Monitor or designee.~~ The possible dosing re-initiation or follow-up schedule for any events meeting either of these criteria will be determined by the Investigator in consultation with the Sponsor Medical Monitor or designee. At the discretion of the Investigator, a decision to hold or permanently stop study drug may be made based on lesser changes in these parameters observed in isolation or in association with other renal-related abnormalities.	The renal function monitoring and stopping rules were updated to align with the modified eligibility criteria based on the collective renal safety and laboratory data currently available across ISIS 757456 clinical studies. The updated language also clarifies the guidelines for Study Drug reinstitution.

Protocol Section	Description of Change	Rationale
Section 8.10.1 Concomitant Therapy	The following are disallowed concomitant therapies: • Other medications for the treatment of HTN (e.g., minoxidil, diazoxide, renin inhibitors) • Medications that may cause hyperkalemia **unless on a stable dose at least 1 month prior to the Screening visit** ~~(e.g., cyclosporine or tacrolimus, pentamidine, trimethoprim-sulfamethoxazole, all heparins)~~ • Use of oral anticoagulants unless stable for 4 weeks prior to the first dose of Study Drug and regular monitoring must be performed, per clinical practice during the study unless the patient is receiving vitamin K agonists. If the patient is receiving vitamin K antagonists (e.g., warfarin) INR should be in therapeutic range, as established by the Investigator, for 4 weeks prior to the first dose • Chronic administration (defined as > 3 days per week for the duration of the trial) of NSAIDs or COX-2 inhibitors (except aspirin for cardiovascular disease provided the total daily dose does not exceed 325 mg) • Use of phosphodiesterase 5 inhibitors (e.g., sildenafil, tadalafil, vardenafil, avanafil) within 72 hours prior to any scheduled visit • ~~Potassium sparing diuretics (e.g., eplerenone, spironolactone, amiloride, triamterene)~~	The disallowed concomitant therapies were updated to reflect the updated exclusion criterion.
Section 9.3.3 Serious Adverse Event	The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE]; ~~OR Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials;~~ the event itself may be of relatively minor medical significance (such as severe headache without any further findings).	Correction. Severity of adverse events and serious adverse events will be graded based on criteria from the Common Terminology Criteria for Adverse Events (CTCAE) and not the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

Protocol Section	Description of Change	Rationale
Appendix A Schedule of Procedures Footnote 7	If the platelet value, ~~serum creatinine,~~ **eGFR, UPCR,** potassium, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) a repeat blood specimen; should be re-drawn as soon as possible (ideally within 7 days either in clinic, by Home Healthcare, or local laboratory) and not meet a stopping rule prior to next dose. **If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15.**	Correction. Added eGFR and UPCR tests to align with safety monitoring rules and clarified how to manage uninterpretable values after Day 1.

PROTOCOL SYNOPSIS

Protocol Title	A Double-Blind, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure
Study Phase	2
Indication	Hypertension
Primary Objective	• To evaluate the effect of ISIS 757456 compared to placebo on seated automated office systolic blood pressure (SBP) from Baseline to Study Day 85 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
Secondary Objectives	• To evaluate the effect of ISIS 757456 on plasma angiotensinogen (AGT) at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate the effect of ISIS 757456 on 24-hour ambulatory blood pressure at Study Day 85 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate the effect of ISIS 757456 on seated automated office SBP at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate the effect of ISIS 757456 on seated automated office diastolic blood pressure (DBP) at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
Exploratory Objectives	• To evaluate the effects of ISIS 757456 administered subcutaneously (SC) (e.g., angiotensin II, renin [Plasma renin activity (PRA); direct renin], urinary AGT) in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate the pharmacokinetics (PK) of ISIS 757456 (as total full length ASO, including fully conjugated, partially conjugated, and unconjugated ISIS 757456) administered SC in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate potential PK / pharmacodynamic (PD) correlation with relevant biomarkers and/or clinical endpoints
Safety Objective	• To evaluate the safety and tolerability of ISIS 757456 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications • To evaluate the effects of ISIS 757456 on patient reported quality of life outcomes
Study Design	Randomized, double blind, placebo controlled, multi-center study
Number of Patients	Approximately 150 patients will be enrolled

PROTOCOL SYNOPSIS (CONTINUED)

Study Population	**Inclusion Criteria** 1. Must have given written informed consent (signed and dated) and any authorizations required by local law and be able to comply with all study requirements 2. Males or females aged 18–80 inclusive and weighing ≥ 50 kg at the time of informed consent 3. Satisfy the following: a. Females: must be non-pregnant and non-lactating, and either: i. surgically sterile (e.g., bilateral tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) ii. post-menopausal (defined as 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and follicle stimulating hormone (FSH) levels in the postmenopausal range for the laboratory involved) b. Males must be abstinent*, surgically sterile (vasectomy with negative semen analysis at Follow-up) or if engaged in sexual relations with a woman of childbearing potential (WOCBP), a highly effective contraceptive method must be used (refer to Section 6.4.1) from the time of signing the informed consent form until at least 20 weeks after the last dose of Study Drug (ISIS 757456 or placebo) or abstinent* * Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception. 4. Body mass index (BMI) ≤ 45.0 kg/m^2 5. Brachial circumference ≥ 22 and ≤ 43 cm (8.7 and 16.9 inches) 6. At Screening, the patient must have been on a stable, maximally tolerated regimen (per Investigator judgement) of 3 or more antihypertensive medications for at least 1 month prior to screening and will be required to maintain this regimen throughout the Study. The combination of antihypertensive medications must be in the following categories: a. angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) b. beta blocker c. calcium channel blocker d. diuretic e. alpha-1 blocker f. centrally acting sympatholytic agent g. direct acting vasodilators (e.g., hydralazine) 7. At Screening Visit 1 and on Screening Visit 2 prior to the 24-hr ambulatory blood pressure monitoring, the average seated automated office BP (using the Sponsor provided BP machine) must be within > 130–≤ 170mmHg systolic and meet the Exclusion Criterion #4 Pulse Pressure requirement. The average is to be derived from 3 assessments taken within 10 minutes. Up to 2 additional tests at each time point are allowed in order to qualify

PROTOCOL SYNOPSIS (CONTINUED)

Study Population (Continued)	**Inclusion Criteria (Continued)** 8. Agree to abstain from alcoholic beverages for at least 24 hours prior to clinic visits 9. Agree to maintain adequate hydration and adhere to a low sodium diet throughout the duration of the study (from the Screening visit onward) 10. Agree to abstain from exercise, smoking, and caffeine use 30 minutes prior to seated automated office BP measurements **Exclusion Criteria** 1. Clinically significant abnormalities in medical history according to Investigator judgement (e.g., major surgery non-inclusive of exclusion criteria 12 within 2 months of Screening, type 1 diabetes mellitus) 2. History of secondary hypertension (HTN) including, but not limited to any of the following: renovascular HTN (unilateral or bilateral renal artery stenosis), coarctation of the aorta, primary hyperaldosteronism, Cushing's disease, pheochromocytoma, polycystic kidney disease, and drug-induced HTN 3. Patient with borderline orthostatic hypotension (assessed at Screening Visit 1), when they assume a standing position (within >1 to ≤3 minutes of standing up), defined as: a. A decrease in SBP of ≥ 15 mmHg Up to 1 additional test may be done once the patient is adequately hydrated if orthostasis is thought to be attributed to volume depletion 4. Patient with a pulse pressure at Screening Visit 1 of > 80 mmHg. Patients with a pulse pressure >80 mmHg at Screening Visit 1 may be allowed in consultation with the Sponsor Medical Monitor or designee. Pulse pressure is calculated by the difference between the office seated SBP and the office seated DBP (seated automated office blood pressure is to be derived by the average of three assessments) 5. The use of the following at time of Screening and during the course of the study: a. Other medications for the treatment of HTN (e.g., minoxidil, diazoxide, renin inhibitors) b. Medications that may cause hyperkalemia unless on a stable dose at least 1 month prior to the Screening visit and no history of hyperkalemia per Investigator judgement c. Use of oral anticoagulants, unless stable for 4 weeks prior to the first dose of Study Drug and regular monitoring must be performed per clinical practice during the study unless the patient is receiving vitamin K agonists. If the patient is receiving vitamin K antagonists (e.g., warfarin) international normalized ratio (INR) should be in therapeutic range, as established by the Investigator, for 4 weeks prior to the first dose d. Chronic administration (defined as > 3 days per week for the duration of the trial) of NSAIDs or COX-2 inhibitors (except aspirin for cardiovascular disease provided the total daily dose does not exceed 325 mg) e. Use of phosphodiesterase 5 inhibitors (e.g., sildenafil, tadalafil, vardenafil, avanafil) within 72 hours prior to any scheduled visit f. Intentionally left blank

PROTOCOL SYNOPSIS (CONTINUED)

Study Population (Continued)	**Exclusion Criteria (Continued)** 6. Treatment with another Study Drug, biological agent, or device within 1 month of Screening, or 5 half-lives of study agent, whichever is longer 7. Previous treatment with an oligonucleotide or other RNA therapeutic (including small interfering ribonucleic acid [siRNA]) within 4 months of Screening if single dose received, or within 12 months of Screening if multiple doses received. This exclusion criterion does not apply to the COVID-19 vaccines which are allowed. COVID-19 vaccination performed prior to study participation or during study participation will be collected in the electronic database. 8. Active infection with human immunodeficiency virus (HIV), hepatitis B (HBV) (as evidenced by a positive test for hepatitis B surface antigen), or hepatitis C (HCV) (diagnosed by initial serological testing and confirmed with RNA testing), or prior treatment for hepatitis C. 9. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be eligible if reviewed by the Sponsor Medical Monitor or designee 10. History of bleeding diathesis, coagulopathy, immune thrombocytopenic purpura (ITP), thrombotic cytopenic purpura (TTP), or any qualitative or quantitative platelet defect 11. Recent history of, or current drug or alcohol abuse 12. Unstable/underlying known cardiovascular disease defined as: a. Any history of congestive heart failure (New York Heart Association [NYHA] Class III-IV) b. Any history of previous myocardial infarction, coronary revascularization, unstable or stable angina pectoris < 1 year prior to screening c. Clinically relevant prolonged QTc (using Fridericia's formula [QTcF]) per Investigator judgement d. Any hemodynamically unstable atrial or ventricular arrhythmias e. Significant uncorrected valvular heart disease f. Any history of stroke or transient ischemic attack < 1 year prior to screening

PROTOCOL SYNOPSIS (CONTINUED)

Study Population (Continued)	**Exclusion Criteria (Continued)** 13. A cardiac valve repair, cardiac device implantation, and/or a hospitalization for heart failure within 3 months of Screening 14. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1 15. Abnormal thyroid function tests at Screening with clinical significance, per Investigator judgment. Patients receiving dose-stable thyroid hormone replacement therapy for at least 3 months prior to screening will be allowed to participate as long as thyroid tests (TSH/T3/T4) show that patient is euthyroid 16. Screening laboratory results as follows, or any other clinically significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion a. Urine protein-creatinine ratio (UPCR) ≥ 0.5 mg/mg b. Intentionally left blank c. A positive test for blood (including trace) on urinalysis that is subsequently confirmed with urine microscopy showing > 5 red blood cells per high-power field and is related to glomerulopathies. In women, this exclusion criterion must be assessed outside of menstrual period. If, in the opinion of the Investigator, the haematuria is not considered related to glomerulopathies the patient may be considered eligible, pending proper follow-up and a discussion with the Sponsor Medical Monitor or designee. Patients with history of bladder cancer must have been treated with curative intent and have not presented recurrence within the prior 5 years d. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) > 1.5 × upper limit of normal (ULN) e. Total bilirubin ≥ 1.5 × ULN (patients with total bilirubin ≥ 1.5 × ULN may be allowed on study if only indirect bilirubin is elevated, ALT/AST is not greater than the ULN, and known to have Gilbert's disease) f. Platelet count < 125,000/mm^3 g. Serum potassium > 5.1 mmol/L h. Estimated glomerular filtration rate (eGFR) of < 30 mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration formula i. Hemoglobin A1c (HbA1c) of > 9% or diabetes mellitus type 2 not well-controlled based on the Investigator's judgement

PROTOCOL SYNOPSIS (CONTINUED)

Study Population (Continued)	**Exclusion Criteria (Continued)** 17. Clinically significant abnormalities upon physical examination which in the Investigator's opinion should exclude the patient from study participation 18. Patient works nighttime shifts (e.g., 11 PM to 7 AM) 19. Have any other conditions, which, in the opinion of the Investigator or Sponsor would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study 20. Unwilling to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator
Treatment Groups	Approximately 150 patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 and randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio.
Study Drug Dosage and Administration	ISIS 757456 (100 mg/mL) and placebo will be supplied in vials of 0.8 mL in a stoppered glass vial. Study Drug (ISIS 757456 or placebo) injection volumes will be 0.8 mL or 1.2 mL for Cohort A and Cohort B, respectively. All Study Drug injections will be administered by qualified personnel at the clinic or by a Home Healthcare Professional.
Rationale for Dose and Schedule Selection	The dose levels of 80 mg and 120 mg were selected based on safety, PK, tolerability and PD data from the ISIS 757456 Phase 1 study in healthy volunteers and ISIS 757456 Phase 2 studies in uncontrolled hypertensive patients (wash out patients and patients on 2 to 3 HTN medications). In the ISIS 757456 Phase 1 study plasma AGT decreased in a dose-dependent manner after 6 weeks of 40 mg and 80 mg once-weekly doses, and the mean reduction of AGT was approximately 46% and 60% (Study Day 43), respectively. In 2 completed Phase 2 studies in patients with controlled and uncontrolled HTN, the mean reduction of AGT was observed up to approximately 54% after 6 weeks and 67% after 8 weeks of dosing of 80 mg once-weekly doses. Although neither study was powered for statistical relevant reductions in BP, a trend in SBP lowering > 5 mmHg was observed in patients treated with ISIS 757456 compared to placebo. Hence, the 80 mg dose level was selected for this study as well. Exposure-response analysis of the Phase 1 and 2 studies suggested a potential for more reductions in plasma AGT with a dose of 120 mg (compared to 80 mg). Hence, the dose level of 120 mg was also selected to evaluate plasma AGT dynamics and to evaluate further, the subsequent changes in SBP reductions. In both Phase 2 trials, including patients on ACEi or ARB therapy, no protocol stopping rules were met for renal, hepatic, platelet, hypotension or hyperkalemia. The proposed 120 mg per week dose is also supported by the 3.5-fold margin from the no observed adverse effect level (NOAEL) observed at 6 mg/kg/wk in the 9-month chronic toxicology study.
Adjustment of Dose and/or Treatment Schedule	An adjustment to the dose frequency from weekly to every 2 weeks or a weekly dose reduction may be permitted for safety or efficacy reasons in consultation with the Sponsor Medical Monitor or designee.

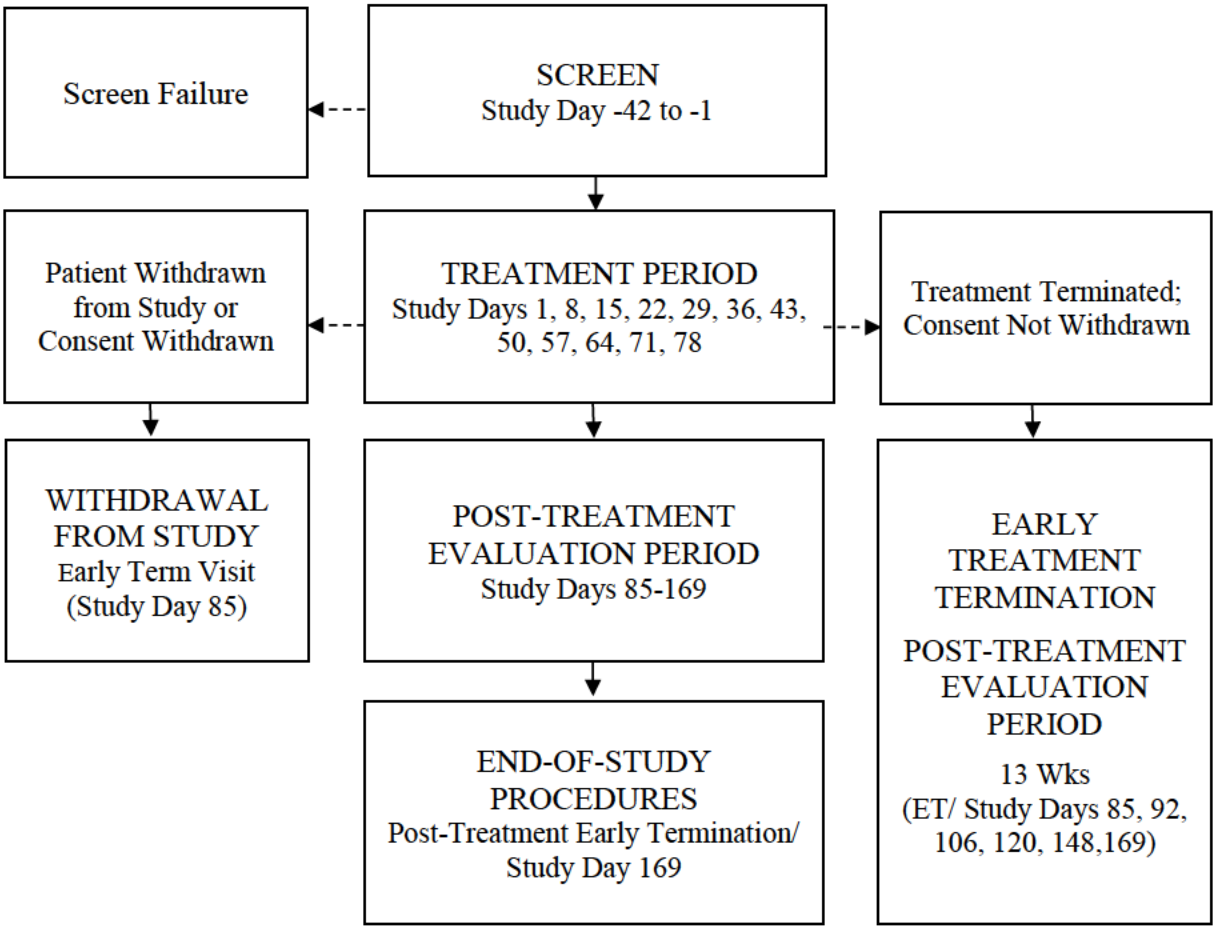
PROTOCOL SYNOPSIS (CONTINUED)

Study Visit Schedule and Procedures	In-clinic BP, blood and urine samples will be collected regularly throughout the study for safety, efficacy, and exploratory analyses. Appendix B shows a list of analytes required for the study. The safety of ISIS 757456 will be monitored in an ongoing fashion throughout the trial. **Screening: Week -6 to Week -1** Laboratory and other study procedures will be performed to assess eligibility during the Screening Period. Patients will be given a hydration and low sodium diet education at the Screening Visit. **Treatment: Week 1 to Week 12** Eligible patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 and randomized 1:1:1 to 80 mg ISIS 757456: 120 mg ISIS 757456: placebo. Patients will receive SC doses of Study Drug on Study Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78. Patients will continue their previous antihypertensive medication regimen throughout the study. The study site will counsel and ask the patient at each visit if (s)he is maintaining a low sodium diet and adequate hydration. The patient is expected to maintain adequate hydration and a low sodium diet throughout the study. Patients that discontinue treatment are encouraged to remain in the study for the Post-Treatment Evaluation Period and will conduct procedures outlined at Study Day 85 visit upon discontinuation of Study Drug. **Post-Treatment: Week 13 to Week 25** Patients are to return to the Study Center for follow-up visits on Study Days 85, 92, 106, 120, 148, and 169. The study site will continue to counsel and ask the patient at each visit if (s)he is maintaining a low sodium diet and adequate hydration in the Post-Treatment Period. The final study visit will be Study Day 169.
Primary Endpoint	• Change in seated automated office SBP from Baseline to Study Day 85
Secondary Endpoints	• Absolute levels and change and percent change in plasma AGT from Baseline to each scheduled, post-baseline visit • Changes from Baseline to Study Day 85 in 24-hour mean SBP and DBP measured by ambulatory blood pressure monitoring (ABPM) • Percentage of patients reaching the goals of seated automated office seated SBP $\leq$ 140 mmHg, DBP $\leq$ 90 mmHg, and both during the study (excluding patients with a baseline SBP of $\leq$ 140 mmHg) • Percentage of patients reaching the goals of automated office seated SBP $\leq$ 130 mmHg, DBP $\leq$ 80 mmHg, and both during the study • Change on seated automated office SBP from Baseline to each scheduled, post-Baseline visit • Change on seated automated office DBP from Baseline to each scheduled, post-Baseline visit

PROTOCOL SYNOPSIS (CONTINUED)

Exploratory Endpoints	• Absolute levels and change and percent change of angiotensin II, renin (PRA; direct renin), urinary AGT from Baseline to each scheduled, post-Baseline visit • Summary of peak and trough ISIS 757456 plasma concentrations • Potential exposure-response analysis using relevant exposure parameters and biomarkers including, but not limited to trough concentration (C_{trough}) and plasma AGT may be performed • Changes from baseline to Study Day 85 and each scheduled post Baseline assessment in the following patient reported outcome questionnaires: EuroQol-5 Dimension 5 Level (EQ-5D-5L), 12-Item Short Form Survey (SF-12), Power Over Pressure- Hypertension Specific Questionnaire (POP-HSQ), Patient Global Impression- Change (PGI-C), and Patient Global Impression- Severity (PGI-S)
Safety Endpoint	• Incidence and severity of treatment-emergent adverse events (TEAE), use of concomitant medications, abnormal findings in laboratory assessments, ECG, and vital signs
Statistical Considerations	Approximately 150 patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 and randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio. The planned sample size will provide approximate 80% of power to demonstrate the primary efficacy endpoint of mean SBP reduction difference of 8 mmHg between each ISIS treated group and pooled placebo based on the assumption of pooled standard deviation of 13 mmHg with the two-sided test and a significant level of 0.05 while taking into account an estimated 10% drop-out rate. The overall type I error rate is at 0.1 level, two-sided. The primary efficacy analysis will be the comparison of change from Baseline to Study Day 85 in SBP. The multiplicity will be controlled by using the sequential testing strategy. All planned analyses will compare ISIS 757456 to pooled placebo. An interim analysis may be conducted after approximately 50% of the patients have been enrolled.
Sponsor	Ionis Pharmaceuticals, Inc.

STUDY DESIGN AND TREATMENT SCHEMA



STUDY GLOSSARY

Abbreviation	Definition
2′-MOE	2′-*O*-(2-methoxyethyl)
ABP	ambulatory blood pressure
ABPM	ambulatory blood pressure monitoring
ACEi	angiotensin-converting enzyme inhibitor
AE	adverse event
AGT	angiotensinogen
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
aPTT	activated partial thromboplastin time
ARB	angiotensin receptor blockers
ARF	acute renal failure
ASGPR	asialoglycoprotein receptor
ASO	antisense oligonucleotide
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
CKD	chronic kidney disease
CMV	cytomegalovirus
CRF	case report form
CRNMB	clinically relevant non-major bleeding
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	trough concentration
DBP	diastolic blood pressure
ECG	electrocardiogram
eCRF	electronic Case Report Form
eGFR	estimated glomerular filtration rate
EQ-5D-5L	EuroQoL-5 Dimension 5 Level
FAS	full analysis set
FSH	follicle stimulating hormone
GalNAc	*N*-acetyl galactosamine
GCP	Good Clinical Practice
HAV	hepatitis A virus
HbA1c	Hemoglobin A1c
HbsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
hs-CRP	CRP measured by high sensitivity assay
HTN	hypertension
hr(s)	hour(s)
ICH	International Conference on Harmonization
IgM	immunoglobulin M
INR	international normalized ratio

IRB	Institutional Review Board
ITP	immune thrombocytopenic purpura
IV	intravenous
MB	major bleeding
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NOAEL	No Observed Adverse Effect Level
NYHA	New York Heart Association
on study	The patient is 'on study' from signing of the informed consent until their last study visit
PD	pharmacodynamic(s)
PGI-C	Patient Global Impression- Change
PGI-S	Patient Global Impression- Severity
PK	pharmacokinetic(s)
POP-HSQ	Power Over Pressure- Hypertension Specific Questionnaire
PPS	per protocol set
PRA	plasma renin activity
PT	prothrombin time
RAAS	renin-angiotensin-aldosterone system
RHTN	resistant hypertension
RNase H1	an ubiquitous endonuclease that specifically hydrolyzes the RNA strand in RNA/DNA hybrids
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
siRNA	small interfering ribonucleic acid
SC	subcutaneous(ly)
SF-12	12-Item Short Form Survey
Study Day 1	defined as the first day Study Drug product is administered to the patient
Study Drug	ISIS 757456 or placebo
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
TTP	thrombotic cytopenic purpura
UACR	urine albumin-creatinine ratio
ULN	upper limit of normal
UPCR	urine protein-creatinine ratio
WBC	white blood cell
WOCBP	woman of childbearing potential

1. OBJECTIVES AND ENDPOINTS

1.1. Objectives

1.1.1. Primary Objective

- To evaluate the effect of ISIS 757456 compared to placebo on seated automated office systolic blood pressure (SBP) from Baseline to Study Day 85 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.1.2. Secondary Objectives

- To evaluate the effect of ISIS 757456 on plasma angiotensinogen (AGT) at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on 24-hour ambulatory blood pressure at scheduled visits in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on seated automated office SBP at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on seated automated office diastolic blood pressure (DBP) at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.1.3. Safety Objectives

- To evaluate the safety and tolerability of ISIS 757456 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.1.4. Exploratory Objectives

- To evaluate the effects of ISIS 757456 administered subcutaneously (SC) (e.g., angiotensin II, renin [Plasma renin activity (PRA); direct renin], urinary angiotensinogen) in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the pharmacokinetics (PK) of ISIS 757456 (as total full-length ASO, including fully conjugated, partially conjugated, and unconjugated ISIS 757456) administered SC in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate potential PK/ pharmacodynamic (PD) correlation with relevant biomarkers and/or clinical endpoints
- To evaluate the effects of ISIS 757456 on patient reported quality of life outcomes

1.2. Study Endpoints

1.2.1. Primary Endpoint

- Change in seated automated office SBP from Baseline to Study Day 85

1.2.2. Secondary Endpoints

- Absolute levels and change and percent change in plasma AGT from Baseline to each scheduled, post-Baseline visit
- Changes from Baseline to Study Day 85 in 24-hour mean SBP and DBP measured by ambulatory blood pressure monitoring (ABPM)
- Percentage of patients reaching the goals of seated automated office seated SBP ≤ 140 mmHg, DBP ≤ 90 mmHg, and both during the study (excluding patients with a baseline SBP of ≤ 140 mmHg)
- Percentage of patients reaching the goals of automated office seated SBP ≤ 130 mmHg, DBP ≤ 80 mmHg, and both during the study
- Change in seated automated office SBP from Baseline to each scheduled, post-Baseline visit
- Change in seated automated office DBP from Baseline to each scheduled, post-Baseline visit

1.2.3. Safety Endpoint

- Incidence and severity of treatment-emergent adverse events (TEAE), use of concomitant medications, abnormal findings in laboratory assessments, electrocardiogram (ECG), and vital signs

1.2.4. Exploratory Endpoints

- Absolute levels and change and percent change of angiotensin II, renin (PRA; direct renin), urinary AGT from Baseline to each scheduled, post-Baseline visit
- Summary of peak and trough ISIS 757456 plasma concentrations
- Potential exposure-response analysis using relevant exposure parameters and biomarkers including, but not limited to trough concentration (C_{trough}) and plasma AGT may be performed
- Changes from Baseline to Study Day 85 and each scheduled post-Baseline assessment in the following patient reported outcome questionnaires: EuroQol-5 Dimension 5 Level (EQ-5D-5L), 12-Item Short Form Survey (SF-12), Power Over Pressure- Hypertension Specific Questionnaire (POP-HSQ), Patient Global Impression- Change (PGI-C), and Patient Global Impression- Severity (PGI-S)

2. BACKGROUND AND RATIONALE

2.1. Overview of Disease

Hypertension (HTN) is defined as failure to achieve BP goal < 140/90 mmHg. HTN is a major contributor to cardiovascular disease (CVD) morbidity and mortality and chronic kidney disease (CKD). Approximately 1.5 M people in the US have myocardial infarction or stroke annually, with ~50% of these major adverse cardiovascular events attributed to HTN (Lawes, Vander Hoorn, and Rodgers 2008; Korsnes et al. 2015). Inadequate BP control can lead to increase cardiovascular risk. Some patients have pseudo-resistance HTN as they are not compliant with their medications or they have white coat HTN. Lowering blood pressure reduces cardiovascular (CV) risk including major CV events, CHD, stoke, heart failure, renal failure, all-cause mortality (Ettehad et al. 2016). Providing sustained and controlled blood pressure with weekly or less administration could benefit patients inadequately controlled with daily administered therapies. Night-time SBP has consistently been shown to be an important predictor of CV risk. A 10 mmHg increase in night-time SBP can increase risk of total CV events, stroke and cardiac mortality in diabetic patients (Draman et al. 2015).

Resistant hypertension (RHTN) is defined as failure to achieve BP goal of < 130/80 mmHg in patients adherent to adequate doses of ≥ 3 medications (1 of which is diuretic) (Judd and Calhoun 2014; Sigmund et al. 2020). In US alone, 70 million adults have HTN, of which 12-15% have RHTN. Among these patients 33% of them have uncontrolled RHTN (Judd and Calhoun 2014). In an analysis of National Health and Nutrition Examination Survey database, these patients are more likely to be black, with diabetes, with CKD Stage 3, with proteinuria and congestive heart failure compared to patients with HTN and without resistant HTN.

2.2. Therapeutic Rationale

AGT is the source of all downstream angiotensin metabolites. Angiotensin II (ang II) is the principal angiotensin of the RAAS cascade (Figure 1) that elicits a multitude of effects that impact the cardiovascular and renal systems. Ang II acts via multiple mechanisms to contribute to hypertension and related disease states such as heart failure and CKD. Such mechanisms include vasoconstriction, renal sodium reabsorption, cell proliferation and dedifferentiation, aldosterone secretion and activation of pro-fibrotic and reactive oxygen species pathways (Carey 2013; Te Riet et al. 2015). AGT is acted upon by renin and as such is the only known substrate for renin. Renin cleavage of AGT generates the *N*-terminal derived decapeptide, angiotensin I (Ang I), and the remaining protein des(AngI)-AGT. Renin cleavage of AGT is the rate-limiting step in Ang II formation, as Ang I is converted to Ang II by ACE in a substrate-limited reaction.

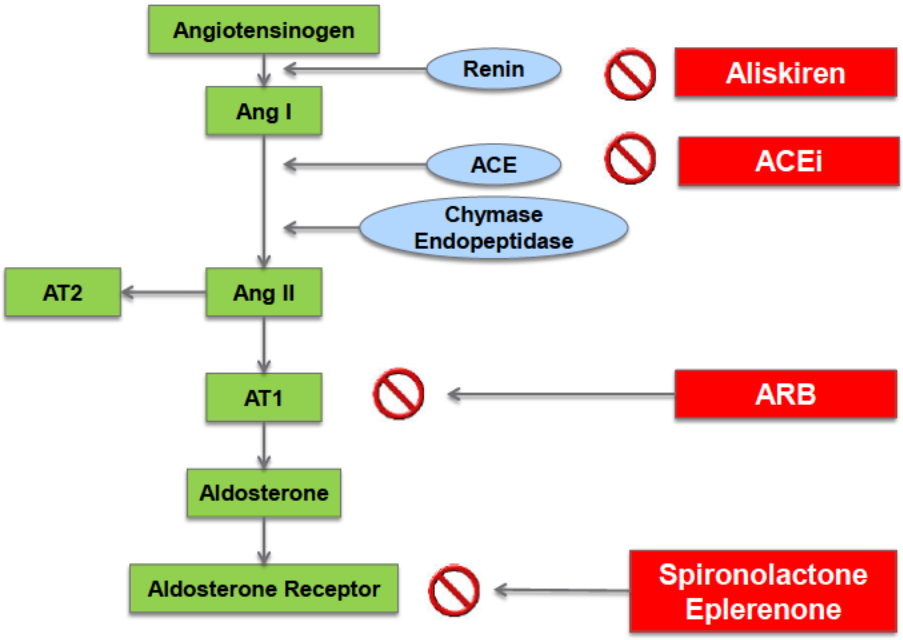
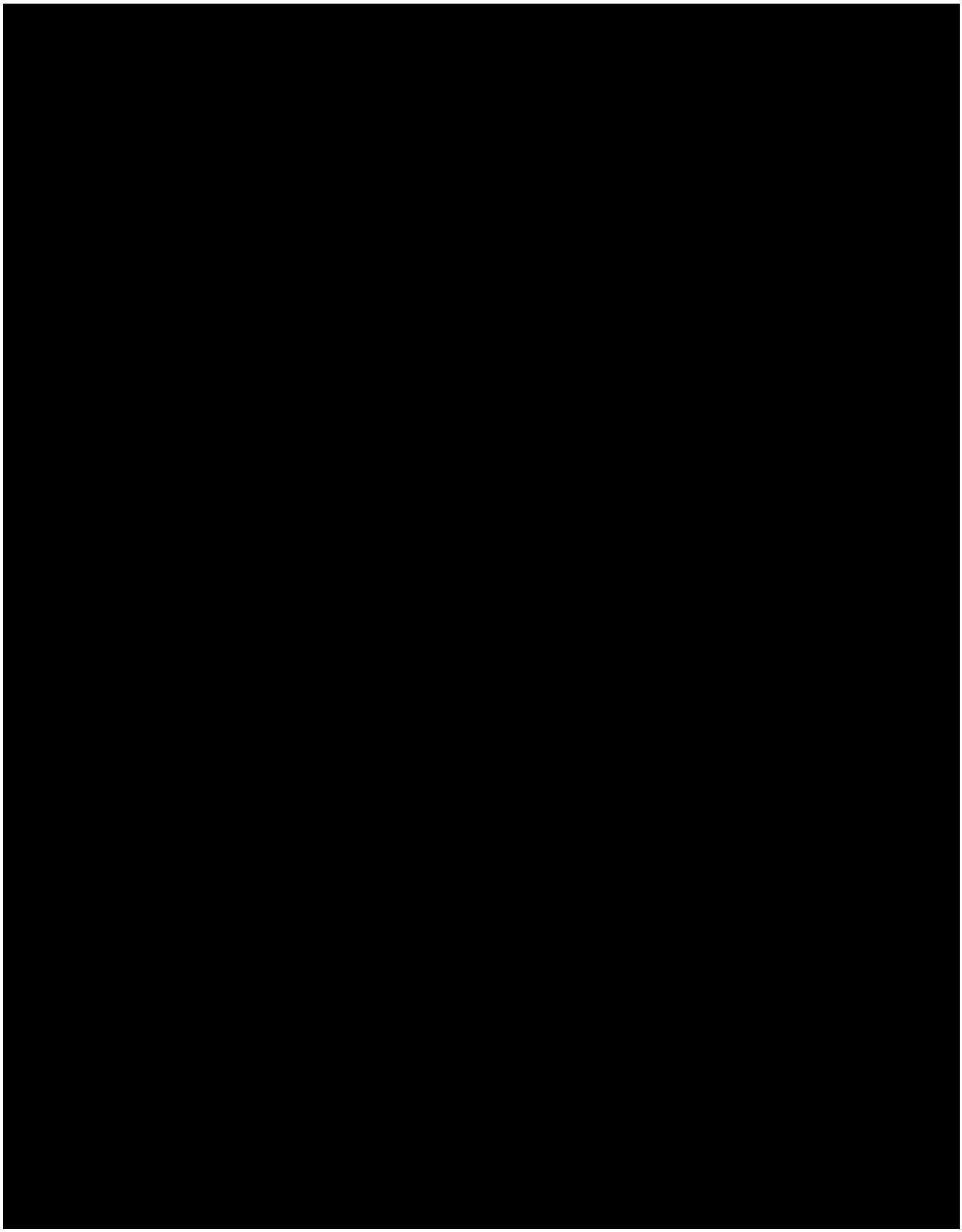


Figure 1: The Renin-Angiotensin-Aldosterone (RAAS) System and Site of Action for Principle Antagonists

Renin-angiotensin-aldosterone system (RAAS) inhibition is well established as a mode of improving HTN (Te Riet et al. 2015) and complications of HTN. While ACEi, angiotensin receptor blockers (ARB) and renin inhibitors are widely used, escape mechanisms are common and lead to incomplete RAAS blockade and limiting the efficacy of these therapies. Efforts to provide better RAAS blockade using 2 or more agents in this pathway have been complicated by hyperkalemia, hypotension, and acute renal failure (ARF). Angiotensinogen is the source of all downstream angiotensin metabolites, therefore, the effect of this antisense oligonucleotide (ASO) occurs upstream of all the known targets for RAAS inhibitors used in clinical practice and could potentially reduce escape mechanisms. The hyperkalemia and ARF of RAAS blockade could be secondary to renal specific reduction of these pathways. The *N*-acetyl galactosamine (GalNAc) ASOs have more prominent effect in the liver. As such, the renal sparing effects of this ASO may result in an improved therapeutic index compared with RAAS inhibitors, especially important in patients with CKD and/or those at submaximal RAAS blockage. It may also allow adequate BP control with fewer drugs in combination, or even as monotherapy, thus improving treatment compliance. Finally, due to its estimated half-life of 2 to 4 weeks, ISIS 757456 will have a superior duration of action vs. all RAAS blockers thereby contributing to the potential of ISIS 757456 having an enhanced pharmacological benefit relative to the standard of care (e.g., weekly administration for ISIS 757456 vs. daily administration for RAAS blockers).

2.3. ISIS 757456



[redacted]

2.3.3. Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 757456 can be found in the Investigator's Brochure.

2.3.4. Clinical Experience

Detailed information concerning the clinical studies conducted with ISIS 757456 can be found in the Investigator's Brochure. A summary is included below.

The safety and tolerability of ISIS 757456 was evaluated in a total of 62 healthy volunteer subjects aged 18–60 in a blinded, placebo-controlled, Phase 1 study ISIS 757456-CS1. Of these 62 subjects, 46 received ISIS 757456 (29 in the single-dose cohorts and 17 in the multiple-dose cohorts), and 16 received placebo. ISIS 757456 was well-tolerated at doses up to 80 mg administered as a single dose and 480 mg total dose (80 mg administered as 6 doses over 6 weeks). ISIS 757456 demonstrated dose-dependent plasma reduction of AGT levels following multiple doses, and there were no dose-dependent clinically meaningful trends in laboratory assessments and no serious adverse events (SAE). The Phase 1 study did not result in hypotension, hyperkalemia or renal changes. The elimination half-life of ISIS 757456 was estimated to be approximately 2-4 weeks.

ISIS 757456-CS2 was a Phase 2 multicenter, double-blind, placebo-controlled study to assess the safety, tolerability and efficacy of ISIS 757456 in patients with controlled BP. The study enrolled 25 patients randomized 2:1 (ISIS 757456 to placebo) to receive 80 mg ISIS 757456 or placebo for 6 weeks. The patients were washed out of their antihypertensive medications prior to Study Day 1 and the BP was confirmed to be uncontrolled after washout. A 54% mean reduction of AGT was observed at an 80 mg weekly dose over 6 weeks. A trend in blood pressure lowering was observed in patients treated with ISIS 757456 compared to placebo. Mean systolic blood pressure was reduced 7.5 mmHg in patients treated with ISIS 757456 and 1.7 mmHg in placebo patients. No rebound hypertension was observed. ISIS 757456 was well-tolerated and there were no clinically meaningful trends in laboratory assessments, including hypotension and hyperkalemia, no adverse events of special interest (AESI), and no related SAEs (Morgan et al. In Press).

ISIS 757456-CS3 was a Phase 2 multicenter, double-blind, placebo-controlled study to assess the safety, tolerability and efficacy of ISIS 757456 in hypertensive patients with uncontrolled BP. The patients continued a stable regimen of 2-3 antihypertensives, one of which was an ACEi or ARB, throughout the duration of the study. The study enrolled 26 patients randomized 2:1 (ISIS 757456 to placebo) to receive 80 mg ISIS 757456 or placebo. The Study Drug was administered once weekly for 8 weeks with a loading dose on Study Day 3. A 67% mean reduction of AGT was observed after the 8 weeks of dosing. A trend in blood pressure lowering was observed in patients treated with ISIS 757456 compared to placebo. Mean systolic blood pressure was reduced 12.8 mmHg in patients treated with ISIS 757456 and 4.6 mmHg in placebo patients. No rebound hypertension was observed. ISIS 757456 was well-tolerated in hypertension patients taking 2 or 3 hypertension medication including an ACEi or an ARB and

there were no clinically meaningful trends in laboratory assessments, including hypotension and hyperkalemia, no AESIs, and no SAEs (Morgan et al. In Press).

2.5. Benefit-Risk Assessment

Detailed information concerning the benefit-risk assessment of ISIS 757456 can be found in the Investigator's Brochure.

2.5.1. Benefit Assessment

This compound has been administered to healthy volunteers, hypertensive patients washed out of their antihypertensive medications, and hypertensive patients on antihypertensive medications. As a GalNAc, specifically due to the mediated uptake to the hepatocytes, ISIS 757456 will primarily affect circulating AGT rather than decrease renal AGT transcription. Therefore, it has the potential of an improved therapeutic index due to the potential of lower occurrence of hyperkalemia and ARF, which are issues with aggressive RAAS blockage in some subjects that may be due, in part, to excessive inhibition of intrarenal Ang II signaling. Based on its MOA of blocking the RAAS pathway it is expected to lower the BP in patients with HTN. While there are other compounds (ACEi, ARB, aldosterone blockers, renin inhibitors) that block different components of this pathway, this compound blocks AGT, the most upstream factor in the pathway. Finally, due to its estimated half-life of 2 to 4 weeks, ISIS 757456 may have a superior

duration of action compared to RAAS blockers thereby contributing to the potential of ISIS 757456 having an enhanced pharmacological benefit relative to the standard of care.

2.5.2. Risk Assessment

The known potential risks to study participants associated with ISIS 757456 are elaborated on in the Guidance for the Investigator section of the Investigator's Brochure. Additional study associated risks are:

Table 1: Known Risks Associated with RAAS Blockade

Potential Risk of Clinical Significance	**Summary of Data/Rationale for Risk**	**Mitigation Strategy**
Hypotension	RAAS blockade may lower BP Duration of effect: Long half-life of the ASO Extent of effect: AGT is the most upstream molecule in RAAS pathway	Eligibility criteria exclude patients at enhanced risk and Weekly BP monitoring Monitoring and stopping rules in place for hypotension
Hyperkalemia	RAAS blockade is known to increase serum potassium	ISIS 757456 is expected to have less effect on the renal RAAS system (less elevation of serum potassium) than ACEi, ARB or direct renin antagonists Most likely to occur in patients with high serum potassium. Eligibility criteria excludes K+ > 5.1 mmol/L and patients on medications that may cause hyperkalemia unless on a stable dose at least 1 month prior to the Screening visit and no known history of hyperkalemia per Investigator judgement. Eligibility criteria exclude patients at enhanced risk Monitoring and stopping rules in place for hyperkalemia
ARF	RAAS blockade is known to cause ARF	Based on liver (hepatocyte) targeting via the GalNAc conjugation, ISIS 757456 is expected to have less effect on the renal RAAS system than ACEi, ARB or direct renin antagonists Monitor renal function closely

3. EXPERIMENTAL PLAN

3.1. Study Design

This will be a Phase 2, double-blind, randomized, placebo-controlled study of ISIS 757456 conducted in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications (see Section 5.1 Inclusion Criteria). Patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 (see Section 10.1).

The 80 mg and 120 mg once-weekly doses are designed to assess the safety, tolerability, and efficacy of Study Drug (ISIS 757456 or placebo) administered over a 12-week period.

All patients will complete a 13-week Post-Treatment Period.

ISIS 757456 (100 mg/mL) and placebo will be supplied in vials of 0.8 mL in a stoppered glass vial. Study Drug (ISIS 757456 or placebo) injection volumes will be 0.8 mL or 1.2 mL for Cohort A and Cohort B, respectively. All Study Drug injections will be administered by qualified personnel at the clinic or by a Home Healthcare Professional (as arranged with the site staff and after appropriate training). Refer to Section 8.1 for additional details.

3.2. Number of Study Centers

This study will be conducted at multiple centers in the United States and Canada.

3.3. Number of Patients

Approximately 150 patients are planned to be enrolled in this study.

3.4. Overall Study Duration and Follow-up

The study will consist of Screening (up to 6 weeks), Treatment (12 weeks), and Post-Treatment Periods (13 weeks). Please refer to the Schedule of Procedures in Appendix A.

Patients may be required to attend additional visits for monitoring of adverse events (AEs) or abnormal investigation results. The frequency of additional monitoring will be determined by the Study Medical Monitor in consultation with the Investigator.

3.4.1. Screening

Patient eligibility for the study will be determined within 6 weeks prior to study entry (Study Day -42 to -1).

3.4.2. Treatment

Patients will be stratified based on based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 (see Section 10.1). Patients will receive a total of 12 SC doses of Study Drug once weekly for 12 weeks (Study Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78).

3.4.3. Post-Treatment

Patients are to return to the Study Center for Post-Treatment visits on Study Days 85, 92, 106, 120, 148, and 169. The final study visit will be Study Day 169.

3.5. End-of-Study

The End-of-Study is defined as the date of the last visit of the last patient.

4. PATIENT ENROLLMENT

4.1. Screening

Before patients may be enrolled into the study, the Sponsor requires a copy of the Study Center's written institutional review board (IRB) approval of the protocol, informed consent form, and all other patient information and/or recruitment material.

Patients must sign the consent form before any screening tests or assessments are performed. At the time of consent, the patient will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including Screening procedures, are performed. At the time of randomization, patients will be assigned a unique patient identification number. This number will be used to identify the patient throughout the trial and must be used on all study documentation related to that patient. The screening number and patient identification number must remain constant throughout the entire trial. Screening numbers and patient identification numbers, once assigned, will not be re-used.

4.2. Randomization

Patients will be randomized at Study Day 1, after all assessments have been completed and after the Investigator has verified that they are eligible per criteria in Section 5.1 and Section 5.2. No patient may begin treatment prior to randomization and assignment of a unique patient identification number.

Patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 (see Section 10.1) and then patients will be randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio.

The Sponsor or designee will prepare the randomization list and utilize an automated IRT (Interactive Response Technology) system.

4.3. Replacement of Patients

Patients who withdraw from the study will not be replaced.

4.4. Unblinding of Treatment Assignment

The Sponsor and all patients, monitors, and Study Center personnel related to the study, will be blinded throughout the study. However, if a patient has suffered a SAE (as defined in Section 9.3.3), and/or when knowledge of the treatment assignment will impact the clinical management of the patient, the Investigator will have the ability to unblind the treatment assignment for that patient. The Sponsor or designee will be informed of the unblinding of a patient within 24 hours. An unblinded randomization schema will be maintained securely at the

Sponsor's designated vendor. In addition, all SUSARs will be unblinded by the Sponsor or designee for the purpose of regulatory reporting (see Section 9.2).

Every reasonable attempt should be made to complete the early termination study procedures and observations (see Appendix A and Appendix B) prior to unblinding, as knowledge of the treatment arm could influence patient assessment.

An unblinded interim analysis may be performed and the results summarized by cohort after approximately 50% of the patients have been enrolled.

5. PATIENT ELIGIBILITY

To be eligible to participate in this study candidates must meet the following eligibility criteria at the time point specified in the individual eligibility criterion listed.

The Sponsor/Designee may be consulted if any questions arise regarding the inclusion or exclusion criteria.

5.1. Inclusion Criteria

1. Must have given written informed consent (signed and dated) and any authorizations required by local law and be able to comply with all study requirements
2. Males or females aged 18–80 inclusive and weighing ≥ 50 kg at the time of informed consent
3. Satisfy the following:
   a. Females: must be non-pregnant and non-lactating, and either:
      i. surgically sterile (e.g., bilateral tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or
      ii. post-menopausal (defined as 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and follicle stimulating hormone (FSH) levels in the postmenopausal range for the laboratory involved)
   b. Males must be abstinent*, surgically sterile (vasectomy with negative semen analysis at follow-up) or if engaged in sexual relations with a woman of childbearing potential (WOCBP), a highly effective contraceptive method must be used (refer to Section 6.4.1) from the time of signing the informed consent form until at least 20 weeks after the last dose of Study Drug (ISIS 757456 or placebo) or abstinent*

   * Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.
4. body mass index (BMI) ≤ 45.0 kg/m^2
5. Brachial circumference ≥ 22 and ≤ 43 cm (8.7 and 16.9 inches)

6. At Screening, the patient must have been on a stable, maximally tolerated regimen (per Investigator judgement) of 3 or more antihypertensive medications for at least 1 month prior to screening and will be required to maintain this regimen throughout the Study. The combination of antihypertensive medications must be in the following categories:
   a. angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB)
   b. beta blocker
   c. calcium channel blocker
   d. diuretic
   e. alpha-1 blocker
   f. centrally acting sympatholytic agent
   g. direct acting vasodilators (e.g., hydralazine)
7. At Screening Visit 1 and on Screening Visit 2 prior to the 24-hr ambulatory blood pressure monitoring, the average seated automated office BP (using the Sponsor provided BP machine) must be within > 130 – ≤ 170 mmHg systolic and meet the Exclusion Criterion 4 Pulse Pressure requirement. The average is to be derived from 3 assessments taken within 10 minutes. Up to 2 additional tests at each time point are allowed in order to qualify
8. Agree to abstain from alcoholic beverages for at least 24 hours prior to clinic visits
9. Agree to maintain adequate hydration and adhere to a low sodium diet throughout the duration of the study (from the Screening visit onward)
10. Agree to abstain from exercise, smoking, and caffeine use 30 minutes prior to seated automated office BP measurements

5.2. Exclusion Criteria

1. Clinically significant abnormalities in medical history according to Investigator judgement (e.g., major surgery non inclusive of exclusion criteria 12 within 2 months of Screening, type 1 diabetes mellitus)
2. History of secondary HTN including, but not limited to any of the following: renovascular HTN (unilateral or bilateral renal artery stenosis), coarctation of the aorta, primary hyperaldosteronism, Cushing's disease, pheochromocytoma, polycystic kidney disease, and drug-induced HTN
3. Patient with borderline orthostatic hypotension (assessed at Screening Visit 1), when they assume a standing position (within > 1 to ≤ 3 minutes of standing up), defined as:
   a. A decrease in SBP of ≥ 15 mmHg

   Up to 1 additional test may be done once the patient is adequately hydrated if orthostasis is thought to be attributed to volume depletion

4. Patient with a pulse pressure at Screening Visit 1 of > 80mmHg. Patients with a pulse pressure >80 mmHg at Screening Visit 1 may be allowed in consultation with the Sponsor Medical Monitor or designee. Pulse pressure is calculated by the difference between the office seated SBP and the office seated DBP (seated automated office blood pressure is to be derived by the average of 3 assessments)
5. The use of the following at time of Screening and during the course of the study:
    a. Other medications for the treatment of HTN (e.g., minoxidil, diazoxide, renin inhibitors)
    b. Medications that may cause hyperkalemia unless on a stable dose at least 1 month prior to the Screening visit and no history of hyperkalemia per Investigator judgement
    c. Use of oral anticoagulants, unless stable for 4 weeks prior to the first dose of Study Drug and regular monitoring must be performed per clinical practice during the study unless the patient is receiving vitamin K agonists. If the patient is receiving vitamin K antagonists (e.g., warfarin) international normalized ratio (INR) should be in therapeutic range, as established by the Investigator, for 4 weeks prior to the first dose
    d. Chronic administration (defined as > 3 days per week for the duration of the trial) of NSAIDs or Cox-2 inhibitors (except aspirin for cardiovascular provided the total daily dose does not exceed 325 mg)
    e. Use of phosphodiesterase 5 inhibitors (e.g., sildenafil, tadalafil, vardenafil, avanafil) within 72 hours prior to any scheduled visit
    f. Intentionally left blank
6. Treatment with another Study Drug, biological agent, or device within 1 month of Screening, or 5 half-lives of study agent, whichever is longer
7. Previous treatment with an oligonucleotide or other RNA therapeutic (including small interfering ribonucleic acid [siRNA]) within 4 months of Screening if single dose received, or within 12 months of Screening if multiple doses received. This exclusion criteria does not apply to the COVID-19 vaccines which are allowed. COVID-19 vaccination performed prior to study participation or during study participation will be collected in the electronic database
8. Active infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV) (as evidenced by a positive test for hepatitis B surface antigen), or hepatitis C (HCV) (diagnosed by initial serological testing and confirmed with RNA testing) or prior treatment for hepatitis C.
9. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be eligible if reviewed by the Sponsor Medical Monitor or designee
10. History of bleeding diathesis, coagulopathy, immune thrombocytopenic purpura (ITP), thrombotic cytopenic purpura (TTP), or any qualitative or quantitative platelet defect

11. Recent history of, or current drug or alcohol abuse
12. Unstable/underlying known cardiovascular disease defined as:
    a. Any history of congestive heart failure (New York Heart Association [NYHA] Class III-IV)
    b. Any history of previous myocardial infarction, coronary revascularization, unstable or stable angina pectoris < 1 year prior to screening
    c. Clinically relevant prolonged QTc (using Fridericia's formula [QTcF]) per Investigator judgement
    d. Any hemodynamically unstable atrial or ventricular arrhythmias
    e. Significant uncorrected valvular heart disease
    f. Any history of stroke or transient ischemic attack < 1 year prior to screening
13. A cardiac valve repair, cardiac device implantation, and/or a hospitalization for heart failure within 3 months of Screening
14. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1
15. Abnormal thyroid function tests at Screening with clinical significance, per Investigator judgement. Patients receiving dose-stable thyroid hormone replacement therapy for at least 3 months prior to screening will be allowed to participate as long as thyroid tests (TSH/T3/T4) show that patient is euthyroid
16. Screening laboratory results as follows, or any other clinically significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion
    a. Urine protein-creatinine ratio (UPCR) ≥ 0.5 mg/mg
    b. Intentionally left blank
    c. A positive test for blood (including trace) on urinalysis that is subsequently confirmed with urine microscopy showing > 5 red blood cells per high power field and is related to glomerulopathies. In women, this exclusion criterion must be assessed outside of menstrual period. If, in the opinion of the Investigator, the haematuria is not considered related to glomerulopathies the patient may be considered eligible, pending proper follow-up and a discussion with the Sponsor Medical Monitor or designee. Patients with history of bladder cancer must have been treated with curative intent and have not presented recurrence within the prior 5 years
    d. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) > 1.5 × upper limit of normal (ULN)
    e. Total bilirubin ≥ 1.5 × ULN (patients with total bilirubin ≥ 1.5 × ULN may be allowed on study if only indirect bilirubin is elevated, ALT/AST is not greater than the ULN, and known to have Gilbert's disease)
    f. Platelet count < 125,000/mm^3
    g. Serum potassium > 5.1 mmol/L

h. Estimated glomerular filtration rate (eGFR) of < 30 mL/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration formula

i. Hemoglobin A1c (HbA1c) of > 9% or diabetes mellitus type 2 not well-controlled based on the Investigator's judgement

17. Clinically significant abnormalities upon physical examination which in the Investigator's opinion should exclude the patient from study participation
18. Patient works nighttime shifts (e.g., 11 PM to 7 AM)
19. Have any other conditions, which, in the opinion of the Investigator or Sponsor would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study
20. Unwilling to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator

6. STUDY PROCEDURES

6.1. Study Schedule

All required study procedures are listed by visit in Appendix A, Appendix B and Appendix C.

6.1.1. Screening

Written informed consent for the study will be obtained prior to the performance of any study-related procedures including screening procedures. A 6-week period is provided for completing screening assessments and determining patient eligibility for the study. Safety labs may be re-tested up to 2 additional times for determination of patient eligibility.

During the Screening Period, patients will undergo a medical history (including cardiovascular disease risk factors) and physical examination including vital signs, orthostatic hypotension assessment, 12-lead ECG, and have blood and urine samples taken for clinical laboratory testing. Patients will be screened for HIV, HepB, and HCV.

During the Screening Period the study site will also educate patients regarding adequate hydration and low sodium diet.

A 24-hour blood pressure (BP) monitoring will be performed during the Screening Period.

The study site will record basic personal details, including name, contact details, gender, height, weight, date of birth, age, ethnicity, and racial origin (to be used only for clinical purposes), as well as information on medical history, and clinical data collected about the patient's participation in the study.

6.1.1.1. 24-Hour Ambulatory Blood Pressure Monitoring

A 24-hour ABPM, conducted with a Sponsor provided machine, will be assessed during the Screening Period and at one additional time point for patients who have received 8 or more doses as detailed in the Appendix A Schedule of Procedures.

The assessment should take place after the Screening Visit 1 blood pressure, pulse pressure, and labs have been resulted, the results are deemed not to be exclusionary, and blood pressure is confirmed not to be exclusionary at Screening Visit 2. Ensure sufficient time during the Screening Period to allow for a repeat (if the first 24-hr monitoring is not successful) prior to the Study Day 1 visit.

For patients who have been administered 8 or more doses of Study Drug, 24-hr ABPM should ideally be initiated in-clinic after all other study procedures have been completed either on Study Day 78 or Day 85 (completed 12 doses) or Treatment Early Termination/Study Day 85 (completed 8-11 doses). If a repeat is necessary, the 24-hr ABPM should be initiated no later than the Study Day 92 visit. Ensure the 24-hr ABPM is not done concurrently with other study procedures.

Please refer to the Study Manual for additional details.

6.1.1.2. Seated Automated Office Blood Pressure Measuring Instructions at Each Visit

The seated automated office blood pressure monitoring is to be conducted with Sponsor provided blood pressure machines throughout the study.

Patient Instructions

- Patient should have abstained from smoking, exercise and caffeine use at least 30 minutes prior to BP measurements
- Patient should be wearing a loose, short-sleeved top. If patient is wearing a long-sleeved or tight garment around the arm, provide a gown or remove the arm from the sleeve
- Patient should be comfortably seated (e.g., with his/her back against the chair, feet flat on the floor)
- Patient's arm should be bent at the elbow and supported by a table

Observer Instructions

- Confirm with that the patient has abstained from smoking, exercise and caffeine use at least 30 minutes prior to measurement
- Place the seated automated office blood pressure cuff on the patient's upper arm at the level of the heart and centered at the midpoint of the humerus
- Leave patient alone for 5 minutes in a quiet setting
- After 5 minutes, return and take 3 readings, with approximately 2-3 minutes between readings
- Document the average of the 3 readings

Screening Visit 1 BP Results

At the Screening Visit 1, measure the BP per instructions above with both the right and left arm. The arm with the highest SBP (that meets inclusion/exclusion criteria) and meets the Pulse Pressure criteria should be used at each subsequent visit.

6.1.1.3. Orthostatic Blood Pressure Assessment

An orthostatic BP assessment will be done at Screening Visit 1. BP and pulse rate will be assessed 2 times (Table 2). The patient will lie down (supine) for at least 5 minutes, then BP and pulse rate will be measured. The patient will then change to a standing position and the BP and pulse rate measurements will be repeated after standing greater than 1 minute, but less than or equal to 3 minutes. Up to 1 additional test may be done once the patient is adequately hydrated if orthostasis is thought to be attributed to volume depletion.

Borderline orthostatic hypotension will be defined as a confirmed (sequence must be repeated to confirm) decrease in SBP $\geq$ 15 mmHg from BP at supine position.

Table 2: Orthostatic Hypotension Assessment

Position	Time in Position	Vital Signs Assessed
Supine	5 minutes	BP and pulse rate after 5 minutes
Standing	> 1 to $\leq$ 3 minutes	BP and pulse rate assessed within > 1 to $\leq$ 3 minutes of standing

6.1.2. Treatment Period

Eligible patients will be administered Study Drug (ISIS 757456 or placebo) on a weekly basis (12 weeks) for a total of 12 doses. Measurement and review of the in-clinic BP should be performed on the same day prior to administering the Study Drug. Of the 12 required study visits during the Treatment Period, 11 selected study visits have the option for Home Healthcare (a Sponsor approved vendor). Home Healthcare assessments and procedures may be conducted by a Home Healthcare professional at specified visits per the Schedule of Procedures (Appendix A) and as arranged by site staff. If 3 consecutive Home Healthcare visits have been conducted, the Sponsor must be consulted prior to the scheduling of a 4th in-home visit. The Home Healthcare visit must be conducted by a Home Healthcare professional that is appropriately trained prior to a Home Healthcare visit. If a visit cannot be performed in the clinic or by a Home Healthcare professional, patient contact (e.g., video call, text, email, etc.) is required by site personnel to assess any AEs, changes in concomitant medications, adequate hydration and low sodium diet counseling.

Safety and clinical laboratory evaluations as well as blood sampling for PK analysis will be performed periodically throughout the Treatment Period (Appendix A, Appendix B, and Appendix C). Any AEs and concomitant medications will be recorded. At each visit, qualified site staff will review vital signs and laboratory results prior to each dosing to ensure compliance with monitoring and stopping rules outlined in Section 8.

The patient is expected to maintain adequate hydration and a low sodium diet throughout the study. The study site will counsel and ask the patient at each in-clinic visit if (s)he is maintaining adequate hydration and a low sodium diet.

Patients who discontinue the Treatment Period early will continue in the study following the post-treatment evaluations (Section 6.1.3).

All safety data including AEs, BP, and concomitant medications will be reviewed by the Sponsor's Medical Monitor or designee on an ongoing basis throughout the trial

6.1.3. Post-Treatment Period

After the last dose (Study Day 78) or last dose for early termination patients, patients will return to the clinic once weekly for the first 2 weeks (Study Days 85, 92) and then on Study Days 106, 120, 148, and 169 for safety assessments.

The study site will continue to counsel and ask the patient at each visit if (s)he is maintaining adequate hydration and a low sodium diet in the Post-Treatment Period.

For patients who have been administered 8 or more doses of Study Drug, the second 24-hr ABPM will be conducted. This 24-hr APBM should ideally be initiated in-clinic after all other study procedures have been completed either on Study Day 78 or Treatment Early Termination/Study Day 85. If a repeat is necessary, the 24-hr ABPM should be initiated no later than the Study Day 92 visit. Ensure the 24-hr ABPM is not done concurrently with other study procedures.

All safety data including AEs, in-clinic BP, and concomitant medications will be reviewed by the Sponsor's Medical Monitor or designee on an ongoing basis throughout the trial.

Of the 6 required study visits during the Post-Treatment Period, 4 selected study visits have the option to be performed outside the clinic by a Sponsor selected Home Health Care professional. Home Healthcare assessments and procedures may be conducted by a Home Healthcare professional at specified visits per the Schedule of Procedures (Appendix A) and as arranged by site staff. If 3 consecutive Home Healthcare visits have been conducted, the Sponsor must be consulted prior to the scheduling of a 4th in-home visit. If a visit cannot be performed in the clinic or by Home Health Care, patient contact (e.g., video call, text, email, etc.) is required by site personnel to assess any AEs, changes in concomitant medications, adequate hydration and low sodium diet counseling.

6.2. Laboratory Assessments

Laboratory analyte samples will be collected throughout the study. A list of these analytes is contained in Appendix B.

If the platelet value, eGFR, UPCR, potassium, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing a repeat blood specimen should be re-drawn as soon as possible (ideally within 7 days, either in clinic, by Home Healthcare or local laboratory). Any uninterpretable platelet count, eGFR, UPCR, liver enzyme, or potassium result must be rechecked and determined not to have met a stopping rule before dosing can continue. If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15. If there is suspicion of EDTA mediated platelet clumping, a repeat platelet count should be collected in a sodium citrate tube as soon as possible. Treatment should be held if there is no evaluable platelet count within 18 days prior to the scheduled dose (Section 8.5.2).

6.3. Patient Reported Outcomes

The assessments listed in the sections below should be, ideally, performed prior to any interventional study procedures, such as blood draws and vital signs. Appropriate instructions will be provided to the patient by site staff.

6.3.1. Patient Global Impression Items

The Patient Global Impression- Change (PGI-C) is a questionnaire which asks the patient to rate the perceived level of improvement (or worsening) in the patient's hypertension since the start of study treatment, using categorical response options. The Patient Global Severity- Change (PGI-S) is a questionnaire which asks the patient to rate his or her current hypertension disease severity, using categorical response options. The assessments may take approximately 3 to 5 minutes each to complete.

6.3.2. EuroQol-5 Dimension 5 Level (EQ-5D-5L)

The EQ-5D-5L is a two-part, self-reported instrument that assesses the patient's health state. The patient must self-rate their level of severity for five dimensions that include mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. In a second part of the assessment, the patient must report their perceived health on a visual analog scale ranging from 0 to 100. The questionnaire may take approximately 3 to 5 minutes to complete.

6.3.3. 12-Item Short Form Survey (SF-12)

Patients will be asked to complete the SF-12, which is a set of generic, coherent, and easily administered quality of life measures. The SF-12 consists of 12 questions that measure 8 health domains: general health, physical functioning, role physical, bodily pain, vitality, social functioning, role emotional, and mental health. The assessment should take approximately 3 to 5 minutes to complete.

6.3.4. Power Over Pressure- Hypertension Specific Questionnaire (POP-HSQ)

The POP-HSQ is a disease specific patient reported questionnaire which assesses the patient's severity and frequency of nonspecific symptoms associated with hypertension and mental health as impacted by hypertension. The assessment may take approximately 15 minutes to complete.

6.4. Restriction on the Lifestyle of Patients

6.4.1. Contraception Requirements

All male and female patients must refrain from sperm/egg donation from the time of signing the informed consent until at least 20 weeks after the patient's last dose of study treatment.

Males must be abstinent[†], or if engaged in sexual relations with a woman of childbearing potential, highly effective contraception must be used from the time of signing the informed consent until at least 20 weeks after the patient's last dose of study treatment.

For the purposes of this study, WOCBP are defined as any female who has experienced menarche, and who does not meet one of the following conditions:

- Postmenopausal: 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved
- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Post-hysterectomy

For the purposes of the study, highly effective contraception is defined as follows:

- For males: surgical sterilization (vasectomy with negative semen analysis at follow-up), a surgically sterile non-pregnant female partner, or the non-pregnant female partner of WOCBP uses a highly effective contraceptive method (defined below)
- For female partners of male subjects: surgical sterilization (e.g., bilateral tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy), hormonal contraception associated with inhibition of ovulation (combined estrogen and progestogen containing, or progestogen-only), intrauterine contraception device or intrauterine hormone-releasing system (IUS).

Male patients with partners that are pregnant must use condoms as barrier method to ensure that the fetus is not exposed to the Study Drug.

†**Note:** Abstinence (i.e., refraining from heterosexual intercourse throughout the duration of study participation) is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.

7. STUDY DRUG

[redacted]

7.2. Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug (ISIS 757456 or placebo) labeled in accordance with specific country regulatory requirements.

7.3. Study Drug Accountability

The study staff is required to document the receipt, dispensing, and return/destruction of Study Drug (ISIS 757456 or placebo) supplies provided by the Sponsor according to Sponsor instruction and in accordance with institutional policy.

8. TREATMENT OF PATIENTS

8.1. Study Drug Administration

Please refer to the Study Drug Manual provided by the Sponsor for more detailed instructions for Study Drug (ISIS 757456 or placebo) preparation and administration.

Table 4: Study Drug Dosing Information

Cohort	[redacted]	**Total Dose**
Cohort A		80 mg ISIS 757456 or placebo SC
Cohort B		120 mg ISIS 757456 or placebo SC

* The administration of Study Drug for doses of 120 mg may be delivered as a single injection or 2 non-contiguous injections. [redacted]

8.2. Other Protocol-Required Drugs

Per eligibility criteria, patients must be on a stable regimen of 3 antihypertensives for at least 1 month prior to screening evaluations and will be required to continue the stable regimen throughout the duration of the study (Refer to Inclusion Criteria Section 5.1).

8.3. Other Protocol-Required Treatment Procedures

There are no other protocol-required treatment procedures.

8.4. Treatment Precautions

There are no treatment precautions required.

8.5. Safety Monitoring Rules

Please refer also to the 'Guidance for Investigator' section of the Investigator's Brochure.

For the purposes of safety monitoring Baseline is defined as:

- The last non-missing measurement prior to the first dose

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations.

Confirmation Guidance: At any time during the study (Treatment or Post-Treatment Periods), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible (ideally within 3 days of the initial collection). For stopping rules, if the initial laboratory result is observed during the Treatment Period, the results from the retest **must be available** prior to administering the next dose of Study Drug (ISIS 757456 or placebo).

Re-dosing Guidance: Patients with initial laboratory test values that reach a stopping rule must not be re-dosed until the re-test results are available. In general, patients who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator and the Sponsor Medical Monitor (or appropriately qualified designee) should confer as to whether additional close monitoring of the patient is appropriate. If any of the stopping criteria described below (refer to Sections 8.6.1 to 8.6.6) are met, the patient will be permanently discontinued from further treatment with Study Drug (ISIS 757456 or placebo), evaluated fully as outlined below and in consultation with the Sponsor Medical Monitor or appropriately qualified designee, and will be followed up in accordance with Section 8.8 of the protocol.

8.5.1. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009. For a definition of Baseline, please refer to guidance in Section 8.5 above.

In the event of an ALT or AST measurement that is > 3 × ULN at any time during the study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above. Additional, confirmatory measurements should also be performed if ALT or AST levels increase to 5 × ULN.

Frequency of Repeat Measurements: Patients with confirmed ALT or AST levels > 3 × ULN should have their liver chemistry tests (ALT, AST, ALP, INR and total bilirubin) retested at least once-weekly until ALT and AST levels become ≤ 1.2 × ULN or 1.2 × baseline value if the baseline value was > ULN.

Further Investigation into Liver Chemistry Elevations: For patients with confirmed ALT or AST levels > 3 × ULN, the following evaluations should be performed:

- Obtain a more detailed history of symptoms and prior and concurrent diseases
- Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Obtain a history for exposure to environmental chemical agents and travel

- Serology for viral hepatitis (hepatitis A virus [HAV] immunoglobulin M [IgM], HBsAg, HCV antibody, cytomegalovirus [CMV] IgM, and EBV antibody panel)
- Serology for autoimmune hepatitis (e.g., antinuclear antibody [ANA], smooth muscle antibody IgG, Liver-Kidney Microsome-1 Antibody, IgG)

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic computed tomography (CT), or magnetic resonance imaging (MRI) scans, may be performed at the discretion of the Investigator, in consultation with the Sponsor Medical Monitor. Repetition of the above evaluations should be considered if a patient's ALT and/or AST levels reach 5 × ULN.

For a definition of Baseline, please refer to guidance in Section 8.5.

8.5.2. Safety Monitoring Rules for Platelet Count Results

Please refer also to Table 5.

Platelet count will be monitored every 2 weeks during the Treatment Period and at the following visits in the Post-Treatment Period: Study Days 85, 92, 106, 120, 148, and 169. The Investigator should review all platelet count results within 48 hours of receipt. If a patient's platelet count falls to 100,000/mm^3 or less, then the patient's platelet counts should be monitored as described in Table 5. In case of platelet reduction to < 50,000/mm^3, the platelet monitoring rule defined in Stopping rules (Section 8.6.3) should be followed.

Treatment should be held if there is no evaluable platelet count within 18 days prior to the scheduled dose. Any uninterpretable platelet count result must be rechecked and determined not to have met a stopping rule before dosing can continue. If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15.

In the event of a platelet count < 75,000/mm^3, additional laboratory investigations may be conducted after discussion between the Investigator and the Sponsor Medical Monitor including: fibrinogen or D-dimer (local lab), complement (total C3, total C4, C5a), peripheral smear, citrated sample for platelets, CBC with reticulocytes and mean platelet volume, coagulation panel (prothrombin time/international normalized ratio [PT/INR], activated partial thromboplastin time [aPTT]), total globulins [IgG and IgM]), serology for hepatitis B virus, HCV, HIV (if not done at Screening), anti-platelet antibodies (specialty lab), and anti-drug antibodies (specialty lab).

Any case of a platelet count < 50,000/mm^3 should be reported to the Sponsor in an expedited fashion (see Section 9.3.4). **The Study Medical Monitor will discuss the assessment and treatment of such subjects with the Investigator.**

Table 5: Monitoring Schedule and Stopping Rules for Platelet Counts

Platelet Count on Rx	Drug Dose	Monitoring
> 100,000/mm^3	No action	Monitor per Schedule of Procedures (Appendix A)
≥ 75,000– ≤ 100,000/mm^3	No action	Monitor every week until 3 successive values > 100,000/mm^3
≥ 50,000– < 75,000/mm^3	Pause dosing When platelet count returns to > 100,000/mm^3 restart dosing **only if approved by Sponsor Medical Monitor**	Monitor at least twice weekly until 3 successive values > 75,000/mm^3 then weekly until 3 values > 100,000/mm^3 If **not** redosing then subsequent monitoring should be per the Schedule of Procedures (Appendix A) If redosing then continue to monitor weekly for the remainder of the Treatment Period Consider discontinuation of antiplatelet agents / non-steroidal anti-inflammatory drug (NSAIDS) / anticoagulant medication while platelet count < 75,000/mm^3
≥ 25,000– < 50,000/mm^3	Permanently discontinue Study Drug	Report to the Sponsor in an expedited fashion Monitor at least twice weekly until 3 successive values > 75,000/mm^3 then weekly until 3 values > 100,000/mm^3. Subsequent monitoring should be per the Schedule of Procedures (Appendix A) Discontinue antiplatelet agents / NSAIDS / anticoagulant medication while platelet count < 75,000/mm^3 if possible
< 25,000/mm^3	Permanently discontinue Study Drug	Report to the Sponsor in an expedited fashion Monitor daily until 3 successive values > 25,000/mm^3 then monitor at least twice weekly until 3 successive values > 75,000/mm^3 then weekly until 3 values > 100,000/mm^3. Subsequent monitoring should be per the Schedule of Procedures (Appendix A) Corticosteroids recommended* Consider need for hospitalization and referral to hematologist Discontinue antiplatelet agents / NSAIDS / anticoagulant medication while platelet count < 75,000/mm^3 if possible

* Recovery in platelet count may be accelerated by administration of high-dose glucocorticoids. Treatment as recommended by the American Society of Hematology (ASH) (2019) guidelines for immune thrombocytopenia (Blood Advances, 10 DECEMBER 2019, Volume 3, Number 23) includes initial therapy with either dexamethasone 40 mg per day for 4 days, or prednisone 0.5 to 2.0 mg/kg per day. Prednisolone or prednisone may be administered for up to 2 to 4 weeks with taper; alternatively, intravenous immunoglobulin (IVIG) may be administered at 0.4 g/kg/d for 5 days, or infusions of 1 g/kg/d for 1-2 days (Provan et al. 2010; Provan et al. 2019).

8.5.3. Safety Monitoring for Minor Bleeding Events

Minor bleeding events are those that do not fulfill the criteria for major bleeding (MB) or clinically relevant, non-major bleeding (CRNMB) events (which are defined in Section 8.6.4), for example excess bruising, petechiae, gingival bleeding on brushing teeth. Additional testing of platelet counts and coagulation parameters (PT/INR/aPTT) should be considered after consultation with the Sponsor Medical Monitor.

8.5.4. Safety Monitoring for Potassium

In the event of a potassium measurement > 5.3 mmol/L at any time during the study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed. If the initial potassium measurement is confirmed to be > 5.3 mmol/L, weekly monitoring will be initiated until levels return to < 5.3 mmol/L.

In the event of a potassium measurement ≥ 5.5 mmol/L, but less than 6.0 mmol/L, study drug is to be held. In the event of a potassium measurement ≥ 6.0 mmol/L refer to Section 8.6.5 Stopping Rule for Potassium. In the event of a potassium measurement ≥ 5.5 mmol/L, the Investigator or designee should assess for symptoms consistent with hyperkalemia (e.g., muscle weakness or paralysis). An ECG may be considered per Investigator judgement to assess electrocardiographic signs of hyperkalemia (delayed conduction, heart block, arrhythmias) which, if conducted, should be discussed with the Sponsor Medical Monitor or designee to determine continued dosing with Study Drug. The investigator or designee should consider treating the patients with therapies that reduce the serum potassium, such as a low-potassium diet, diuretics, or a reduction or cessation of medications that can increase the serum potassium (ACEi/ARB). Serum potassium should be measured frequently until documented to be < 5.5 mmol/L. Dosing with Study Drug may continue after a confirmed potassium measurement < 5.5 mmol/L with Investigator and Sponsor Medical Monitor or designee consultation.

8.5.5. Safety Monitoring for Blood Pressure

If the patient is symptomatic (i.e., dizziness, light-headedness, clammy skin, fatigue, blurry vision), at any point in the study, the patient should contact the Study Center immediately or should a patient experience a BP measurement of ≤ 90 mmHg (systolic) 2 additional measurements must be done within a 30-minute period. If the confirmed average of the 2 additional SBP measurements is ≤ 90 mmHg and/or the patient remains symptomatic, dosing of a patient with Study Drug will be paused. Oral and/or IV hydration should be instituted. The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Sponsor Medical Monitor after the SBP has normalized and/or patient is no longer symptomatic and will be based on the reversibility of initiating factors (e.g., decreased oral intake, cold/flu, other illness). If the patient remains symptomatic or if the SBP does not normalize Study Drug should be permanently discontinued (refer to Section 8.6.6).

8.6. Stopping Rules

For the purposes of the stopping rules, Baseline is defined as:

- Temporary stopping rules for renal function tests – Baseline is defined as the average of the pre-dose test closest to Day 1 and Day 1
- Stopping rules for platelets – Baseline is defined as the last non-missing measurement prior to the first dose

8.6.1. Stopping Rules for Liver Chemistry Elevations

In the event of laboratory results meeting the following criteria, **and the event is without an alternative explanation as discussed with the Sponsor Medical Monitor,** dosing of a patient with Study Drug (ISIS 757456 or placebo) will be stopped permanently; values that are not confirmed due to failure to retest or missing lab values will be presumed confirmed:

1. ALT or AST > 8 × ULN, which is confirmed
2. ALT or AST > 5 × ULN, which is confirmed and persists for ≥ 2 weeks
3. ALT or AST > 3 × ULN, which is confirmed **and** total bilirubin > 2 × ULN or INR > 1.5
4. ALT or AST > 3 × ULN, which is confirmed, **and** the new appearance (i.e., onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN)

8.6.2. Stopping Rules for Renal Function Test Results

1. Confirmed 30% decline in eGFR from Baseline eGFR values [eGFR should be calculated using the CKD-EPI creatinine equation (Levey et al. 2009)]
2. Confirmed proteinuria: Absolute UPCR value ≥ 1000 mg/g

The possible dosing re-initiation or follow-up schedule for any events meeting either of these criteria will be determined by the Investigator **in consultation with** the Sponsor Medical Monitor or designee.

At the discretion of the Investigator, a decision to hold or permanently stop study drug may be made based on lesser changes in these parameters observed in isolation or in association with other renal-related abnormalities.

8.6.3. Stopping Rule for Platelet Count Results

See Table 5 for an overview of monitoring and stopping rules for platelet counts.

- In the event of a confirmed platelet count less than 50,000/mm^3, dosing of a patient with Study Drug (ISIS 757456 or placebo) will be stopped permanently
  - The platelet count should be tested at least twice weekly until 3 successive values above 75,000/mm^3
  - Then tested weekly until 3 values above 100,000/mm^3
  - Subsequent monitoring should follow the Schedule of Procedures (Appendix A)

- In the event of any platelet count less than 25,000/mm^3, dosing of the patient with Study Drug will be stopped permanently
  - Platelet count should be monitored daily until 3 successive values above 25,000/mm^3
  - Then monitor at least twice weekly until 3 successive values above 75,000/mm^3
  - Then monitor weekly until 3 successive values above 100,000/mm^3
  - Then monitor per the Schedule of Procedures (Appendix A)
  - Administration of corticosteroids is recommended for patients whose platelet count is less than 25,000/mm^3, with referral to a hematologist.

In the event of a platelet count < 75,000/mm^3 and ≥ 50,000/mm^3, and in the absence of MB or CRNMB (defined below), dosing with Study Drug should be suspended temporarily until the platelet count has recovered to > 100,000/mm^3. The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Study Medical Monitor and will be based on factors such as the original rate of decline in the patient's platelet count, whether any bleeding events were experienced, and the speed of recovery of platelet count after interruption of dosing. If dosing is reinitiated, platelet count must be measured twice weekly (consider more frequent monitoring if risk factors for bleeding are present) until 3 successive values above 75,000/mm^3 then weekly until the end of study. Treatment should be held if there is no evaluable platelet count within 18 days prior to the scheduled dose. Any uninterpretable platelet count result must be rechecked and determined not to have met a stopping rule before dosing can continue.

Labs to Be Performed in the Event of a Platelet Count Less than 50,000/mm^3

The following is a list of recommended lab analyses for patients who have any occurrence of platelet count less than 50,000/mm^3. Archived samples prior to Day 1 of investigational treatment may also be analyzed in order to determine the patient's baseline conditions.

Table 6: Recommended Laboratory Analyses

To Be Performed at Local Lab
Peripheral smear (should be performed locally, fixed, and sent to central lab for review)
Fibrinogen split products or D-dimer on fresh blood
To Be Performed at Central Lab
Citrated sample for platelets
Coagulation panel (PT/INR, aPTT)
CBC with reticulocytes
Total globulins: IgG and IgM
Complement: total C3, total C4, C5a
Serology for:
HBV, HCV, HIV (if not done recently for screening)
To Be Performed at Specialty Lab(s)
Antiplatelet antibodies
Anti-ASO antibody

Note: These labs may be performed in the event of a platelet count less than 75,000/mm^3 after discussion with the Study Medical Monitor. Additional lab tests may be considered in order to ascertain potential causative conditions for significant platelet count decreases / thrombocytopenia.

8.6.4. Bleeding Events

8.6.4.1. Definition of Major Bleeding Events (Schulman and Kearon 2005)

1. Fatal bleeding, and/or
2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarterial or pericardial, or intramuscular with compartment syndrome
3. Clinically overt bleeding leading to transfusion of ≥ 2 units of packed red blood cells or whole blood or a fall in hemoglobin of 2.0 mg/dL (1.24 mmol/L) or more within 24 hours

8.6.4.2. Definition of Clinically Relevant Non-Major Bleeding Events

Clinically relevant non-major bleeding is defined as overt bleeding not meeting the criteria for MB but that resulted, for example, in medical examination, intervention, or had clinical consequences for a patient.

8.6.4.3. Definition of Minor Bleeding Events

Minor bleeding events are those that do not fulfill the criteria for MB or CRNMB (defined above), for example excess bruising, petechiae, gingival bleeding on brushing teeth.

8.6.5. Stopping Rule for Potassium

In the event of confirmed potassium of ≥ 6.0 mmol/L dosing of a patient with Study Drug will be stopped permanently. Investigator or designee should assess for symptoms (e.g., muscle weakness or paralysis) and electrocardiographic signs of hyperkalemia (delayed conduction, heart block, arrhythmias). The Investigator or designee should consider treating the patients with therapies that reduce the serum potassium, such as a low-potassium diet, diuretics, or a reduction or cessation of medicines that can increase the serum potassium (ACEi/ARB). Additional treatments for hyperkalemia (e.g., intravenous [IV] calcium, insulin and glucose, gastrointestinal cation exchangers) can be considered per investigator judgment. The Investigator may consider obtaining a nephrology consultation with consideration for dialysis to treat hyperkalemia. Additional consultation of the Investigator with the Sponsor Medical Monitor may be considered to individualize treatment plan.

8.6.6. Stopping Rule for Blood Pressure

1. If the patient remains symptomatic (i.e., dizziness, light-headedness, clammy skin, fatigue, blurry vision) and/or in the event of the average of 2 additional BP measurements ≤ 90 mmHg (systolic) after instituting oral or IV hydration, Study Drug should be permanently discontinued, and the patient should be referred to urgent care or the emergency room.

   Any other antihypertensives or other medications that could affect BP should be stopped, if feasible. The use of IV angiotensin II or vasopressors should be considered in patients that remain symptomatic and are hemodynamically unstable. The patient will continue to be followed per protocol in the study. Follow-up for patients that screen fail due to BP inclusion criteria (see Section 5.1) will not be necessary.

2. Should a patient experience a BP measurement > 180 mmHg (systolic) or > 115 mmHg (diastolic) 2 additional measurements must be done within a 30-minute period and the average must be calculated. If the average of the 2 additional measurements is > 180 mmHg (systolic) or > 115 mmHg (diastolic), they will then be withdrawn from further blinded, randomized treatment. Study Drug is to be permanently discontinued unless the hypertensive event is transient and dependent on reversible initiating factors (e.g., stressful event). The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Sponsor Medical Monitor after the patient's BP has normalized. Additional antihypertensive medication(s) may be added per Investigator and Sponsor Medical Monitor judgement if the patient has discontinued Study Drug. If the patient has not discontinued Study Drug, changes to the stable regimen of antihypertensives may result in withdrawal of the patient from the Treatment Period. The patient will continue to be followed per protocol in the study. Follow-up for patients that screen fail due to BP inclusion criteria (see Section 5.1) will not be necessary.

Table 7: Blood Pressure Stopping and Monitoring Rules

Confirmed Blood Pressure	Dosing	Additional Actions
SBP ≤ 90 mmHg* and/or patient is symptomatic (e.g., decreased oral intake, cold/flu, other illness)	• Pause	• Institute oral and/or IV hydration. If hypotension or symptoms of hypotension persist proceed to stopping rules below • The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Sponsor Medical Monitor after the SBP has normalized and/or patient is no longer symptomatic and will be based on the reversibility of initiating factors (e.g., decreased oral intake, cold/flu, other illness)
SBP ≤ 90 mmHg* and/or the patient remains symptomatic after instituting oral and/or IV hydration and there is no other reason contributing to hypotensive event	• Permanently Discontinue	• The patient should be referred to urgent care or the emergency room • Any other antihypertensives or other medications that could affect BP should be stopped, if feasible • The use of IV angiotensin II or vasopressors should be considered in patients that remain symptomatic and are hemodynamically unstable • The patient will continue to be followed per protocol in the study. Follow-up for patients that screen fail due to BP inclusion criteria (see Section 5.1) will not be necessary
SBP > 180 mmHg* or DBP > 115 mmHg*	• Pause if BP elevation is thought to be transient, reversible and related to external initiating factors (e.g., stressful event) • Permanently discontinue if no other reason contributing to hypertensive event	• The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Sponsor Medical Monitor after the patient's BP has normalized • Additional antihypertensive medication(s) may be added per Investigator and Sponsor Medical Monitor judgement if the patient has discontinued Study Drug. If the patient has not discontinued Study Drug, changes to the stable regimen of antihypertensives may result in withdrawal of the patient from the Treatment Period • The patient will continue to be followed per protocol in the study. Follow-up for patients that screen fail due to BP inclusion criteria (see Section 5.1) will not be necessary

* BP measurements must be confirmed by the average of 2 additional measurements done within a 30-minute period

8.7. Adjustment of Dose and/or Treatment Schedule

An adjustment to the dose frequency from weekly to every 2 weeks or a weekly dose reduction may be permitted for safety or efficacy reasons in consultation with the Sponsor Medical Monitor or designee.

8.8. Discontinuation of Study Drug

A patient must permanently discontinue study treatment for any of the following:

- The patient becomes pregnant. Report the pregnancy according to instructions in Section 9.5.4
- The patient withdraws consent
- The patient experiences an AE that necessitates permanent discontinuation of Study Drug
- The patient develops laboratory test abnormalities that meet any of the stopping rules listed in Sections 8.6.1 to 8.6.3
- The patient experiences an AE that necessitates unblinding of the Investigator

The reason for discontinuation of Study Drug must be recorded in the electronic Case Report Form (eCRF) and source documentation.

Patients who early terminate from treatment will complete the Post-Treatment study procedures per the Appendix A.

For patients withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination of study procedures and observations at the time of withdrawal (see Appendix A) and ideally within 2 weeks from the last dose of Study Drug.

8.9. Withdrawal of Patients from the Study Procedures

Patients must be withdrawn from study procedures for any of the following:

- Withdrawal of consent
- The patient is unwilling or unable to comply with the protocol

Other reasons for withdrawal of patients from study procedures might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from study, must be recorded in the eCRF.

Any patient who withdraws consent to participate in the study will be removed from further treatment and study observation immediately upon the date of request. These patients should be

encouraged to complete the early termination study procedures and observations at the time of withdrawal (Appendix A).

For patients withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination of study procedures and observations at the time of withdrawal (see Appendix A) and ideally within 2 weeks from the last dose of Study Drug.

8.10. Concomitant Therapy and Procedures

The use of concomitant therapies or procedures defined below must be recorded on the patient's eCRF. Adverse events related to administration of these therapies or procedures must also be documented on the appropriate eCRF.

8.10.1. Concomitant Therapy

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications, and vitamin supplements) administered between Screening and the Post-Treatment Evaluation Period.

Allowed Concomitant Therapy

Any other medications deemed necessary by the Investigator are allowed except those listed in the disallowed concomitant therapy.

COVID-19 vaccines are allowed during study participation. However, because the vaccines are associated with local injection site reactions and constitutional symptoms, the vaccination should NOT be dosed on the same day of Study Drug injection and avoid the same site of any Study Drug injection. If a patient has a reaction from a COVID-19 vaccination, ensure patient recovers prior to administering the next Study Drug injection (even if it incurs a delay).

Disallowed Concomitant Therapy

The following are disallowed concomitant therapies:

- Other medications for the treatment of HTN (e.g., minoxidil, diazoxide, renin inhibitors)
- Medications that may cause hyperkalemia unless on a stable dose at least 1 month prior to the Screening visit
- Use of oral anticoagulants unless stable for 4 weeks prior to the first dose of Study Drug and regular monitoring must be performed, per clinical practice during the study unless the patient is receiving vitamin K agonists. If the patient is receiving vitamin K antagonists (e.g., warfarin) INR should be in therapeutic range, as established by the Investigator, for 4 weeks prior to the first dose
- Chronic administration (defined as > 3 days per week for the duration of the trial) of NSAIDs or COX-2 inhibitors (except aspirin for cardiovascular disease provided the total daily dose does not exceed 325 mg)
- Use of phosphodiesterase 5 inhibitors (e.g., sildenafil, tadalafil, vardenafil, avanafil) within 72 hours prior to any scheduled visit

Changes to the stable regimen of antihypertensives allowed at Screening may result in withdrawal of the patient from the Treatment Period.

8.10.2. Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between Screening and the Post-Treatment Evaluation Period.

8.11. Treatment Compliance

Compliance with treatment dosing is to be monitored and recorded in the eCRF by Study Center staff.

9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING

9.1. Sponsor Review of Safety Information

Safety information will be collected, reviewed, and evaluated by the Sponsor or designee in accordance with the applicable Ionis and/or designee SOPs throughout the conduct of the clinical trial.

9.2. Regulatory Reporting Requirements

The Sponsor or designee is responsible for regulatory submissions and reporting to the Investigators of SAEs including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH GCP. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

Institutional Review Boards will be notified of any SAE according to applicable regulations.

In addition to the Investigator's assessment of relatedness, the Sponsor or designee will evaluate the available information and perform an independent assessment of all reported SAEs and determine if there is a reasonable possibility that the Study Drug (ISIS 757456 or placebo) is causally related to a reported SAE and, therefore, meets the definition of an SUSAR. While the Sponsor's independent causality assessment may differ from the Investigator's assessment, the country-specific regulatory requirements must be followed for expedited reporting of SUSAR to local regulatory authorities.

Appropriate personnel at the Sponsor or designee will unblind SUSARs for the purpose of regulatory reporting. The Sponsor or designee will submit SUSARs to Regulatory Agencies in blinded or unblinded fashion according to local law. The Sponsor or designee will submit SUSARs to Investigators in a blinded fashion.

For the purpose of regulatory reporting of SUSARs, there are no "expected" AEs in this study population. Investigator's Brochure (provided separately) for expected AEs.

9.3. Definitions

9.3.1. Adverse Event

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

An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at Baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from Study Drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies).

9.3.2. Adverse Drug Reaction and Suspected Unexpected Adverse Drug Reaction

Adverse Drug Reaction (ADR)

In the *pre-approval* clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not have been established, ADR is defined as follows:

> All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

The phrase "responses to a medicinal product" means that a causal relationship between the medicinal product and the AE has been determined by the Sponsor as at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Suspected Unexpected Adverse Drug Reaction

A suspected unexpected ADR is any ADR, the nature or severity of which is not consistent with the applicable product information, e.g., Investigator's Brochure for an unapproved medicinal (investigational) product.

A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

9.3.3. Serious Adverse Event

A SAE is any AE that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event

  An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly or birth defect in the offspring of the patient (whether the patient is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE]; the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

9.3.4. Adverse Event of Special Interest

Adverse events of special interest (AESI), including both serious or non-serious events, is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor could be appropriate.

For the purpose of this study, severe reductions in platelet count < 50,000/mm^3 accompanied by an MB event or CRNMB event, or platelet count of < 25,000/mm^3 independent of an MB or CRNMB event are considered as AEs of special interest. AESI must be reported to the Sponsor within 24 hours to ensure timely submission as the 15-day expedited report by the Sponsor to regulatory agencies.

Adverse events of special interest are required to be reported by the Investigator to the Sponsor immediately, no more than 24 hours of the Investigator's first knowledge of the event for expedited reporting to Regulatory Authorities.

9.4. Monitoring and Recording Adverse Events

Any pre-existing conditions or signs and/or symptoms present in a patient prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible. Before a diagnosis is confirmed, all symptoms should be reported as separate AEs.

9.4.1. Serious Adverse Events

In the interest of patient safety, and in order to fulfill regulatory requirements**,** all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor or designee within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the patient signs the informed consent form and stop at the end of the patient's Follow-up Period which is defined as Study Day 169. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message.

Once the patient is randomized to study drug, SAEs should be reported using electronic SAE electronic data capture system (EDC) whenever possible. In situations where electronic SAE submission is unavailable or of an SAE occurs prior to the patient's randomization, a **paper** Serious Adverse Event Form should be completed, and a copy should be faxed or emailed to the Sponsor or designee. For SAEs reported after randomization, information submitted on paper form should be entered into EDC as soon as the system becomes available. The SAE reporting instruction, including the fax number and email address can be found in the Investigator site file for the study.

Detailed follow-up information should be actively sought and promptly submitted electronically or on a Serious Adverse Event Form if reporting by paper before randomization.

All SAEs will be followed until resolution. Serious Adverse Events that remain ongoing past the patient's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the patient's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

9.4.2. Non-Serious Adverse Events

The recording of non-serious AEs will begin after the patient signs the informed consent form and will stop at the end of the patient's Follow-up Period, which is defined as Study Day 169. The Investigator will monitor each patient closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

9.4.3. Evaluation of Adverse Events (Serious and Non-Serious)

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form.

9.4.3.1. Relationship to the Study Drug

The event's relationship to the Study Drug (ISIS 757456 or placebo) is characterized by one of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug, e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the patient's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug (ISIS 757456 or placebo) administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug (ISIS 757456 or placebo) administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- **Not Related:** The event can be readily explained by the patient's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

9.4.3.2. Severity

The severity of AEs and SAEs relating to laboratory tests and AEs at the injection site will be graded based on criteria from the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017 (refer to Appendix D). Any AE not listed in Appendix D will be graded as follows:

- **Mild:** The event is easily tolerated by the patient and does not affect the patient's usual daily activities
- **Moderate:** The event causes the patient more discomfort and interrupts the patient's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the patient's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in Section 9.3.3).

9.4.3.3. Action Taken with Study Drug

Action taken with Study Drug (ISIS 757456 or placebo) due to the event is characterized by one of the following.

- **None:** No changes were made to Study Drug (ISIS 757456 or placebo) administration and dose
- **Not Applicable:** SAE/AE was reported during Screening Period prior to Study Drug administration
- **Permanently Discontinued:** Study Drug was discontinued and not restarted

- **Temporarily Interrupted, Restarted – Same Dose:** Dosing and/or dosing frequency was temporarily interrupted/changed or delayed due to the AE and restarted at the same dose
- **Reduced Dose:** Dosing was reduced, temporarily interrupted, or delayed due to the AE and restarted at the next lower dose or reduced dosing frequency

9.4.3.4. Treatment Given for Adverse Event

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form. Treatment should also be recorded on the concomitant treatment or ancillary procedures eCRF, as appropriate.

9.4.3.5. Outcome of the Adverse Event

If the event is a non-serious AE, then the event's outcome is characterized by one of the following:

- **AE Persists:** Patient terminates from the trial and the AE continues
- **Recovered:** Patient recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is an SAE, then the event's outcome is characterized by one of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Patient has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE eCRF (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Patient recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Recovered with Sequelae:** The signs/symptoms of the reported SAE have improved but not completely resolved, and a new baseline for the patient is established since full recovery is not expected
- **Fatal:** Patient died (the date of death should be entered as the SAE resolution date)
- **Unknown:** The outcome of the reported SAE is not available, e.g., patient is lost to follow-up

9.4.3.6. Follow-up of Adverse Event

Investigator Follow-Up

During the Study Period, the Investigator should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable, the patient is lost to follow-up, or the

patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to Study Drug or related to study procedures until a final outcome can be reported.

Resolution of AE (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

Investigator should follow-up or support the Sponsor's effort to follow up with all pregnancies reported during the study from either the study patient or the female partner of male study patient until pregnancy outcome is available.

Sponsor Follow-Up

For SAEs, AESI , and pregnancy cases in patients who has completed or terminated study, the Sponsor or a designee should follow-up by telephone, fax, email, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of these case.

9.5. Procedures for Handling Special Situations

9.5.1. Abnormalities of Laboratory Tests

Clinically significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Sponsor Medical Monitor. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents and should include the Investigator's assessment of clinical significance of out of range/abnormal laboratory values.

9.5.2. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the patient is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the patient's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the patient's consent to participate in the study and the timing of the procedure or treatment

- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

9.5.3. Medication Errors

Study Drug (ISIS 757456 or placebo) errors (for example, overdose, underdose, and administration error) should be reported as a medication error on the Protocol Deviation CRF. A brief description should be provided when reporting the event, including whether the patient was symptomatic or asymptomatic, and whether the event was accidental or intentional.

Dosing details should also be captured on the Dosing CRF. If the patient takes a dose of Study Drug that exceeds protocol specifications and the patient is symptomatic, then an AE of medication error should be reported per Section 9.4, and all symptoms should be included in the event description.

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment should be reported as an AE of medication error. All symptoms associated with an overdose or incorrect administration of Study Drug should be recorded on the Adverse Event eCRF. The event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

Should an overdose occur, the Investigator or designee should refer to the Guidance to Investigator's section of the Investigator's Brochure and contact the Sponsor or designee within 24 hours.

9.5.4. Contraception and Pregnancy

Male patients must continue to use appropriate contraception with their partners, or refrain from sexual activity, as described in Section 6.4.1.

If a male patient makes or believes that he has made someone pregnant during the study, then the Study Center staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination should be reported by designating as 'Follow-up' on the Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the patient's responsibility.

Male patients: The progress of the pregnancy of a male patient's partner should be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, the Study Center and Sponsor may follow-up with the mother and may request access to the mother and infant's medical records to obtain additional information relevant to the pregnancy progress and outcome. A longer follow-up may be required if a newborn child experiences a medical condition. Follow-up will be performed to the extent permitted by the applicable regulations and privacy considerations, e.g., partner ICF may be required.

10. STATISTICAL CONSIDERATIONS

10.1. Stratification, Subsets, and Covariates

Patients will be stratified on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2. In general, the covariates will include stratification factors and baseline measurement.

10.2. Sample Size Considerations

Approximately 150 patients will be stratified based on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2 and randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio. The planned sample size will provide approximate 80% of power to demonstrate the mean SBP reduction difference of 8 mmHg between each ISIS treated group and pooled placebo based on the assumption of pooled standard deviation of 13 mmHg with the two-sided test and a significant level of 0.05 while taking into account an estimated 10% drop-out rate. The overall type I error rate is at 0.1 level, two-sided.

10.3. Populations

Full Analysis Set (FAS): All randomized patients who have received at least 1 injection of Study Drug (ISIS 757456 or placebo) and who have at least 1 post-Baseline efficacy measurements.

Per Protocol Set (PPS): All FAS patients who received at least 10 of the 12 doses of Study Drug, did not alter Screening antihypertensive medications during the Treatment Period and prior to Study Day 85, and have no significant protocol deviations that would be expected to affect efficacy assessments.

Safety Set: All patients who are randomized and receive at least 1 dose of Study Drug.

PK Set: All patients who are randomized and receive at least 1 dose of Study Drug and have at least 1 evaluable PK sample.

10.4. Definition of Baseline

The baseline for plasma AGT will be define as the average of all values prior to the first dose of Study Drug.

The baseline for all other assessments will be defined as the last non-missing measurement prior to the first dose.

10.5. Interim Analysis and Multiplicity

An interim analysis may be conducted after approximately 50% of the patients have been enrolled.

For the final analysis, the multiplicity of primary and key secondary endpoints comparisons will be controlled by using sequential testing strategy in the following testing sequence:

- Comparison of change from baseline to Study Day 85 in SBP between each ISIS 757456 treated group and pooled placebo in the PPS

- Comparison of percent change from baseline to Study Day 85 in AGT between each ISIS 757456 treated group and pooled placebo in the PPS
- Comparison of change from baseline to Study Day 85 in SBP from 24 hr mean ABPM between each ISIS 757456 treated group and pooled placebo in the PPS
- Comparison of portion of patients reaching the goals of seated automated SBP ≤ 140 mmHg and DBP ≤ 90 mmHg at Day 85 between each ISIS 757456 treated group and pooled placebo in the PPS

The statistical comparison will be conducted with 2-sided alpha level of 0.05 for each ISIS 757456 treated group verse pooled placebo. If an efficacy endpoint comparison in the sequence of an ISIS treated group is not statistically significant, then the subsequent endpoints analyses will be considered as exploratory for that ISIS 757456 treated group. Details will be provided in the Statistical Analysis Plan (SAP).

10.6. Planned Methods of Analysis

All case report form (CRF) data, lab data transfers, and any outcomes derived from the data may be provided in the patient data listings. Patient data listings will be presented for all patients randomized in the study. Descriptive summary statistics including n, mean, median, standard deviation, standard error, interquartile range (25^{th} percentile, 75^{th} percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by treatment group. Where appropriate, p-values will be reported. The placebo patients from both cohorts will be pooled. All primary and secondary endpoints will be assessed on the Full Analysis Set and Per-Protocol Set, with the latter being the basis for the primary efficacy analysis. All safety assessments will be performed on the Safety Set.

10.6.1. Demographic and Baseline Characteristics

Demographic and Baseline characteristics will be summarized using descriptive statistics by treatment group. Patient randomization will be summarized by cohort and treatment group. The patient disposition will be summarized. All patients enrolled will be included in a summary of patient disposition.

10.6.2. Safety Analysis

The safety analysis will be conducted on the Safety Population.

Treatment duration and amount of Study Drug (ISIS 757456 or placebo) received will be summarized by treatment group. Patient incidence rates of all AEs will be tabulated by MedDRA™ system organ class, and by Preferred Term. Narratives of treatment emergent deaths, SAEs and AEs of special interest, including early withdrawals due to AEs, will also be provided.

All treatment-emergent AEs and SAEs as well as all treatment-emergent AEs and SAEs potentially related to Study Drug (ISIS 757456 or placebo) will be summarized.

Laboratory tests to ensure patient safety including chemistry panel, complete blood count with differential, coagulation panel, complement etc., will be summarized by study visits for each treatment group. These safety variables will also be presented as change and percent change

from Baseline over time after Study Drug (ISIS 757456 or placebo) administration, as appropriate.

Vital sign and ECG measures will be tabulated by treatment group. In addition, the number of patients who experience abnormalities in clinical laboratory evaluations will be summarized by treatment group.

10.6.3. Efficacy Analysis

The primary analysis will be the comparison of change from Baseline to Study Day 85 in SBP between each ISIS 757456 treated group and pooled placebo group in the PPS. The data will be analyzed using analysis of covariance (ANCOVA) with treatment, and randomization stratification factor (Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2), and baseline measure (SBP) as independent variables. In the case data departs substantially from normality, the nonparametric test will be employed instead.

For the dichotomous endpoints, logistic regression model will be used.

The secondary efficacy analyses will be performed in a similar way to the primary analysis, which include:

- Comparison of change from Baseline to Study Day 85 in SBP and DBP from 24 hr mean ABPM between each ISIS 757456 treated group and pooled placebo group in the PPS and FAS
- Comparison of change and percentage change from Baseline to each schedule post Baseline visit in plasma AGT between each ISIS 757456 treated group and pooled placebo group in PPS and FAS
- Comparison of proportion of patients reached the goal of SBP $\leq$ 140 mmHg, DBP $\leq$ 90 mmHg, and both for each scheduled visit between each ISIS 757456 treated group and pooled placebo group in PPS and FAS, excluding patients with baseline SBP $\leq$ 140 mmHg
- Comparison of proportion of patients reached the goal of SBP $\leq$ 130 mmHg, DBP $\leq$ 80 mmHg, and both for each scheduled visit between each ISIS 757456 treated group and pooled placebo group in PPS and FAS
- Comparison of change from Baseline to each schedule post-Baseline visit in SBP between each ISIS 757456 treated group and pooled placebo group in PPS and FAS
- Comparison of change from Baseline to each schedule post Baseline visit in DBP between each ISIS 757456 treated group and pooled placebo group in PPS and FAS

10.6.4. Pharmacokinetic Analysis

Plasma ISIS 757456 concentrations at trough during the Treatment Period and concentrations observed during the Post-Treatment Evaluation Period will be listed by dose, study day, time point, and summarized using descriptive statistics.

Other plasma PK parameters, as appropriate, may be determined or calculated at the discretion of the PK scientist.

Metabolite identification and profiling may be conducted on select plasma samples.

Additional details regarding the PK analysis will be described in the Statistical Analysis Plan (SAP).

Analysis of potential exposure-response relationship between plasma AGT and ISIS 757456 exposure (such as C_{trough}) will be conducted. Relationships between other relevant biomarkers and clinical endpoints with PK measures may also be explored, if deemed appropriate.

Population PK and PK/PD analysis may be performed using PK data from this Study, and/or combined with other ISIS 757456 clinical PK/PD data later in the development timeline.

10.6.5. Exploratory Analyses

The change and percent change from Baseline to each schedule post-Baseline visit in angiotensin II and renin (PRA; direct renin) will be compared between each ISIS 757456 treated group and pooled placebo group in PPS and FAS. The data will be analyzed in a similar way to the primary analysis.

Change from Baseline to each schedule post-Baseline visit in SF-12 scores (domain, physical component summary, and mental component summary) and EQ-5D-5L (dimension, visual acuity score, index score) will be compared between each ISIS 757456 treated group and pooled placebo group in PPS and FAS.

Proportion of improvement from baseline to each scheduled post Baseline in PGI-S and PGI-C will be compared between each ISIS 757456 treated group and pooled placebo group in PPS and FAS.

Proportion of each questionnaire over time in POP-HSQ will be compared between each ISIS 757456 treated group and pooled placebo group in PPS and FAS.

The analyses will be detailed in the SAP.

11. INVESTIGATOR'S REGULATORY OBLIGATIONS

11.1. Informed Consent

The written informed consent document should be prepared in the language(s) of the potential patient population, based on an English version provided by the Sponsor.

Before a patient's participation in the trial, the Investigator is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any Study Drug (ISIS 757456 or placebo) is administered. The patient must be given sufficient time to consider whether to participate in the study.

The acquisition of informed consent and the patient's agreement or refusal to notify his/her primary care physician should be documented in the patient's medical records and the informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion (not necessarily an Investigator). The original signed informed consent form should be retained in the Study Master File and in any other locations

required by institutional policy, and a copy of the signed consent form should be provided to the patient.

11.2. Ethical Conduct of the Study

All applicable regulations and guidelines of current Good Clinical Practice (GCP), the Declaration of Helsinki as well as the demands of national drug and data protection laws and other applicable regulatory requirements must be followed.

11.3. Independent Ethics Committee/Institutional Review Board

A copy of the protocol; proposed informed consent form, other written patient information, and any proposed advertising material must be submitted to the IRB for written approval. A copy of the written approval of the protocol and informed consent form must be received by the Sponsor before recruitment of patients into the study and shipment of Study Drug. A copy of the written approval of any other items/materials that must be approved by the Study Center or IRB must also be received by the Sponsor before recruitment of patients into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IRB for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document. The Investigator should notify the IRB of deviations from the protocol in accordance with ICH GCP. The Investigator should also notify the IRB of SAEs occurring at the Study Center and other AE reports received from the Sponsor, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IRB approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IRB submissions and the IRB continuance of approval must be sent to the Sponsor.

11.4. Patient Confidentiality

The Investigator must ensure that the patient's confidentiality is maintained. On the CRFs or other documents submitted to the Sponsor, patients should be identif

ied by initials (if permitted by local law) and a patient identification number only. Documents that are not for submission to the Sponsor (e.g., signed informed consent forms) should be kept in strict confidence by the Investigator.

In compliance with Federal and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB direct access to review the patient's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the patient to permit named representatives to have access to his/her study-related records without violating the confidentiality of the patient.

12. ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1. Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor. Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent document. The regulatory authority and IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the patients or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IRB to the Sponsor.

12.2. Study Termination

The Sponsor reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator should notify the IRB in writing of the trial's completion or early termination and send a copy of the notification to the Sponsor or designee.

12.3. Study Documentation and Storage

An eCRF utilizing an Electronic Data Capture (EDC) application will be used for this study.

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the patient's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, imaging, and correspondence. In this study, eCRF may not be used as source documents.

The Investigator and Study Center staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with ICH GCP, suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. Elements should include:

- Patient files containing completed CRFs, informed consents, and supporting copies of source documentation
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IRB and the Sponsor
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, final Study Drug product reconciliation, and all drug-related correspondence

In addition, all original source documents supporting entries in the CRFs must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor.

12.4. Study Monitoring

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., CRFs and other pertinent data) provided that patient confidentiality is respected.

The Sponsor monitor or designee is responsible for inspecting the CRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to patient medical records and other study-related records needed to verify the entries on the CRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department. Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on patient data received by the Sponsor. During this review, patient data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor.

The Principal Investigator will sign and date the indicated places on the CRF. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the CRF, the data queries, and the Study Center notifications, and agrees with the content.

12.5. Language

Case report forms must be completed in English. Generic names and trade names are acceptable for concomitant medications. Combination medications should be recorded using their trade name.

All written information and other material to be used by patients and investigative staff must use vocabulary and language that are clearly understood.

12.6. Compensation for Injury

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Patients will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

13. REFERENCES

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APPENDIX A. SCHEDULE OF PROCEDURES

Appendix A Schedule of Procedures

	Screen		Treatment Period (12 Weeks)												Post-Treatment Period (13 Weeks)					
Study Week			Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9	Wk10	Wk11	Wk12	Wk13	Wk14	Wk16	Wk18	Wk22	Wk25
Study Day	D-42 to D-1		D1	D8	D15	D22	D29	D36	D43	D50	D57	D64	D71	D78	Treatment Early Term[9]/D85	D92	D106	D120	D148	PT Early Term[9]/ D169
Visit Window (Days)	NA		NA	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 2	± 5	± 5	± 5
Scheduled Visit #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Option for Home Health Care Visit[10]				X	X	X	X	X	X	X	X	X	X	X[14]		X[14]	X	X	X	
Informed Consent	X																			
Inclusion/Exclusion	X	X	X[a]																	
Medical History	X																			
Hydration and Low Sodium Diet Education	X	← Continuous Counseling & Monitoring →																		
CVD Risk Factors	X																			
Body Weight and Height[8]	X		X												X					X
Physical Exam[1]	X		X												X					X
ECG (12-Lead)	X														X					
EQ-5D-5L, SF-12, POP-HSQ[15]			X						X						X		X			
PGI-S[15]			X												X					
PGI-C[15]															X					
HTN Medication Compliance			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (clinic)[2]	X	X	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X	X	X	X	X	X
HIV, Hep B & C	X																			
FSH[3]	X																			
Pregnancy Test[4]	X																			
Chemistry Panel[7, 10]	X		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	X	X	X	X	X
Archived Urine Sample[6]	X		X[a]				X[a]				X[a]				X	X	X	X	X	X
TSH, FT3, FT4	X																			
Hematology[7]	X		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	X	X	X	X	X
Urinalysis[10]	X[16]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	X	X	X	X	X

Appendix A Schedule of Procedures (Continued)

	Screen		Treatment Period (12 Weeks)												Post-Treatment Period (13 Weeks)					
Study Week			**Wk1**	**Wk2**	**Wk3**	**Wk4**	**Wk5**	**Wk6**	**Wk7**	**Wk8**	**Wk9**	**Wk10**	**Wk11**	**Wk12**	**Wk13**	**Wk14**	**Wk16**	**Wk18**	**Wk22**	**Wk25**
Study Day	**D-42 to D-1**		**D1**	**D8**	**D15**	**D22**	**D29**	**D36**	**D43**	**D50**	**D57**	**D64**	**D71**	**D78**	**Treatment Early Term[9]/D85**	**D92**	**D106**	**D120**	**D148**	**PT Early Term[9]/ D169**
Visit Window (Days)	**NA**		**NA**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**± 5**	**± 5**	**± 5**
Scheduled Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Option for Home Health Care Visit[10]				X	X	X	X	X	X	X	X	X	X	X[14]		X[14]	X	X	X	
Plasma AGT	X		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	X	X	X	X	X
Exploratory	X		X[a]						X[a]						X		X	X	X	X
24-hr ABPM		X[11]												X[13]						
Study Drug Administration[12]			X	X	X	X	X	X	X	X	X	X	X	X						
hs-CRP			X[a]												X					X
PT, INR, aPTT	X																			
AEs	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PK Blood Sampling[5]			X[b]		X[a]		X[a]		X[a]		X[a]		X[a]	X[b]	X	X	X	X	X	X
Anti-drug Antibody (ADA) Testing			X[a]		X[a]		X[a]				X[a]				X					X
Archived Serum Sample[6]			X		X		X				X		X		X			X		X
Patient Contact	If an in-clinic visit or Home Healthcare visit cannot be conducted, patient contact is required by site personnel to assess AEs, concomitant medications, adequate hydration and low sodium diet counseling (e.g., video call, text, email, etc.)																			

Note: D = Day, Wk = Week

[1] Full physical exam to be given at Screening and abbreviated physical exam to be given during Treatment and Post-Treatment Period as indicated to assess changes from Screening

[2] Vital Signs (clinic): Blood pressure (SBP/DPB; sitting), Orthostatic assessment (supine and standing, required at Screening Visit 1), heart rate, respiratory rate, temperature. Vital sign assessments will be reviewed by the study doctor at each visit prior to dosing

[3] Women who are not surgically sterile, and women who require confirmation of menopause at Screening per Inclusion Criterion 3

[4] Women who are not surgically sterile. Serum test to be done

[5] Refer to Appendix C for PK sampling schedule

[6] Stored for follow-up exploration of laboratory findings and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc.) in this or subsequent clinical studies of ISIS 757456

[7] If the platelet value, eGFR, UPCR, potassium, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) a repeat blood specimen; should be re-drawn as soon as possible (ideally within 7 days either in clinic, by Home Healthcare, or local laboratory) and not meet a stopping rule prior to next dose. If Day 1 results are uninterpretable, Day 8 dosing may proceed, however, an interpretable result is required (and must be reviewed) before study drug dosing on Day 15.

Appendix A Schedule of Procedures (Continued)

[8] Height at Screening only
[9] Patients who terminate treatment or post-treatment early from the study should be encouraged to participate in an early termination visit, at which time the Study D85 or Study D169 assessments should be conducted, respectively
[10] Home Healthcare assessments and procedures may be conducted by a Home Healthcare professional at specified visits and as arranged by the site staff. If 3 consecutive Home Healthcare visits have been conducted, the Sponsor must be consulted prior to the scheduling of a 4th in-home visit
[11] 24-hr ABPM should take place after the visit 1 Screening labs have been resulted and the results are deemed not to be exclusionary. Ensure sufficient time during the Screening Period to allow for a repeat (if the first 24-hr monitoring is not successful) prior to the Study Day 1 visit
[12] Study Drug may be administered by a trained Home Healthcare Professional
[13] For patients who have been administered 8 or more doses of Study Drug, a 24-hr ABPM should ideally be initiated in-clinic after all study procedures have been completed either on Study D78 or Treatment Early Termination/Study D85. If a repeat is necessary, the 24-hr ABPM should be initiated no later than the Study D92 visit. Ensure the 24-hr APBM is not done concurrently with other study procedures
[14] The study visit must occur in-clinic if the 24-hour ambulatory blood pressure is initiated
[15] Ideally should be completed prior to any interventional study procedures (e.g., blood draws, vital signs)
[16] Urine angiotensinogen is not required at Screening

Time (time is in reference to Study Drug (ISIS 757456 or placebo) administration)
[a] Pre-dose
[b] Pre-dose, optional 2-hr post-dose

APPENDIX B. LIST OF LABORATORY ANALYTES

Appendix B List of Laboratory Analytes

Based on emerging data from this or future studies, additional tests not listed below may be performed on stored samples to better characterize the profile of ISIS 757456 or other similar oligonucleotides.

Clinical Chemistry Panel
- Sodium
- Potassium
- Chloride
- Bicarbonate
- Total protein
- Albumin
- Calcium
- Magnesium
- Phosphorus
- Glucose
- BUN
- Creatinine
- Creatinine Clearance
- Uric Acid
- Total bilirubin
- Direct (conjugated) bilirubin
- Indirect (unconjugated) bilirubin
- ALT
- AST
- ALP
- Creatinine kinase
- GGT

Thyroid Panel
- TSH
- Free T4
- Free T3

Screening Tests
- Hepatitis B surface antigen
- Hepatitis C antibody
- HIV antibody
- FSH (women only, if applicable)
- Serum βhCG (women only, if applicable)
- Hemoglobin A1c

Coagulation
- aPTT (sec)
- PT (sec)
- INR

Efficacy
- Plasma AGT

Pharmacokinetics[1]
- ISIS 757456 concentrations in plasma

ADA
- Anti-ISIS 757456 antibodies[3]

Hematology
- Red blood cells
- Hemoglobin
- Hematocrit
- MCV, MCH, MCHC
- Platelets
- White blood cells (WBC)
- WBC Differential (% and absolute)
- Neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- Monocytes

Exploratory
- Angiotensin II
- Renin (plasma renin activity)
- Direct renin
- Plasma aldosterone[3]
- NT- pro B-type natriuretic peptide
- NT-pro Atrial natriuretic peptide[3]
- Endothelin I[3]

Inflammatory
- hs-CRP

Urinalysis
- Color
- Appearance
- Specific gravity
- pH
- Protein/Creatinine Ratio (UPCR)
- Protein
- Blood
- Ketones
- Urobilinogen
- Glucose
- Bilirubin
- Leukocyte esterase
- Nitrate
- Microscopic examination[2]
- Albumin
- Creatinine
- Protein
- A/C Ratio (UACR)
- Angiotensinogen

[1] Plasma PK samples may also be used for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, or to assess other actions of ISIS 757456 with plasma constituents

[2] Will be performed on abnormal findings unless otherwise specified

[3] May be analyzed

APPENDIX C. PK SAMPLING SCHEDULE

Appendix C PK Sampling Schedule

PK Sampling Schedule

Study Day	**D1**	**D8**	**D15**	**D22**	**D29**	**D36**	**D43**	**D50**	**D57**	**D64**	**D71**	**D78**	**D85**	**D92**	**D106**	**D120**	**D148**	**D169**
Time Relative to Dose of Study Drug	Pre-dose, optional 2 hrs post-D1 SC injection		Pre-dose		Pre-dose		Pre-dose		Pre-dose		Pre-dose	Pre-dose, optional 2 hrs post-D78 SC injection	Anytime	Anytime	Anytime	Anytime	Anytime	Anytime
Number of Samples Collected	2		1		1		1		1		1	2	1	1	1	1	1	1

SC = subcutaneous
D = Day

APPENDIX D. GRADING SCALE FOR ADVERSE EVENTS RELATING TO LABORATORY ABNORMALITIES

Appendix D Grading Scale for Adverse Events Relating to Laboratory Abnormalities

The following grading recommendations for adverse events relating to lab test abnormalities are based upon the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017.

Adverse Event	Mild	Moderate	Severe
Hematology			
aPTT prolonged	>ULN - 1.5 x ULN	>1.5 - 2.5 x ULN	>2.5 x ULN; bleeding
Eosinophils increased[1]	>ULN and >Baseline	-	Steroids Initiated
Fibrinogen decreased	<1.0 - 0.75 x LLN; if abnormal, <25% decrease from baseline	<0.75 - 0.5 x LLN; if abnormal, 25 - <50% decrease from baseline	<0.5 x LLN; if abnormal, ≥50% decrease from baseline
Hemoglobin decreased (Anemia)	Hemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN - 6.2 mmol/L; <LLN - 100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 - 80g/L	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated
Hemoglobin increased**	Increase in >0 - 2 g/dL above ULN or above baseline if baseline is above ULN	Increase in >2 - 4 g/dL above ULN or above baseline if baseline is above ULN	Increase in >4 g/dL above ULN or above baseline if baseline is above ULN
INR increased	>1.2 - 1.5; >1 - 1.5 times above baseline if on anticoagulation	>1.5 - 2.5; >1.5 - 2.5 x baseline if on anticoagulation; monitoring only indicated	>2.5; >2.5 x baseline if on anticoagulation; dose adjustment indicated
Lymphocyte count decreased	<LLN - 800/mm^3; <LLN - 0.8 x 10^9/L	<800 - 500/mm^3; <0.8 - 0.5 x 10^9 /L	<500 /mm^3; <0.5 x 10^9 /L
Lymphocyte count increased	-	>4000/mm^3 - 20,000/mm^3	>20,000/mm^3
Neutrophil count decreased	<LLN - 1500/mm^3; <LLN - 1.5 x 10^9 /L	<1500 - 1000/mm^3; <1.5 - 1.0 x 10^9 /L	<1000/mm^3; <1.0 x 10^9 /L
Platelet count decreased	<LLN - 75,000/mm^3; <LLN - 75.0 x 10^9 /L	<75,000 - 50,000/mm^3; <75.0 - 50.0 x 10^9 /L	<50,000/mm^3; <50.0 x 10^9 /L
White blood cell decreased	<LLN - 3000/mm^3; <LLN - 3.0 x 10^9 /L	<3000 - 2000/mm^3; <3.0 - 2.0 x 10^9 /L	<2000/mm^3; <2.0 x 10^9 /L
Chemistry			
Acidosis	pH <normal, but >=7.3	-	pH <7.3
Alanine aminotransferase increased	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Alkaline phosphatase increased	>ULN - 2.5 x ULN if baseline normal 2.0 - 2.5 x baseline if baseline abnormal	>2.5 - 5.0 x ULN if baseline normal >2.5 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline was abnormal
Alkalosis	pH >normal, but ≤7.5	-	pH >7.5
Aspartate aminotransferase increased	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Blood bilirubin increased	>ULN - 1.5 x ULN if baseline normal >1.0 - 1.5 x baseline if baseline abnormal	>1.5 - 3.0 x ULN if baseline normal >1.5 - 3.0 x baseline if baseline abnormal	>3.0 x ULN if baseline normal >3.0 x baseline if baseline abnormal
Cardiac troponin I increased	Levels above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer	-	Levels consistent with myocardial infarction as defined by the manufacturer

Adverse Event	Mild	Moderate	Severe
Cardiac troponin T increased	Levels above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer	-	Levels consistent with myocardial infarction as defined by the manufacturer
CD4 lymphocytes decreased	<LLN - 500/mm^3; <LLN - 0.5 x 10^9 /L	<500 - 200/mm^3; <0.5 - 0.2 x 10^9 /L	<200/mm^3; <0.2 x 10^9 /L
CPK increased*	>ULN - <6 ULN	6 - 10 x ULN	>10 x ULN
Creatinine increased**	>ULN - 1.5 x ULN if baseline normal > 1.0 - 1.5 x baseline if baseline abnormal	>1.5 - 3.0 x ULN if baseline normal >1.5 - 3.0 x baseline if baseline abnormal	>3.0 x ULN if baseline normal >3.0 x baseline if baseline abnormal
GGT increased	>ULN - 2.5 x ULN if baseline normal 2.0 - 2.5 x baseline if baseline abnormal	>2.5 - 5.0 x ULN if baseline normal >2.5 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Hypercalcemia	Corrected serum calcium of >ULN - 11.5 mg/dL; >ULN - 2.9 mmol/L; Ionized calcium >ULN - 1.5 mmol/L	Corrected serum calcium of >11.5 - 12.5 mg/dL; >2.9 - 3.1 mmol/L; Ionized calcium >1.5 - 1.6 mmol/L; symptomatic	Corrected serum calcium of >12.5 mg/dL; >3.1 mmol/L; Ionized calcium >1.6 mmol/L; hospitalization indicated
Hyperglycemia††	Fasting glucose value ≥126 mg/dL (7.0 mmol/L)	Change in daily management to maintain fasting blood glucose <126 mg/dL (7.0 mmol/L); e.g. addition of oral antiglycemic agent; workup for diabetes	Insulin therapy initiated; hospitalization indicated
Hyperkalemia	>ULN - 5.5 mmol/L	>5.5 - 6.0 mmol/L; intervention initiated	>6.0; hospitalization indicated
Hypermagnesemia	>ULN - 3.0 mg/dL; >ULN - 1.23 mmol/L	-	>3.0 mg/dL; >1.23 mmol/L
Hypernatremia	>ULN - 150 mmol/L	>150 - 155 mmol/L; intervention initiated	>155 mmol/L; hospitalization indicated
Hyperphosphatemia	Laboratory finding only and intervention not indicated	Noninvasive intervention indicated	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated
Hyperuricemia	>ULN without physiologic consequences	-	>ULN with physiologic consequences
Hypoalbuminemia	<LLN - 3 g/dL; <LLN - 30 g/L	<3 - 2 g/dL; <30 - 20 g/L	<2 g/dL; <20 g/L
Hypocalcemia	Corrected serum calcium of <LLN - 8.0 mg/dL; <LLN - 2.0 mmol/L; Ionized calcium <LLN - 1.0 mmol/L	Corrected serum calcium of <8.0 - 7.0 mg/dL; <2.0 - 1.75 mmol/L; Ionized calcium <1.0 - 0.9 mmol/L; symptomatic	Corrected serum calcium of <7.0 mg/dL; <1.75 mmol/L; Ionized calcium <0.9 mmol/L; hospitalization indicated
Hypoglycemia‡	≥54 mg/dL - <70 mg/dL ≥3.0 mmol/L - <3.9 mmol/L	<54 mg/dL (3.0 mmol/L) AND no assistance required to actively administer carbohydrates, glucagon, or take other corrective actions	Requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions
Hypokalemia	<LLN - 3.0 mmol/L	symptomatic with <LLN - 3.0 mmol/L; intervention indicated	<3.0 mmol/L; hospitalization indicated
Hypomagnesemia	<LLN - 1.2 mg/dL; <LLN - 0.5 mmol/L	<1.2 - 0.9 mg/dL; <0.5 - 0.4 mmol/L	<0.9 mg/dL; <0.4 mmol/L
Hyponatremia	<LLN - 130 mmol/L	125-129 mmol/L and asymptomatic	125-129 mmol/L symptomatic; 120-124 mmol/L regardless of symptoms
Hypophosphatemia	Laboratory finding only and intervention not indicated	Oral replacement therapy indicated	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated
Lipase increased	>ULN - 1.5 x ULN	>1.5 - 2.0 x ULN; >2.0 - 5.0 x ULN and asymptomatic	>2.0 x ULN with signs or symptoms
Serum amylase increased	>ULN - 1.5 x ULN	>1.5 - 2.0 x ULN; >2.0 - 5.0 x ULN and asymptomatic	>2.0 x ULN with signs or symptoms

Adverse Event	Mild	Moderate	Severe
Urine			
Proteinuria Adults	1+ proteinuria; urinary protein ≥ULN - <1.0 g/24 hrs	2+ and 3+ proteinuria; urinary protein 1.0 - 3.4 g/24 hrs;	4+ proteinuria; Urinary protein ≥3.5 g/24 hrs;
Children	-	Urine P/C (Protein/Creatinine) ratio 0.5 - 1.9	Urine P/C >1.9
Hematuria	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; urinary catheter or bladder irrigation indicated	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective invasive intervention indicated
Adverse Events at the Injection Site			
Adverse events at the injection site**	An event at the injection site (e.g. erythema, tenderness, itching) that is easily tolerated by the subject and does not affect the subject's usual daily activities	- Persistent (>24 hours) pain, phlebitis or edema; OR - Lipodystrophy, hair growth or alopecia, OR - Prolonged (>1 month) hypo/hyperpigmentation	- Ulceration or necrosis; severe tissue damage; operative intervention indicated, OR - Any event at the injection site that is incapacitating

†Grading for this parameter is derived from the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Sept 2007

*Grading for this parameter is derived from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0, Nov 2014

††Modified for consistency with ADA "Standards of Medical Care in Diabetes - 2018" Diabetes Care 2018;41(Suppl. 1):S13–S27. https://doi.org/10.2337/dc18-S002

‡Modified for consistency with ADA "Glycemic Targets: Standards of Medical Care in Diabetes - 2018", Diabetes Care 2018;41(Suppl. 1):S55–S64. https://doi.org/10.2337/dc18-S006

**Adapted from the original CTCAE V5.0 scale

Protocol

Version:	1
Version Date:	05 Nov 2021
Title:	757456-CS4 Amendment 4: A DB, Placebo-Controlled, Phase 2 Study to Assess the Safety, Tolerability & Efficacy of IONIS-AGT-LRX, an Antisense Inhibitor of Angiotensinogen Production Administered SC for 12 Wks to Hypertensive Pts with

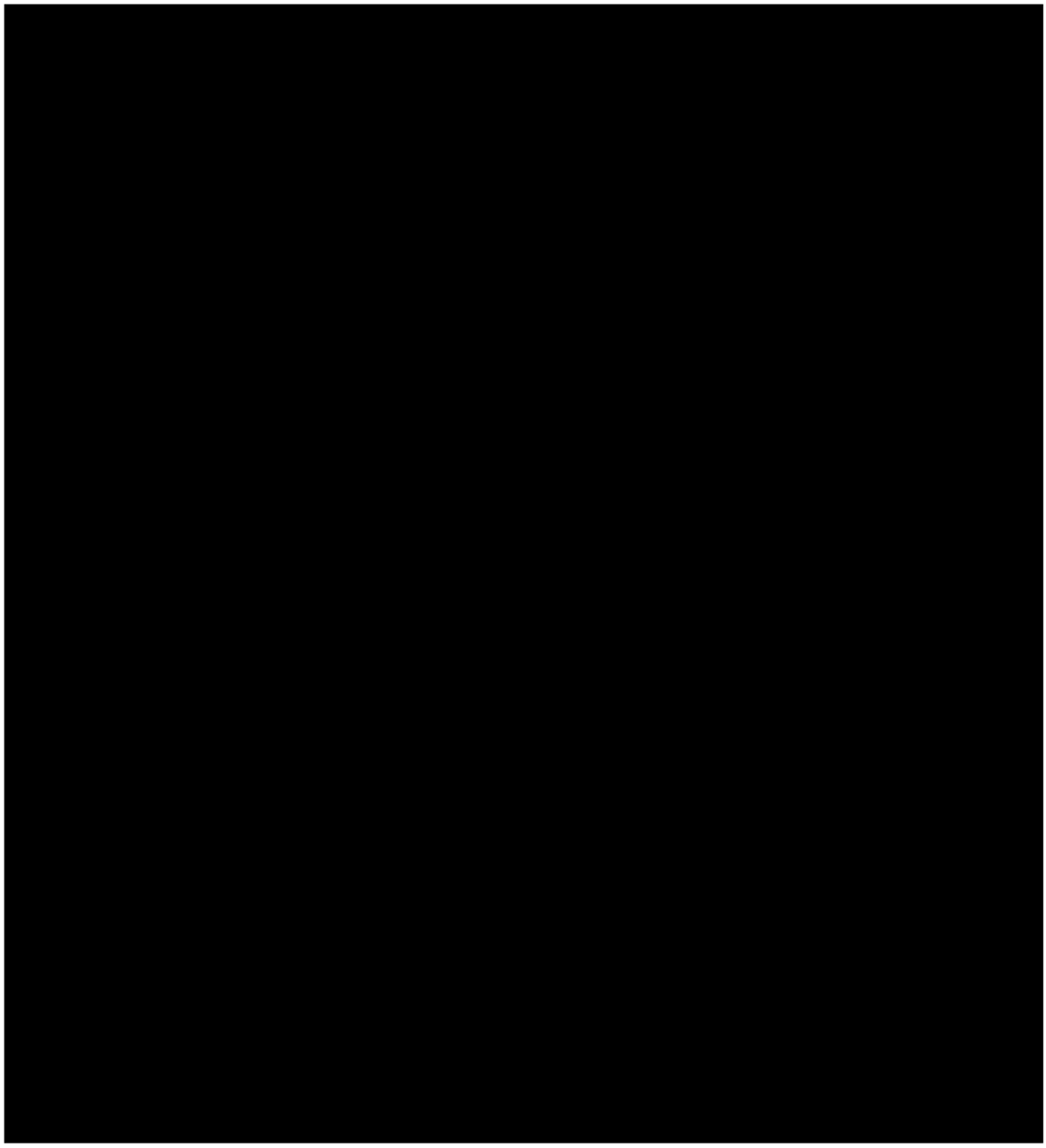
Statistical Analysis Plan

ISIS 757456-CS4

A Double-Blind, Placebo-controlled, Phase 2 Study to Assess the Safety, Tolerability and Efficacy of IONIS-AGT-L_{RX}, an Antisense Inhibitor of Angiotensinogen Production Administered Subcutaneously for 12 Weeks to Hypertensive Patients with Uncontrolled Blood Pressure

Date: 15 August 2022

Version: 2.0



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REVISION HISTORY

Version No.	Effective Date	Summary of Changes
1.0	28 March 2022	New Document
2.0	1 July 2022	Updated language for Section 3.5, Exploratory Analyses endpoints for PGI-C, PGI-S and POP-HSQ. • Section 3.2.1, for the continuous endpoints, Van Elteren test with treatment and randomization stratification factor will be conducted if the data departs substantially from normality. Changed from rank ANCOVA to Van Elteren test. • Section 3.7.3, modified potassium (K) categories to "Absolute levels ≥ 5.5–< 6, confirmed"; "Absolute levels ≥ 6, confirmed". Removed Platelet count first category "Confirmed Platelet count > 100,000/mm^3". Clarified local lab data will be included when derive confirmed abnormal results.

ABBREVIATIONS

In addition to the study glossary provided in the protocol, here are additional abbreviations provided for the study SAP.

Abbreviation	Definition
ABP	ambulatory blood pressure
ABPM	ambulatory blood pressure monitoring
AE	adverse event
AGT	angiotensinogen
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
ANCOVA	Analysis of Covariance
BMI	body mass index
BP	blood pressure
CI	confidence interval
CRF	case report form
CVD	cardiovascular disease
CSR	clinical study report
DBP	diastolic blood pressure
ECG	electrocardiogram
eCRF	electronic Case Report Form
eGFR	estimated glomerular filtration rate
EQ-5D-5L	EuroQoL-5 Dimension 5 Level
FAS	full analysis set
HTN	hypertension
LCRIS	Local cutaneous reaction at injection site
LLQ	lower limit of quantitation
MMRM	mixed models with repeated measures
PD	pharmacodynamic(s)
PGI-C	Patient Global Impression- Change
PGI-S	Patient Global Impression- Severity
PK	pharmacokinetic(s)
POP-HSQ	Power Over Pressure- Hypertension Specific Questionnaire
PPS	per protocol set
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SF-12	12-Item Short Form Survey
TEAE	treatment-emergent adverse event
UACR	urinary albumin/creatinine ratio
ULN	upper limit of normal

1. INTRODUCTION

This document provides a description of the study organization, study procedures, and the plan for the statistical analysis of the study data. Section 1 discusses study design, objectives, and endpoints; Section 2 provides the study procedures; Section 3 provides the detailed plan for the statistical analyses.

The purpose of this plan is to provide specific guidelines from which the analysis will proceed. Any deviations from these guidelines will be documented in the clinical study report (CSR).

Within this document, the terms 'patient' and 'subject' are both used to describe the individual who enrolls in this study.

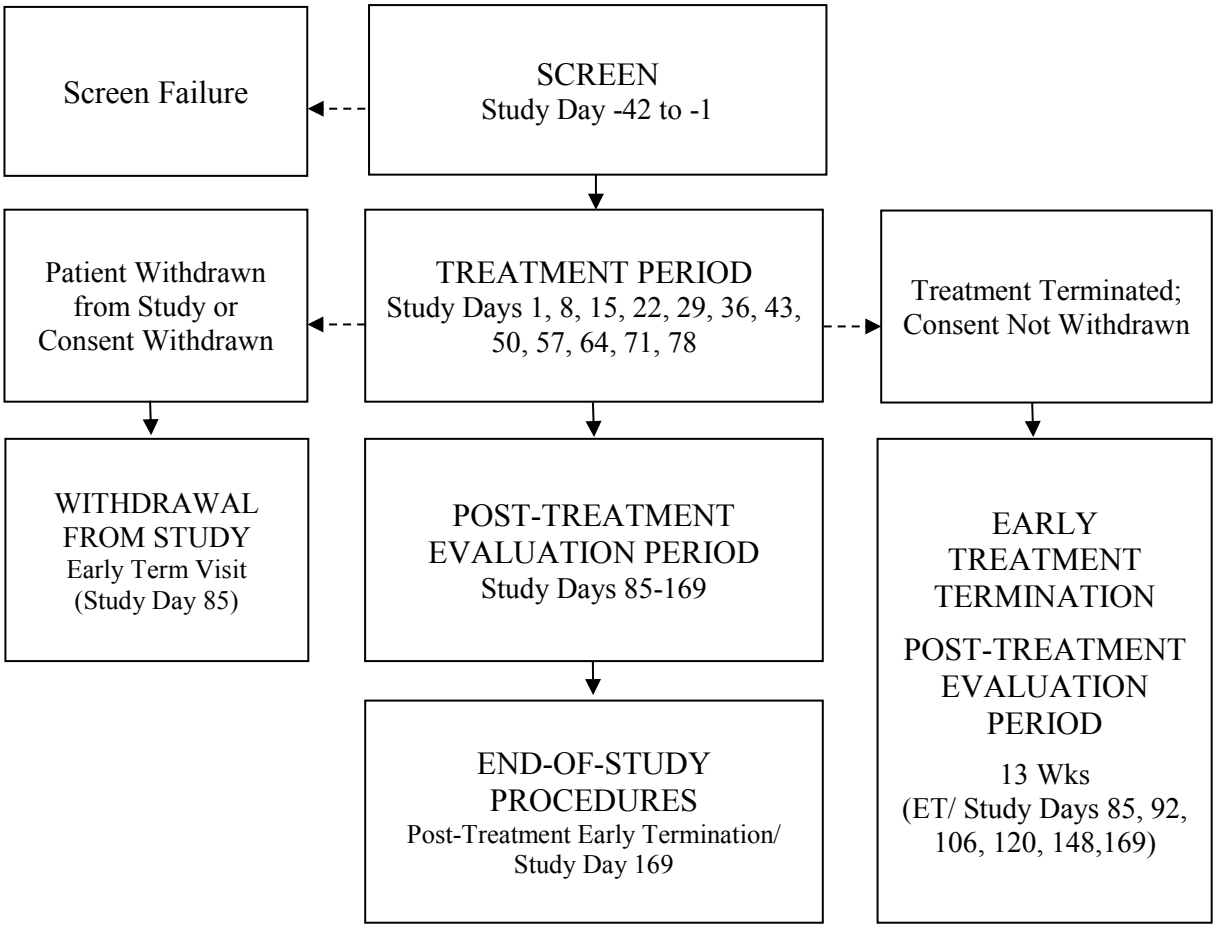
1.1. Study Overview

This is a Phase 2, double-blind, randomized, placebo-controlled study of ISIS 757456 conducted in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications. Eligible patients will be stratified based on a screening eGFR result (<60 vs. ≥ 60 mL/min/1.73 m2) and randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A (80 mg ISIS 757456 or placebo) or Cohort B (120mg ISIS 757456 or placebo)). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio. Patients will receive SC doses of Study Drug on Study Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78 during treatment period. Patients will continue their antihypertensive medication regimen throughout the study. The patient is expected to maintain adequate hydration and a low sodium diet throughout the study. After the Treatment Period, all patients will then complete a 13-week Post-Treatment Period.

This study will be conducted at multiple centers in the United States. Approximately 150 patients are planned to be enrolled in this study. Study Drug (ISIS 757456 or placebo) injection volumes will be 0.8 mL or 1.2 mL for cohort A or Cohort B, respectively. All Study Drug injections will be administered by qualified personnel at the clinic or by a home healthcare professional (as arranged with the site staff and after appropriate training).

The study will consist of Screening, Treatment, and Post-treatment. The overall length of a subject's participation will be approximately 31 weeks (up to 6 weeks for Screening, a 12-week Treatment Period, and a 13-week Post-Treatment Evaluation Period).

The study design and treatment schema are depicted as follows:



1.2. Objectives

1.2.1. Primary Objective

To evaluate the effect of ISIS 757456 compared to placebo on seated automated office systolic blood pressure (SBP) from Baseline to Study Day 85 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.2.2. Secondary Objectives

- To evaluate the effect of ISIS 757456 on plasma angiotensinogen (AGT) concentration at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on 24-hour ambulatory blood pressure at scheduled visits in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on seated automated office SBP at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the effect of ISIS 757456 on seated automated office diastolic blood pressure (DBP) at each scheduled visit in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.2.3. Safety Objectives

To evaluate the safety and tolerability of ISIS 757456 in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications

1.2.4. Exploratory Objectives

- To evaluate the effects of ISIS 757456 administered subcutaneously (SC) (e.g., angiotensin II, renin [Plasma renin activity (PRA); direct renin], urinary angiotensinogen) in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate the pharmacokinetics (PK) of ISIS 757456 (as total full-length ASO, including fully conjugated, partially conjugated, and unconjugated ISIS 757456) administered SC in uncontrolled hypertensive patients on ≥ 3 antihypertensive medications
- To evaluate potential PK/ pharmacodynamic (PD) correlation with relevant biomarkers and/or clinical endpoints
- To evaluate the effects of ISIS 757456 on patients reported quality of life outcomes

1.3. Endpoints

1.3.1. Primary Endpoint

Change in seated automated office SBP from baseline to Study Day 85

1.3.2. Secondary Endpoints

- Absolute levels and change and percent change in plasma AGT concentration from baseline to each scheduled, post-baseline visit
- Changes from Baseline to Study Day 85 in 24-hour mean SBP and DBP measured by ambulatory blood pressure monitoring (ABPM)
- Percentage of patients reaching the goals of seated automated office SBP ≤ 140 mmHg, DBP ≤ 90 mmHg, and both for each scheduled, post-Baseline visit (excluding patients with a baseline SBP of ≤ 140 mmHg)
- Percentage of patients reaching the goals of automated office seated SBP ≤ 130 mmHg, DBP ≤ 80 mmHg, and both for each scheduled, post-Baseline visit
- Change on seated automated office SBP from Baseline to each scheduled, post-Baseline visit
- Change on seated automated office DBP from Baseline to each scheduled, post-Baseline visit

1.3.3. Safety Endpoints

Incidence and severity of treatment-emergent adverse events (TEAE), use of concomitant medications, abnormal findings in laboratory assessments, electrocardiogram (ECG), and vital signs

1.3.4. Exploratory Endpoints

- Absolute levels and change and percent change of angiotensin II, renin (PRA; direct renin), urinary AGT concentration from Baseline to each scheduled, post-Baseline visit
- Summary of peak and trough ISIS 757456 plasma concentrations
- Potential exposure-response analysis using relevant exposure parameters and biomarkers including, but not limited to trough concentration (C_{trough}) and plasma AGT may be performed
- Changes from Baseline to Study Day 85 and each scheduled post-Baseline assessment in the following patient reported outcome questionnaires: EuroQol-5 Dimension 5 Level (EQ-5D-5L), 12-Item Short Form Survey (SF-12), Power Over Pressure- Hypertension Specific Questionnaire (POP-HSQ), Patient Global Impression- Change (PGI-C), and Patient Global Impression-Severity (PGI-S)

- Change from Baseline to Study Day 85 in daytime SBP and DBP from 24 hr ABPM
- Change from Baseline to Study Day 85 in nighttime SBP and DBP from 24 hr ABPM
- Change from Baseline to Day 85 in eGFR
- Change from Baseline to Day 85 in urinary albumin/creatinine ratio (UACR)
- Primary endpoint, selective secondary and exploratory endpoints will be also analyzed using a sub-population of patients with baseline 24 hr ABPM SBP > 130

2. PROCEDURES

2.1. General Overview of Procedures

Ionis Pharmaceuticals, Inc. (or designee) will review all study data including source documents, case report forms, and laboratory reports. The study site will enter subject source data into the case report form. Central laboratory data will be transferred electronically from Medpace Reference Laboratories (MRL) Inc. for those analytes noted in the MRL Laboratory Services Agreement, PK Plasma concentration data will be from Pharmaceutical Product Development (PPD) Inc., and immunogenicity data will be from Charles River Laboratories in Montreal (CR-SEN) to Ionis Pharmaceuticals, Inc. Additionally, plasma AGT, Ang II, aldosterone and PRA data analyzed by Attoquant Diagnostics GmbH will be transferred to Ionis via separate Data Transfer Agreement (DTA).

2.2. Randomization & Treatment Allocation

Patients will be randomized after all screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in Clinical Study Protocol Sections 5.1 and 5.2. No subject may begin treatment prior to randomization and assignment of a unique subject identification number.

Eligible patients will be stratified based on a screening eGFR result (< 60 vs. $\geq$ 60 mL/min/1.73 m^2) and then patients will be randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized in a 2:1 to receive ISIS 757456 or placebo as outlined in Clinical Study Protocol Section 4.2. The Sponsor or designee will prepare the randomization list and utilize an automated IRT (Interactive Response Technology) system for randomization assignment.

2.3. Conduct

The study will be conducted in accordance with current Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) guidelines, the World Medical Association Declaration of Helsinki guidelines, the Food and Drug Administration (FDA) Code of Federal Regulations, and all other local regulatory requirements.

2.4. Data Monitoring

2.4.1. Safety Data Monitoring

Ionis Pharmaceuticals, Inc. (or designee) is responsible for processing all reported adverse events (AEs). All serious adverse events (SAEs), reported to Ionis Pharmaceuticals, Inc. (or designee), are reviewed according to standard operating procedures. The medical monitor will review all AEs and SAEs on an ongoing basis throughout the study. Ionis Pharmaceuticals, Inc. (or designee) will prepare and submit safety reports to the health authorities worldwide in accordance with local requirements. If it becomes necessary to communicate new safety information, Ionis Pharmaceuticals, Inc. (or designee) will also prepare a safety notification letter and transmit it to study site.

2.5. Data Management

An electronic case report form (eCRF) utilizing an Electronic Data Capture (EDC) application will be used for this Study.

2.5.1. Case Report Form (CRF) Data

Clario (Formerly BioClinica) (or designee) is responsible for creating the EDC data entry screens, database and edit checks using definitions developed by Ionis Pharmaceuticals, Inc. Ionis Pharmaceuticals, Inc. is responsible for the review, data management querying and locking of the database.

Data are single-entered into the EDC system by the investigator site staff. Programmed edit checks (computer logic that checks the validity of the data entered and also prompts for missing data that is expected to be entered) are run and automatic queries are generated. Ionis Pharmaceuticals, Inc. reviews all data for accuracy and validity and generates additional queries in the EDC system when necessary. The data is corrected or an explanation concerning the query is provided in the EDC system. After all data is entered, source data verified, reviewed and queried the database is closed, the data is then reviewed by Ionis Pharmaceuticals, Inc. and additional queries may be generated if necessary. After all queries are resolved and PI signature are received, the database is locked.

2.5.2. Laboratory Data

Ionis Pharmaceuticals, Inc. is responsible for the format of the laboratory electronic data transfers, the transfer schedule, and the review of the clinical laboratory data. Investigator sites have access to safety data via lab reports sent directly from the laboratory and online using the MRL ClinTrack® database. The laboratory data will be stored as SAS datasets.

Plasma AGT, Ang II, aldosterone, and PRA by Attoquant Diagnostics GmbH will be transferred to Ionis via separate Data Transfer Agreement (DTA).

2.5.3. Pharmacokinetics (PK) and Immunogenicity (IM) Data

Ionis Pharmaceuticals, Inc. is responsible for the management and review of the plasma drug ISIS 757456 concentration data and immunogenicity sample analysis. This review process involves reconciling the patient and visit identifiers (e.g. patient demographics) with the clinical data collected in the EDC system. The final PK data will be stored as CSV and/or EXCEL files per the Analysis Plan.

3. ANALYSIS PLAN

3.1. Statistical Design Summary

This is a Phase 2, randomized, placebo-controlled, multi-center study. Approximated 150 patients will be stratified based on a screening eGFR result (< 60 vs. ≥ 60 mL/min/1.73 m^2) and randomized to 1 of 2 dose cohorts in a 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio.

All planned analyses will compare each ISIS 757456 treatment group to pooled Placebo. The overall study type I error is at 0.1 level, two-sided. The multiplicity of study treatment groups comparison and study endpoints testing sequence will be further described in Section 3.4.2.

The planned sample size will provide approximate 80% of power to demonstrate the mean SBP reduction difference of 8 mmHg between each ISIS 757456 treated group and pooled placebo based on the assumption of pooled standard deviation of 13 mmHg with the two-sided test and a significant level of 0.05.

An interim analysis may be conducted after approximately 50% of patients have been enrolled. The unblinding information dissemination during this interim analysis will be controlled and described in the request to unblind data for study analysis document to ensure the trial integrity. Please refer to Section 3.3.2 for further details.

The primary analysis will take place after all patients complete Treatment Period (Day 85) assessments and the database has been locked. The final analysis will be performed after all patients have completed or have had the opportunity to complete the study Day 169 assessments (such as patients who have terminated early) and the database has been locked.

The primary endpoint is the change from baseline to Study Day 85 in seated automated office SBP and will be analyzed by analysis of covariance (ANCOVA) with treatment, randomization stratification factor and baseline measure (SBP) as independent variables. The statistical inferences will be summarized by treatment group. Analyses will assess the hypothesis there are difference in the SBP change from baseline at Day 85 between each ISIS 757456 treatment group and pooled placebo. The estimated treatment effect with 95% CI and p-values will be provided.

Plasma AGT concentration from Attoquant will be used for AGT analysis.

3.2. General Overview of Analyses

3.2.1. Statistical Methods

The study outcomes will be summarized by treatment group. Descriptive summary statistics including number of patients, mean, median, standard deviation, standard error of mean, 25th percentile, 75th percentile, minimum, and maximum for continuous variables, and counts and percentages for categorical variables will be provided to summarize most data. The efficacy endpoints will be assessed on the full analysis set (FAS) and per-protocol set (PPS) with the latter being the basis for the primary efficacy analysis. The safety analyses will be performed on the Safety Set. Pharmacokinetic analysis will be conducted in the PK Set.

All planned analyses will compare each ISIS 757456 treatment group to pooled placebo.

All statistical tests will be conducted using 2-sided tests with 5% Type I error rate unless otherwise stated. P-values will be provided to assess the null hypothesis that there is no statistical difference between an ISIS 757456 treatment group and pooled placebo in study endpoints. The stratification factor (screening eGFR status [<60 vs. ≥ 60 mL/min/1.73 m^2]), treatment received, and baseline measure are included in the Analysis of Covariate (ANCOVA) model as independent variables. For the continuous endpoints, the ANCOVA model will be applied or Van Elteren test with treatment and randomization stratification factor if the data departs substantially from normality. The normality assumption will be assessed by the Shapiro-Wilk test based on the residuals from ANCOVA modeling. Least Square mean (LSM) difference with 95% CI and p-values between treatment groups in study endpoints will be provided based on ANCOVA model. For the dichotomous endpoints, logistic regression model with treatment, randomization stratification factor and baseline measure (SBP) as independent variables will be used instead.

To evaluate treatment difference across scheduled post-baseline visits, an additional mixed model with repeated measures (MMRM) analysis may be utilized. Besides stratification factor, visit, treatment received, baseline measure, and treatment-by-visit interaction will be included in the model, which visit as an ordinally categorical variable. An unstructured covariance will be used initially, with correlation coefficients being greater for visits that are closer. Other covariance structures, such as first-order autoregressive covariance structure based on the assumption of the measurements over time are expected to be correlated, may be considered based on model fitting consideration. The adjusted mean with 95% CI for each treatment group and 95% CIs for the mean difference between pooled placebo and each ISIS 757456 treatment groups at each scheduled time point will be provided.

Additional covariates, such as baseline characteristics, may be considered as an additional covariate in the comparison statistical modeling and for subgroup summary for the exploratory analysis purpose.

Laboratory outcomes with lower limit of quantitation (LLQ) or less will be imputed as LLQ in the summary tables and figures.

PK parameters will be summarized using include number of patients, mean, standard deviation, coefficient of variation (CV), geometric mean, median, minimum, and maximum.

Additional subject listings including case report form (CRF) data and derived outcomes from the data may be presented.

Baseline Definition

The baseline for plasma AGT concentration from Attoquant will be defined as the average of all values prior to the first dose of Study Drug. The baseline for all other assessments will be defined as the last non-missing measurement prior to the first dose.

Analytical Visits

In general, all post-baseline data will be summarized using the visit labels provided in the data. Multiple results with the same visit label will be averaged for continuous variables, and the worst result will be used for categorical variables. Results with visit labels as "Unscheduled" will not be included in by-visit summary tables and figures except for determining baseline and the incidence of abnormality lab summary including, but not limited to, AST, ALT, potassium, eGFR, urine protein/creatinine ratio, and platelet specified in Section 3.7.4, but will be presented in data listings. Other situations, such as required additional confirmation, will be described separately in the related endpoint analysis approach.

3.2.2. Subject Population Analyzed

The following analysis populations are defined for this study:

- Full Analysis Set (FAS): All randomized patients who have received at least 1 injection of Study Drug (ISIS 757456 or Placebo) and who have at least 1 post-Baseline primary efficacy measurements.
- Per Protocol Set (PPS): All FAS patients who received at least 10 of the 12 doses of Study Drug, did not alter antihypertensive medications during the Treatment Period and prior to Study Day 85, and have no significant protocol deviations that would be expected to affect efficacy assessment. Significant protocol deviations are defined as those deviations from the protocol likely to impact the perceived efficacy and/or safety of study treatments, for example, other antihypertensive medication usage compliance during the study. After all data are entered, reviewed, and queried, the database is closed and sent to clinical group for review and identification of significant protocol deviations before unblinding.
- Safety Set: All patients who are randomized and receive at least 1 dose of Study Drug.
- Pharmacokinetics (PK) Set: All patients who are randomized and receive at least 1 dose of ISIS 757456 and have at least 1 evaluable PK sample.

ln addition to the above analysis sets, it is recognized that some data displays will be provided for the number of patients in "All Screened", "Screening Failures" and "All Randomized" but no data analysis will be executed in these populations. A by-patient listing based on all randomized will be provided.

3.2.3. Handling of Missing Data

Unless otherwise specified, missing values will not be imputed.

Patients with missing data for a scheduled assessment time point will be excluded from the summary for that time point.

Number of patients with missing data and missing data related to COVID-19 public health emergency may be provided.

3.2.4. Disposition of Subjects

The number of patients screened, screen failures, the number and percentage of patients randomized, dosed, completed the study treatment, discontinued from treatment early and reason, completed post-treatment follow-up, and discontinued from post-treatment follow-up and reason will be tabulated by treatment group.

A by-patient listing will also be provided.

Additional by-patient listing will be provided to describe screen failures, treatment/post-treatment follow-up termination information related COVID-19 public health emergency.

3.2.5. Demographic and Baseline Characteristics

Demographic and Baseline characteristics (e.g., age, gender, ethnicity, race, cardiovascular disease (CVD) risk factors, tobacco/nicotine use, number of antihypertensive medications received, diuretic use (Y/N), CVD history, diabetic status, weight, height, BMI) will be summarized using descriptive statistics by treatment group.

Additionally, the following additional baseline characteristics will be summarized:

- Age (yrs): < 65, ≥ 65
- BMI (Kg/m^2): < 30, ≥ 30
- eGFR ($mL/min/1{,}73\ m^2$): ≥ 60, < 60
- heart rate
- Antihypertensive medications received by category (RAS inhibitor, beta-blocker, calcium channel blocker, diuretic, alpha-1 blocker, centrally acting sympatholytic agent, vasodilators, aldosterone antagonists)
- ≥ 3 anti-HTN med with diuretic
- Number anti-HTN medications 3, 4, ≥ 5
- SBP and DBP (mmHg) at baseline
- 24 hr ABPM SBP at baseline >130 SBP
- Daytime ABPM SBP at baseline > 135
- Plasma AGT
- HbA1c

- eGFR
- urinary albumin/creatinine ratio (UACR): normal: < 30 mg/g was considered normal; micro-albuminuria: 30-300 mg/g; macro-albuminuria: > 300 mg/g
- CV Risk

For race summary, if multiple races are recorded in the database, then 'Multiple Race' will be used in the summary table. The listing will display the specific race values.

BMI will be computed using the formula:
BMI = (weight in kilograms) / [screening height in cm / 100]^2

Listing of medical history will be provided.

3.2.6. Protocol Deviations

Protocol deviations will be classified to major or minor based on the study protocol deviation process plan. Protocol deviations will be provided in the data listings. Additional tables may be provided to summarize the protocol deviations related to COVID-19 public health emergency.

A listing of all patients affected by the COVID-19 public health emergency related study disruption by subject number identifier and by investigational site, and a description of how the individual's participation was altered will be provided.

3.2.7. Stratification, Subsets, and Covariates

Eligible Patients will be stratified on Screening eGFR result of < 60 or ≥ 60 mL/min/1.73 m^2. In the case of errors in stratification, the analyses should be based on the actual stratification, regardless of IRT entry.

The following patient demographic and baseline characteristics may be considered as additional covariates in the comparison statistical modeling for exploratory analysis purposes:

- Age group (< 65 years vs. ≥ 65 years)
- Race (Caucasian vs non-Caucasian)
- Gender
- Obesity status
- Number of antihypertensive medications received
- Diabetic status
- UACR categories
- Two or more CV risk factors
- 24 hr ABPM SBP > 130 mmHg
- Chronic kidney disease status defined by eGFR (> 90 mL/min, 60-90 mL/min, and 45-59 mL/min)

3.2.8. Scoring of Questionnaires

EuroQoL-5 Dimensional 5-level Questionnaire (EQ-5D-5L)

EQ-5D-5L is a standardized measure of health status developed by the EuroQoL Group to provide a simple, generic measure of health for clinical and economic appraisal. The EQ-5D-5L consists of the EQ-5D-5L descriptive system and the EQ visual analog scale (VAS).

The EQ-5D-5L descriptive system comprises five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), and the response to each dimension is scored as: 1 = no problems/pain/anxious, 2 = slight problems/pain/anxious, 3 = moderate problems/pain/anxious, 4 = severe problems/pain/anxious, 5 = unable/extreme. Lower score indicates worsening of QoL.

The EQ VAS is used as a quantitative measure of health outcome as judged by the individual respondent. The EQ VAS records a self-rating of health status on a vertical VAS anchored at 100 (best imaginable health state) at the top and 0 (worst imaginable health state) at the bottom.

Additionally, the EQ-5D health state index score reflects how good or bad a health state is according to the preferences of the general population of a country/region will be derived and summarized. If any of five dimensions is missing, then the index value will be set as missing. The details of EQ-5D health state index score derivation algorithm is described in Appendix 1.

Patient Global Impression

Patient Global Impression of Severity (PGI-S) is a single-item scale instrument asking patients to rate the severity of their health condition on a scale of (1) none, (2) mild, (3) moderate, (4) severe, (5) very severe.

Patient Global Impression of Change (PGI-C) is a single-item scale, which aims to evaluate all aspects of patients' health and determine if there has been an improvement or not in the patients' overall change in health condition. Patients rate their change as (1) much better, (2) a little better, (3) no change, (4) a little worse, (5) much worse.

Power Over Pressure - Hypertension Specific Questionnaire (POP-HSQ)

The POP-HSQ (Schmider, et. al 2017) comprising questionnaire A and questionnaire B (what symptoms have you experienced in the last 4 weeks). In questionnaire A, patients rate their health state in part 1 to part 4 and their blood pressure management and impact in part 5 to part 7. In questionnaire B, patients provide the experienced symptoms' frequency and intensity.

Short Form (SF) -12

The SF-12 comprising 12 items that yield 8 healthy domains profile of physical and mental well-being. These 8 domains are: health domains: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH). Each health domain score contributes to the physical component summery (PCS, including PF, RP, GH and BP) and a mental component summary (MCS, including MH, RE, SF, and VT). Other measures can be derived from SF-12 data, including SF-6D preference-based utility index and the medical expenditure prediction to help understand the economic impact of a patient's condition and treatment. The norm-based score for each domain and components will be calculated using Optum's SF36v2/SF-12v2 scoring software

operated by Ionis data management function personal. Higher scores are associated with a better quality of life (QOL).

3.3. Primary Analysis

3.3.1. Primary Efficacy Analysis

The primary analysis will be the comparison of change from Baseline to Study Day 85 (Study Week 13) in seated automated office SBP between each ISIS 757456 treatment dose group and pooled placebo group in the PPS. The data will be analyzed as described in Section 3.2.1. Least square mean (LSM) estimated treatment difference with 95% CI and p value will be provided. Additional MMRM analysis will be conducted to assess the treatment effect.

Additional covariates, such as baseline characteristics, may be considered, in the comparison statistical modeling for exploratory analyses.

Additional analysis summary based on the FAS will also be provided.

3.3.2. Sample Size consideration

Approximately 150 patients will be stratified and randomized to 1 of 2 dose cohorts in 1:1 ratio (Cohort A or Cohort B). Within each dose cohort, patients will be further randomized to receive ISIS 757456 or placebo in a 2:1 ratio.

With the assumptions of mean seated automated office SBP reduction difference of 8 mmHg between an ISIS treated group and pooled placebo with the common standard deviation of 13 mmHg, there are at least 86 eligible patients (n = 43 for an ISIS 757456 treated group and n = 43 for pooled placebo) are needed to provide approximate 80% of power to demonstrate the mean difference between an ISIS treated group and pooled placebo by ANCOVA with the two-sided significant level of 0.05. Additional patients are included while considering an estimated approximate 10% drop-out rate.

The overall type I error rate is controlled at 0.1 level, two-sided.

3.4. Secondary Efficacy Analyses

3.4.1. Secondary Efficacy Endpoints

The secondary efficacy analyses will be performed using statistical methods described in Section 3.2.1. ANCOVA model will be used for continuous endpoints, logistic regression model will be used for dichotomous endpoints. Secondary endpoints include:

1. Comparison of percent change from baseline to Study Day 85 in AGT between an ISIS 757456-treated group and pooled-placebo in the PPS
2. Comparison of change from baseline to Study Day 85 in SBP from 24-hrs mean ABPM between an ISIS 757456-treated group and pooled-placebo in the PPS
3. Comparison of proportion of patients reaching the goals of seated automated office SBP $\leq$ 140 mmHg and DBP $\leq$ 90 mmHg at Study Day 85 between an ISIS 757456-treated group and pooled-placebo in the PPS, excluding patients with baseline SBP $\leq$ 140 mmHg

4. Comparison of absolute value, change and percent change from Baseline to each scheduled post-Baseline visit in plasma AGT between each ISIS 757456-treated group and pooled-placebo group in PPS and FAS
5. Comparison of change from Baseline to Study Day 85 in SBP and DBP from 24 hr mean ABPM between each ISIS 757456-treated group and pooled-placebo group in the PPS and FAS
6. Comparison of patient incidence in reaching the goals of seated automated office SBP ≤ 140 mmHg, DBP ≤ 90 mmHg, and both for each scheduled, post-Baseline visit between each ISIS 757456-treated group and pooled-placebo group in PPS and FAS, excluding patients with Baseline SBP ≤ 140 mmHg
7. Comparison of patient incidence in reaching the goals of seated automated office SBP ≤ 130 mmHg, DBP ≤ 80 mmHg, and both for each scheduled, post-Baseline visit between each ISIS 757456-treated group and pooled-placebo group in PPS and FAS
8. Comparison of change from Baseline to each scheduled post-Baseline visit in seated automated office SBP between each ISIS 757456-treated group and pooled-placebo group in PPS and FAS
9. Comparison of change from Baseline to each scheduled post-Baseline visit in seated automated office DBP between each ISIS 757456-treated group and pooled-placebo group in PPS and FAS

Figures contain the summary (mean with standard error of mean) by scheduled visit overtime based on PPS will be provided. Spaghetti plots demonstrating each individual's efficacy assessment over time by treatment group will be provided.

3.4.2. Multiplicity

The multiplicity of primary and the first three secondary endpoints will be controlled by using sequential (closed) testing strategy in the following testing sequence for interim analysis (if applicable) and final analysis, which is also illustrated in Figure 1 below.

- Comparison of change from baseline to Study Day 85 in in seated automated office SBP between an ISIS 757456-treated group and pooled-placebo in the PPS (primary)
- Comparison of percent change from baseline to Study Day 85 in AGT between an ISIS 757456-treated group and pooled-placebo in the PPS (secondary 1)
- Comparison of change from baseline to Study Day 85 in SBP from 24-hrs mean ABPM between an ISIS 757456-treated group and pooled-placebo in the PPS (secondary 2)
- Comparison of portion of patients reaching the goals of seated automated office SBP ≤ 140 mmHg and DBP ≤ 90 mmHg at Study Day 85 between an ISIS 757456-treated group and pooled-placebo in the PPS, excluding patients with baseline SBP ≤ 140 mmHg (secondary 3)

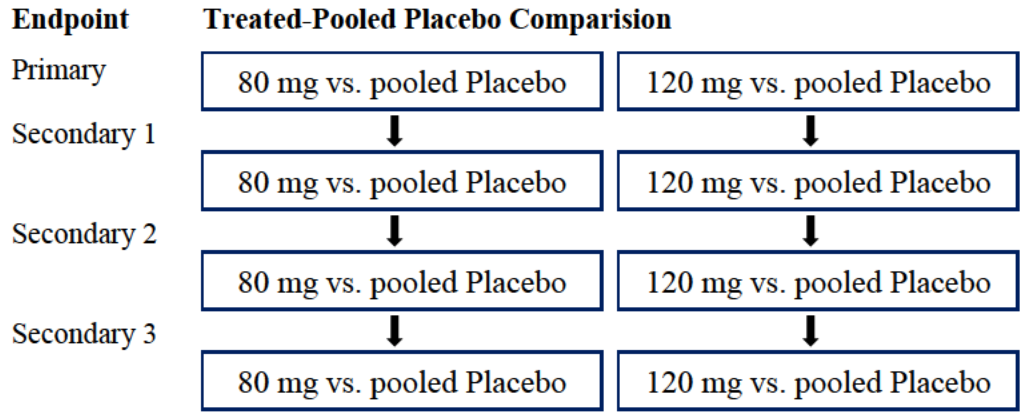


Figure 1: Testing Strategy illustration

In each dose cohort, testing of secondary endpoints will be performed only if the treatment comparison of the primary endpoint is statistically significant. All comparisons between treated group and the pooled placebo for secondary endpoints will be considered as exploratory analyses if the primary endpoint is not statistically significant ($p > 0.05$, or 0.049 if interim analysis is conducted). If a secondary efficacy endpoint in the sequence is not statistically significant, then the subsequent endpoints outcomes will be considered as exploratory.

3.4.3. Interim Analysis

An interim analysis may be conducted after approximately 50% of the planned patients have been enrolled. All study primary and secondary efficacy endpoints will be summarized for the interim analysis. The unblinding information dissemination during this interim analysis will be controlled and described in the Request to Unblind Treatment Assignment document to ensure the trial integrity. If conducted, the interim analysis for primary and secondary endpoints will test the significance at alpha level of 0.001, two-sided, and the alpha level for the final analysis of primary and secondary endpoints will be 0.049, two-sided, between an ISIS 757456 treated group and placebo. The overall type I error rate will be controlled at 0.1, two-sided.

3.5. Exploratory Analyses

The exploratory efficacy analyses will be conducted using statistical methods described in Section 3.2.1. ANCOVA model will be used for continuous endpoints, logistic regression model will be used for dichotomous endpoints using PPS and FAS.

- Comparison of absolute levels, change and percent change from baseline to each scheduled post-Baseline visit in angiotensin II, renin (Plasma renin activity [PRA]; direct renin), and urinary AGT will be conducted between each ISIS 757456-treated group and pooled-placebo group in the PPS and FAS.
- Comparison of absolute scores, change from baseline to each scheduled post-Baseline visit in SF-12 scores (8 domains, PCS, and MCS), and EQ-5D-5L (5 dimensions, VAS, and index score) will be conducted between each ISIS 757456-treated group and pooled-placebo group.

- Proportion of non-worsening from baseline to each scheduled post-Baseline PGI-C and PGI-S will be compared between each ISIS 757456-treated group and pooled-placebo group. PGI-C will be categorized by non-worsening and worsening, where non-worsening includes categories of "Much better", "A little better" and "No change". PGI-S will be categorized by non-worsening and worsening, where non-worsening defined as ≥ 0 point improvement in the change from baseline score. The adjusted odds ratio (each ISIS 757456-treated dose group vs. pooled-placebo group) with 95% CI from logistic regression model will be provided.
- Comparison of change from Baseline to Study Day 85 in daytime SBP and DBP from 24 hr ABPM will be conducted between each ISIS 757456-treated group and pooled-placebo group
- Comparison of change from Baseline to Study Day 85 in nighttime SBP and DBP from 24 hr ABPM will be conducted between each ISIS 757456-treated group and pooled-placebo group
- Comparison of change from Baseline to Day 85 in eGFR will be conducted between each ISIS 757456-treated group and pooled-placebo group
- Comparison of change from Baseline to Day 85 in UACR will be conducted between each ISIS 757456-treated group and pooled-placebo group
- Primary endpoint, selective secondary and exploratory endpoints will be also analyzed using a sub-population of patients with Baseline 24 hr ABPM SBP > 130
- POP-HSQ scores will be listed.

Additional POP-HSQ summaries and analysis will be conducted, details are described in POP-HSQ SAP. Other exploratory PK endpoints will be further described in the Section 3.6.

3.6. Pharmacokinetic (PK) and Immunogenicity (IM) Analysis

3.6.1. PK Analysis

The plasma pharmacokinetics of ISIS 757456 (as total full-length oligonucleotides or ISIS 757456-equivalent, ISIS 757456-eq.) will be assessed following SC administration(s). PK analysis summary will be based on PK set.

Metabolite identification and profiling may be determined in some of the collected plasma samples and will be reported separately.

Plasma Concentration Data of Total Full-Length Oligonucleotides

Plasma concentrations of ISIS 757456 (ISIS 757456eq.), along with the scheduled (nominal) and actual sample times (i.e., time from SC dosing) will be listed (when applicable) for each patient, by treatment group, actual dose, subject IM status and study day. In addition, percent differences between scheduled and actual sampling times will also be listed for all patients, as well as percent differences between actual administered dose and nominal dose.

ISIS 757456 concentrations will be summarized using descriptive statistics by treatment, dose, cohort, study day, and scheduled time point, without and with stratification by IM status. Plasma

concentrations below the lower limit of quantification (LLOQ) will be indicated by "BLQ". For the purpose of calculating typical descriptive statistics (n, mean, SD, SE, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for plasma concentrations, all BLQ values will be set to zero. Mean plasma concentrations that are BLQ will be presented as "BLQ", and the SD and %CV will be reported as not applicable. Other stratifications may also be performed if deemed warranted to properly interpret the pharmacokinetic analysis. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose (> 30%).

Plasma Pharmacokinetic Parameters

The plasma PK of ISIS 757456 (as total full-length oligonucleotides) will be assessed following the SC administration. Non-compartmental PK analysis of ISIS 757456 (total full-length oligonucleotides) will be carried out on each individual subject data set using Phoenix WinNonlin version 8.0 or higher (Pharsight Corporation, Mountain View, CA). For calculation of PK parameters, all BLQ values will be set to zero. The plasma PK parameters for ISIS 757456eq. will be calculated based on actual sampling times. Since only trough and post-treatment follow up PK samples (and optional samples 2 hours post-dose on Day 1 and Day 78) are to be collected, only the following PK parameters will be calculated:

- C_{trough}
- $t_{1/2\lambda z}$

The terminal half-life shall be determined over a time interval equal to at least $1.5 \times t_{1/2\lambda z}$ (Span > 1.5). In addition, a minimum of three data points in the elimination phase will be used to define λ_z, and the coefficient of determination (r^2_adjusted) shall be greater than or equal to 0.8 for the estimate to be accepted. Other plasma PK parameters, as appropriate, may be determined or calculated at the discretion of the PK scientist.

Plasma PK parameters will be listed by treatment, dose, subject ID, subject IM status, and study day; and appropriately summarized using descriptive statistics (n, mean, SD, SE, %CV, geometric mean, geometric %CV, median, minimum, and maximum) by treatment and study day. Additionally, subject IM status stratified plasma PK parameters will be similarly summarized. Other stratifications may also be performed if deemed warranted at the discretion of the pharmacokineticist and/or biostatistician.

3.6.2. Pharmacokinetic/Pharmacodynamic Exposure-Response Analysis

Exposure-response correlations may be explored graphically between plasma exposure (e.g., $C_{trough,}$), and selected PD measures (e.g., plasma AGT level), other relevant biomarkers (such as plasma renin activity, angiotensin II, etc.) and relevant clinical endpoints (such as systolic blood pressure). In addition, the relationship between serum AGT level with plasma concentrations (C_{trough}) of ISIS 757456 eq. may be further evaluated with an inhibitory effect E_{max} model. The analysis may include stratification by IM status as well.

Population PK and PKPD analysis may be performed using the PK and PD data from this Study, and/or combined with other ISIS 757456 clinical PK/PD data from any previous and future studies in the development timeline.

3.6.3. IM Analysis

Samples collected at pre-dose on Study Days 1 (baseline), 15, 29, 57 and anytime on Days 85 and 169 in the post-treatment period will be analyzed for anti-ISIS 757456 antibodies.

Sample Level ADA data

An evaluable sample will be designated 'IM positive' based on both positive screening and confirmation assay results (i.e., confirmed positive result), and otherwise will be deemed 'IM negative'. Sample IM results (screen positive/negative, confirmed positive/negative or unevaluable, and when applicable, titer of anti-ISIS 757456 antibodies) before, during, and after treatment with study drug (sample IM status) will be listed by treatment, dose, and day of collection.

The sample ADA incidence (number) and incidence rate (percent) at each evaluated study time point will be determined and appropriately summarized by treatment and dose as the total number of and percentage of evaluated subjects with sample ADA negative, positive, and unknown status. Furthermore, titer over time will be also appropriately summarized (using descriptive statistics) as median, quartiles (25% and 75%), and range by treatment and dose.

Subject Level ADA Data

Subject's overall ADA status will be defined as 'Positive' if they have at least one confirmed positive sample result at any time during the treatment or post-treatment evaluation periods; 'Negative' if all evaluated ADA sample results during the treatment and post-treatment evaluation periods are ADA negative and they have at least one evaluable ADA result collected post study drug treatment. Otherwise, a study subject will be assigned 'Unknown' ADA status.

Furthermore, subjects with overall positive ADA status will be further classified into different ADA types based on their baseline ADA status and change in ADA titer post treatment as described below (Shankar et al. 2014):

- Treatment-Emergent ADA: sum of treatment-induced ADA and treatment-boosted ADA as described below:
  - Treatment-Induced ADA: ADA developed de novo (seroconversion) following biologic drug administration (i.e., formation of ADA any time after the initial drug administration in a subject without pre-existing ADA, i.e., baseline negative ADA)
  - Treatment-Boosted ADA: pre-existing ADA that were boosted to a higher level following biologic drug administration (i.e., any time after the initial drug administration the ADA titer is greater than the baseline titer by a factor of 8-fold or more)
- Treatment-Unaffected ADA: pre-existing ADA that were not affected (boosted) following biologic drug administration (i.e., any time after the initial drug administration the ADA titer is 4-fold or less)
- ADA type would be not applicable (NA) if the subject's overall ADA status is negative.

Other subject level IM parameters to be calculated/defined may include but not limited to:

- Subject ADA Status at Baseline: "Positive" if the subject has Week 1 Day 1 pre-dose sample (baseline) tested as confirmed positive; "Negative" if the subject has Week 1 Day 1 pre-dose sample (baseline) tested as confirmed negative; "Unknown" if the subject has Week 1 Day 1 pre-dose sample (baseline) unevaluable.
- Onset of ADA: i.e., the first day ADA positive sample observed, will be calculated by: the date of first sample has “positive” sample IM status - first dose date +1
- Last Positive ADA Study Day: defined as the last positive ADA sample observed from the start of study drug treatment and will be calculated by: the date of last sample has “positive” sample IM status - first dose date +1
- Last IM Sampling Study Day: defined as the last ADA sample collected from the start of study drug treatment and will be calculated by: the date of last sample collected - first dose date +1
- Peak titer: the highest titer observed for the subject
- Time to peak titer: the time to reach peak titer will be calculated by: the date of first peak titer observed- first dose date +1
- Total number of ADA Positive Samples: the total number of ADA samples being confirmed positive for the subject
- Total number of ADA Samples evaluated: the total number of ADA samples being collected and analyzed successfully with reportable results for the subject

Lastly, subjects with positive ADA status may further be classified as being transient or persistent ADA response, if there are sufficient number of subjects with transient ADA status. Transient and persistent ADA definitions are defined below and based on (Shankar et al. 2014):

Transient ADA response:

- Treatment-induced ADA detected only at one sampling time point during the treatment or follow-up observation period (excluding the last sampling time point, which will be considered persistent unless shown to be undetectable at a later time) or
- Treatment-induced ADA detected at two or more sampling time points during the treatment (including follow-up period if any), where the first and last ADA-positive samples (irrespective of any negative samples in between) are separated by a period less than 16 weeks, and the subject’s last sampling time point is ADA-negative.

Persistent ADA response:

- Treatment-induced ADA detected at two or more sampling time points during the treatment (including follow-up period if any), where the first and last ADA-positive samples (irrespective of any negative samples in between) are separated by a period of 16 weeks or longer or

- Treatment-induced ADA detected only at the last sampling time point of the study treatment period or at a sampling time point with less than 16 weeks before an ADA-negative last sample.

The subject level ADA prevalence, incidence, and positive ADA response being transient or persistent (if applicable) will be calculated as the number and the proportion (percent) of the study population during the study period by treatment and dose. Subject level IM parameters (as described above) will be listed by treatment and dose for all evaluable subjects, and also appropriately summarized (using descriptive statistics) as median, quartiles (25% and 75%) and range, by treatment and dose.

Evaluation of IM Impact on PK, PD, Efficacy and Safety

The impact of IM on PK and PD will be evaluated by stratifying plasma PK parameters, plasma trough and post-treatment ISIS 757456 concentrations and PD biomarker levels by subject ADA status, summarized using typical descriptive statistics, and presented graphically and/or in tables. The impact of IM may also be evaluated on selected clinical efficacy end points and safety measures.

Lastly, other stratifications (e.g., based on antibody titer, onset of ADA, etc.) of selected PK, efficacy and safety assessments may also be performed if deemed warranted at the discretion of the pharmacokineticist, medical monitor, and/or biostatistician.

3.7. Safety Analyses

The safety analysis will be conducted on the Safety Set.

3.7.1. Exposure

Total amount of study drug (Placebo, ISIS 757456 80 mg or ISIS 757456 120 mg) received (mg), total number of doses administered, and treatment duration will be summarized by the treatment group.

The treatment duration will be defined as the total number of days a subject is known to be followed on study calculated as follows:

$$\text{Treatment duration} = \text{Last dose date} - \text{Date of first dose} + 1$$

3.7.2. Adverse Events

The number and incidence of adverse events (AEs) will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) version 24.0 preferred term (PT) and system organ class (SOC) for:

Any treatment emergent adverse events (TEAEs).

Serious TEAEs (Treatment-emergent SAEs).

Related TEAEs and SAEs. Related is defined as “Related”, “Possible”, or missing relationship to study drug.

Any TEAEs by severity. At each severity level of subject summarization, a subject is classified according to the highest severity if the subject reported one or more events. AEs with missing severity will be categorized as "Missing" for this summary.

TEAEs leading to permanent study drug discontinuation.

Serious and non-serious AEs that lead to study discontinuation or investigational drug discontinuation will be listed separately. Non-treatment emergent adverse event will be included and be noted in the subject AE listing.

TEAEs will be defined as those adverse events that either start or worsen in severity on or after the date/time of first dose of study treatment.

In addition, if severity of an AE changes during the study, a separate AE will be recorded for each severity on the AE CRF. "first" and "second" AE will be identify based on AE start date. AE start date of the second record is AE stop date of first record. These linked events will be identified based on "Formlink" dataset and compared pairwise, and consider two cases, where the AE severity (mild/moderate/severe) are compared between the two records in the pair.

Case 1: The first AE record in the pair occurs before first dosing, and the second AE record occurs after first dosing.

If the AE severity or seriousness of the second record is worse than that of the first record, then only the second AE is deemed as a TEAE. Otherwise, neither record is considered as TEAE.

Case 2: Both AE records in the pair occur after first dosing.

Only the worst AE will be deemed as one TEAE.

All TEAEs identified based on the rules above will be summarized in the event number analysis.

The most conservative approach will be used to determine if the event occurs after the treatment. For example, if the onset date or resolution date of an AE is prior to the first study treatment date, it will be considered to have occurred prior to the study period. If the onset or resolution date of an AE is a partial date with only month or year available or complete missing, then the event is assumed to be within the study period unless the year is prior to the year of the first study treatment date, or if in the same year, the month is prior to the month of the first study treatment date.

Addition summary of hypotension and hyperkalemia incidence will be provided.

Local Cutaneous Reactions at the Injection Site

Local cutaneous reaction at injection site (LCRIS) is defined as (A) moderate or severe adverse events with the PTs Injection site erythema, Injection site swelling, Injection site pruritus, or Injection site pain that started on the day of injection, persisted for at least two days or ongoing; or (B) any AE at the Study Drug injection site, regardless of severity, that leads to discontinuation of study drug, where AE at the Study Drug injection site is the principal reason for discontinuation.

Percentage of injections leading to LCRIS will be calculated as follows for each subject: (A/B)*100, where A = number of injections with an LCRIS, and B = total number of injections. Doses that are split across multiple injections are counted as a single injection

LCRIS will be summarized using the MedDRA coding system, by SOC/PT. Percentage of the injections leading to LCRIS at injection site will also be summarized.

LCRIS will be listed by preferred term.

Flu-like Reactions

Flu-like reactions are defined as adverse events with PTs including either (A) Influenza like illness or (B) Pyrexia or Feeling hot or Body temperature increased, plus at least two of the following symptoms with the PTs: Chills, Myalgia, or Arthralgia, starting on day of injection or the next day.

Percentage of injections leading to flu-like reactions will be summarized using the descriptive statistics.

Percentage of injections leading to flu-like reactions will be calculated as follows for each subject: (A/B)*100, where A = number of injections leading to flu-like reactions, and B = total number of injections.

FLRs will be summarized using the MedDRA coding system by SOC/PT. Percentage of the injections leading to FLRs at injection site will also be summarized.

FLRs will be listed by preferred term.

AE of Special Interest (AESI): Platelet reduction

Per protocol, severe reductions in platelet count < 50,000/mm^3 accompanied by a clinically relevant bleeding event or platelet count of < 25,000/mm^3 independent of a clinically relevant bleeding event are considered as AESI. The AEs meeting the AESI criteria will be captured in the AE CRF page with a check box to indicate.

AESI will be summarized using the MedDRA coding system, by SOC/PT. A listing of AESI will also be generated.

3.7.3. Laboratory Measurements

Chemistry including thyroid panel and inflammatory, hematology, coagulation, and urinalysis (result, change and percent change from baseline) will be summarized by each treatment group and each post-baseline visit. Listing of laboratory assessments in Chemistry, Hematology and urinalysis will be provided. Local laboratory data will also be provided in the listings separately.

For alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the number and percent of subjects with post-Baseline results falling in each of the following categories (using available central and local laboratory assessments) will be tabulated:

ALT or AST > 3 × ULN, confirmed

ALT or AST > 5 × ULN, confirmed

ALT or AST > 3 × ULN and serum total bilirubin > 2 × ULN, confirmed or INR > 1.5

ALT > 3 × ULN at any time during post-baseline or AST > 3 × ULN at any time during post-Baseline and with total bilirubin (TBL) > 2 × ULN at any time during post-baseline

For potassium (K), the number and percent of subjects falling in each of the following categories (using available central and local laboratory assessments) during post-baseline will be tabulated:

- Absolute levels ≥ 5.5–< 6 mmol/L, confirmed
- Absolute levels ≥ 6 mmol/L, confirmed

For platelet, the number and percentage of patients falling in each of the following categories (using available central and local laboratory assessments) based on post-baseline assessments will be provided:

- Confirmed Platelet count 75,000 to ≤ 100,000/mm^3
- Confirmed Platelet count 50,000 to < 75,000/mm^3
- Confirmed Platelet count 25,000 to < 50,000/mm^3
- Confirmed Platelet count < 25,000/mm^3
- Confirmed ≥ 30% Platelet count decrease from baseline; further classifying by the lower limit of normal (Above or below)

The number and percent of subjects with post-baseline results falling in the following categories (using available central and local laboratory assessments) will be also tabulated:

- Confirmed 30% decline in eGFR from Baseline eGFR values [eGFR should be calculated using the CKD-EPI creatinine equation (Levey et al. 2009)]
- Confirmed proteinuria: Absolute UPCR value ≥ 1000 mg/g

A confirmed value is based on a consecutive lab value performed on a different day to, but within 7 days of, the initial value. If there are multiple results on the same day, no matter from the same lab vendor or different lab vendors, then the worst value will be utilized in the analysis. If the repeated value is in the same or worse category, then the initial value is considered confirmed. If the consecutive value is in a better category, then the initial value is confirmed using the consecutive value category. If values that are not confirmed due to failure to retest or missing lab values, then the initial value is presumed confirmed.

Additional analysis to explore the possible thrombocytopenia incidence can also be found in AESI described in Section 3.7.2.

Subjects with more than one confirmed value will be counted exactly once under the worst confirmed category.

3.7.4. 2.12.4 Vital Signs

Vital signs include heart rate, respiratory rate, body temperature, BMI and systolic and diastolic blood pressure. Except for systolic and diastolic blood pressure, which are efficacy endpoints for this study and will be summarized separately, other vital signs will be summarized by treatment group for vital sign values as well as the change and percent change from baseline at each post-Baseline visit.

3.7.5. 12-Lead Electrocardiograms (ECG)

Safety 12 lead ECG will be performed at the visits indicated in the protocol Schedule of Procedures.

The ECG data will include ventricular rate (VR), PR interval, QRS duration, QT, and corrected QT intervals (QT corrected using the Fridericia's formula), and overall interpretation.

For the continuous variables above, descriptive statistics (n, mean, standard error, standard deviation, median, 25th percentile, 75th percentile, minimum and maximum) of results at study screening and study Week 13 visit, as well as the change and percent change from Baseline, will be presented in summary tables; for the categorical responses to overall interpretation.

All the ECG data collected will be listed.

3.7.6. Prior and Concomitant Medications

Prior and Concomitant medications will be coded using WHO Drug dictionary (Global B3 March 2021) and summarized by ATC class, generic name and treatment group.

Prior medications include medications that started prior to the first dose of study medication regardless of whether they continued while on treatment or not. Concomitant medications include medications that patients are exposed to on or after the first dose of study medication.

Additional summary of HTN medications uses at baseline and new initiation of HTN medication during the study will be summarized by ATC class and treatment group.

Listings will be provided.

4. REFERENCES

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APPENDIX 1. EQ-5D HEALTH STATE INDEX SCORE DERIVATION ALGORITHM

To derive this index value, the response of each dimension will be first translated into a weight based on the value sets issued by EuroQoL group (see table x). The index value then can be calculated as **1- sum of weights from these 5 dimensions' response**. The possible best value is 1 for both regions and the possible worst value is -0.285 for UK region and -0.573 for US region. If there is a missing response from a dimension, then the index value will not be calculated and be treated as missing. For this study, all the other non-US counties will utilize UK county weight instead. The display of the index value is with 3 digits of decimal points.

Table 1: Weight of EQ-5D-5L Response by Region

				EQ-5D-5L Dimension		
Region	**Response**	**Mobility**	**Self-Care**	**Usual Activities**	**Pain/ Discomfort**	**Anxiety/ Depression**
UK (16/11/2020)	1) No problem	0	0	0	0	0
	2) Slight problem	0.058	0.05	0.05	0.063	0.078
	3) Moderate problems	0.076	0.08	0.063	0.084	0.104
	4) Severe problems	0.207	0.164	0.162	0.276	0.285
	5) Unable to	0.274	0.203	0.184	0.335	0.289
US (16/11/2020)	1) No problem	0	0	0	0	0
	2) Slight problem	0.096	0.089	0.068	0.06	0.057
	3) Moderate problems	0.122	0.107	0.101	0.098	0.123
	4) Severe problems	0.237	0.22	0.255	0.318	0.299
	5) Unable to	0.322	0.261	0.255	0.414	0.321

Sources: https://euroqol.org/support/tools/analysis-tools/index-value-set-calculators/

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