STATISTICAL ANALYSIS PLAN
ALN-AGT01-002 (KARDIA-1)

Protocol Title:	A Randomized, Double-blind, Placebo-Controlled, Dose-Ranging Multicenter Study to Evaluate the Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension
Short Title:	A Study to Evaluate Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension (KARDIA-1)
Study Drug:	Zilebesiran (ALN-AGT01)
Protocol Date:	Original protocol, 09 April 2021 Amendment 1, 20 April 2021 Amendment 2, 09 June 2021 Amendment 3, 09 December 2021 Amendment 4, 22 March 2022 Amendment 5, 04 November 2022
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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABPM	Ambulatory blood pressure monitoring
ADA	Anti-drug antibody(ies)
AE	Adverse event
AGT	Angiotensinogen
ALT	Alanine aminotransferase
AngI/II	Angiotensin I/II
AST	Aspartate aminotransferase
AUC	Area under the curve
DB	Double-blind
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
eDISH	Evaluation of drug-induced serious hepatotoxicity
EOT	End of treatment
ET	Early termination
FAS	Full analysis set
HbA1c	Hemoglobin A1c
HBPM	Home blood pressure monitoring
HLT	High level term
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISR	Injection site reaction
LFT	Liver function test
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed more for repeated measurement
OLE	Open-label extension

Abbreviation	Definition
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred term
Q3M	Once every 3 months
Q6M	Once every 6 months
RAAS	Renin-angiotensin-aldosterone system
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SC	Subcutaneous(ly)
SD	Standard deviation
SOC	System Organ Class
ULN	Upper limit of normal
ULOQ	Upper limit of quantification

1. INTRODUCTION

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacokinetic (PK) and pharmacodynamic (PD) data summaries and statistical analyses in support of the clinical study report (CSR) for Study ALN-AGT01-002 (KARDIA-1). This SAP is finalized prior to treatment unblinding and conducting the primary analysis, which will occur after all patients complete the Month 6 visit or withdraw from the study prior to the Month 6 visit. Changes to planned analyses specified in this SAP made after database lock will be documented in the CSR.

Table, figure, and listing (TFL) mocked shells and specifications are contained in a separate document.

2. STUDY DESIGN

2.1. General Study Design

This is a Phase 2, randomized, double-blind, placebo-controlled, dose-ranging, multicenter study designed to evaluate the safety, efficacy, and pharmacodynamics (PD) of zilebesiran (ALN-AGT01), administered subcutaneously (SC), in patients with mild-to-moderate hypertension. A schematic of the study design is provided in Figure 1. Before randomization, patients will discontinue prior antihypertensive medications (if taking) for a Washout period of at least 2 weeks (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers).

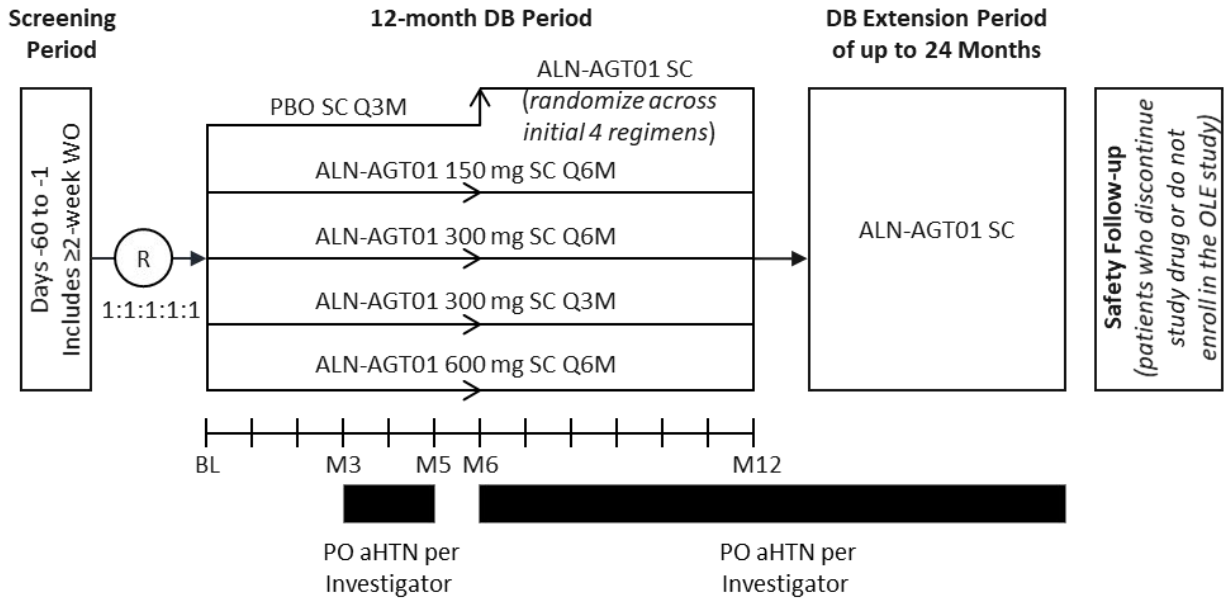
Patients who complete the Screening period and meet all inclusion/exclusion criteria for enrollment will receive zilebesiran or placebo for the first 6 months of the 12-month Double-blind (DB) treatment period.

Starting at Month 3, conventional oral antihypertensives may be added per Investigator judgement for elevated blood pressure. Oral antihypertensives (if taking) will be temporarily held from Month 5 to Month 6 to assess the effect of zilebesiran alone (vs placebo) at Month 6. During this 4-week period, blood pressure will be carefully monitored by home blood pressure monitoring and oral antihypertensive medications restarted if indicated. Patients may resume conventional oral antihypertensives at Month 6 per Investigator judgement.

Patients randomized to placebo will be re-randomized at Month 6 to 1 of the 4 initial zilebesiran regimens until the end of the DB period. Patients randomized to zilebesiran regimens will remain on their originally assigned regimens through Month 12.

After the 12-month DB treatment period, patients may be eligible to participate in a zilebesiran open-label extension (OLE) study. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 24 additional months until the OLE study is open and then transition.

Figure 1 Study Design



Abbreviations: aHTN=antihypertensive medications; DB=double-blind; M=month; OLE=open-label extension; PBO=placebo; PO=per os (oral); Q3M=once every 3 months; Q6M=once every 6 months; R=randomization; SC=subcutaneous; WO=washout.
Note: Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, 24, 30, or 36 (whichever visit occurs first).
Note: Patients who were previously taking antihypertensives at screening should undergo a washout of these medications for at least 2 weeks during the Screening period (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers).

2.2. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the effect of ALN-AGT01 on SBP as assessed by ABPM at Month 3	• Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
Secondary	
Through Month 6 • To evaluate the effect of ALN-AGT01 on blood pressure assessed by ABPM • To evaluate the effect of ALN-AGT01 on office blood pressure • To characterize the PD effects of ALN-AGT01	Key Secondary Endpoints • Change from baseline at Month 3 in office SBP • Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM • Change from baseline at Month 6 in office SBP • Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6 Other Secondary Endpoints • Time-adjusted change from baseline in 24-hour mean SBP and DBP, assessed by ABPM • Change from baseline in 24-hour mean DBP, assessed by ABPM • Change from baseline in office SBP and DBP • Change in serum AGT • Change in daytime and nighttime blood pressure (including dipping pattern)
Exploratory	
• To evaluate the effect of ALN-AGT01, over time, on other measures of blood pressure reduction (through Month 12)	• Change in SBP and DBP assessed by ABPM • Change in office SBP and DBP • Office blood pressure and ABPM control and response rates • Proportion of patients with oral antihypertensive use • Change in SBP and DBP assessed by HBPM

Objectives	Endpoints
	• Change in pulse pressure assessed by ABPM and office blood pressure
• To characterize the PD effects of ALN-AGT01 (after Month 6)	• Change in serum AGT
• To characterize the plasma PK of ALN-AGT01	• Plasma concentrations of ALN-AGT01 and its metabolite AS(N-1)3′ ALN-AGT01
• To assess the effect of ALN-AGT01 on exploratory biomarkers of the RAAS	• Change from baseline in plasma renin concentration, aldosterone, AngI, and AngII
• To evaluate the immunogenicity of ALN-AGT01	• Incidence and titers of ADA
• To assess the effect of ALN-AGT01 on body weight, BMI, and morphometric measurements	• Change from baseline in body weight, BMI, waist circumference, and waist-to-hip ratio
• To assess the effect of ALN-AGT01 on metabolic syndrome parameters	• Change from baseline in HbA1c, fasting plasma glucose, insulin, and serum lipid profile
• To correlate blood pressure measurements obtained with a wearable, noninvasive, cuffless device to those obtained by standard cuff-based methods	• Correlation of blood pressure values obtained with a wearable device versus ABPM, HBPM, and office measurements
• To assess the long-term treatment effect of ALN-AGT01 (through Month 36)	• Change from baseline in SBP and DBP assessed by office blood pressure and HBPM
Safety	
• To evaluate the safety of ALN-AGT01 in patients with mild to moderate hypertension	• Frequency of AEs

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibody; AE=adverse event; AGT=angiotensinogen; Ang=angiotensin; BMI=body mass index; DBP=diastolic blood pressure; ECG=electrocardiogram; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; PD=pharmacodynamic; PK=pharmacokinetic; RAAS=renin-angiotensin-aldosterone system; SBP=systolic blood pressure.

2.3. Study Procedure

The Schedule of Assessments is provided in Table 2.

2.4. Randomization Methodology

Using the Interactive Response Technology (IRT), patients will be randomized 1:1:1:1:1 to the following arms during the first 6 months of the 12-month DB period:

- Placebo SC once every 3 months
- 150 mg zilebesiran SC once every 6 months
- 300 mg zilebesiran SC once every 6 months
- 300 mg zilebesiran SC once every 3 months
- 600 mg zilebesiran SC once every 6 months

Patients initially randomized to placebo will be re-randomized 1:1:1:1 at Month 6 to 1 of the 4 initial zilebesiran regimens.

Patients who enter the DB Extension period will continue their current blinded dosing regimen from the DB period.

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (mean 24-hour SBP < or ≥145 mmHg).

Each patient will be uniquely identified in the study by a combination of the site number and patient identification number. Upon signing the informed consent form (ICF), the patient will be assigned a patient identification number by the IRT system. The Investigator or his/her designee will randomize the patient in IRT after confirming that the patient fulfills all the inclusion criteria and none of the exclusion criteria. The Investigator or his/her designee will re-randomize the patient in IRT at Month 6 to assign placebo patients to 1 of the 4 initial zilebesiran dose groups in a way that maintains the study blind for the patient.

2.5. Blinding

The Sponsor will be blinded to study drug treatment through Month 6 of the 12-month DB period. During the course of the study, serum AGT, plasma PK, and treatment assignment using dummy IDs will be made available to a small, independent pharmacometrics team at the Sponsor that will not be involved in the conduct or oversight of the study. After the last patient completes the Month 3 visit and prior to the last patient's Month 6 visit, SBP data from a limited number of patients (about one-third) will be made available to this small, independent pharmacometrics team for preliminary PK/PD modeling. After the database lock to support the analysis of Month 6 data is complete, all other Sponsor personnel will be unblinded to treatment assignment.

Blinded doses of study drug will be administered under the supervision of the Investigator (refer to Protocol Section 5.2.2). All patients will receive the same volume and number of injections regardless of treatment assignment (patients receiving once every 6-month zilebesiran regimens will receive placebo SC at dosing visits at which they do not receive zilebesiran to maintain the blind). Because zilebesiran may be slightly visually distinguishable from placebo, all blinded study drug doses will be prepared, and the syringe(s) will be masked by a site pharmacist or delegate prior to administration by a blinded healthcare professional. See the Pharmacy Manual for additional details.

Details about the specifics of the blinding aspects throughout the entire study are available in the Randomization and Blinding Plan.

Any unplanned/emergency unblinding occurring during the DB Period will be documented and reported in the CSR.

Refer to the study Randomization and Blinding Plan for more details.

2.6. Determination of Sample Size

Assuming a standard deviation (SD) in the range of 15 to 20 mmHg in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM, a maximum mean difference in effect size between zilebesiran and placebo of 10 mmHg, and a 15% dropout rate at Month 3, a sample size of 375 patients (75 per treatment group) provides at least 84% power to detect a dose response signal in SBP reduction among all zilebesiran doses and placebo at the 2-sided significance level of 0.05. In addition, this sample size provides at least 80% power to detect a 10 mmHg difference between an individual zilebesiran dose versus placebo with a 2-sided significance level of 0.05.

3. ANALYSIS POPULATIONS

During the study, 16 patients were randomized from Ukraine in January and February 2022 before a geographic conflict started in February 2022. In response to this conflict, Regulatory Authorities advised Sponsors to refer to guidance documents issued during the COVID-19 pandemic with respect to the handling of clinical trial sites in Ukraine. In line with these guidance documents, and due to challenges in data collection and cleaning and verification of such data, data from patients enrolled at sites in Ukraine will be excluded from the analysis sets defined below. Data from these patients will be provided in listings.

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. All by-treatment analyses based on the FAS will be according to the randomized treatment arm.
- Safety Analysis Set: All patients who received any amount of study drug, grouped according to the treatment actually received.
- PK Analysis Set: All patients who received at least 1 full dose of ALN-AGT01 and have at least 1 nonmissing postdose PK assessment.
- PD Analysis Set: All patients who received at least 1 full dose of study drug. All by-treatment analyses based on the PD Analysis Set will be grouped according to the treatment actually received.
- All Zilebesiran Treated Set: All patients who received any amount of 1 of the 4 ALN-AGT01 dosing regimens, including patients who took ALN-AGT01 during the 6-month placebo-controlled period and patients who initially took placebo and then switched to ALN-AGT01 after the Month 6 visit.

For the primary analysis for 6-month placebo-controlled DB period, the primary population used to evaluate efficacy will be the FAS. Safety will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

The All Zilebesiran Treated Set will be used to summarize the efficacy and safety of zilebesiran throughout the entire DB period (including DB extension period).

3.1. Randomization and Treatment Errors

For patients who were not treated, not randomized, or received incorrect treatment, the following rules will be used:

- Randomized but not treated: they will be excluded from the FAS and Safety Analysis Set for efficacy and safety evaluations as actual treatment is missing.
- Treated but not randomized: they will be excluded from the efficacy analyses since randomized treatment is missing but will be reported under the treatment actually received for all safety analyses.
- Randomized but took incorrect treatment: they will be reported under their randomized treatment arm for all efficacy analyses. But for safety analyses, a patient will be included under the active treatment arm if the patient is randomized to placebo arm and received an active dose by mistake.

4. GENERAL STATISTICAL CONSIDERATIONS

4.1. General Considerations

In general, data will be summarized for each planned analysis defined in Section 4.7 separately.

For Month 3 endpoints, zilebesiran 300mg Q3M and 300mg Q6M will be pooled together.

Categorical variables will be summarized using counts and percentages. Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), standard error of mean (SEM), median, first quartile (Q1), third quartile (Q3), minimum, and maximum.

The day of the first dose of study drug administered is defined as Day 1. The Study Day of a time point of interest is calculated as follows:

- If on or after Day 1, Study Day = date of interest – date of the first dose of study drug + 1
- If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection (LLOQ) will be replaced by the LLOQ. Any assessment collected and recorded as greater than the upper limit of quantification (ULOQ) will be replaced by the ULOQ.

All descriptive summaries will be presented by treatment arm.

Statistical analyses will be conducted using SAS software Version 9.4 or newer or R version 3.6 or newer.

4.2. Blood Pressure Collection and Handling

4.2.1. 24-Hour Ambulatory Blood Pressure Monitoring

24-hour ambulatory blood pressure monitoring (ABPM) is programmed to take readings every 20 minutes during the day (6 am to 9:59 pm) and every 30 minutes during the night (10 pm to 5:59 am). An ABPM will be considered adequate if (1) the number of successful daytime readings is ≥33, (2) the number of successful nighttime readings is ≥11, and (3) no more than 3 hours are not represented (i.e., 3 sections of 60 minutes where 0 valid readings were obtained). If the ABPM recording is inadequate, the patient will be provided 1 opportunity to repeat the recording within 7 days from the end time of the invalid ABPM.

To summarize the 24-hour ABPM, hourly adjusted mean will be calculated. Hourly mean and 24-hour mean will be calculated by two steps:

1. Calculate the hourly mean: average of BP by each hour of the day (e.g., mean of BP measurements from 16:00 to 16:59). If there is no reading in a specific hour, this hour will not be included in calculation.
2. Calculate the 24-hour mean: average of the hourly means.

4.2.2. Office Blood Pressure

The mean office BP in the sitting position and office BP in standing position will be used for the analysis. Office BP in the sitting position will be collected with a set of 4 replicates. The average of the last 3 replicates will be calculated and used for analysis. If there are multiple sets of assessments collected on the same day, the last set will be used for the analysis. Office BP collected in standing position will be used to derive orthostatic hypotension.

4.2.3. Home Blood Pressure Monitoring

Home blood pressure will be measured both pre and post randomization. To establish baseline, each patient should measure HBPM during the week (with at least 3 successful readings) immediately prior to randomization. After Day 1, HBPM will be measured at least once per week. Four sequential blood pressure measurements at 1-minute intervals will be recorded. The mean sitting blood pressure for the visit will be defined by the average of the last 3 individual measurements. HBPM will be summarized by weekly average.

Details of the blood pressure collection are in Study Protocol Section 10.

4.3. Multiple Comparisons/Multiplicity Procedure

The overall familywise error rate will be controlled at $\alpha=0.05$ for the primary and key secondary endpoints as shown in Table 1

Table 1 Multiplicity Procedure

Test Step[a]	Endpoint	Comparison	Success criteria
1	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	Dunnett's Procedure	If the global hypothesis is rejected at 0.05 alpha level, then at least one zilebesiran dose is superior to Placebo
2	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
3	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05
4	Change from baseline at Month 3 in office SBP	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
5	Change from baseline at Month 3 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
6	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo	Nominal p-value < 0.05
7	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05
8	Change from baseline at Month 6 in office SBP	300 mg Q3M vs placebo	Nominal p-value < 0.05
9	Change from baseline at Month 6 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
10	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	300 mg Q3M vs placebo	Nominal p-value < 0.05
11	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	600 mg Q6M vs placebo	Nominal p-value < 0.05

[a] If the MCP criterion is satisfied in a given step, the hypothesis test is deemed statistically significant, and the next step will be evaluated; otherwise all hypotheses in the given and subsequent steps are deemed not statistically significant
[b] 300mg Q3M and 300mg Q6M will be pooled together for the test

4.4. Handling of Missing Data

No imputation will be done for missing values for the primary analysis of the primary endpoint. Results based on mixed model with repeated measurements (MMRM) are valid based on missing at random (MAR) assumption.

4.5. Baseline Definitions

For the primary analysis of 6-month placebo-controlled period, for office BP, the baseline is the average of the mean sitting office BP value on Day 1 prior to receiving the first dose of study drug and last non-missing value prior to Day 1 during screening visit. For HBPM, the baseline will be the average of all assessments during last week prior to receiving the first dose of study drug. For all other endpoints including ABPM, baseline is the last non-missing value (including unscheduled visit) prior to receiving the first dose of study drug.

For final analyses, patients initially randomized to placebo and re-randomized at Month 6 will be summarized in two ways:

From Day 1. Baseline definition is the same as for the primary analysis for the 6-month placebo-controlled period.

From the start of zilebesiran dosing at Month 6: Baseline is the last assessment prior to Month 6 dosing.

For patients initially randomized to zilebesiran dosing regimen, baseline remains the same as the one for the primary analysis.

4.6. Randomization Stratification Factors

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (24-hour mean SBP < or ≥145 mmHg). Stratification factors are recorded in the IRT database. Key data is integrated into the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database (EDC). In the presence of stratification errors, the stratification used in analysis may not match that in the IRT. A comparison of the number and percentage of patients in each randomization stratification factor in IRT versus the clinical database will be summarized by randomized treatment arm and overall.

4.7. Planned Analyses and Data Cutoffs

4.7.1. Primary Analysis

The primary analysis will be performed after the last randomized patient has completed Month 6 Visit or otherwise discontinued the study. For the primary analysis, as this study will be ongoing with some patients in the 12-month DB period, DB extension period or safety follow-up period, the study database will undergo an interim database lock with the last Month 6 visit date as the

cutoff date (i.e., data in EDC will be cleaned, frozen and electronically signed by investigators; external laboratory data will be cleaned and will undergo quality assurance). Data will be summarized in the CSR. Additional details regarding the interim database locks will be documented in the study Data Management Plan.

The primary analysis will include data on, or prior to, this prespecified cutoff date. For assessments with starting/ending dates (e.g., adverse events [AEs], medications), the starting date will be compared with the pre-specified cutoff date. Data records with starting dates after the specified data cutoff date will be excluded.

Data collected during the 6-month double-blind period (i.e. up to Month 6 visit dates) will be analyzed based on the methodology described in this SAP, while the data after Month 6 visit dates will be mainly summarized by descriptive statistics.

4.7.2. Final Analysis

After all patients reach the end of the study, the database will undergo a final database lock, and the data will be summarized in the CSR. The analyses for the 6-Month DB period will not be repeated.

A patient is considered to have reached the end of the study if the patient:

- has completed at least the Month 12 visit and enrolled in the OLE study, or
- has completed the Safety Follow-up visits for patients who discontinue study drug or do not enroll in the OLE study, or withdrawal from the study.

5. STATISTICAL ANALYSES

5.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized for all randomized patients and by randomized treatment arm and overall:

- Randomized
- Treated
- Completed the placebo-controlled 6-month double-blind (DB) treatment period
- Discontinuation and primary reason for discontinuation of treatment during 6-month DB period
- Discontinuation of study and primary reason for discontinuation of study during the 6-month DB period
- Completed the 12-month double-blind (DB) treatment period
- Completed the DB extension period
- Completed the study
- Discontinuation of treatment and primary reason for discontinuation of treatment after Month 6 during DB and DB extension period

- Discontinuation of study and primary reason for discontinuation of study after Month 6 during DB and DB extension period

In addition to the primary reason for discontinuation of treatment and withdrawal from study, patients will also be categorized if discontinuation/withdrawal was due to COVID-19.

5.2. Demographics and Baseline Characteristics

Demographic and baseline disease characteristics will be summarized by treatment arm and overall for Full Analysis Set, and also presented in listings.

Age at consent, height, weight, body mass index (BMI), 24-hour mean ABPM and office BP will be summarized using descriptive statistics. Sex, race, ethnicity, and country will be summarized by presenting the frequencies and percentages of patients in each category.

5.3. Medical History

Medical history and prior procedures reported will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 26.0 or newer. Unique patients who report medical history events will be summarized by MedDRA system organ class (SOC), high level term (HLT) and preferred term (PT).

5.4. Protocol Deviations

Protocol deviations will be classified by medical review prior to each planned analysis database lock, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry, 2013).

The Sponsor or designee will be responsible for producing the protocol deviation file. This file will include a description of each protocol deviation and classification as major or minor. The protocol deviations will be reviewed and finalized prior to the planned analysis database lock.

All protocol deviations and major protocol deviations will be summarized and listed.

5.5. Study Drug Exposure and Compliance

The following variables will be summarized by descriptive statistics and/or frequency tabulation:

- Duration of exposure, defined as: date of last exposure – date of first dose +1. Date of last exposure is the earliest date of the following:
  - date of last dose + the length of dosing interval, i.e.,
    - date of last zilebesiran dose + 169 days for the following treatment arms: zilebesiran 150 mg Q6M, 300 mg Q6M and 600 mg Q6M,
    - date of last dose + 85 days for the following treatment arms: placebo and 300 mg Q3M.
  - date of end of study
  - date of analysis data cutoff

- Number of doses received; as continuous and/or categorical variable
- Number of missed doses; as a categorical variable
- Total exposure (patient years)

5.6. Prior and Concomitant Medications

Medications will be coded using the World Health Organization (WHO) Drug Dictionary, version WHO-DD Global B3, March 2021 or newer. Unique patients who reported medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 3 class (or level 2 if not available) and PT. Summaries will be provided for prior and concomitant medications separately.

Prior medications are those medications with start date prior to the first dose of study drug.

Concomitant medications are medications, other than the study drug, administered at or after the first dose of study drug, as well as medications that started prior to the first dose of study drug and are ongoing after the first dose of study drug.

If the medication start date is on or after the date of first dose of study drug, the medication will be summarized as a concomitant medication even if the medication end date is missing.

If the end date of a medication is missing or incomplete, such that it cannot be determined whether it is after the first dose of study drug, it will be counted as a concomitant medication.

For missing or partial dates for medications, the imputation of start and end dates is described in Section 7.2.

5.7. Efficacy Analyses

5.7.1. Primary Endpoint

5.7.1.1. Definition of Estimand

The primary objective of the study is to evaluate the effect of zilebesiran on SBP as assessed by ABPM at Month 3. Primary estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150 mg Q6M, 300 mg Q6M, 300 mg Q3M and 600 mg Q6M. (300 mg Q6M and Q3M will be pooled together)
- Target population: patients with mild-to-moderate hypertension
- Endpoint: Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Population-level summary: Least square mean difference between zilebesiran and placebo in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Intercurrent events strategy: Rescue medication taken before Month 3 visit will be considered as an intercurrent event. For patients who require rescue medication, given the expected number of such patients at Month 3 is low, hypothetical strategy will be used, i.e., ABPM assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone. List of rescue medication ATC code is in Section 7.4.

5.7.1.2. Primary Analysis

The primary endpoint is the change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM. The hypothesis of the dose response relationship for the primary endpoint will be tested as:

- $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_0$ (there is no dose response relationship)
- H_a $\mu_1 \neq \mu_0$ or $\mu_2 \neq \mu_0$ or $\mu_3 \neq \mu_0$ (at least one zilebesiran dose is different from placebo).

Where μ_0 is the mean change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM in placebo arm; and μ_1, μ_2, and μ_3 are the mean changes from baseline at Month 3 in 24-hour mean SBP in zilebesiran 150mg Q6M, 300mg Q3M and Q6M pooled, 600mg Q6M arms, respectively.

The above hypothesis will be tested using the Dunnett's procedure based on Mixed model for repeated measurements (MMRM). The MMRM model will include treatment, visit, treatment-by-visit interaction, race (black; all other races) as fixed factors, baseline 24-hour mean SBP assessed by ABPM as a covariate. Unstructured covariance matrix will be used. Least square mean difference between zilebesiran and placebo, 95% confidence interval (CI) and p-values (both unadjusted and adjusted by Dunnett's procedure) will be generated.

5.7.1.3. Sensitivity Analyses

For the sensitivity analysis, treatment policy strategy will be used for patients who require rescue medication. All collected blood pressure measurements will be analyzed by the same MMRM model as the primary analysis, regardless of the rescue medication.

Due to the expected long-acting effect of zilebesiran, the commonly used control-based Pattern Mixture Model (PMM) will not be used to assess the missing not at random (MNAR) assumption.

5.7.1.4. Other Analyses

To demonstrate zilebesiran consistently controls blood pressure over the 24-hour period, hourly mean change from baseline at Month 3 in SBP assessed by ABPM will be plotted for each treatment group.

5.7.2. Secondary Endpoints

5.7.2.1. Key Secondary Endpoints

Secondary objective of the study is to evaluate the effect of zilebesiran on blood pressure assessed by ABPM and office blood pressure through Month 6. The estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150mg Q6M, 300mg Q6M, 300mg Q3M and 600mg Q6M
- Target population: patients with mild-to-moderate hypertension
- Endpoint:
  - Change from baseline at Month 3 in mean sitting office SBP

  - Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM
  - Change from baseline at Month 6 in mean sitting office SBP
  - Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medication at Month 6

- Population-level summary: Least square mean difference between zilebesiran and placebo in each of the change from baseline endpoints. Odds ratio for the binary endpoint.
- Intercurrent events strategy: For patients who require rescue medication,
  - for change from baseline endpoints: given the expected number of such patients at Month 3 is low and the protocol requires washout of these medications between Month 5 and Month 6, the hypothetical strategy will be used, i.e, blood pressure assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone.
  - for binary endpoint, composite strategy will be used. Patients with rescue medication are considered as not meeting the BP control and response criteria.

For change from baseline endpoints, MMRM model will be used as the primary analysis. The model will include treatment, visit, treatment-by-visit interaction, race (black, all other races) as fixed factors, corresponding baseline as a covariate. Least square (LS) mean difference of each zilebesiran dose group compared with placebo dose group, 95% CI and p-value will be generated. For change from baseline at Month 6 in office SBP, as a significant proportion of patients are expected to receive rescue medication, data collected at Month 4 and 5 visits will be excluded from MMRM model.

These endpoints will also be analyzed by the same MMRM model using the treatment policy strategy. I.e., all collected data will be analyzed regardless of the rescue medication. For change from baseline at Month 6 in mean sitting office SBP, Data collected at Month 4 and 5 Visits will be included as well.

For the proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without rescue medication at Month 6, logistic regression will be used. The model will include treatment as a factor and baseline 24-hour mean SBP as a covariate. Patients without Month 6 ABPM data will be considered as not meeting the criteria.

Multiplicity adjustment will be applied across primary and these secondary endpoints. Details of multiplicity control are in Section 4.3.

5.7.2.2. Other Secondary Endpoints

Other blood pressure related secondary endpoints are:

- Change from baseline at Month 3 in 24-hour mean DBP assessed by ABPM
- Change from baseline at Month 6 in 24-hour mean DBP assessed by ABPM

- Change from baseline at Month 3 in mean sitting office DBP
- Time adjusted change from baseline through Month 3 in mean sitting office SBP/DBP
- Change from baseline at Month 6 in mean sitting office DBP
- Time adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM
- Time adjusted change from baseline through Month 6 in mean sitting office SBP/DBP
- Change from baseline in daytime/nighttime mean SBP/DBP by ABPM at each visit. Daytime is defined as 6 am to 9:59 pm and nighttime is defined as 10 pm to 5:59 am.

Time-adjusted change is defined as the area under the curve (AUC) of BP change from baseline divided by the duration of the time period. It leads to the weighted average of change from baseline to each scheduled visit. Details of the definition and calculation are in Section 7.2.

In general, these endpoints will be analyzed using two approaches, similar as primary endpoint and key secondary endpoints. Blood pressure collected while patients are on and within 2 weeks after stopping any rescue medication will be censored. All collected BP data will also be analyzed.

Each endpoint will be analyzed by the MMRM model described in Section 5.7.1.2 with the corresponding baseline as a covariate.

In addition, ABPM dipping pattern, defined as ratio of nighttime to daytime mean SBP will be calculated. The number and proportion of patients will be summarized by treatment and by visit into 3 categories: 1) <0.9; 2) ≥0.9 to <1; 3) ≥1.

The secondary endpoint of percent change in serum AGT at each visit will be summarized by treatment using descriptive statistics.

5.7.3. Exploratory Endpoints

The blood pressure related exploratory endpoints through Month 12 are:

- Change from baseline in 24-hour mean SBP/DBP assessed by ABPM by each visit
- Change from baseline in mean sitting office SBP/DBP by each visit
- Change in pulse pressure assessed by ABPM and office blood pressure
- BP control and response rate by each visit, defined as
  - Office SBP < 140 mmHg and/or reduction from baseline ≥ 20 mmHg without additional antihypertensive medication
  - 24-hour mean DBP < 85 mmHg and/or reduction from baseline ≥ 10 mmHg assessed by ABPM without additional antihypertensive medication
  - Office DBP < 90 mmHg and/or reduction from baseline ≥ 10 mmHg without additional antihypertensive medication

- Proportion of patients with rescue medication use by each visit
- Change from baseline in SBP/DBP by HBPM

To assess the long-term treatment effect of zilebesiran through Month 36, change from baseline in SBP/DBP assessed by ABPM, office BP and HBPM will be summarized.

Body weight, metabolic related exploratory endpoints are:

- Change from baseline in body weight/body mass index (BMI)/waist circumference/waist-to-hip ratio by each visit
- Change from baseline in HbA1c/fasting glucose/insulin/serum lipids by each visit

Exploratory endpoints will be summarized using descriptive statistics based on all observed data. Missing data will not be imputed.

For BP control and response rate, data will be summarized based on observed data

1. for all patients.
2. for patients whose baseline BP is above the threshold.

5.7.4. Evaluation of Subgroups

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age (<65; >=65)
- Sex
- Race (black; all other races)
- Baseline 24-hour mean SBP assessed by ABPM (<145 mmHg, >=145 mmHg)
- eGFR (<60; >=60 mL/min/1.73m^2)

Subgroup analyses will be performed for the primary endpoint and key secondary endpoints using the MMRM within each subgroup. Model will include treatment, visit, treatment-by-visit interaction, race ([black, all other races], when race is not the subgroup to be analyzed) as fixed factors, baseline value as a covariate. Point estimate of treatment effect and 95% confidence interval are to be generated for each subgroup. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

5.8. Pharmacodynamic Analyses

In addition to serum AGT, the PD parameters include plasma renin concentration, aldosterone, AngI and AngII. Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment group. In addition to serum AGT percent reduction analyses, the AGT maximum and mean percentage reductions over the 6-month placebo-controlled DB period will be summarized using descriptive statistics.

5.9. Pharmacokinetic Analyses

Plasma concentrations of zilebesiran and its metabolite will be summarized descriptively. Descriptive statistics for zilebesiran and its metabolite plasma concentrations will include the number of patients, mean, SD, coefficient of variation, geometric mean, geometric mean coefficient of variation, median, minimum, and maximum.

Additional analysis may be done as needed.

5.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive anti-drug antibody (ADA) assay results at baseline and at any time during the 6-month placebo-controlled DB period, as well as treatment-emergent ADA during the 12-month DB period, will be summarized. Treatment-emergent ADA consist of treatment-induced ADA and treatment-boosted ADA, as defined below:

- Treatment-induced ADA: Confirmed positive ADA developed de novo after drug administration in patients without preexisting (baseline) confirmed positive ADA
- Treatment-boosted ADA: Confirmed positive ADA after drug administration with ADA titer > 4x baseline ADA titer in patients with preexisting (baseline) confirmed positive ADA

5.11. Safety Analyses

5.11.1. Adverse Events

Adverse events (AEs) will be classified by the MedDRA coding system (Version 26.0 or newer) and displayed in tables and data listings using SOC and PT.

Treatment-emergent AEs (TEAEs) will be summarized for the 6-month placebo-controlled DB period and for the zilebesiran treatment period separately. For the 6-month DB period, TEAE is defined as any AE occurring or worsening on or after the first dose of study drug and through Month 6 Visit (prior to Month 6 dosing).

For zilebesiran treatment period, TEAE is defined as any AE occurring or worsening after the first dose of zilebesiran through 169 days following the last dose of zilebesiran. TEAE will be summarized by zilebesiran dosing regimen.

Treatment related AEs are counted as TEAE.

AE occurred or worsening during safety follow-up period will be listed and may be summarized if needed.

Because any worsening AE is reported as a new AE with higher severity, programmatical comparison of severity is not needed for the classification of TEAE. For missing or partial dates for AEs, the imputation of start and end date can be found in Section 7.3 . Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first dose of study drug.

AEs will be summarized by the numbers and percentages of patients and number of events reporting a given AE. An overall table of TEAEs will include:

- any AE,
- any AE related to study drug,
- any serious AE (SAE),
- any SAE related to study drug,
- any AE leading to study drug discontinuation,
- any drug-related AE leading to study drug discontinuation,
- any AE leading to study drug interruption,
- any drug-related AE leading to study drug interruption,
- any AE leading to death.

Tabulations by SOC and PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- AEs by maximum severity,
- Treatment-related AEs by maximum severity,
- Severe AEs,
- SAEs,
- AEs leading to treatment discontinuation.

Tabulations by PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- SAEs.

In addition, for the zilebesiran treatment period (includes the 12-month DB period and DB extension period) both exposure-adjusted event rate (EAER) and exposure-adjusted incidence rate (EAIR) will be calculated.

EAER is defined as number of events/total duration of zilebesiran exposure * 100.

EAIR is defined as number of patients with event/cumulative follow-up time * 100, where the follow-up time for each patient is defined as either:

- the first dose of zilebesiran to the time of the first event occurrence for the patients with the event, or
- the total duration of exposure for the patients without the event.

A patient contributes only once to the frequency for a given AE (overall, by SOC, by preferred term). Patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence. An AE with missing severity will be assumed to be severe. An AE with missing study drug relatedness will be assumed to be related.

Listings of all deaths (if any), SAEs, and AEs leading to treatment discontinuation will be provided.

AEs of Clinical Interest

AEs of special interest or AEs mapping to certain standardized MedDRA queries (SMQs) will be summarized by SOC and PT. Other SMQs or AE groupings may be evaluated.

Injection Site Reactions [ISRs]: AEs mapping to the High-Level Term (HLT)= "Injection Site Reactions" using MedDRA dictionary will be included in the summary. Frequency (percentages) of patients with ISRs by SOC and PT will be presented by treatment. A separate listing will be generated to display all patients who reported ISRs. In addition, a table of the number (%) of patients with signs and symptoms reported due to ISRs will be generated.

Hepatic AEs, including Liver Function Test (LFT) abnormalities: Analysis of hepatic AEs will include AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms).

Acute renal failure/hyperkalemia/hypotension

Acute renal failure AEs will include AEs mapping to the Standardized MedDRA Query (SMQ). The adjudicated renal events will be summarized or listed.

Hyperkalemia AEs will be through Customized MedDRA Query (CMQ) search. PT terms are listed in Section 7.5 .

Hypotension AEs include AEs mapping to the FDA Medical Queries (FMQ) hypotension search (includes narrow terms), and additional terms potentially related to hypotension. List of additional terms is in Section 7.5. Frequency (percentages) of these AEs will be summarized by SOC and PT. Separate listings will be generated of all patients reporting these events.

Additional summaries of AEs mapping to a COVID-19 custom query are described in Section 5.13.2.

5.11.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

For each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies), descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit. These by-visit tables will use central laboratory data only.

Select clinical laboratory parameters may be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 or above. Shift summary from baseline CTCAE grade to maximum (worst) post-baseline grade will be presented for all graded parameters with directionality specified (e.g., hyper or hypo). To determine the

worst post-baseline value, all scheduled and unscheduled test results will be used. For hematology and serum chemistry, frequency tables of potentially clinically significant (PCS) abnormalities will be provided.

All laboratory data (both central and local) will be provided in data listings. Out-of-range laboratory results will be presented in a separate listing with proper flags. Local laboratory data, if available, will also be flagged.

Liver Function Tests

A frequency table and a shift table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN

In separate evaluation of drug-induced serious hepatotoxicity (eDISH) figures, the peak total bilirubin (as multiple of ULN) at any time post-baseline will be plotted against the peak ALT, AST, ALT or AST level and at any time post-baseline.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

Renal function
Estimated glomerular filtration rate (eGFR) will be calculated from serum creatinine (SCr) based on the Modification of Diet in Renal Disease (MDRD) Formula:

- Conventional units – eGFR (mL/min/1.73 m^2) = 175 × (SCr[mg/dL])$^{-1.154}$ × (age)$^{-0.203}$ and (× 0.742, if female) and (× 1.212, if African American)
- SI units – eGFR (mL/min/1.73 m^2) = 175 × (SCr[µmol/L]/88.4)$^{-1.154}$ × (age)$^{-0.203}$ and (× 0.742, if female) and (× 1.212, if African American)

5.11.3. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm and overall interpretation.

Post-baseline overall interpretation (normal vs. abnormal) will be summarized in frequency table by treatment and visit.

All ECG data for each patient will be provided in a data listing.

5.11.4. Vital Signs

For vital signs except blood pressure, descriptive statistics for actual values and change from baseline will be provided by treatment and visit for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

Abnormal vital signs will be summarized by treatment arm. The abnormality is defined as: mean sitting office SBP > 160 mmHg; Increment from baseline > 40 mmHg; <90 mmHg; decrement from baseline >40 mmHg;

mean sitting office DBP > 100 mmHg, increment from baseline >20 mmHg; < 50 mmHg; decrement from baseline > 20 mmHg.

In addition, orthostatic hypotension will be summarized by treatment arm. Orthostatic hypotension is defined as office SBP drop at least 20mmHg or office DBP drop at least 10 mmHg from sitting position to standing position.

5.11.5. Evaluation of Subgroups

AE summary tables will be separately generated for each of the subgroups as defined for the primary efficacy endpoint (see Section 5.7.4).

5.12. Interim Analysis

No interim analysis is planned for this study.

5.13. COVID-19

Additional data are collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidance (including FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

5.13.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 5.1.

Impact on study participation due to COVID-19, including missing visits, visit location changes, study drug dosing changes and missing doses, will be listed.

Impact on study participation due to COVID-19 will be presented in data listings at patient and visit level.

5.13.2. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will be presented. AEs mapping to the COVID-19 custom query will be summarized by SOC and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

6. CHANGES FROM PLANNED ANALYSES

Original SAP

Section of the SAP	Summary of change from protocol	Rationale
—	—	—

Amendment 1

Section of the SAP	Summary of change from original SAP	Rationale
Section 3	Ukraine patients handling	During the study conduct, 16 patients were randomized from Ukraine in January/February 2022 before geographic conflict started. Due to the challenge of data collection and cleaning, these patients will be excluded from the analysis sets defined below. Data from these patients will be listed only.
Section 4.5	ABPM baseline definition: Original SAP defines ABPM baseline as the one used for eligibility. In SAP Amendment 1, baseline ABPM definition is updated to use the last non-missing assessments prior to receiving study drug.	Per schedule of assessment table, ABPM is only measured once during screening. It will be used for both enrollment eligibility and baseline for efficacy analysis. However, unscheduled ABPM assessments during screening were also performed in some patients. It is more appropriate to use the last one prior to randomization as the baseline for efficacy analyses.
Section 4.7	Remove Month 12 analysis as planned analysis	There is no planned CSR when all patients complete 12-month DB period. Therefore, formal analysis is not needed.
Section 5.7.2.2	Add ABPM dipping definition	ABPM dipping pattern was listed as a secondary endpoint. Details of the endpoint are specified.
Section 5.11.2	eGFR calculation	eGFR will be derived for analysis. Formula is provided.
Section 7.4	Add list of rescue antihypertensive medication ATC code	Rescue antihypertensive medication is important for the primary analyses of the primary endpoint and several key-secondary endpoints. The list of medication ATC code is added.

Amendment 2

Section of the SAP	Summary of change from SAP Amendment 1	Rationale
Section 5.7.2	Clarify for BP control and response endpoint, missing data is considered as non-responder.	Clarification

Section 5.7.3	Remove the correlation between wearable device and other BP assessments	During the study conduct, data collected by wearable device is very limited.
Section 5.7.3	Clarify BP control and response rate analysis	Clarification
Section 5.11.1	Clarify both exposure adjusted event rate and incidence rate will be summarized for AEs during zilebesiran treatment period	Clarification
Section5.11.1	Add additional safety analyses based on AE for: acute renal failure, hyperkalemia, and hypotension	Acute renal failure, hyperkalemia and hypotension are potential risks for RAAS inhibitors.

7. APPENDICES

7.1. Protocol Schedule of Assessments

Schedule of assessments are listed in Table 2.

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site		Double-blind Period[a]													DB Extension Period[a]	M36/EOT/ET	Safety Follow-up
Study Visit (Month)	**Screening Period**		**W2**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M6.5**	**M7**	**M8**	**M9**	**M12**			**Q6M post last dose of study drug**
Study Day (±Visit Window)	**D-60 to -1**	**D1**	**D15±2**	**D29 ±2**	**D57 ±7**	**D85 ±7**	**D113 ±7**	**D141 ±7**	**D169 ±7**	**D183 ±7**	**D197 ±7**	**D225 ±7**	**D253 ±7**	**D337±7**	**Q3M ±14**	**D1009±14**	**±14**
Informed consent	X																
Medical history	X																
Demographics	X																
Inclusion/exclusion criteria	X																
Oral antihypertensive medication washout of at least 4 weeks	X																
Serum pregnancy test/FSH screening	X																
Vital signs and office blood pressure[c,d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
24-hour ABPM[c,e]	X			X		X			X		X		X	X		X[g]	
HBPM[c,f]	X	At least once per week															
Optional exploratory wearable blood pressure measurements	X					X											
Full physical exam	X	X												X		X	
Neurological evaluation and symptom-directed physical exam						X			X				X		X		X
Height, body weight, and BMI	X	X				X			X					X	X	X	X
Single 12-Lead ECG	X	X												X		X	

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site		Double-blind Period[a]													DB Extension Period[a]	M36/EOT/ET	Safety Follow-up
Study Visit (Month)	**Screening Period**		**W2**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M6.5**	**M7**	**M8**	**M9**	**M12**			**Q6M post last dose of study drug**
Study Day (±Visit Window)	**D-60 to -1**	**D1**	**D15±2**	**D29 ±2**	**D57 ±7**	**D85 ±7**	**D113 ±7**	**D141 ±7**	**D169 ±7**	**D183 ±7**	**D197 ±7**	**D225 ±7**	**D253 ±7**	**D337±7**	**Q3M ±14**	**D1009±14**	**±14**
Serum chemistry[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Hematology, urinalysis, coagulation[c]	X	X				X			X				X	X	X	X	X
LFTs[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
24-hour urine for aldosterone, sodium, and creatinine	X					X			X					X			
Spot urine for albumin and creatinine	X	X				X			X				X	X	X	X	
Fasting glucose, insulin, lipid panel, and HbA1c	X	X				X			X					X	X[h]	X	X
Randomization		X							X								
Plasma for PK		X							X								
Immunogenicity (ADA)[i]		X				X			X				X	X	X	X	X
Serum AGT		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
RAAS biomarkers: renin and aldosterone		X	X	X	X	X			X					X	X	X	
RAAS biomarkers: AngI/II		X				X			X					X			
Optional exploratory biomarkers (urine, plasma, serum)		X		X		X			X				X	X	X	X	
Waist circumference and waist-to-hip ratio		X				X			X					X		X	X
Exploratory DNA sample (optional)		X															

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	M36/EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	D1009±14	±14
Urine pregnancy test[b]		X				X			X				X	X	X	X	
Temporary hold of oral antihypertensives								X									
Study drug administration		X				X			X				X	X	X		
AEs	Continuous																
Concomitant medications	Continuous																

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibodies; AGT=angiotensinogen; AE=adverse event; Ang=angiotensin; BMI=body mass index; D=day; DNA=deoxyribonucleic acid; ECG=electrocardiogram; EOT=end of treatment; ET=early termination; FSH=follicle-stimulating hormone; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; ICF=informed consent form; LFT=liver function test; M=month; OLE=open-label extension; PD=pharmacodynamics; PK=pharmacokinetics; Q3M=once every 3 months; Q6M=once every 6 months; RAAS=renin-angiotensin-aldosterone system; SAE=serious adverse event; W=week.

Notes:

- When scheduled at the same time points and where feasible, the assessments of vital signs and blood sample collections for RAAS biomarkers (renin and aldosterone, and AngI/II) should be performed before physical examinations and 12-lead ECGs.
- Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may receive a dose of ALN-AGT01 at the Month 12 visit and continue their current blinded dosing in the DB Extension period for up to 24 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, 24, 30 or 36 (whichever visit occurs first). Patients who rollover at Month 12 should complete all assessments scheduled for the Month 12 visit except for study drug administration. Patients who rollover at Months 18, 24, 30 or 36 should complete the EOT visit instead of the assessments scheduled at those visits.
- Patients who do not enroll in the OLE study will be asked to perform Safety Follow-up visits q6M after the last dose of study drug. During this Follow-up period, HBPM monitoring may continue at the discretion of the Investigator. The ADA sample should only be collected at the first Follow-up visit during the Follow-up period.
- Patients who discontinue study drug prior to the Month 6 visit will be asked to complete the remainder of scheduled study visits and assessments (except for study drug administration) through Month 6. If a patient discontinues study drug after the Month 6 visit, EOT/ET assessments should be performed.

Footnotes:

[a] All assessments, except for postdose PK sample collection, are to be performed prior to dosing at dosing visits.

[b] When applicable, pregnancy test results must be known prior to dosing.

[c] Clinical laboratory assessments and blood pressure measurements taken for eligibility must be performed after at least 4 weeks of washout.
[d] Office blood pressure must be measured before the patient takes oral antihypertensive medications.
[e] ABPM recordings associated with dosing visits must be obtained within 7 days before the dosing visit and results reviewed before dosing. ABPM should only be collected at Months 18 and 24 for patients in the DB Extension period.
[f] HBPM must be measured in the morning upon waking. HBPM should be measured daily between Months 5 and 6 if oral antihypertensives are temporarily held. HBPM is not required at times when ABPM is being assessed.
[g] ABPM and collection of RAAS biomarkers should only be performed as part of ET assessments if the patient discontinues the study prior to Month 12, and ABPM should only be performed at ET if the patient and has not had an ABPM within the last 3 months. These assessments should not be performed at Month 36.
[h] Fasting plasma laboratory samples should only be collected at Month 18.[i] The ADA sample should only be collected at the first Follow-Up visit during the Follow-up period.

7.2. Time-Adjusted Change from Baseline

Time-adjusted change from baseline is the area under the curve (AUC) divided by time interval. It leads to a weighted average of all scheduled change from baseline during that time interval.

E.g., ABPM is assessed at Month 1, 3 and 6. Time-adjusted change from baseline through Month 6 in 24-hour mean SBP assessed by ABPM is:

1. AUC is calculated as: $\left[\frac{1}{2}(y_1 + y_3) * 2 + \frac{1}{2}(y_3 + y_6) * 3\right]$
2. Time interval is 5 months, from Month 1 to Month 6. AUC divided by time interval is

$$AUC/5 = 0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$$

Where y_1, y_3 and y_6 are the 24-hour mean ABPM at Month 1, 3, and 6.

Table 3 listed all time-adjusted endpoints and the weights of the assessments.

Table 3 Time-Adjusted Endpoints

Time-adjusted Endpoint	Weighted average
Time-adjusted change from baseline through Month 3 in 24-hour mean SBP/DBP assessed by ABPM	$0.5 * y_1 + 0.5 * y_3$
Time-adjusted change from baseline through Month 3 in office SBP/DBP	$0.25 * y_1 + 05 * y_2 + 0.25 * y_3$
Time-adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM	$0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$
Time-adjusted change from baseline through Month 6 in office SBP/DBP	$0.1 * y_1 + 0.2 * y_2 + 0.2 * y_3 + 0.2 * y_4 + 0.2 * y_5 + 0.1 * y_6$

7.3. Missing or Partial Dates

7.3.1. Prior and Concomitant Medications

For medications with partial start or end dates: the first day/month will be imputed for start date, and the last day/month will be imputed for end date. For medications with a completely missing start date, the medications will be considered as started one day prior to the first dose of study drug. If an imputed start date is after the collected end date, the end date will be used as the imputed start date. For medications with a completely missing end date or an imputed end date that is after the earliest date of: end of study date, data cutoff date or date of death, the latter (i.e., the earliest date of: end of study date, data cutoff date or date of death) will be used as the imputed end date.

7.3.2. Adverse Events

For records with fully or partially missing AE onset date, conventions for the imputation is as below:

- AE onset dates with missing day and non-missing month will be imputed to occur on the first day of the non-missing month, except for AEs occurring in the first month of dosing, in which case the date will be the first day of dosing.
- AE onset dates with missing month will be imputed to occur on the first day of the non-missing year (i.e., January 1), except for AEs occurring in the first year of dosing, in which case the date will be the first day of dosing.
- If year of the AE start date is missing, the onset date will be imputed as the first day of dosing, except if it can be unequivocally determined (from the partial or complete stop date) that the event occurred prior to the first dose of study drug, in which case the AE onset date will not be imputed.
- If an imputed onset date is after the collected AE end date, the end date will be used as the imputed onset date.

7.3.3. Others

For other incomplete dates, unless otherwise specified, the following conventions will be used for the calculation of duration (e.g., time in years since diagnosis):

- Missing day: the first day of the month will be used.
- Missing month: the January 1 of the non-missing year will be used.
- Missing year: no duration will be calculated.

7.4. List of Rescue Antihypertensive Medication ATC Code

The ATC codes for rescue medications are:

- C02 - Antihypertensives
- C03 - Diuretics
- C07 - Beta blocking agents
- C08 - Calcium channel blockers
- C09 - Agents acting on the renin-angiotensin system

7.5. Preferred Terms for Hyperkalemia and Potentially Related to Hypotension

Hyperkalemia:

Hyperkalemia
Blood potassium increased
Blood potassium abnormal

Potential Hypotension:

Blood pressure fluctuation
Labile blood pressure
Orthostatic intolerance
Circulatory collapse
Distributive shock
Dizziness
Dizziness exertional
Dizziness postural
Hypoperfusion
Peripheral circulatory failure
Presyncope
Procedural shock
Shock
Shock symptom
Syncope
Cardiovascular insufficiency
Hypotensive crisis
Hypotensive transfusion reaction
Orthostatic hypotension
Vasolplegia syndrome
Blood pressure abnormal
Blood pressure ambulatory abnormal
Blood pressure ambulatory decreased
Blood pressure diastolic abnormal
Blood pressure immeasurable
Blood pressure orthostatic abnormal
Blood pressure orthostatic decreased
Blood pressure systolic decreased
Blood pressure systolic inspiratory decreased
Mean arterial pressure decreased
Loss of consciousness

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ALN-AGT01-002 SAP Amend2

STATISTICAL ANALYSIS PLAN
ALN-AGT01-002 (KARDIA-1)

Protocol Title:	A Randomized, Double-blind, Placebo-Controlled, Dose-Ranging Multicenter Study to Evaluate the Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension
Short Title:	A Study to Evaluate Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension (KARDIA-1)
Study Drug:	Zilebesiran (ALN-AGT01)
Protocol Date:	Original protocol, 09 April 2021 Amendment 1, 20 April 2021 Amendment 2, 09 June 2021 Amendment 3, 09 December 2021 Amendment 4, 22 March 2022
SAP Date:	Original SAP: 29 March 2022 Amendment 1: 11 October 2022
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: +1-617-551-8200

APPROVAL SIGNATURE PAGE

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABPM	Ambulatory blood pressure monitoring
ADA	Anti-drug antibody(ies)
AE	Adverse event
AGT	Angiotensinogen
ALT	Alanine aminotransferase
AngI/II	Angiotensin I/II
AST	Aspartate aminotransferase
AUC	Area under the curve
DB	Double-blind
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
eDISH	Evaluation of drug-induced serious hepatotoxicity
EOT	End of treatment
ET	Early termination
FAS	Full analysis set
HbA1c	Hemoglobin A1c
HBPM	Home blood pressure monitoring
HLT	High level term
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISR	Injection site reaction
LFT	Liver function test
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed more for repeated measurement
OLE	Open-label extension

Abbreviation	Definition
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred term
Q3M	Once every 3 months
Q6M	Once every 6 months
RAAS	Renin-angiotensin-aldosterone system
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SC	Subcutaneous(ly)
SD	Standard deviation
SOC	System Organ Class
ULN	Upper limit of normal
ULOQ	Upper limit of quantification

1. INTRODUCTION

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacokinetic (PK) and pharmacodynamic (PD) data summaries and statistical analyses in support of the clinical study report (CSR) for Study ALN-AGT01-002 (KARDIA-1). This SAP is finalized prior to treatment unblinding and conducting the primary analysis, which will occur after all patients complete the Month 6 visit or withdraw from the study prior to the Month 6 visit. Changes to planned analyses specified in this SAP made after database lock will be documented in the CSR.

Table, figure, and listing (TFL) mocked shells and specifications are contained in a separate document.

2. STUDY DESIGN

2.1. General Study Design

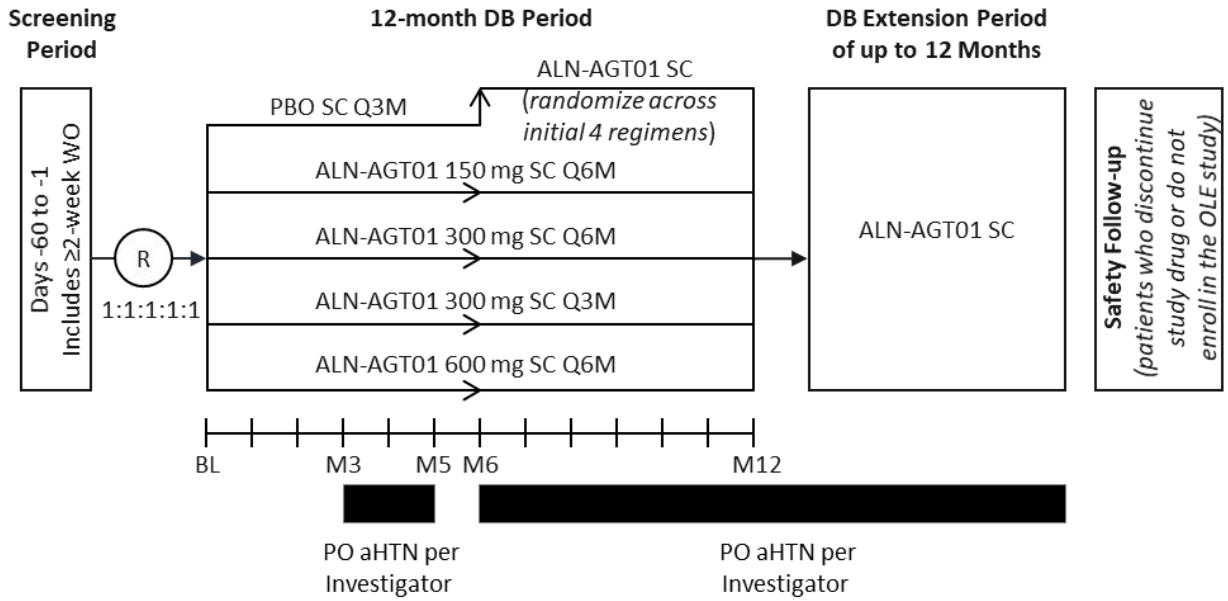
This is a Phase 2, randomized, double-blind, placebo-controlled, dose-ranging, multicenter study designed to evaluate the safety, efficacy, and pharmacodynamics (PD) of zilebesiran (ALN-AGT01), administered subcutaneously (SC), in patients with mild-to-moderate hypertension. A schematic of the study design is provided in Figure 1. Before randomization, patients will discontinue prior antihypertensive medications (if taking) for a Washout period of at least 2 weeks (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers). Patients who complete the Screening period and meet all inclusion/exclusion criteria for enrollment will receive zilebesiran or placebo for the first 6 months of the 12-month Double-blind (DB) treatment period.

Starting at Month 3, conventional oral antihypertensives may be added per Investigator judgement for elevated blood pressure. Oral antihypertensives (if taking) will be temporarily held from Month 5 to Month 6 to assess the effect of zilebesiran alone (vs placebo) at Month 6. During this 4-week period, blood pressure will be carefully monitored by daily home blood pressure monitoring and medications restarted if indicated. Patients may resume conventional oral antihypertensives at Month 6 per Investigator judgement.

Patients randomized to placebo will be re-randomized at Month 6 to 1 of the 4 initial zilebesiran regimens until the end of the DB period. Patients randomized to zilebesiran regimens will remain on their originally assigned regimens through Month 12.

After the 12-month DB treatment period, patients may be eligible to participate in a zilebesiran open-label extension (OLE) study. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition.

Figure 1 Study Design



Abbreviations: aHTN=antihypertensive medications; DB=double-blind; M=month; OLE=open-label extension; PBO=placebo; PO=per os (oral); Q3M=once every 3 months; Q6M=once every 6 months; R=randomization; SC=subcutaneous; WO=washout.
Note: Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, or 24 (whichever visit occurs first).
Note: Patients who were previously taking antihypertensives at screening should undergo a washout of these medications for at least 2 weeks during the Screening period (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers).

2.2. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the effect of ALN-AGT01 on SBP as assessed by ABPM at Month 3	• Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
Secondary	
Through Month 6 • To evaluate the effect of ALN-AGT01 on blood pressure assessed by ABPM • To evaluate the effect of ALN-AGT01 on office blood pressure • To characterize the PD effects of ALN-AGT01	Key Secondary Endpoints • Change from baseline at Month 3 in office SBP • Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM • Change from baseline at Month 6 in office SBP • Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6 Other Secondary Endpoints • Time-adjusted change from baseline in 24-hour mean SBP and DBP, assessed by ABPM • Change from baseline in 24-hour mean DBP, assessed by ABPM • Change from baseline in office SBP and DBP • Change in serum AGT • Change in daytime and nighttime blood pressure (including dipping pattern)
Exploratory	
• To evaluate the effect of ALN-AGT01, over time, on other measures of blood pressure reduction (through Month 12)	• Change in SBP and DBP assessed by ABPM • Change in office SBP and DBP • Office blood pressure and ABPM control and response rates • Proportion of patients with oral antihypertensive use • Change in SBP and DBP assessed by HBPM

Objectives	Endpoints
	• Change in pulse pressure assessed by ABPM and office blood pressure
• To characterize the PD effects of ALN-AGT01 (after Month 6)	• Change in serum AGT
• To characterize the plasma PK of ALN-AGT01	• Plasma concentrations of ALN-AGT01 and its metabolite AS(N-1)3′ ALN-AGT01
• To assess the effect of ALN-AGT01 on exploratory biomarkers of the RAAS	• Change from baseline in plasma renin concentration, aldosterone, AngI, and AngII
• To evaluate the immunogenicity of ALN-AGT01	• Incidence and titers of ADA
• To assess the effect of ALN-AGT01 on body weight, BMI, and morphometric measurements	• Change from baseline in body weight, BMI, waist circumference, and waist-to-hip ratio
• To assess the effect of ALN-AGT01 on metabolic syndrome parameters	• Change from baseline in HbA1c, fasting plasma glucose, insulin, and serum lipid profile
• To correlate blood pressure measurements obtained with a wearable, noninvasive, cuffless device to those obtained by standard cuff-based methods	• Correlation of blood pressure values obtained with a wearable device versus ABPM, HBPM, and office measurements
• To assess the long-term treatment effect of ALN-AGT01 (through Month 24)	• Change from baseline in SBP and DBP assessed by office blood pressure and HBPM
Safety	
• To evaluate the safety of ALN-AGT01 in patients with mild to moderate hypertension	• Frequency of AEs

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibody; AE=adverse event; AGT=angiotensinogen; Ang=angiotensin; BMI=body mass index; DBP=diastolic blood pressure; ECG=electrocardiogram; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; PD=pharmacodynamic; PK=pharmacokinetic; RAAS=renin-angiotensin-aldosterone system; SBP=systolic blood pressure.

2.3. Study Procedure

The Schedule of Assessments is provided in Table 2.

2.4. Randomization Methodology

Using the Interactive Response Technology (IRT), patients will be randomized 1:1:1:1:1 to the following arms during the first 6 months of the 12-month DB period:

- Placebo SC once every 3 months
- 150 mg zilebesiran SC once every 6 months
- 300 mg zilebesiran SC once every 6 months
- 300 mg zilebesiran SC once every 3 months
- 600 mg zilebesiran SC once every 6 months

Patients initially randomized to placebo will be re-randomized 1:1:1:1 at Month 6 to 1 of the 4 initial zilebesiran regimens.

Patients who enter the DB Extension period will continue their current blinded dosing regimen from the DB period.

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (mean 24-hour SBP < or ≥145 mmHg).

Each patient will be uniquely identified in the study by a combination of the site number and patient identification number. Upon signing the informed consent form (ICF), the patient will be assigned a patient identification number by the IRT system. The Investigator or his/her designee will randomize the patient in IRT after confirming that the patient fulfills all the inclusion criteria and none of the exclusion criteria. The Investigator or his/her designee will re-randomize the patient in IRT at Month 6 to assign placebo patients to 1 of the 4 initial zilebesiran dose groups in a way that maintains the study blind for the patient.

2.5. Blinding

The Sponsor, all site personnel (except for the site pharmacist or delegate), and patients will be blinded to study drug treatment through Month 6 of the 12-month DB period. During the course of the study, serum AGT, plasma PK, and treatment assignment using dummy IDs will be made available to a small, independent pharmacometrics team at the Sponsor that will not be involved in the conduct or oversight of the study. After the last patient completes the Month 3 visit and prior to the last patient's Month 6 visit, SBP data from a limited number of patients (about on-third) will be made available to this small, independent pharmacometrics team for preliminary PK/PD modeling. After the database lock to support the analysis of Month 6 data is complete, all other Sponsor personnel will be unblinded to treatment assignment, but the site personnel (except for the site pharmacist) and patients will remain blinded to treatment assignment until after the analysis of Month 12 data is complete. The Sponsor and all site personnel will be blinded to serum AGT results until their respective unblinding. Serum AGT results will not be reported to site personnel until the last patient completes the assessments at the Month 12 visit.

Blinded doses of study drug will be administered under the supervision of the Investigator (refer to Protocol Section 5.2.2). All patients will receive the same volume and number of injections regardless of treatment assignment (patients receiving once every 6-month zilebesiran regimens will receive placebo SC at dosing visits at which they do not receive zilebesiran to maintain the blind). Because zilebesiran may be slightly visually distinguishable from placebo, all blinded study drug doses will be prepared, and the syringe(s) will be masked by a site pharmacist or delegate prior to administration by a blinded healthcare professional. See the Pharmacy Manual for additional details.

Details about the specifics of the blinding aspects throughout the entire study are available in the Randomization and Blinding Plan.

Any unplanned/emergency unblinding occurring during the DB Period will be documented and reported in the CSR.

Refer to the study Randomization and Blinding Plan for more details.

2.6. Determination of Sample Size

Assuming a standard deviation (SD) in the range of 15 to 20 mmHg in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM, a maximum mean difference in effect size between zilebesiran and placebo of 10 mmHg, and a 15% dropout rate at Month 3, a sample size of 375 patients (75 per treatment group) provides at least 84% power to detect a dose response signal in SBP reduction among all zilebesiran doses and placebo at the 2-sided significance level of 0.05. In addition, this sample size provides at least 80% power to detect a 10 mmHg difference between an individual zilebesiran dose versus placebo with a 2-sided significance level of 0.05.

3. ANALYSIS POPULATIONS

During the study, 16 patients were randomized from Ukraine in January and February 2022 before a geographic conflict started in February 2022. In response to this conflict, Regulatory Authorities advised Sponsors to refer to guidance documents issued during the COVID-19 pandemic with respect to the handling of clinical trial sites in Ukraine. In line with these guidance documents, and due to challenges in data collection and cleaning and verification of such data, data from patients enrolled at sites in Ukraine will be excluded from the analysis sets defined below. Data from these patients will be provided in listings.

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. All by-treatment analyses based on the FAS will be according to the randomized treatment arm.
- Safety Analysis Set: All patients who received any amount of study drug, grouped according to the treatment actually received.
- PK Analysis Set: All patients who received at least 1 full dose of ALN-AGT01 and have at least 1 nonmissing postdose PK assessment.
- PD Analysis Set: All patients who received at least 1 full dose of study drug. All by-treatment analyses based on the PD Analysis Set will be grouped according to the treatment actually received.
- All Zilebesiran Treated Set: All patients who received any amount of 1 of the 4 ALN-AGT01 dosing regimens, including patients who took ALN-AGT01 during the 6-month placebo-controlled period and patients who initially took placebo and then switched to ALN-AGT01 after the Month 6 visit.

For the primary analysis for 6-month placebo-controlled DB period, the primary population used to evaluate efficacy will be the FAS. Safety will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

The All Zilebesiran Treated Set will be used to summarize the efficacy and safety of zilebesiran throughout the entire DB period (including DB extension period).

3.1. Randomization and Treatment Errors

For patients who were not treated, not randomized, or received incorrect treatment, the following rules will be used:

- Randomized but not treated: they will be excluded from the FAS and Safety Analysis Set for efficacy and safety evaluations as actual treatment is missing.
- Treated but not randomized: they will be excluded from the efficacy analyses since randomized treatment is missing but will be reported under the treatment actually received for all safety analyses.
- Randomized but took incorrect treatment: they will be reported under their randomized treatment arm for all efficacy analyses. But for safety analyses, a patient will be included under the active treatment arm if the patient is randomized to placebo arm and received an active dose by mistake.

4. GENERAL STATISTICAL CONSIDERATIONS

4.1. General Considerations

In general, data will be summarized for each planned analysis defined in Section 4.7 separately.

For Month 3 endpoints, zilebesiran 300mg Q3M and 300mg Q6M will be pooled together.

Categorical variables will be summarized using counts and percentages. Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3), minimum, and maximum.

The day of the first dose of study drug administered is defined as Day 1. The Study Day of a time point of interest is calculated as follows:

- If on or after Day 1, Study Day = date of interest – date of the first dose of study drug + 1
- If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection (LLOQ) will be replaced by the LLOQ. Any assessment collected and recorded as greater than the upper limit of quantification (ULOQ) will be replaced by the ULOQ.

All descriptive summaries will be presented by treatment arm.

Statistical analyses will be conducted using SAS software Version 9.4 or newer or R version 3.6 or newer.

4.2. Blood Pressure Collection and Handling

4.2.1. 24-Hour Ambulatory Blood Pressure Monitoring

24-hour ambulatory blood pressure monitoring (ABPM) is programmed to take readings every 20 minutes during the day (6 am to 9:59 pm) and every 30 minutes during the night (10 pm to 5:59 am). An ABPM will be considered adequate if (1) recording time is at least 22 hours (2) the number of successful daytime readings is ≥33, (3) the number of successful nighttime readings is ≥11, and (4) no more than 3 hours are not represented (ie, 3 sections of 60 minutes where 0 valid readings were obtained). If the ABPM recording is inadequate, the patient will be provided 1 opportunity to repeat the study within 4 days.

To summarize the 24-hour ABPM, hourly adjusted mean will be calculated. Hourly adjusted mean will be calculated by two steps:

1. Calculate the hourly mean: average of BP by each hour of the day (e.g., mean of BP measurements from 16:00 to 16:59). If there is no reading in a specific hour, this hour will not be included in calculation.
2. Calculate the 24-hour mean: average of the hourly means.

4.2.2. Office Blood Pressure

The office BP in the sitting position will be used for the analysis. Office BP will be collected with a set of 4 replicates. The average of the last 3 replicates will be calculated and used for analysis. Office BP collected in standing position will be listed.

4.2.3. Home Blood Pressure Monitoring

Home blood pressure will be measured both pre and post randomization. To establish baseline, each patient should measure HBPM during the week (with at least 3 successful readings) immediately prior to randomization. After Day 1, HBPM will be measured at least once per week. Four sequential blood pressure measurements at 1-minute intervals will be recorded. The seated blood pressure for the visit will be defined by the average of the last 3 individual measurements. HBPM will be summarized by weekly average.

Details of the blood pressure collection are in Study Protocol Section 10.

4.3. Multiple Comparisons/Multiplicity Procedure

The overall familywise error rate will be controlled at α=0.05 for the primary and key secondary endpoints as shown in Table 1

Table 1 Multiplicity Procedure

Test Step[a]	Endpoint	Comparison	Success criteria
1	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	Dunnett's Procedure	If the global hypothesis is rejected at 0.05 alpha level, then at least one zilebesiran dose is superior to Placebo
2	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
3	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05
4	Change from baseline at Month 3 in office SBP	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
5	Change from baseline at Month 3 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
6	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo	Nominal p-value < 0.05
7	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05
8	Change from baseline at Month 6 in office SBP	300 mg Q3M vs placebo	Nominal p-value < 0.05
9	Change from baseline at Month 6 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
10	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	300 mg Q3M vs placebo	Nominal p-value < 0.05
11	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	600 mg Q6M vs placebo	Nominal p-value < 0.05

[a] If the MCP criterion is satisfied in a given step, the hypothesis test is deemed statistically significant and the next step will be evaluated; otherwise all hypotheses in the given and subsequent steps are deemed not statistically significant
[b] 300mg Q3M and 300mg Q6M will be pooled together for the test

4.4. Handling of Missing Data

No imputation will be done for missing values for the primary analysis of the primary endpoint. Results based on mixed model with repeated measurements (MMRM) are valid based on missing at random (MAR) assumption. A sensitivity analysis of the primary endpoint using an imputation method will be performed (details described in Section 5.7.1.3). For all analyses using MMRM, no explicit imputation of missing values will be done.

4.5. Baseline Definitions

For the primary analysis of 6-month placebo-controlled period, for office BP, baseline is the average of the office BP value on Day 1 prior to receive the first dose of study drug and last non-missing value prior to Day 1 during screening visit. For HBPM, baseline will be the average of all assessments during last week prior to receive the first dose of study drug. For all other endpoints including ABPM, baseline is the last non-missing value (including unscheduled visit) prior to receive the first dose of study drug.

For final analyses, patients initially randomized to placebo and re-randomized at Month 6 will be summarized in two ways:

From Day 1. Baseline is the same as the one for the primary analysis.

From the start of zilebesiran dosing at Month 6. Baseline will be the last assessment prior to Month 6 dosing.

For patients initially randomized to zilebesiran dosing regimen, baseline remains the same as the one for the primary analysis.

4.6. Randomization Stratification Factors

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (mean 24-hour SBP < or ≥145 mmHg). Stratification factors are recorded in the IRT database. Key data is integrated into the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database (EDC). In the presence of stratification errors, the stratification used in analysis may not match that in the IRT. A comparison of the number and percentage of patients in each randomization stratification factor in IRT versus the clinical database will be summarized by randomized treatment arm and overall.

4.7. Planned Analyses and Data Cutoffs

4.7.1. Primary Analysis

The primary analysis will be performed after the last randomized patient has completed Month 6 Visit or otherwise discontinued the study. For the primary analysis, as this study will be ongoing with some patients in the 12-month DB period, DB extension period or safety follow-up period,

the study database will undergo an interim database lock at the Month 6 cutoff dates (i.e., data in EDC will be cleaned, frozen and electronically signed by investigators; external laboratory data will be cleaned and will undergo quality assurance). Data will be summarized in the CSR. Additional details regarding the interim database locks will be documented in the study Data Management Plan.

The primary analysis will include data on, or prior to, this prespecified cutoff date. For assessments with starting/ending dates (e.g., adverse events [AEs], medications), the starting date will be compared with the pre-specified cutoff date. Data records with starting dates after the specified data cutoff date will be excluded.

4.7.2. Final Analysis

After all patients reach the end of the study, the database will undergo a final database lock, and the data will be summarized in the CSR.

A patient is considered to have reached the end of the study if the patient:

- has completed at least the Month 12 visit and enrolled in the OLE study, or
- has completed the Safety Follow-up visits for patients who discontinue study drug or do not enroll in the OLE study, or withdrawal from the study.

5. STATISTICAL ANALYSES

5.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall:

- Randomized
- Treated
- Completed the placebo-controlled 6-month double-blind (DB) treatment period
  - Primary reason for discontinuation of treatment during 6-month DB period.
- Completed the 12-month double-blind (DB) treatment period
- Discontinuation of treatment and primary reason for discontinuation of treatment
- Withdrawal from study and primary reason for withdrawal from study
- Completed the DB extension period
- Completed the study
- Rollover to the separate OLE study

In addition to the primary reason for discontinuation of treatment and withdrawal from study, patients will also be categorized if discontinuation/withdrawal was due to COVID-19.

5.2. Demographics and Baseline Characteristics

Demographic and baseline disease characteristics will be summarized by treatment arm and overall for Full Analysis Set, and also presented in listings.

Age at consent, height, weight, body mass index (BMI), 24-hour mean ABPM and office BP will be summarized using descriptive statistics. Sex, race, ethnicity, and country will be summarized by presenting the frequencies and percentages of patients in each category.

5.3. Medical History

Medical history and prior procedures reported will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 23.1 or newer. Unique patients who report medical history events will be summarized by MedDRA system organ class (SOC), high level term (HLT) and preferred term (PT).

5.4. Protocol Deviations

Protocol deviations will be classified by medical review prior to each planned analysis database lock, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry, 2013).

The Sponsor or designee will be responsible for producing the protocol deviation file. This file will include a description of each protocol deviation and classification as major or minor. The protocol deviations will be reviewed and finalized prior to the planned analysis database lock.

All protocol deviations and major protocol deviations will be summarized and listed.

5.5. Study Drug Exposure and Compliance

The following variables will be summarized by descriptive statistics and/or frequency tabulation:

- Duration of exposure, defined as: date of last exposure – date of first dose +1. Date of last exposure is the earliest date of the following:
  - date of last dose + the length of dosing interval, i.e.,
    - date of last dose + 169 days for zilebesiran 150 mg Q6M, 300 mg Q6M and 600 mg Q6M,
    - date of last dose + 85 days for placebo and 300 mg Q3M.
  - date of end of study
  - date of analysis data cutoff
- Number of doses received; as continuous and/or categorical variable
- Number of missed doses; as a categorical variable
- Total exposure (patient years)

5.6. Prior and Concomitant Medications

Medications will be coded using the World Health Organization (WHO) Drug Dictionary, version WHO-DD Global B3, March 2021 or newer. Unique patients who reported medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 3 class (or level 2 if not available) and PT. Summaries will be provided for prior and concomitant medications separately.

Prior medications are those medications with start date prior to the first dose of study drug.

Concomitant medications are medications, other than the study drug, administered at or after the first dose of study drug, as well as medications that started prior to the first dose of study drug and are ongoing after the first dose of study drug.

If the medication start date is on or after the date of first dose of study drug, the medication will be summarized as a concomitant medication even if the medication end date is missing.

If the end date of a medication is missing or incomplete, such that it cannot be determined whether it is after the first dose of study drug, it will be counted as a concomitant medication.

For missing or partial dates for medications, the imputation of start and end dates is described in Section 7.2

5.7. Efficacy Analyses

5.7.1. Primary Endpoint

5.7.1.1. Definition of Estimand

The primary objective of the study is to evaluate the effect of zilebesiran on SBP as assessed by ABPM at Month 3. Primary estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150 mg Q6M, 300 mg Q6M, 300 mg Q3M and 600 mg Q6M. (300 mg Q6M and Q3M will be pooled together)
- Target population: patients with mild-to-moderate hypertension
- Endpoint: Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Population-level summary: Least square mean difference between zilebesiran and placebo in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Intercurrent events strategy: Rescue medication taken before Month 3 visit will be considered as an intercurrent event. For patients who require rescue medication, given the expected number of such patients at Month 3 is low, hypothetical strategy will be used, i.e., ABPM assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone. List of rescue medication ATC code is in Section 7.4.

5.7.1.2. Primary Analysis

The primary endpoint is the change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM. The hypothesis of the dose response relationship for the primary endpoint will be tested as:

- $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_0$ (there is no dose response relationship)
- H_a $\mu_1 \neq \mu_0$ or $\mu_2 \neq \mu_0$ or $\mu_3 \neq \mu_0$ (at least one zilebesiran dose is different from placebo).

Where μ_0 is the mean change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM in placebo arm; and μ_1, μ_2, and μ_3 are the mean changes from baseline at Month 3 in 24-hour mean SBP in zilebesiran 150mg Q6M, 300mg Q3M and Q6M pooled, 600mg Q6M arms, respectively.

The above hypothesis will be tested using the Dunnett's procedure based on Mixed model for repeated measurements (MMRM). The MMRM model will include treatment, visit, treatment-by-visit interaction, race (black; all other races) as fixed factors, baseline 24-hour mean SBP assessed by ABPM as a covariate. Unstructured covariance matrix will be used. Least square mean difference between zilebesiran and placebo, 95% confidence interval (CI) and adjusted p-values will be generated.

5.7.1.3. Sensitivity Analyses

For the sensitivity analysis, treatment policy strategy will be used for patients who require rescue medication. All collected blood pressure measurements will be analyzed by the same MMRM model as the primary analysis, regardless of the rescue medication.

Due to the expected long-acting effect of zilebesiran, the commonly used control-based Pattern Mixture Model (PMM) will not be used to assess the missing not at random (MNAR) assumption.

5.7.1.4. Other Analyses

To demonstrate zilebesiran consistently controls blood pressure over the 24-hour period, hourly mean change from baseline at Month 3 in SBP assessed by ABPM will be plotted for each treatment group.

5.7.2. Secondary Endpoints

5.7.2.1. Key Secondary Endpoints

Secondary objective of the study is to evaluate the effect of zilebesiran on blood pressure assessed by ABPM and office blood pressure through Month 6. The estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150mg Q6M, 300mg Q6M, 300mg Q3M and 600mg Q6M
- Target population: patients with mild-to-moderate hypertension
- Endpoint:
  - Change from baseline at Month 3 in office SBP

  - Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM
  - Change from baseline at Month 6 in office SBP
  - Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medication at Month 6

- Population-level summary: Least square mean difference between zilebesiran and placebo in each of the endpoints
- Intercurrent events strategy: For patients who require rescue medication, given the expected number of such patients at Month 3 is low and the protocol requires washout of these medications between Month 5 and Month 6, the hypothetical strategy will be used, i.e, blood pressure assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone.

MMRM model will be used as the primary analysis. The model will include treatment, visit, treatment-by-visit interaction, race (black, all other races) as fixed factors, corresponding baseline as a covariate. Least square (LS) mean difference of each zilebesiran dose group compared with placebo dose group, 95% CI and p-value will be generated. In addition, LS mean difference between 300 Q3M and 600 Q6M groups and the 95% CI will be calculated. For change from baseline at Month 6 in office SBP, as a significant proportion of patients are expected to receive rescue medication, data collected at Month 4 and 5 visits will be excluded from MMRM model.

For the proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without rescue medication at Month 6, logistic regression will be used. The model will include treatment as a factor and baseline 24-hour mean SBP as a covariate.

Multiplicity adjustment will be applied across primary and these secondary endpoints. Details of multiplicity control are in Section 4.3.

These endpoints will also be analyzed by the same MMRM model using the treatment policy strategy. I.e., all collected data will be analyzed regardless of the rescue medication. For change from baseline at Month 6 in office SBP, Data collected at Month 4 and 5 Visits will be included as well.

5.7.2.2. Other Secondary Endpoints

Other blood pressure related secondary endpoints are:

- Change from baseline at Month 3 in 24-hour mean DBP assessed by ABPM
- Change from baseline at Month 6 in 24-hour mean DBP assessed by ABPM
- Change from baseline at Month 3 in office DBP
- Time adjusted change from baseline through Month 3 in office SBP/DBP
- Change from baseline at Month 6 in office DBP

- Time adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM
- Time adjusted change from baseline through Month 6 in office SBP/DBP
- Change from baseline in daytime/nighttime SBP/DBP by ABPM at each visit. Daytime is defined as 6 am to 9:59 pm and nighttime is defined as 10 pm to 5:59 am.

Time-adjusted change is defined as the area under the curve (AUC) of BP change from baseline divided by the duration of the time period. It leads to the weighted average of change from baseline to each scheduled visit. Details of the definition and calculation are in Section 7.2.

In general, these endpoints will be analyzed using two approaches, similar as primary endpoint and key secondary endpoints. Blood pressure collected while patients are on and within 2 weeks after stopping any rescue medication will be censored. All collected BP data will also be analyzed.

Each endpoint will be analyzed by the MMRM model described in Section 5.7.1.2 with the corresponding baseline as a covariate.

In addition, ABPM dipping pattern, defined as ratio of nighttime to daytime SBP will be calculated. The number and proportion of patients will be summarized by treatment and by visit into 3 categories: 1) <0.9; 2) ≥0.9 to <1; 3) ≥1.

The secondary endpoint of percent change in serum AGT at each visit will be summarized by treatment using descriptive statistics.

5.7.3. Exploratory Endpoints

The blood pressure related exploratory endpoints through Month 12 are:

- Change from baseline in 24-hour mean SBP/DBP assessed by ABPM by each visit
- Change from baseline in office SBP/DBP by each visit
- Change in pulse pressure assessed by ABPM and office blood pressure
- BP response rate by each visit, defined as
  - Office SBP < 140 mmHg and/or reduction from baseline ≥ 20 mmHg without additional antihypertensive medication
  - 24-hour mean DBP < 85 mmHg and/or reduction from baseline ≥ 10 mmHg assessed by ABPM without additional antihypertensive medication
  - Office DBP < 90 mmHg and/or reduction from baseline ≥ 10 mmHg without additional antihypertensive medication
- Proportion of patients with rescue medication use by each visit
- Change from baseline in SBP/DBP by HBPM
- Correlation of blood pressure obtained by wearable device versus ABPM, office BP and HBPM.

To assess the long-term treatment effect of zilebesiran through Month 24, change from baseline in SBP/DBP assessed by ABPM, office BP and HBPM will be summarized.

Body weight, metabolic related exploratory endpoints are:

- Change from baseline in body weight/body mass index (BMI)/waist circumference/waist-to-hip ratio by each visit
- Change from baseline in HbA1c/fasting glucose/insulin/serum lipids by each visit

Exploratory endpoints will be summarized using descriptive statistics based on all observed data. Missing data will not be imputed.

5.7.4. Evaluation of Subgroups

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age (<65; >=65)
- Sex
- Race (black; all other races)
- Baseline 24-hour mean SBP assessed by ABPM (<145 mmHg, >=145 mmHg)
- eGFR (<60; >=60 mL/min/1.73m^2)

Subgroup analyses will be performed for the primary endpoint using the MMRM within each subgroup. Model will include treatment, visit, treatment-by-visit interaction, race ([black, all other races], when race is not the subgroup to be analyzed) as fixed factors, baseline 24-hour mean SBP assessed by ABPM as a covariate. Point estimate of treatment effect and 95% confidence interval are to be generated for each subgroup. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

5.8. Pharmacodynamic Analyses

In addition to serum AGT, the PD parameters include plasma renin concentration, aldosterone, AngI and AngII. Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment group. In addition to serum AGT percent reduction analyses, the AGT maximum and mean percentage reductions over the 6-month placebo-controlled DB period will be summarized using descriptive statistics.

5.9. Pharmacokinetic Analyses

Plasma concentrations of zilebesiran and its metabolite will be summarized descriptively. Descriptive statistics for zilebesiran and its metabolite plasma concentrations will include the number of patients, mean, SD, coefficient of variation, geometric mean, geometric mean coefficient of variation, median, minimum, and maximum.

Additional analysis may be done as needed.

5.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive anti-drug antibody (ADA) assay results at baseline and at any time during the 6-month placebo-controlled DB period, as well as treatment-emergent ADA during the 12-month DB period, will be summarized. Treatment-emergent ADA consist of treatment-induced ADA and treatment-boosted ADA, as defined below:

- Treatment-induced ADA: Confirmed positive ADA developed de novo after drug administration in patients without preexisting (baseline) confirmed positive ADA
- Treatment-boosted ADA: Confirmed positive ADA after drug administration with ADA titer > 4x baseline ADA titer in patients with preexisting (baseline) confirmed positive ADA

5.11. Safety Analyses

5.11.1. Adverse Events

Adverse events (AEs) will be classified by the MedDRA coding system (Version 23.1 or newer) and displayed in tables and data listings using SOC and PT.

Treatment-emergent AEs (TEAEs) will be summarized for the 6-month placebo-controlled DB period and for the zilebesiran treatment period separately. For the 6-month DB period, TEAE is defined as any AE occurring or worsening on or after the first dose of study drug and through Month 6 Visit (prior to Month 6 dosing).

For zilebesiran treatment period, TEAE is defined as any AE occurring or worsening after the first dose of zilebesiran through 169 days following the last dose of zilebesiran. TEAE will be summarized by zilebesiran dosing regimen.

AE occurred or worsening during safety follow-up period will be listed and may be summarized if needed.

Because any worsening AE is reported as a new AE with higher severity, programmatical comparison of severity is not needed for the classification of TEAE. For missing or partial dates for AEs, the imputation of start and end date can be found in Section 7.2. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first dose of study drug.

AEs will be summarized by the numbers and percentages of patients reporting a given AE. An overall table of TEAEs will include:

- any AE,
- any AE related to study drug,
- any serious AE (SAE),
- any SAE related to study drug,
- any AE leading to study drug discontinuation,

- any drug-related AE leading to study drug discontinuation,
- any AE leading to death.

Tabulations by SOC and PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- AEs by maximum severity,
- Treatment-related AEs by maximum severity,
- Severe AEs,
- SAEs,
- AEs leading to treatment discontinuation.

Tabulations by PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- SAEs.

A patient contributes only once to the count for a given AE (overall, by SOC, by preferred term). Patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence. An AE with missing severity will be assumed to be severe. An AE with missing study drug relatedness will be assumed to be related.

Listings of all deaths (if any), SAEs, and AEs leading to treatment discontinuation will be provided.

AEs of Clinical Interest

AEs of special interest or AEs mapping to certain standardized MedDRA queries (SMQs) will be summarized by SOC and PT. Other SMQs or AE groupings may be evaluated.

Injection Site Reactions [ISRs]: AEs mapping to the High-Level Term (HLT)= "Injection Site Reactions" using MedDRA dictionary will be included in the summary. Frequency (percentages) of patients with ISRs by SOC and PT will be presented by treatment. A separate listing will be generated to display all patients who reported ISRs. In addition, a table of the number (%) of patients with signs and symptoms reported due to ISRs will be generated.

Hepatic AEs, including Liver Function Test (LFT) abnormalities: Analysis of hepatic AEs will include AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms). Frequency (percentages) of hepatic AEs will be summarized by SOC and PT. A separate listing will be generated of all patients reporting these events.

All AEs will be presented in patient data listings. AEs mapping to the SMQs as described above will also be listed.

Additional summaries of AEs mapping to a COVID-19 custom query are described in Section 5.13.2.

5.11.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

For each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies), descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit. These by-visit tables will use central laboratory data only.

Select clinical laboratory parameters may be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 or above. Shift summary from baseline CTCAE grade to maximum (worst) post-baseline grade will be presented for all graded parameters with directionality specified (e.g., hyper or hypo). To determine the worst post-baseline value, all scheduled and unscheduled test results will be used. For hematology and serum chemistry, frequency tables of potentially clinically significant (PCS) abnormalities will be provided.

All laboratory data (both central and local) will be provided in data listings. Out-of-range laboratory results will be presented in a separate listing with proper flags. Local laboratory data, if available, will also be flagged.

Liver Function Tests

A frequency table and a shift table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN

In separate evaluation of drug-induced serious hepatotoxicity (eDISH) figures, the peak total bilirubin (as multiple of ULN) at any time post-baseline will be plotted against the peak ALT, AST, ALT or AST level and at any time post-baseline.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

Estimated glomerular filtration rate (eGFR) will be calculated from serum creatinine (SCr) based on the Modification of Diet in Renal Disease (MDRD) Formula:

- Conventional units – eGFR (mL/min/1.73 m^2) = 175 × (SCr[mg/dL])$^{-1.154}$ × (age)$^{-0.203}$ and (× 0.742, if female) and (× 1.212, if African American)

- SI units – eGFR (mL/min/1.73 m^2) = 175 × (SCr[µmol/L]/88.4)$^{-1.154}$ × (age)$^{-0.203}$ and (× 0.742, if female) and (× 1.212, if African American)

5.11.3. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm and overall interpretation.

Post-baseline overall interpretation (normal vs. abnormal) will be summarized in frequency table by treatment and visit.

All ECG data for each patient will be provided in a data listing.

5.11.4. Vital Signs

For vital signs except blood pressure, descriptive statistics for actual values and change from baseline will be provided by treatment and visit for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

5.11.5. Evaluation of Subgroups

AE summary tables will be separately generated for each of the subgroups as defined for the primary efficacy endpoint (see Section 5.7.4).

5.12. Interim Analysis

No interim analysis is planned for this study.

5.13. COVID-19

Additional data are collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidance (including FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

5.13.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 5.1.

Impact on study participation due to COVID-19, including missing visits, visit location changes, study drug dosing changes and missing doses, will be summarized descriptively overall and by visit on the patient level, and overall on the event level. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings at patient and visit level.

5.13.2. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will be presented. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

6. CHANGES FROM PLANNED ANALYSES

Original SAP

Section of the SAP	Summary of change from protocol	Rationale
—	—	—

Amendment 1

Section of the SAP	Summary of change from protocol	Rationale
Section 3	Ukraine patients handling	During the study conduct, 16 patients were randomized from Ukraine in January/February 2022 before geographic conflict started. Due to the challenge of data collection and cleaning, these patients will be excluded from the analysis sets defined below. Data from these patients will be listed only.
Section 4.5	ABPM baseline definition: Original SAP defines ABPM baseline as the one used for eligibility. In SAP Amendment 1, baseline ABPM definition is updated to use the last non-missing assessments prior to receive study drug.	Per schedule of assessment table, ABPM is only measured once during screening. It will be used for both enrollment eligibility and baseline for efficacy analysis. However, unscheduled ABPM assessments during screening were also performed in some patients. It is more appropriate to use the last one prior to randomization as the baseline for efficacy analyses.
Section 4.7	Remove Month 12 analysis as planned analysis	There is no planned CSR when all patients complete 12-month DB period. Therefore, formal analysis is not needed.
Section 5.7.2.2	Add ABPM dipping definition	ABPM dipping pattern was listed as a secondary endpoint. Details of the endpoint are specified.
Section 5.11.2	eGFR calculation	eGFR will be derived for analysis. Formula is provided.
Section 7.4	Add list of rescue antihypertensive medication ATC code	Rescue antihypertensive medication is important for the primary analyses of the primary endpoint and several key-secondary endpoints. The list of medication ATC code is added.

7. APPENDICES

7.1. Protocol Schedule of Assessments

Schedule of assessments are listed in Table 2.

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site		Double-blind Period[a]															Safety Follow-up
Study Visit (Month)	**Screening Period**		**W2**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M6.5**	**M7**	**M8**	**M9**	**M12**	**DB Extension Period[a]**	**EOT/ET**	**Q6M post last dose of study drug**
Study Day (±Visit Window)	**D-60 to -1**	**D1**	**D15±2**	**D29 ±2**	**D57 ±7**	**D85 ±7**	**D113 ±7**	**D141 ±7**	**D169 ±7**	**D183 ±7**	**D197 ±7**	**D225 ±7**	**D253 ±7**	**D337±7**	**Q3M ±14**	**M24±14**	**±14**
Informed consent	X																
Medical history	X																
Demographics	X																
Inclusion/exclusion criteria	X																
Oral antihypertensive medication washout of at least 4 weeks	X																
Serum pregnancy test/FSH screening	X																
Vital signs and office blood pressure[c,d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
24-hour ABPM[c,e]	X			X		X			X		X		X	X		X[g]	
HBPM[c,f]	X	At least 3 times/week															
Optional exploratory wearable blood pressure measurements	X					X											
Full physical exam	X	X												X		X	
Neurological evaluation and symptom-directed physical exam						X			X				X		X		X
Height, body weight, and BMI	X	X				X			X					X	X	X	X
Single 12-Lead ECG	X	X												X		X	

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	M24±14	±14
Serum chemistry[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Hematology, urinalysis, coagulation[c]	X	X				X			X				X	X	X	X	X
LFTs[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
24-hour urine for aldosterone, sodium, and creatinine	X					X			X					X			
Spot urine for albumin and creatinine	X	X				X			X				X	X	X	X	
Fasting glucose, insulin, lipid panel, and HbA1c	X	X				X			X					X	X[h]	X	X
Randomization		X							X								
Plasma for PK		X							X								
Immunogenicity (ADA)		X				X			X				X	X	X	X	X
Serum AGT		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
RAAS biomarkers: renin and aldosterone		X	X	X	X	X			X					X	X	X	
RAAS biomarkers: AngI/II		X				X			X					X			
Optional exploratory biomarkers (urine, plasma, serum)		X		X		X			X				X	X	X	X	
Waist circumference and waist-to-hip ratio		X				X			X					X		X	X
Exploratory DNA sample (optional)		X															

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	M24±14	±14
Urine pregnancy test[b]		X				X			X				X	X	X	X	
Temporary hold of oral antihypertensives								X									
Study drug administration		X				X			X				X	X	X		
AEs	Continuous																
Concomitant medications	Continuous																

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibodies; AGT=angiotensinogen; AE=adverse event; Ang=angiotensin; BMI=body mass index; D=day; DNA=deoxyribonucleic acid; ECG=electrocardiogram; EOT=end of treatment; ET=early termination; FSH=follicle-stimulating hormone; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; ICF=informed consent form; LFT=liver function test; M=month; OLE=open-label extension; PD=pharmacodynamics; PK=pharmacokinetics; Q3M=once every 3 months; Q6M=once every 6 months; RAAS=renin-angiotensin-aldosterone system; SAE=serious adverse event; W=week.

Notes:

- When scheduled at the same time points and where feasible, the assessments of vital signs and blood sample collections should be performed before physical examinations and 12-lead ECGs.
- Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may receive a dose of ALN-AGT01 at the Month 12 visit and continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, or 24 (whichever visit occurs first). Patients who rollover at Month 12 should complete all assessments scheduled for the Month 12 visit except for study drug administration. Patients who rollover at Months 18 or 24 should complete the EOT visit instead of the assessments scheduled at those visits.
- Patients who do not enroll in the OLE study will be asked to perform Safety Follow-up visits q6M after the last dose of study drug. During this Follow-up period, HBPM monitoring may continue at the discretion of the Investigator. The ADA sample should only be collected at the first Follow-up visit during the Follow-up period.
- Patients who discontinue study drug prior to the Month 6 visit will be asked to complete the remainder of scheduled study visits and assessments (except for study drug administration) through Month 6. If a patient discontinues study drug after the Month 6 visit, EOT/ET assessments should be performed.

Footnotes:

[a] All assessments, except for postdose PK sample collection, are to be performed prior to dosing at dosing visits.

[b] When applicable, pregnancy test results must be known prior to dosing.

[c] Clinical laboratory assessments and blood pressure measurements taken for eligibility must be performed after at least 4 weeks of washout.

[d] Office blood pressure must be measured before the patient takes oral antihypertensive medications.
[e] ABPM recordings associated with dosing visits should be obtained within 7 days before the dosing visit and results reviewed before dosing. ABPM should only be collected at Months 18 and 24 for patients in the DB Extension period.
[f] HBPM must be measured in the morning upon waking. HBPM should be measured daily between Months 5 and 6 if oral antihypertensives are temporarily held. HBPM is not required at times when ABPM is being assessed.
[g] ABPM and collection of RAAS biomarkers should only be performed as part of ET assessments if the patient discontinues the study prior to Month 12, and ABPM should only be performed at ET if the patient and has not had an ABPM within the last 3 months. These assessments should not be performed at Month 24.
[h] Fasting plasma laboratory samples should only be collected at Month 18.

7.2. Time-Adjusted Change from Baseline

Time-adjusted change from baseline is the area under the curve (AUC) divided by time interval. It leads to a weighted average of all scheduled change from baseline during that time interval.

E.g., ABPM is assessed at Month 1, 3 and 6. Time-adjusted change from baseline through Month 6 in 24-hour mean SBP assessed by ABPM is:

1. AUC is calculated as: $\left[\frac{1}{2}(y_1 + y_3) * 2 + \frac{1}{2}(y_3 + y_6) * 3\right]$
2. Time interval is 5 months, from Month 1 to Month 6. AUC divided by time interval is

$$AUC/5 = 0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$$

Where y_1, y_3 and y_6 are the 24-hour mean ABPM at Month 1, 3, and 6.

Table 3 listed all time-adjusted endpoints and the weights of the assessments.

Table 3 Time-Adjusted Endpoints

Time-adjusted Endpoint	Weighted average
Time-adjusted change from baseline through Month 3 in 24-hour mean SBP/DBP assessed by ABPM	$0.5 * y_1 + 0.5 * y_3$
Time-adjusted change from baseline through Month 3 in office SBP/DBP	$0.25 * y_1 + 05 * y_2 + 0.25 * y_3$
Time-adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM	$0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$
Time-adjusted change from baseline through Month 6 in office SBP/DBP	$0.1 * y_1 + 0.2 * y_2 + 0.2 * y_3 + 0.2 * y_4 + 0.2 * y_5 + 0.1 * y_6$

7.3. Missing or Partial Dates

7.3.1. Prior and Concomitant Medications

For medications with partial start or end dates: the first day/month will be imputed for start date, and the last day/month will be imputed for end date. For medications with a completely missing start date, the medications will be considered as started one day prior to the first dose of study drug. If an imputed start date is after the collected end date, the end date will be used as the imputed start date. For medications with a completely missing end date or an imputed end date that is after the earliest date of: end of study date, data cutoff date or date of death, the latter (i.e., the earliest date of: end of study date, data cutoff date or date of death) will be used as the imputed end date.

7.3.2. Adverse Events

For records with fully or partially missing AE onset date, conventions for the imputation is as below:

- AE onset dates with missing day and non-missing month will be imputed to occur on the first day of the non-missing month, except for AEs occurring in the first month of dosing, in which case the date will be the first day of dosing.
- AE onset dates with missing month will be imputed to occur on the first day of the non-missing year (i.e., January 1), except for AEs occurring in the first year of dosing, in which case the date will be the first day of dosing.
- If year of the AE start date is missing, the onset date will be imputed as the first day of dosing, except if it can be unequivocally determined (from the partial or complete stop date) that the event occurred prior to the first dose of study drug, in which case the AE onset date will not be imputed.
- If an imputed onset date is after the collected AE end date, the end date will be used as the imputed onset date.

7.3.3. Others

For other incomplete dates, unless otherwise specified, the following conventions will be used for the calculation of duration (e.g., time in years since diagnosis):

- Missing day: the first day of the month will be used.
- Missing month: the January 1 of the non-missing year will be used.
- Missing year: no duration will be calculated.

7.4. List of Rescue Antihypertensive Medication ATC Code

The ATC codes for rescue medications are:

- C02 - Antihypertensives
- C03 - Diuretics
- C07 - Beta blocking agents
- C08 - Calcium channel blockers
- C09 - Agents acting on the renin-angiotensin system

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ALN-AGT01-002 SAP Amend1

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ALN-AGT01-002 SAP Amend1

STATISTICAL ANALYSIS PLAN
ALN-AGT01-002 (KARDIA-1)

Protocol Title:	A Randomized, Double-blind, Placebo-Controlled, Dose-Ranging Multicenter Study to Evaluate the Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension
Short Title:	A Study to Evaluate Efficacy and Safety of ALN-AGT01 in Patients with Mild-to-Moderate Hypertension (KARDIA-1)
Study Drug:	Zilebesiran (ALN-AGT01)
Protocol Date:	Original protocol, 09 April 2021 Amendment 1, 20 April 2021 Amendment 2, 09 June 2021 Amendment 3, 09 December 2021 Amendment 4, 22 March 2022
SAP Date:	Original SAP: 29 March 2022
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: +1-617-551-8200

APPROVAL SIGNATURE PAGE

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABPM	Ambulatory blood pressure monitoring
ADA	Anti-drug antibody(ies)
AE	Adverse event
AGT	Angiotensinogen
ALT	Alanine aminotransferase
AngI/II	Angiotensin I/II
AST	Aspartate aminotransferase
AUC	Area under the curve
DB	Double-blind
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
eDISH	Evaluation of drug-induced serious hepatotoxicity
EOT	End of treatment
ET	Early termination
FAS	Full analysis set
HbA1c	Hemoglobin A1c
HBPM	Home blood pressure monitoring
HLT	High level term
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISR	Injection site reaction
LFT	Liver function test
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed more for repeated measurement

Abbreviation	Definition
OLE	Open-label extension
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred term
Q3M	Once every 3 months
Q6M	Once every 6 months
RAAS	Renin-angiotensin-aldosterone system
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SC	Subcutaneous(ly)
SD	Standard deviation
SOC	System Organ Class
ULN	Upper limit of normal
ULOQ	Upper limit of quantification

1. INTRODUCTION

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacokinetic (PK) and pharmacodynamic (PD) data summaries and statistical analyses in support of the clinical study report (CSR) for Study ALN-AGT01-002 (KARDIA-1). This SAP is finalized prior to treatment unblinding and conducting the primary analysis, which will occur after all patients complete the Month 6 visit or withdraw from the study prior to the Month 6 visit. Changes to planned analyses specified in this SAP made after database lock will be documented in the CSR.

Table, figure, and listing (TFL) mocked shells and specifications are contained in a separate document.

2. STUDY DESIGN

2.1. General Study Design

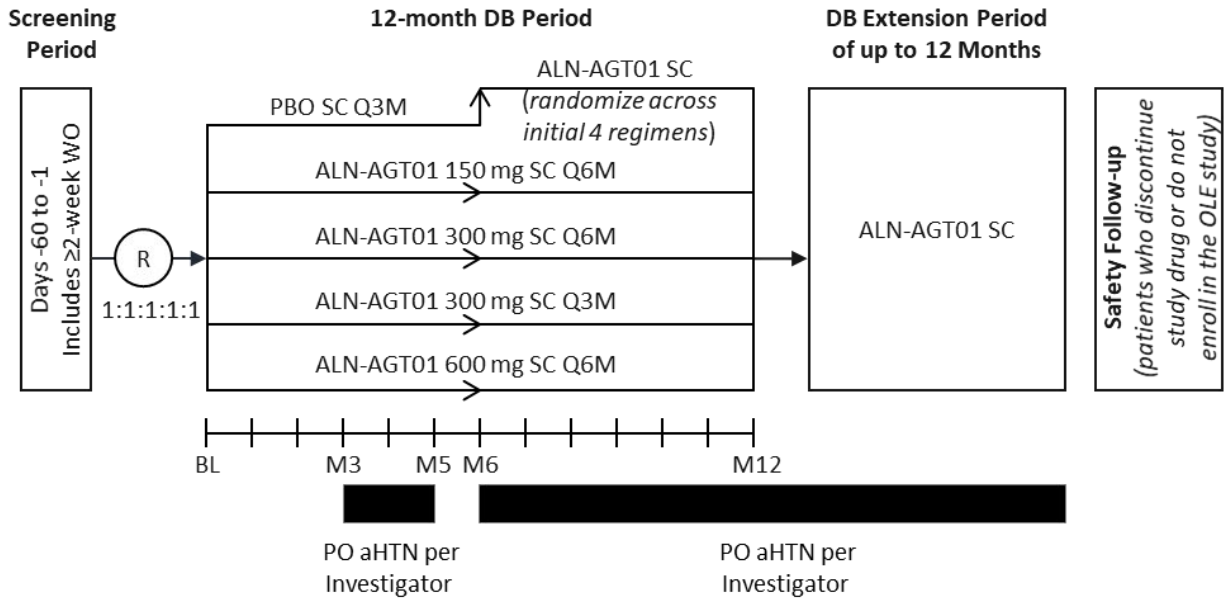
This is a Phase 2, randomized, double-blind, placebo-controlled, dose-ranging, multicenter study designed to evaluate the safety, efficacy, and pharmacodynamics (PD) of zilebesiran (ALN-AGT01), administered subcutaneously (SC), in patients with mild-to-moderate hypertension. A schematic of the study design is provided in Figure 1. Before randomization, patients will discontinue prior antihypertensive medications (if taking) for a Washout period of at least 2 weeks (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers). Patients who complete the Screening period and meet all inclusion/exclusion criteria for enrollment will receive zilebesiran or placebo for the first 6 months of the 12-month Double-blind (DB) treatment period.

Starting at Month 3, conventional oral antihypertensives may be added per Investigator judgement for elevated blood pressure. Oral antihypertensives (if taking) will be temporarily held from Month 5 to Month 6 to assess the effect of zilebesiran alone (vs placebo) at Month 6. During this 4-week period, blood pressure will be carefully monitored by daily home blood pressure monitoring and medications restarted if indicated. Patients may resume conventional oral antihypertensives at Month 6 per Investigator judgement.

Patients randomized to placebo will be re-randomized at Month 6 to 1 of the 4 initial zilebesiran regimens until the end of the DB period. Patients randomized to zilebesiran regimens will remain on their originally assigned regimens through Month 12.

After the 12-month DB treatment period, patients may be eligible to participate in a zilebesiran open-label extension (OLE) study. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition.

Figure 1 Study Design



Abbreviations: aHTN=antihypertensive medications; DB=double-blind; M=month; OLE=open-label extension; PBO=placebo; PO=per os (oral); Q3M=once every 3 months; Q6M=once every 6 months; R=randomization; SC=subcutaneous; WO=washout.
Note: Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, or 24 (whichever visit occurs first).
Note: Patients who were previously taking antihypertensives at screening should undergo a washout of these medications for at least 2 weeks during the Screening period (4 weeks of washout is required for long-acting antihypertensive medications, such as long-acting diuretics or calcium channel blockers).

2.2. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the effect of ALN-AGT01 on SBP as assessed by ABPM at Month 3	• Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
Secondary	
Through Month 6 • To evaluate the effect of ALN-AGT01 on blood pressure assessed by ABPM • To evaluate the effect of ALN-AGT01 on office blood pressure • To characterize the PD effects of ALN-AGT01	Key Secondary Endpoints • Change from baseline at Month 3 in office SBP • Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM • Change from baseline at Month 6 in office SBP • Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6 Other Secondary Endpoints • Time-adjusted change from baseline in 24-hour mean SBP and DBP, assessed by ABPM • Change from baseline in 24-hour mean DBP, assessed by ABPM • Change from baseline in office SBP and DBP • Change in serum AGT • Change in daytime and nighttime blood pressure (including dipping pattern)
Exploratory	
• To evaluate the effect of ALN-AGT01, over time, on other measures of blood pressure reduction (through Month 12)	• Change in SBP and DBP assessed by ABPM • Change in office SBP and DBP • Office blood pressure and ABPM control and response rates • Proportion of patients with oral antihypertensive use • Change in SBP and DBP assessed by HBPM • Change in pulse pressure assessed by ABPM and office blood pressure

Objectives	Endpoints
• To characterize the PD effects of ALN-AGT01 (after Month 6)	• Change in serum AGT
• To characterize the plasma PK of ALN-AGT01	• Plasma concentrations of ALN-AGT01 and its metabolite AS(N-1)3′ ALN-AGT01
• To assess the effect of ALN-AGT01 on exploratory biomarkers of the RAAS	• Change from baseline in plasma renin concentration, aldosterone, AngI, and AngII
• To evaluate the immunogenicity of ALN-AGT01	• Incidence and titers of ADA
• To assess the effect of ALN-AGT01 on body weight, BMI, and morphometric measurements	• Change from baseline in body weight, BMI, waist circumference, and waist-to-hip ratio
• To assess the effect of ALN-AGT01 on metabolic syndrome parameters	• Change from baseline in HbA1c, fasting plasma glucose, insulin, and serum lipid profile
• To correlate blood pressure measurements obtained with a wearable, noninvasive, cuffless device to those obtained by standard cuff-based methods	• Correlation of blood pressure values obtained with a wearable device versus ABPM, HBPM, and office measurements
• To assess the long-term treatment effect of ALN-AGT01 (through Month 24)	• Change from baseline in SBP and DBP assessed by office blood pressure and HBPM
Safety	
• To evaluate the safety of ALN-AGT01 in patients with mild to moderate hypertension	• Frequency of AEs

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibody; AE=adverse event; AGT=angiotensinogen; Ang=angiotensin; BMI=body mass index; DBP=diastolic blood pressure; ECG=electrocardiogram; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; PD=pharmacodynamic; PK=pharmacokinetic; RAAS=renin-angiotensin-aldosterone system; SBP=systolic blood pressure.

2.3. Study Procedure

The Schedule of Assessments is provided in Table 2.

2.4. Randomization Methodology

Using the Interactive Response Technology (IRT), patients will be randomized 1:1:1:1:1 to the following arms during the first 6 months of the 12-month DB period:

- Placebo SC once every 3 months
- 150 mg zilebesiran SC once every 6 months

- 300 mg zilebesiran SC once every 6 months
- 300 mg zilebesiran SC once every 3 months
- 600 mg zilebesiran SC once every 6 months

Patients initially randomized to placebo will be re-randomized 1:1:1:1 at Month 6 to 1 of the 4 initial zilebesiran regimens.

Patients who enter the DB Extension period will continue their current blinded dosing regimen from the DB period.

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (mean 24-hour SBP < or ≥145 mmHg).

Each patient will be uniquely identified in the study by a combination of the site number and patient identification number. Upon signing the informed consent form (ICF), the patient will be assigned a patient identification number by the IRT system. The Investigator or his/her designee will randomize the patient in IRT after confirming that the patient fulfills all the inclusion criteria and none of the exclusion criteria. The Investigator or his/her designee will re-randomize the patient in IRT at Month 6 to assign placebo patients to 1 of the 4 initial zilebesiran dose groups in a way that maintains the study blind for the patient.

2.5. Blinding

The Sponsor, all site personnel (except for the site pharmacist or delegate), and patients will be blinded to study drug treatment through Month 6 of the 12-month DB period. During the course of the study, serum AGT, plasma PK, and treatment assignment using dummy IDs will be made available to a small, independent pharmacometrics team at the Sponsor that will not be involved in the conduct or oversight of the study. After the last patient completes the Month 3 visit and prior to the last patient's Month 6 visit, a limited amount (ie, one-third) of SBP data will be made available to this small, independent pharmacometrics team for preliminary PK/PD modeling. After the database lock to support the analysis of Month 6 data is complete, all other Sponsor personnel will be unblinded to treatment assignment, but the site personnel (except for the site pharmacist) and patients will remain blinded to treatment assignment until after the analysis of Month 12 data is complete. The Sponsor and all site personnel will be blinded to serum AGT results until their respective unblinding. Serum AGT results will not be reported to site personnel until the last patient completes the assessments at the Month 12 visit.

Blinded doses of study drug will be administered under the supervision of the Investigator (see Protocol Section 5.2.2). All patients will receive the same volume and number of injections regardless of treatment assignment (patients receiving once every 6-month zilebesiran regimens will receive placebo SC at dosing visits at which they do not receive zilebesiran to maintain the blind). Because zilebesiran may be slightly visually distinguishable from placebo, all blinded study drug doses will be prepared, and the syringe(s) will be masked by a site pharmacist or delegate prior to administration by a blinded healthcare professional. See the Pharmacy Manual for additional details.

Details about the specifics of the blinding aspects throughout the entire study are available in the Randomization and Blinding Plan.

Any unplanned/emergency unblinding occurring during the DB Period will be documented and reported in the CSR.

Refer to the study Randomization and Blinding Plan for more details.

2.6. Determination of Sample Size

Assuming a standard deviation (SD) in the range of 15 to 20 mmHg in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM, a maximum mean difference in effect size between zilebesiran and placebo of 10 mmHg, and a 15% dropout rate at Month 3, a sample size of 375 patients (75 per treatment group) provides at least 84% power to detect a dose response signal in SBP reduction among all zilebesiran doses and placebo at the 2-sided significance level of 0.05. In addition, this sample size provides at least 80% power to detect a 10 mmHg difference between an individual zilebesiran dose versus placebo with a 2-sided significance level of 0.05.

3. ANALYSIS POPULATIONS

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. All by-treatment analyses based on the FAS will be according to the randomized treatment arm.
- Safety Analysis Set: All patients who received any amount of study drug, grouped according to the treatment actually received.
- PK Analysis Set: All patients who received at least 1 full dose of ALN-AGT01 and have at least 1 nonmissing postdose PK assessment.
- PD Analysis Set: All patients who received at least 1 full dose of study drug. All by-treatment analyses based on the PD Analysis Set will be grouped according to the treatment actually received.
- All Zilebesiran Treated Set: All patients who received any amount of 1 of the 4 ALN-AGT01 dosing regimens, including patients who took ALN-AGT01 during the 6-month placebo-controlled period and patients who initially took placebo and then switched to ALN-AGT01 after the Month 6 visit.

For the primary analysis for 6-month placebo-controlled DB period, the primary population used to evaluate efficacy will be the FAS. Safety will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

The All Zilebesiran Treated Set will be used to summarize the efficacy and safety of zilebesiran throughout the entire DB period (including DB extension period).

3.1. Randomization and Treatment Errors

For patients who were not treated, not randomized, or received incorrect treatment, the following rules will be used:

- Randomized but not treated: they will be excluded from the FAS and Safety Analysis Set for efficacy and safety evaluations as actual treatment is missing.
- Treated but not randomized: they will be excluded from the efficacy analyses since randomized treatment is missing but will be reported under the treatment actually received for all safety analyses.
- Randomized but took incorrect treatment: they will be reported under their randomized treatment arm for all efficacy analyses. But for safety analyses, a patient will be included under the active treatment arm if the patient is randomized to placebo arm and received an active dose by mistake.

4. GENERAL STATISTICAL CONSIDERATIONS

4.1. General Considerations

In general, data will be summarized for each planned analysis defined in Section 4.7 separately.

For Month 3 endpoints, zilebesiran 300mg Q3M and 300mg Q6M will be pooled together.

Categorical variables will be summarized using counts and percentages. Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3), minimum, and maximum.

The day of the first dose of study drug administered is defined as Day 1. The Study Day of a time point of interest is calculated as follows:

- If on or after Day 1, Study Day = date of interest – date of the first dose of study drug + 1
- If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection (LLOQ) will be replaced by the LLOQ. Any assessment collected and recorded as greater than the upper limit of quantification (ULOQ) will be replaced by the ULOQ.

All descriptive summaries will be presented by treatment arm.

Statistical analyses will be conducted using SAS software Version 9.4 or newer or R version 3.6 or newer.

4.2. Blood Pressure Collection and Handling

4.2.1. 24-Hour Ambulatory Blood Pressure Monitoring

24-hour ambulatory blood pressure monitoring (ABPM) is programmed to take readings every 20 minutes during the day (6 am to 9:59 pm) and every 30 minutes during the night (10 pm to 5:59 am). An ABPM will be considered adequate if (1) recording time is at least 22 hours (2) the number of successful daytime readings is ≥33, (3) the number of successful nighttime readings is

≥11, and (4) no more than 3 hours are not represented (ie, 3 sections of 60 minutes where 0 valid readings were obtained). If the ABPM recording is inadequate, the patient will be provided 1 opportunity to repeat the study within 4 days.

To summarize the 24-hour ABPM, hourly adjusted mean will be calculated. Hourly adjusted mean will be calculated by two steps:

1. Calculate the hourly mean: average of BP by each hour of the day (e.g., mean of BP measurements from 16:00 to 16:59). If there is no reading in a specific hour, this hour will not be included in calculation.
2. Calculate the 24-hour mean: average of the hourly means.

4.2.2. Office Blood Pressure

The office BP in the sitting position will be used for the analysis. Office BP will be collected with a set of 4 replicates. The average of the last 3 replicates will be calculated and used for analysis. Office BP collected in standing position will be listed.

4.2.3. Home Blood Pressure Monitoring

Home blood pressure will be measured both pre and post randomization. To establish baseline, each patient should measure HBPM during the week (with at least 3 successful readings) immediately prior to randomization. After Day 1, HBPM will be measured at least once per week. Four sequential blood pressure measurements at 1-minute intervals will be recorded. The seated blood pressure for the visit will be defined by the average of the last 3 individual measurements. HBPM will be summarized by weekly average.

Details of the blood pressure collection are in Study Protocol Section 10.

4.3. Multiple Comparisons/Multiplicity Procedure

The overall familywise error rate will be controlled at α=0.05 for the primary and key secondary endpoints as shown in Table 1

Table 1 Multiplicity Procedure

Test Step [a]	Endpoint	Comparison	Success criteria
1	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	Dunnett's Procedure	If the global hypothesis is rejected at 0.05 alpha level, then at least one zilebesiran dose is superior to Placebo
2	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
3	Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05

Test Step [a]	Endpoint	Comparison	Success criteria
4	Change from baseline at Month 3 in office SBP	300 mg Q3M vs placebo [b]	Nominal p-value < 0.05
5	Change from baseline at Month 3 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
6	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	300 mg Q3M vs placebo	Nominal p-value < 0.05
7	Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM	600 mg Q6M vs placebo	Nominal p-value < 0.05
8	Change from baseline at Month 6 in office SBP	300 mg Q3M vs placebo	Nominal p-value < 0.05
9	Change from baseline at Month 6 in office SBP	600 mg Q6M vs placebo	Nominal p-value < 0.05
10	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	300 mg Q3M vs placebo	Nominal p-value < 0.05
11	Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medications at Month 6	600 mg Q6M vs placebo	Nominal p-value < 0.05

[a] If the MCP criterion is satisfied in a given step, the hypothesis test is deemed statistically significant and the next step will be evaluated; otherwise all hypotheses in the given and subsequent steps are deemed not statistically significant
[b] 300mg Q3M and 300mg Q6M will be pooled together for the test

4.4. Handling of Missing Data

No imputation will be done for missing values for the primary analysis of the primary endpoint. Results based on mixed model with repeated measurements (MMRM) are valid based on missing at random (MAR) assumption. A sensitivity analysis of the primary endpoint using an imputation method will be performed (details described in Section 5.7.1.3). For all analyses using MMRM, no explicit imputation of missing values will be done.

4.5. Baseline Definitions

For the primary analysis of 6-month placebo-controlled period, for office BP, baseline is the average of the office BP value on Day 1 prior to receive the first dose of study drug and last non-missing value prior to Day 1 during screening visit. For 24-hour ABPM, baseline is the

measurement used for eligibility. For HBPM, baseline will be the average of all assessments during last week prior to receive the first dose of study drug. For all other endpoints, baseline is the last non-missing value (including unscheduled visit) prior to receive the first dose of study drug.

For Month 12 and final analyses, patients initially randomized to placebo and re-randomized at Month 6 will be summarized in two ways:

- From Day 1. Baseline is the same as the one for the primary analysis
- From the start of zilebesiran dosing at Month 6. Baseline will be the last assessment prior to Month 6 dosing.

For patients initially randomized to zilebesiran dosing regimen, baseline remains the same as the one for the primary analysis.

4.6. Randomization Stratification Factors

Randomization will be stratified by race (black vs all other races) and by baseline blood pressure (mean 24-hour SBP < or ≥145 mmHg). Stratification factors are recorded in the IRT database. Key data is integrated into the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database (EDC). In the presence of stratification errors, the stratification used in analysis may not match that in the IRT. A comparison of the number and percentage of patients in each randomization stratification factor in IRT versus the clinical database will be summarized by randomized treatment arm and overall.

4.7. Planned Analyses and Data Cutoffs

4.7.1. Primary Analysis

The primary analysis will be performed after the last randomized patient has completed Month 6 Visit or otherwise discontinued the study. For the primary analysis, as this study will be ongoing with some patients in the 12-month DB period, DB extension period or safety follow-up period, the study database will undergo an interim database lock at the Month 6 cutoff dates (ie, data in EDC will be cleaned, frozen and electronically signed by investigators; external laboratory data will be cleaned and will undergo quality assurance). Additional details regarding the interim database locks will be documented in the study Data Management Plan.

The primary analysis will include data on, or prior to, this prespecified cutoff date. For assessments with starting/ending dates (e.g., adverse events [AEs], medications), the starting date will be compared with the pre-specified cutoff date. Data records with starting dates after the specified data cutoff date will be excluded.

4.7.2. Month 12 Analysis

The analysis after the end of double-blind period is the Month 12 analysis. The analysis will be performed after all randomized patients have completed the Month 12 Visit or otherwise withdrawn from the study.

The database will undergo an interim database lock, and the data will be summarized in the CSR.

4.7.3. Final Analysis

After all patients reach the end of the study, the database will undergo a final database lock, and the data will be summarized in the CSR.

A patient is considered to have reached the end of the study if the patient:

- has completed at least the Month 12 visit and enrolled in the OLE study, or
- has completed the Safety Follow-up visits for patients who discontinue study drug or do not enroll in the OLE study, or withdrawal from the study.

5. STATISTICAL ANALYSES

5.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall:

- Randomized
- Treated
- Completed the placebo-controlled 6-month double-blind (DB) treatment period
  - Primary reason for discontinuation of treatment during 6-month DB period.
- Completed the 12-month double-blind (DB) treatment period
- Discontinuation of treatment and primary reason for discontinuation of treatment
- Withdrawal from study and primary reason for withdrawal from study
- Completed the DB extension period
- Completed the study
- Rollover to the separate OLE study

In addition to the primary reason for discontinuation of treatment and withdrawal from study, patients will also be categorized if discontinuation/withdrawal was due to COVID-19.

5.2. Demographics and Baseline Characteristics

Demographic and baseline disease characteristics will be summarized by treatment arm and overall for Full Analysis Set, and also presented in listings.

Age at consent, height, weight, body mass index (BMI), 24-hour mean ABPM and office BP will be summarized using descriptive statistics. Sex, race, ethnicity, and country will be summarized by presenting the frequencies and percentages of patients in each category.

5.3. Medical History

Medical history and prior procedures reported will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 23.1 or newer. Unique patients who report medical

history events will be summarized by MedDRA system organ class (SOC), high level term (HLT) and preferred term (PT).

5.4. Protocol Deviations

Protocol deviations will be classified by medical review prior to each planned analysis database lock, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry, 2013).

The Sponsor or designee will be responsible for producing the protocol deviation file. This file will include a description of each protocol deviation and classification as major or minor. The protocol deviations will be reviewed and finalized prior to the planned analysis database lock.

All protocol deviations and major protocol deviations will be summarized and listed.

5.5. Study Drug Exposure and Compliance

The following variables will be summarized by descriptive statistics and/or frequency tabulation:

- Duration of exposure, defined as: date of last exposure – date of first dose +1. Date of last exposure is the earliest date of the following:
  - date of last dose + the length of dosing interval, i.e.,
    - date of last dose + 169 days for zilebesiran 150 mg Q6M, 300 mg Q6M and 600 mg Q6M,
    - date of last dose + 85 days for placebo and 300 mg Q3M.
  - date of end of study
  - date of analysis data cutoff
- Number of doses received; as continuous and/or categorical variable
- Number of missed doses; as a categorical variable
- Total exposure (patient years)

5.6. Prior and Concomitant Medications

Medications will be coded using the World Health Organization (WHO) Drug Dictionary, version WHO-DD Global B3, March 2021 or newer. Unique patients who reported medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 3 class (or level 2 if not available) and PT. Summaries will be provided for prior and concomitant medications separately.

Prior medications are those medications with start date prior to the first dose of study drug.

Concomitant medications are medications, other than the study drug, administered at or after the first dose of study drug, as well as medications that started prior to the first dose of study drug and are ongoing after the first dose of study drug.

If the medication start date is on or after the date of first dose of study drug, the medication will be summarized as a concomitant medication even if the medication end date is missing.

If the end date of a medication is missing or incomplete, such that it cannot be determined whether it is after the first dose of study drug, it will be counted as a concomitant medication.

For missing or partial dates for medications, the imputation of start and end dates is described in Section 7.2

5.7. Efficacy Analyses

5.7.1. Primary Endpoint

5.7.1.1. Definition of Estimand

The primary objective of the study is to evaluate the effect of zilebesiran on SBP as assessed by ABPM at Month 3. Primary estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150 mg Q6M, 300 mg Q6M, 300 mg Q3M and 600 mg Q6M. (300 mg Q6M and Q3M will be pooled together)
- Target population: patients with mild-to-moderate hypertension
- Endpoint: Change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Population-level summary: Least square mean difference between zilebesiran and placebo in change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM
- Intercurrent events strategy: Rescue medication taken before Month 3 visit will be considered as an intercurrent event. For patients who require rescue medication, given the expected number of such patients at Month 3 is low, hypothetical strategy will be used, i.e., ABPM assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone.

5.7.1.2. Primary Analysis

The primary endpoint is the change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM. The hypothesis of the dose response relationship for the primary endpoint will be tested as:

- $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_0$ (there is no dose response relationship)
- H_a $\mu_1 \neq \mu_0$ or $\mu_2 \neq \mu_0$ or $\mu_3 \neq \mu_0$ (at least one zilebesiran dose is different from placebo).

Where μ_0 is the mean change from baseline at Month 3 in 24-hour mean SBP assessed by ABPM in placebo arm; and μ_1, μ_2, and μ_3 are the mean changes from baseline at Month 3 in 24-hour mean SBP in zilebesiran 150mg Q6M, 300mg Q3M and Q6M pooled, 600mg Q6M arms, respectively.

The above hypothesis will be tested using the Dunnett's procedure based on Mixed model for repeated measurements (MMRM). The MMRM model will include treatment, visit, treatment-by-visit interaction, race (black; all other races) as fixed factors, baseline 24-hour mean SBP assessed by ABPM as a covariate. Unstructured covariance matrix will be used. Least square mean difference between zilebesiran and placebo, 95% confidence interval (CI) and adjusted p-values will be generated.

5.7.1.3. Sensitivity Analyses

For the sensitivity analysis, treatment policy strategy will be used for patients who require rescue medication. All collected blood pressure measurements will be analyzed by the same MMRM model as the primary analysis, regardless of the rescue medication.

Due to the expected long-acting effect of zilebesiran, the commonly used control-based Pattern Mixture Model (PMM) will not be used to assess the missing not at random (MNAR) assumption.

5.7.1.4. Other Analyses

In addition to analyses for the primary estimand, treatment policy strategy will also be used for patients who require rescue medication. All collected primary endpoint assessments will be analyzed by the same MMRM model as the primary analysis, regardless of use of rescue medication.

To demonstrate zilebesiran consistently controls blood pressure over the 24-hour period, hourly mean change from baseline at Month 3 in SBP assessed by ABPM will be plotted for each treatment group.

5.7.2. Secondary Endpoints

5.7.2.1. Key Secondary Endpoints

Secondary objective of the study is to evaluate the effect of zilebesiran on blood pressure assessed by ABPM and office blood pressure through Month 6. The estimand is defined as:

- Treatment condition: monotherapy including placebo, zilebesiran 150mg Q6M, 300mg Q6M, 300mg Q3M and 600mg Q6M
- Target population: patients with mild-to-moderate hypertension
- Endpoint:
  - Change from baseline at Month 3 in office SBP
  - Change from baseline at Month 6 in 24-hour mean SBP assessed by ABPM
  - Change from baseline at Month 6 in office SBP
  - Proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without additional antihypertensive medication at Month 6

- Population-level summary: Least square mean difference between zilebesiran and placebo in each of the endpoints
- Intercurrent events strategy: For patients who require rescue medication, given the expected number of such patients at Month 3 is low and the protocol requires washout of these medications between Month 5 and Month 6, the hypothetical strategy will be used, i.e, blood pressure assessed while patients are on and within 2 weeks after stopping any rescue medication will be censored. This will provide the estimated treatment effect of zilebesiran alone.

MMRM model will be used as the primary analysis. The model will include treatment, visit, treatment-by-visit interaction, race (black, all other races) as fixed factors, corresponding baseline as a covariate. Least square (LS) mean difference of each zilebesiran dose group compared with placebo dose group, 95% CI and p-value will be generated. In addition, LS mean difference between 300 Q3M and 600 Q6M groups and the 95% CI will be calculated. For change from baseline at Month 6 in office SBP, as a significant proportion of patients are expected to receive rescue medication, data collected at Month 4 and 5 visits will be excluded from MMRM model.

For the proportion of patients with 24-hour mean SBP assessed by ABPM <130 mmHg and/or reduction ≥20 mmHg without rescue medication at Month 6, logisitic regression will be used. The model will include treatment as a factor and baseline 24-hour mean SBP as a covariate.

Multiplicity adjustment will be applied across primary and these secondary endpoints. Details of multiplicity control are in Section 4.3.

These endpoints will also be analyzed by the same MMRM model using the treatment policy strategy. I.e., all collected data will be analyzed regardless of the rescue medication. For change from baseline at Month 6 in office SBP, Data collected at Month 4 and 5 Visits will be included as well.

5.7.2.2. Other Secondary Endpoints

Other blood pressure related secondary endpoints are:

- Change from baseline at Month 3 in 24-hour mean DBP assessed by ABPM
- Change from baseline at Month 6 in 24-hour mean DBP assessed by ABPM
- Change from baseline at Month 3 in office DBP
- Time adjusted change from baseline through Month 3 in office SBP/DBP
- Change from baseline at Month 6 in office DBP
- Time adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM
- Time adjusted change from baseline through Month 6 in office SBP/DBP
- Change from baseline in daytime/nighttime SBP/DBP by ABPM at each visit. Daytime is defined as 6 am to 9:59 pm and nighttime is defined as 10 pm to 5:59 am.

Time-adjusted change is defined as the area under the curve (AUC) of BP change from baseline divided by the duration of the time period. It leads to the weighted average of change from baseline to each scheduled visit. Details of the definition and calculation are in Section 7.2.

In general, these endpoints will be analyzed using two approaches, similar as primary endpoint and key secondary endpoints. Blood pressure collected while patients are on and within 2 weeks after stopping any rescue medication will be censored. All collected BP data will also be analyzed.

Each endpoint will be analyzed by the MMRM model described in Section 5.7.1.2 with the corresponding baseline as a covariate.

The secondary endpoint of percent change in serum AGT at each visit will be summarized by treatment using descriptive statistics.

5.7.3. Exploratory Endpoints

The blood pressure related exploratory endpoints through Month 12 are:

- Change from baseline in 24-hour mean SBP/DBP assessed by ABPM by each visit
- Change from baseline in office SBP/DBP by each visit
- Change in pulse pressure assessed by ABPM and office blood pressure
- BP response rate by each visit, defined as
  - Office SBP < 140 mmHg and/or reduction from baseline ≥ 20 mmHg without escape antihypertensive medication
  - 24-hour mean DBP < 85 mmHg and/or reduction from baseline ≥ 10 mmHg assessed by ABPM without escape antihypertensive medication
  - Office DBP < 90 mmHg and/or reduction from baseline ≥ 10 mmHg without escape antihypertensive medication
- Proportion of patients with rescue medication use by each visit
- Change from baseline in SBP/DBP by HBPM
- Correlation of blood pressure obtained by wearable device versus ABPM, office BP and HBPM.

To assess the long-term treatment effect of zilebesiran through Month 24, change from baseline in SBP/DBP assessed by ABPM, office BP and HBPM will be summarized.

Body weight, metabolic related exploratory endpoints are:

- Change from baseline in body weight/body mass index (BMI)/waist circumference/waist-to-hip ratio by each visit
- Change from baseline in HbA1c/fasting glucose/insulin/serum lipids by each visit

Exploratory endpoints will be summarized using descriptive statistics based on all observed data. Missing data will not be imputed.

5.7.4. Evaluation of Subgroups

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age (<65; >=65)
- Sex
- Race (black; all other races)
- Baseline 24-hour mean SBP assessed by ABPM (<145 mmHg, >=145 mmHg)
- eGFR (<60; >=60 mL/min)

Subgroup analyses will be performed for the primary endpoint using the MMRM within each subgroup. Model will include treatment, visit, treatment-by-visit interaction, race ([black, all other races], when race is not the subgroup to be analyzed) as fixed factors, baseline 24-hour mean SBP assessed by ABPM as a covariate. Point estimate of treatment effect and 95% confidence interval are to be generated for each subgroup. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

5.8. Pharmacodynamic Analyses

In addition to serum AGT, the PD parameters include plasma renin concentration, aldosterone, AngI and AngII. Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment group. In addition to serum AGT percent reduction analyses, the AGT maximum and mean percentage reductions over the 6-month placebo-controlled DB period will be summarized using descriptive statistics.

5.9. Pharmacokinetic Analyses

Plasma concentrations of zilebesiran and its metabolite will be summarized descriptively. Descriptive statistics for zilebesiran and its metabolite plasma concentrations will include the number of patients, mean, SD, coefficient of variation, geometric mean, geometric mean coefficient of variation, median, minimum, and maximum.

Additional analysis may be done as needed.

5.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive anti-drug antibody (ADA) assay results at baseline and at any time during the 6-month placebo-controlled DB period, as well as treatment-emergent ADA during the 12-month DB period, will be summarized. Treatment-emergent ADA consist of treatment-induced ADA and treatment-boosted ADA, as defined below:

- Treatment-induced ADA: Confirmed positive ADA developed de novo after drug administration in patients without preexisting (baseline) confirmed positive ADA

- Treatment-boosted ADA: Confirmed positive ADA after drug administration with ADA titer > 4x baseline ADA titer in patients with preexisting (baseline) confirmed positive ADA

5.11. Safety Analyses

5.11.1. Adverse Events

Adverse events (AEs) will be classified by the MedDRA coding system (Version 23.1 or newer) and displayed in tables and data listings using SOC and PT.

Treatment-emergent AEs (TEAEs) will be summarized for the 6-month placebo-controlled DB period and for the zilebesiran treatment period separately. For the 6-month DB period, TEAE is defined as any AE occurring or worsening on or after the first dose of study drug and through Month 6 Visit (prior to Month 6 dosing).

For zilebesiran treatment period, TEAE is defined as any AE occurring or worsening after the first dose of zilebesiran. TEAE will be summarized by zilebesiran dosing regimen.

AE occurred or worsening during DB extension period and safety follow-up period will be listed and maybe summarized if needed.

Because any worsening AE is reported as a new AE with higher severity, programmatical comparison of severity is not needed for the classification of TEAE. For missing or partial dates for AEs, the imputation of start and end date can be found in Section 7.2. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first dose of study drug.

AEs will be summarized by the numbers and percentages of patients reporting a given AE. An overall table of TEAEs will include:

- any AE,
- any AE related to study drug,
- any serious AE (SAE),
- any SAE related to study drug,
- any AE leading to study drug discontinuation,
- any drug-related AE leading to study drug discontinuation,
- any AE leading to death.

Tabulations by SOC and PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- AEs by maximum severity,
- Treatment-related AEs by maximum severity,

- Severe AEs,
- SAEs,
- AEs leading to treatment discontinuation.

Tabulations by PT will be produced for the following:

- AEs,
- Treatment-related AEs,
- SAEs.

A patient contributes only once to the count for a given AE (overall, by SOC, by preferred term). Patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence. An AE with missing severity will be assumed to be severe. An AE with missing study drug relatedness will be assumed to be related.

Listings of all deaths (if any), SAEs, and AEs leading to treatment discontinuation will be provided.

AEs of Clinical Interest

AEs of special interest or AEs mapping to certain standardized MedDRA queries (SMQs) will be summarized by SOC and PT. Other SMQs or AE groupings may be evaluated.

Injection Site Reactions [ISRs]: AEs mapping to the High-Level Term (HLT)= "Injection Site Reactions" using MedDRA dictionary will be included in the summary. Frequency (percentages) of patients with ISRs by SOC and PT will be presented by treatment. A separate listing will be generated to display all patients who reported ISRs. In addition, a table of the number of patients with at least 1 ISR, total number of doses (any dose split into multiple injections will be considered 1 dose), total number of doses with ISRs and corresponding % of injections with ISR with the most common signs and symptoms reported due to ISRs will be generated.

Hepatic AEs, including Liver Function Test (LFT) abnormalities: Analysis of hepatic AEs will include AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms). Frequency (percentages) of hepatic AEs will be summarized by SOC and PT. A separate listing will be generated of all patients reporting these events.

All AEs will be presented in patient data listings. AEs mapping to the SMQs as described above will also be listed.

Additional summaries of AEs mapping to a COVID-19 custom query are described in Section 5.13.2.

5.11.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

For each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies), descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit. These by-visit tables will use central laboratory data only.

Select clinical laboratory parameters may be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 or above. Shift summary from baseline CTCAE grade to maximum (worst) post-baseline grade will be presented for all graded parameters with directionality specified (e.g., hyper or hypo). To determine the worst post-baseline value, all scheduled and unscheduled test results will be used. For hematology and serum chemistry, frequency tables of potentially clinically significant (PCS) abnormalities will be provided.

All laboratory data (both central and local) will be provided in data listings. Out-of-range laboratory results will be presented in a separate listing with proper flags. Local laboratory data, if available, will also be flagged.

Liver Function Tests

A frequency table and a shift table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN

In separate evaluation of drug-induced serious hepatotoxicity (eDISH) figures, the peak total bilirubin (as multiple of ULN) at any time post-baseline will be plotted against the peak ALT, AST, ALT or AST level and at any time post-baseline.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

5.11.3. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm and overall interpretation.

Post-baseline overall interpretation (normal vs. abnormal) will be summarized in frequency table by treatment and visit.

All ECG data for each patient will be provided in a data listing.

5.11.4. Vital Signs

For vital signs except blood pressure, descriptive statistics for actual values and change from baseline will be provided by treatment and visit for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

5.11.5. Evaluation of Subgroups

AE summary tables will be separately generated for each of the subgroups as defined for the primary efficacy endpoint (see Section 5.7.4).

5.12. Interim Analysis

No interim analysis is planned for this study.

5.13. COVID-19

Additional data are collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidance (including FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

5.13.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 5.1.

Impact on study participation due to COVID-19, including missing visits, visit location changes, study drug dosing changes and missing doses, will be summarized descriptively overall and by visit on the patient level, and overall on the event level. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings at patient and visit level.

5.13.2. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will be presented. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

6. CHANGES FROM PLANNED ANALYSES

Original SAP

Section of the SAP	Summary of change from protocol	Rationale
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7. APPENDICES

7.1. Protocol Schedule of Assessments

Schedule of assessments are listed in Table 2.

Table 2 Schedule of Assessments

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	M24±14	±14
Informed consent	X																
Medical history	X																
Demographics	X																
Inclusion/exclusion criteria	X																
Oral antihypertensive medication washout of at least 4 weeks	X																
Serum pregnancy test/FSH screening	X																
Vital signs and office blood pressure[c,d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
24-hour ABPM[c,e]	X			X		X			X		X		X	X		X[g]	
HBPM[c,f]	X	At least 3 times/week															
Optional exploratory wearable blood pressure measurements	X					X											
Full physical exam	X	X												X		X	
Neurological evaluation and symptom-directed physical exam						X			X				X		X		X
Height, body weight, and BMI	X	X				X			X					X	X	X	X
Single 12-Lead ECG	X	X												X		X	

Shading indicates visits that must be performed at the site		Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)	**Screening Period**		**W2**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M6.5**	**M7**	**M8**	**M9**	**M12**			**Q6M post last dose of study drug**
Study Day (±Visit Window)	**D-60 to -1**	**D1**	**D15±2**	**D29 ±2**	**D57 ±7**	**D85 ±7**	**D113 ±7**	**D141 ±7**	**D169 ±7**	**D183 ±7**	**D197 ±7**	**D225 ±7**	**D253 ±7**	**D337±7**	**Q3M ±14**	**M24±14**	**±14**
Serum chemistry[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Hematology, urinalysis, coagulation[c]	X	X				X			X				X	X	X	X	X
LFTs[c]	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
24-hour urine for aldosterone, sodium, and creatinine	X					X			X					X			
Spot urine for albumin and creatinine	X	X				X			X				X	X	X	X	
Fasting glucose, insulin, lipid panel, and HbA1c	X	X				X			X					X	X[h]	X	X
Randomization		X							X								
Plasma for PK		X							X								
Immunogenicity (ADA)		X				X			X				X	X	X	X	X
Serum AGT		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
RAAS biomarkers: renin and aldosterone		X	X	X	X	X			X					X	X	X	
RAAS biomarkers: AngI/II		X				X			X					X			
Optional exploratory biomarkers (urine, plasma, serum)		X		X		X			X				X	X	X	X	
Waist circumference and waist-to-hip ratio		X				X			X					X		X	X
Exploratory DNA sample (optional)		X															
Urine pregnancy test[b]		X				X			X				X	X	X	X	
Temporary hold of oral antihypertensives								X									

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	M24±14	±14
Study drug administration		X				X			X				X	X	X		
AEs	Continuous																
Concomitant medications	Continuous																

Shading indicates visits that must be performed at the site	Screening Period	Double-blind Period[a]													DB Extension Period[a]	EOT/ET	Safety Follow-up
Study Visit (Month)			W2	M1	M2	M3	M4	M5	M6	M6.5	M7	M8	M9	M12			Q6M post last dose of study drug
Study Day (±Visit Window)	D-60 to -1	D1	D15±2	D29 ±2	D57 ±7	D85 ±7	D113 ±7	D141 ±7	D169 ±7	D183 ±7	D197 ±7	D225 ±7	D253 ±7	D337±7	Q3M ±14	M24±14	±14

Abbreviations: ABPM=ambulatory blood pressure monitoring; ADA=anti-drug antibodies; AGT=angiotensinogen; AE=adverse event; Ang=angiotensin; BMI=body mass index; D=day; DNA=deoxyribonucleic acid; ECG=electrocardiogram; EOT=end of treatment; ET=early termination; FSH=follicle-stimulating hormone; HbA1c=hemoglobin A1C; HBPM=home blood pressure monitoring; ICF=informed consent form; LFT=liver function test; M=month; OLE=open-label extension; PD=pharmacodynamics; PK=pharmacokinetics; Q3M=once every 3 months; Q6M=once every 6 months; RAAS=renin-angiotensin-aldosterone system; SAE=serious adverse event; W=week.

Notes:

- When scheduled at the same time points and where feasible, the assessments of vital signs and blood sample collections should be performed before physical examinations and 12-lead ECGs.
- Patients may be eligible to participate in an ALN-AGT01 OLE study upon completion of the Month 12 predose assessments. If an individual patient reaches Month 12 prior to availability of the OLE study, they may receive a dose of ALN-AGT01 at the Month 12 visit and continue their current blinded dosing in the DB Extension period for up to 12 additional months until the OLE study is open and then transition. Once the OLE study is open for enrollment, eligible patients may rollover into the OLE study at Months 12, 18, or 24 (whichever visit occurs first). Patients who rollover at Month 12 should complete all assessments scheduled for the Month 12 visit except for study drug administration. Patients who rollover at Months 18 or 24 should complete the EOT visit instead of the assessments scheduled at those visits.
- Patients who do not enroll in the OLE study will be asked to perform Safety Follow-up visits q6M after the last dose of study drug. During this Follow-up period, HBPM monitoring may continue at the discretion of the Investigator. The ADA sample should only be collected at the first Follow-up visit during the Follow-up period.
- Patients who discontinue study drug prior to the Month 6 visit will be asked to complete the remainder of scheduled study visits and assessments (except for study drug administration) through Month 6. If a patient discontinues study drug after the Month 6 visit, EOT/ET assessments should be performed.

Footnotes:

[a] All assessments, except for postdose PK sample collection, are to be performed prior to dosing at dosing visits.
[b] When applicable, pregnancy test results must be known prior to dosing.
[c] Clinical laboratory assessments and blood pressure measurements taken for eligibility must be performed after at least 4 weeks of washout.
[d] Office blood pressure must be measured before the patient takes oral antihypertensive medications.
[e] ABPM recordings associated with dosing visits should be obtained within 7 days before the dosing visit and results reviewed before dosing. ABPM should only be collected at Months 18 and 24 for patients in the DB Extension period.
[f] HBPM must be measured in the morning upon waking. HBPM should be measured daily between Months 5 and 6 if oral antihypertensives are temporarily held. HBPM is not required at times when ABPM is being assessed.
[g] ABPM and collection of RAAS biomarkers should only be performed as part of ET assessments if the patient discontinues the study prior to Month 12, and ABPM should only be performed at ET if the patient and has not had an ABPM within the last 3 months. These assessments should not be performed at Month 24.
[h] Fasting plasma laboratory samples should only be collected at Month 18.

7.2. Time-Adjusted Change from Baseline

Time-adjusted change from baseline is the area under the curve (AUC) divided by time interval. It leads to a weighted average of all scheduled change from baseline during that time interval.

E.g., ABPM is assessed at Month 1, 3 and 6. Time-adjusted change from baseline through Month 6 in 24-hour mean SBP assessed by ABPM is:

1. AUC is calculated as: $\left[\frac{1}{2}(y_1 + y_3) * 2 + \frac{1}{2}(y_3 + y_6) * 3\right]$
2. Time interval is 5 months, from Month 1 to Month 6. AUC divided by time interval is

$$AUC/5 = 0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$$

Where y_1, y_3 and y_6 are the 24-hour mean ABPM at Month 1, 3, and 6.

Table 3 listed all time-adjusted endpoints and the weights of the assessments.

Table 3 Time-Adjusted Endpoints

Time-adjusted Endpoint	Weighted average
Time-adjusted change from baseline through Month 3 in 24-hour mean SBP/DBP assessed by ABPM	$0.5 * y_1 + 0.5 * y_3$
Time-adjusted change from baseline through Month 3 in office SBP/DBP	$0.25 * y_1 + 05 * y_2 + 0.25 * y_3$
Time-adjusted change from baseline through Month 6 in 24-hour mean SBP/DBP assessed by ABPM	$0.2 * y_1 + 0.5 * y_3 + 0.3 * y_6$
Time-adjusted change from baseline through Month 6 in office SBP/DBP	$0.1 * y_1 + 0.2 * y_2 + 0.2 * y_3 + 0.2 * y_4 + 0.2 * y_5 + 0.1 * y_6$

7.3. Missing or Partial Dates

7.3.1. Prior and Concomitant Medications

For medications with partial start or end dates: the first day/month will be imputed for start date, and the last day/month will be imputed for end date. For medications with a completely missing start date, the medications will be considered as started one day prior to the first dose of study drug. If an imputed start date is after the collected end date, the end date will be used as the imputed start date. For medications with a completely missing end date or an imputed end date that is after the earliest date of: end of study date, data cutoff date or date of death, the latter (i.e., the earliest date of: end of study date, data cutoff date or date of death) will be used as the imputed end date.

7.3.2. Adverse Events

For records with fully or partially missing AE onset date, conventions for the imputation is as below:

- AE onset dates with missing day and non-missing month will be imputed to occur on the first day of the non-missing month, except for AEs occurring in the first month of dosing, in which case the date will be the first day of dosing.
- AE onset dates with missing month will be imputed to occur on the first day of the non-missing year (i.e., January 1), except for AEs occurring in the first year of dosing, in which case the date will be the first day of dosing.
- If year of the AE start date is missing, the onset date will be imputed as the first day of dosing, except if it can be unequivocally determined (from the partial or complete stop date) that the event occurred prior to the first dose of study drug, in which case the AE onset date will not be imputed.
- If an imputed onset date is after the collected AE end date, the end date will be used as the imputed onset date.

7.3.3. Others

For other incomplete dates, unless otherwise specified, the following conventions will be used for the calculation of duration (e.g., time in years since diagnosis):

- Missing day: the first day of the month will be used.
- Missing month: the January 1 of the non-missing year will be used.
- Missing year: no duration will be calculated.

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