CLINICAL PROTOCOL

Protocol Title:	A Randomized, Double-blind, Placebo-controlled, Dose-ranging, Multicenter Phase 2 Study to Evaluate the Safety, Efficacy, and Tolerability of MLS-101 in Subjects With Uncontrolled Hypertension
Protocol Number:	MLS-101-201
Investigational Product:	MLS-101
Study Phase:	2
IND Number:	151496
NCT Number:	NCT05001945
Study Sponsor:	Mineralys Therapeutics, Inc. 150 N. Radnor Chester Rd. – Suite F200 Radnor, PA 19087
SAE Reporting:	Email: Safety-Inbox.biotech@iqvia.com Phone: +1 919-313-7111 Fax: +1 919-313-1412
Original Protocol Date:	06 February 2021
Amendment 1 Date:	15 April 2021
Amendment 2 Date:	01 June 2021
Amendment 3 Date:	24 August 2021
Amendment 4 Date:	10 January 2022
Amendment 5 Date:	17 February 2022
Amendment 6 Date:	07 July 2022

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1. SYNOPSIS

Name of Sponsor/Company:
Mineralys Therapeutics, Inc.

Name of Investigational Product:
MLS-101: [redacted]

Name of Active Ingredient:
MLS-101

Title of Study: A Randomized, Double-blind, Placebo-controlled, Dose-ranging, Multicenter Phase 2 Study to Evaluate the Safety, Efficacy, and Tolerability of MLS-101 in Subjects With Uncontrolled Hypertension

Number of Study Center(s): Approximately 50 centers in the United States for Part 1 and approximately 10 centers in the United States for Part 2

Study Period:	**Phase of Development:**
Date first subject enrolled: 30 July 2021 Estimated date last subject last visit: Q3 2022	Phase 2

Objectives and Endpoints:

Objectives:

Primary:

- To characterize the effect of MLS-101 on blood pressure (BP) at 5 dosing regimens versus placebo when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment

Secondary:

- To investigate the safety and tolerability of MLS-101 at 5 dosing regimens versus placebo when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment
- To investigate the pharmacokinetic (PK) profile of MLS-101 at 5 dosing regimens when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment
- To investigate the pharmacodynamic (PD) parameters of MLS-101 at 5 dosing regimens when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment

Endpoints:

Efficacy

Primary:

- Change in office-measured (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) systolic blood pressure (SBP) from baseline to the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)

Secondary:

- Change in 24-hour ambulatory blood pressure monitoring (ABPM) parameters (systolic and diastolic) from baseline to the end of Study Week 7 (ie, Study Day 49 ± 2)

- Change in office-measured (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) SBP and diastolic blood pressure (DBP) from baseline to the end of Study Weeks 1, 2, 3, 4, 5, 6 and 7 (± 2 days); change in office-measured DBP from baseline to the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)
- Proportion of subjects who achieve office-measured BP of ≤ 130/80 mmHg by the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)

Safety

- Incidence and severity of all spontaneously reported adverse events (AEs)
- Changes in vital signs (SBP, DBP, body temperature, heart rate, and respiratory rate)
- Changes in electrocardiogram parameters (including cardiac intervals: PR, QRS, QT, and corrected QT interval using Fridericia's formula)
- Changes in clinical laboratory assessments (hematology, chemistry, coagulation, and urinalysis)
- Change in office-measured SBP from Study Week 8 (end-of-treatment period) to Study Week 12 for Part 1 and Study Week 10 for Part 2 (end of follow-up)

Pharmacokinetics

- PK parameters, including, if feasible, area under the plasma concentration versus time curve (AUC), maximum plasma concentration (C_{max}), time to maximum concentration (T_{max}), and half-life ($t_{1/2}$) will be summarized descriptively for Randomization (baseline) and Study Weeks 1, 4, and 8

Pharmacodynamics

- Change in plasma 11-deoxycortisol and plasma renin activity (PRA) from baseline to the end of Study Weeks 4 and 12 for Part 1 and end of Study Weeks 4 and 10 for Part 2 (end of follow-up)
- Change in serum aldosterone, cortisol, and 11-deoxycorticosterone concentration from baseline to the end of Study Weeks 4 and 12 for Part 1 and end of Study Weeks 4 and 10 for Part 2 (end of follow-up)

Methodology:

This is a 2-part Phase 2 randomized, double-blind, placebo-controlled, dose-ranging, multicenter study designed to evaluate the effect of orally administered MLS-101 on BP for the treatment of uncontrolled hypertension (hypertensive despite receiving ≥ 2 antihypertensives) when used as add-on therapy to stable background treatment in male and female subjects ≥ 18 years of age. Stable background treatment must include ≥ 2 antihypertensives (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses and stable for at least 4 weeks prior to signing the Screening/main study informed consent form (ICF). Background therapy may be adjusted at the investigator's discretion.

Subjects with a history consistent with inadequately controlled hypertension, (ie, SBP ≥ 135 mmHg on a stable treatment regimen of ≥ 2 antihypertensives), will undergo pre-Screening laboratory tests for PRA and aldosterone following their agreement to enter pre-Screening and completion of a pre-Screening ICF that has been approved by the Institutional Review Board (IRB). In Part 1 of the study, the value of PRA must be ≤ 1 ng/mL/h based on morning measurement. If the value of PRA > 1 ng/mL/h based on morning measurement, then subjects may be eligible to enter Part 2 of the study. The value for aldosterone must be ≥ 1 ng/dL based on morning measurement for both Part 1 and Part 2. Alternative target PRA and/or aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during conduct of the trial based on ongoing review of PRA and serum aldosterone values. Subjects with PRA and serum aldosterone values meeting the stipulated guidance criteria will then enter the study Screening process. If PRA and/or

serum aldosterone do not meet the target guidance values during pre-Screening, the PRA and aldosterone measurements may be repeated 1 time and the pre-Screening window will reset to 2 weeks for Part 1 or 4 weeks for Part 2. If guidance criteria are revised, then previously pre-screened individuals who meet the revised criteria may be considered for entry into the Screening process with consent of the sponsor, and the pre-Screening window will reset to 2 weeks for Part 1 and 4 weeks for Part 2.

The current estimate is that approximately 1100 subjects will undergo up to 2 weeks of pre-Screening for Part 1 and 4 weeks of pre-Screening for Part 2. It is estimated that approximately 270 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in for Part 1 after completing a Screening/main study ICF that has been approved by the IRB. Assuming an approximately 40% screen failure rate, a total of approximately 160 subjects will meet all eligibility requirements to qualify for enrollment in Part 1 of the study. For Part 2, it is estimated that approximately 60 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in for Part 2 after completing a Screening/main study ICF that has been approved by the IRB. Assuming an approximately 40% screen failure rate, a total of 36 subjects will meet all eligibility requirements to qualify for enrollment in Part 2 of the study.

At the Screening/start of Placebo Run-in visit (V2/V3), subjects will complete the Screening assessments and begin a single-blind (subjects blinded to treatment allocation) run-in period (up to 2 weeks) of twice-daily (BID) oral treatment with placebo in Part 1 and once daily (QD) oral treatment with placebo in Part 2, while continuing to remain on stable doses of their background antihypertensive medications. Using an automated oscillometric sphygmomanometer device, SBP and DBP will be measured 5 times after approximately 5 minutes of rest in the seated position according to the American Heart Association (AHA) Guidelines (Muntner 2019). The average of the last 2 of 5 unattended measurements of SBP and DBP, respectively, will be used for the analysis. Subjects may also be provided with an automated digital oscillometric home BP device to measure BP at home at the investigator's discretion. In addition to sitting measurements, standing BP will be measured at the Screening/start of Placebo Run-in visit, Randomization, Study Week 1, and Study Week 8 with an automated office blood pressure (AOBP) device according to the procedure described in Appendix 12.5. Subjects will return to the research facility at the start of Week 2 of the run-in period (V4) for protocol-defined assessments including BP measurements.

At the second clinic visit of the Placebo Run-in period, subjects will be evaluated for eligibility based on Screening data, and if eligible, will continue Week 2 of Placebo Run-in. Subjects will be given an ABPM device and instructions on how to perform the ABPM procedure at home by the investigator. The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) in Part 2 and Study Week 7 (ie, Visit 13; Study Day 49 ± 2) in Parts 1 and 2. Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study Weeks 4 and 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to coronavirus disease 2019 (COVID-19), subject exposure to COVID-19, or subject testing positive for COVID-19. If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at Study Week 8. In addition, subjects will be given either a spot urine collection kit for Part 1 or a 24-hour urine collection kit for Part 2 on the second visit of the placebo-run in period (Visit 4) to take home for use on Study Day 1. In Part 1 of the study, first morning urine will be collected prior to morning dose of study drug on Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels. In Part 2 of the study, 24-hour urine collection will be performed on Study Day 1 and again at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone levels. After completion of the single-blind, Placebo Run-in period, subjects will participate in a telephone visit on Study Day 0 (Visit 5) in which they will be reminded of the instructions by the investigator or study

coordinator on how to perform the ABPM procedure and the spot/24-hour urine collection at home. Subjects will return to the clinic on Study Day 1, and those who continue to meet all eligibility requirements and complete all baseline procedures will be randomized to MLS-101 or placebo and undergo up to 8 weeks of treatment and either 4 weeks of follow-up in Part 1 or 2 weeks of follow-up in Part 2.

A planned interim analysis (Section 9.5) was conducted for Part 1 of the study and all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) were reviewed by a Data and Safety Monitoring Board (DSMB) (Section 8.3.8). A second interim analysis at the end of Part 1 to review all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) will be conducted. This analysis will also include Part 2 subjects that have completed week 4 at the time of data cut off. An additional interim analysis may be performed to support drug development decisions.

NOTE: In the previous version of the protocol, subjects in Part 1 of the study were randomized into 6 equal treatment groups (1:1:1:1:1:1) to 12.5 mg BID, 25 mg BID, 12.5 mg QD, 50 mg QD, 100 mg QD, or placebo. After a review of the clinical data at the December 2021 interim analysis, it was decided that the 2 lowest dose levels (12.5 mg QD and 12.5 mg BID) would be dropped due to lack of consistent meaningful reduction of blood pressure. Effective with Amendment 4, subjects will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo.

For Part 1, approximately 120 of 160 enrolled subjects ≥ 18 years of age will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo; each treatment group will consist of approximately 30 subjects stratified by BP (seated SBP ≤ 160 mmHg and seated SBP > 160 mmHg). Approximately 40 of 160 enrolled subjects were enrolled and completed the study in the 2 low dose cohorts that were discontinued with Amendment 4 as described above.

For Part 2, approximately 36 enrolled subjects ≥ 18 years of age will be randomized (5:1) to either 100 mg QD MLS-101 or placebo such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.

Subjects will orally administer the assigned study drug (MLS-101 or placebo) according to the assigned dosing regimen for 8 weeks beginning on Study Day 1. All subjects in Part 1 (regardless of dosing group) will receive BID dosing to preserve the integrity of the blind; active drug is administered as the morning dose for all QD dose groups. All subjects in Part 2 will receive QD dosing to be administered in the morning. Subjects will return to the research facility or be seen by the clinical investigator or approved home health care professional at the end of Study Weeks 1, 2, 3, 4, 5, 6, 7, and 8 (± 2 days) for protocol-defined efficacy and safety assessments and procedures, assessment of AEs, and confirmation of compliance with study drug usage. Subjects will also complete a telephone visit and BP check at home approximately 3 days post last dose of study drug. Subjects will attend up to 14 full clinic visits, including a pre-Screening visit, a Screening/start of Placebo Run-in visit, a second visit during Placebo Run-in, a clinic visit to initiate the ABPM procedure, a Randomization visit, 8 weekly visits during double-blind treatment, and an end-of-study visit scheduled 4 weeks after the last study treatment in Part 1 and 2 weeks after the last study treatment in Part 2 for final efficacy and safety assessments. Subjects should be in a fasted state for all study visits in Part 2 unless there is a medical reason not to fast as determined by the investigator.

Safety Criteria for Dose Adjustment or Stopping Treatment:

If, at any time during the study, a subject meets 1 or more of the clinical or laboratory exclusion criteria for the study (eg, seated SBP ≥ 175 mmHg or DBP ≥ 100 mmHg, serum potassium > 5.2 mEq/L, serum cortisol < 3 mcg/dL), laboratory test values will be confirmed using a local lab and BP measurements will be repeated. If laboratory exclusion criteria are met, the subject's dose of study drug may be temporarily withheld, adjusted, or stopped by the investigator in consultation with the medical monitor. The subject will receive appropriate antihypertensive treatment if clinically

indicated, and will remain in the study for further safety monitoring. Guidelines for dose adjustment/stopping in the management of hyperkalemia are included in Section 6.5.1. All subjects will be given dietary counseling to avoid foods high in potassium during the study (Appendix 12.6). Guidelines for the management of hypotension are included in Section 6.5.2. In addition, serum cortisol levels will be monitored throughout the study, and all subjects will be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every study visit. Based on changes in cortisol levels, an adrenocorticotropic hormone (ACTH) stimulation test (Appendix 12.7) may be performed and the subject's dose of study drug may be stopped. Guidelines for potential dose discontinuation in the management of adrenal insufficiency are included in Section 6.5.3.

Subjects who wish to stop treatment for any reason may stop at any time during the study. If the study treatment is permanently discontinued, the subject will remain in the study to complete all study assessments as described in the schedule of assessments (SoA) (Table 1 and Table 2) through Study Week 8 (end-of-treatment visit). Subjects should also be encouraged to return for a final follow-up visit 4 weeks after the Study Week 8 visit for Part 1 and 2 weeks after the Study Week 8 visit for Part 2, at which the end-of-study assessments will be performed.

An overview of the study design is presented in the Study Schema (Figure 1). Safety and efficacy assessments and study procedures are outlined in the SoA (Table 1 and Table 2). Detailed descriptions of study assessments are provided in Section 4.8 and Section 8.

Number of Subjects (planned):

[redacted]

Eligibility Criteria:

Inclusion Criteria

1. Male and nonpregnant, nonlactating female subjects ≥ 18 years of age. Fertile male and female subjects and their partners must agree to use either highly effective or acceptable methods of contraception as defined in Appendix 12.8 from Screening to 90 days post last dose of study drug
2. Written informed consent, Health Insurance Portability and Accountability Act authorization, and local patient privacy required documentation for this study have been obtained
3. Average of the AOBP measurements taken at Screening/start of the Placebo Run-in Visit and Randomization with SBP ≥ 130 mmHg
4. Background antihypertensive treatment of ≥ 2 drugs (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses for at least 4 weeks prior to signing the Screening/main study ICF
5. For Part 1, inclusion based on morning pre-Screening visit measurement of PRA, and the value for PRA must be ≤ 1 ng/mL/h. For Part 2, inclusion based on morning pre-Screening visit measurement of PRA, and the value of PRA must be > 1 ng/mL/h. Alternative target PRA criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of PRA values.
6. Inclusion based on morning pre-Screening visit measurement of serum aldosterone for Part 1 and pre-Screening visit measurement of aldosterone for Part 2. The value for aldosterone must be ≥ 1 ng/dL based on morning measurement. Alternative target aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of serum aldosterone values.
7. Serum cortisol ≥ 18 mcg/dL (morning measurement) or serum cortisol > 3 and < 18 mcg/dL (morning measurement) at Screening/start of Placebo Run-in visit if there is no evidence of

adrenal insufficiency based on normal ACTH stimulation test results. In the event that ACTH stimulation testing cannot be performed due to logistical challenges, including supply chain limitations to availability of synthetic ACTH, then subjects may be entered into the trial if morning serum cortisol is > 3 mcg/dL and they fulfill the criteria for no recent use of exogenous corticosteroid medications in dosages that could potentially cause adrenal suppression (ie, Exclusion Criteria 16a).

8. Women of childbearing potential must have a negative serum pregnancy test prior to Randomization
9. Willing and able to comply with the study instructions and attend all scheduled study visits

Exclusion Criteria

1. Concomitant use of epithelial sodium channel inhibitors or mineralocorticoid receptor antagonists, including, but not limited to amiloride, triamterene, spironolactone, eplerenone
2. Use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) in combination (ie, concomitant ACE inhibitors plus ARB therapy) as background antihypertensive treatment
3. Subjects with hypokalemia, ie, serum potassium < 3.0 mEq/L at Screening
4. Subjects with hyperkalemia, ie, serum potassium > 5.2 mEq/L at Screening for Part 1 and > 4.8 mEq/L at Screening for Part 2
5. Subjects with serum cortisol < 3 mcg/dL based on morning measurement at Screening
6. Subjects with serum sodium < 135 mEq/L at Screening
7. Subjects with estimated glomerular filtration rate < 60 mL/min/1.73m^2 at Screening
8. Subjects with type 1 or uncontrolled (hemoglobin A1c ≥ 9%) type 2 diabetes mellitus
9. Subjects with body mass index > 40 kg/m^2
10. Subjects with unstable angina who are taking short-acting oral or sublingual nitroglycerin or who have history of myocardial infarction or stroke within 6 months of Screening, sustained atrial fibrillation, ie, lasting > 12 months, or permanent atrial fibrillation; paroxysmal atrial fibrillation that terminates spontaneously or with intervention within 7 days is allowed. Chronic long-acting oral nitrates that have been stable at their prescribed doses for at least 2 weeks prior to signing the Screening/main study ICF are allowed.
11. Subjects with office SBP ≥ 175 mmHg or DBP ≥ 100 mmHg for Part 1 and SBP ≥ 160 mmHg or DBP ≥ 100 mmHg for Part 2 (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) at Pre-Screening, Screening/Start of Placebo Run-in, or Randomization
12. Subjects with a decrease in SBP ≥ 20 mmHg or DBP ≥ 10 mmHg from sitting to standing position at Screening (may be repeated during Screening at the investigator's discretion)
13. Subjects who, in the opinion of the investigator, have suspected nonadherence to antihypertensive treatment
14. Subjects who, in the opinion of the investigator, have any major medical illness or symptoms of a clinically significant illness that may limit their ability to complete the study or influence subject safety and/or the study outcome (eg, active malignancy, HIV infection)
15. Subjects who, in the opinion of the investigator, have any acute or chronic medical or psychiatric condition or laboratory abnormality that would make them unsuitable for participation in this study or that would place the subject at undue risk (eg, history of drug, alcohol, or other substance abuse) or who have other factors limiting the ability of the subject to cooperate and to comply with this protocol

16. Subjects undergoing treatment with any of the following medications:
    a. Chronically administered oral or topical corticosteroids within 3 months of Screening or during study participation. Short-term (ie, ≤ 2 weeks) of topical corticosteroids are allowed if taken ≥ 1 month prior to Randomization.
    b. Sympathomimetic decongestants within 1 week of Screening or during study participation
    c. Theophylline unless treatment has been stable at optimum dose for at least 4 weeks prior to Screening and remains stable during study participation
    d. Regular use of phosphodiesterase type 5 inhibitors within 3 months of Screening or during study participation
    e. Chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs), other than low dose aspirin (81-325 mg), where chronic use is defined as > 3 consecutive or nonconsecutive days of treatment per week, within 2 weeks of Screening. Note: intermittent use of NSAIDs is strongly discouraged; if required, NSAIDs must not be taken more than a total of 2 days during the study (defined as the period from Randomization to the end of study period). If analgesics are required, acetaminophen is recommended.
    f. Intramuscular steroids within 3 months of Screening or during study participation
    g. Estrogen- or progesterone-containing oral contraceptives or implantable progesterone devices
    h. Strong cytochrome P450 3A (CYP3A) and CYP3A4 inhibitors (eg, clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir)
    i. Strong CYP3A and CYP3A4 inducers (eg, apalutamide, carbamazepine, enzalutamide, fosphenytoin, lumacaftor, mitotane, phenobarbital, phenytoin, primidone, rifampin, St. John's Wort)
17. Subjects with known hypersensitivity to MLS-101 or any of the excipients
18. Subjects who are night-shift workers
19. Subjects who are unable to comply with protocol requirements, including treatment compliance and instructions for ABPM
20. Subjects who are currently enrolled in an investigational drug or device study or have used an investigational drug or an investigational device treatment within 4 weeks of Screening (Note: Subjects who are enrolled in a long-term follow-up study and are not actively receiving an investigational drug or device treatment may be eligible for participation in this study)

Investigational Product, Dosage, and Mode of Administration:

MLS-101 will be administered orally in the dosage 25 mg BID, 50 mg QD, or 100 mg QD in Part 1 and orally in the dosage 100 mg QD in Part 2.

Duration of Subject Participation Including Follow-up:

For Part 1, subjects will participate in up to 2 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 4-week follow-up period, for a total duration of up to 16 weeks. For Part 2, subjects will participate in up to 4 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 2-week follow-up period, for a total duration of up to 16 weeks.

Reference Therapy, Dosage and Mode of Administration:

Placebo will be administered orally in the dosage form of a tablet and will mirror the color, shape, and packaging of the respective MLS-101 tablet.

Statistical Methods:

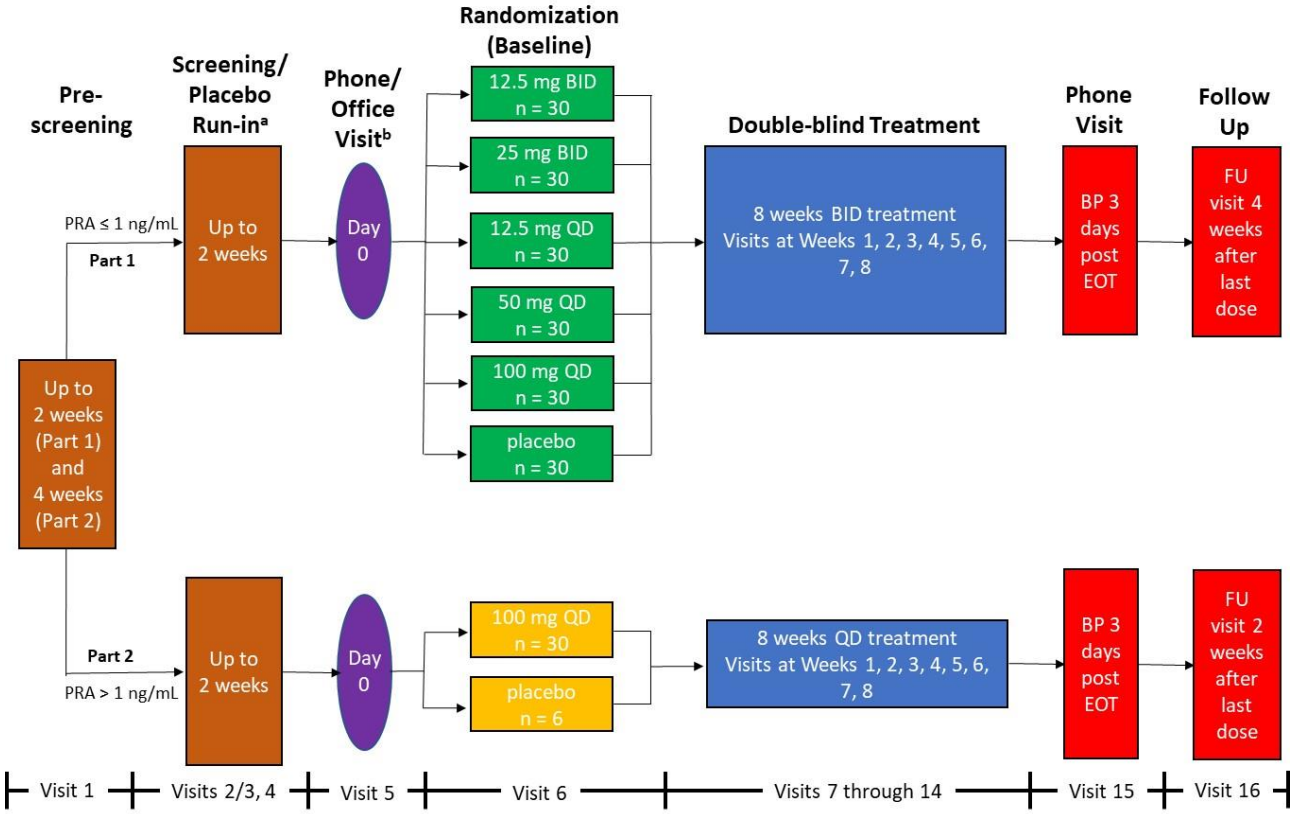
The primary estimand in the study aims to answer the research question on the treatment effect of investigational therapy versus placebo in addition to standard of care on the change in office-measured blood pressure from baseline to Week 8. Data summaries for this study will be primarily descriptive in nature. Both safety and efficacy will focus on subjects receiving at least 1 dose of study treatment. Endpoints measured as categorical outcomes (including binary endpoints) will be summarized as frequencies and percentages. Continuous endpoints will be summarized using the following descriptive statistics: sample size, mean, median, quartiles and standard deviation. For efficacy endpoints, confidence intervals and tests of significance will use a 10% (2-sided) significance level. Given the exploratory nature of this study, no adjustments for multiplicity will be used.

Sample Size

This study has been designed to provide preliminary information regarding the appropriate dose and frequency of dosing for MLS-101 with respect to changes in SBP after 8 weeks of treatment. The sample size has been determined to provide estimates of the placebo-adjusted mean change in SBP from baseline for each dose that are of adequate precision assuming a 90% 2-sided confidence interval and an effect size of 10.0. With 30 subjects planned per arm in Part 1, the mean placebo adjusted change from baseline in SBP for each dose will be estimated with a precision of ± 3.8, assuming a standard deviation of 9 in the subject level changes in SBP from baseline. All analyses based on Part 2 of the study will be exploratory in nature; no formal sample size considerations will be made for Part 2.

The study schema for Study MLS-101-201 is presented in Figure 1. The SoA are presented in Table 1 and Table 2 for Part 1 and Part 2 of the study, respectively. Detailed descriptions of study assessments are provided in Section 4.8 and Section 8.

Figure 1: MLS-101-201 Study Schema



ABPM = ambulatory blood pressure monitoring; BP = blood pressure; BID = twice daily; EOT = end of treatment; FU = follow up; PRA = plasma renin activity; QD = once daily

[a] If Screening results are available, inclusion/exclusion evaluation will be performed. If subject is not eligible based on Screening results, they will not continue to Visit 4. If Screening results are not available, subject proceeds to Visit 4. If Screening results are not available at Visit 4, subject should attend Visit 5 to determine final eligibility. If eligible based on Screening results, ABPM assessment can begin at Visit 5.

[b] The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1). Alternatively, sites may choose to schedule an office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. Training for the ABPM procedure can be done at an office visit or via phone.

Table 1: Schedule of Assessments for Part 1

Study Periods	Pre-Screen	Screening/Start of Placebo Run-in Week 1	Single-blind Placebo Run-in Week 2	Phone or Office Visit	Random-ization[a]	Double-blind Treatment								Phone Visit	Follow Up
Study Visit (V)	V1	V2/V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Study Week	-4 to -2	-2	-1	0	0	1	2	3	4	5	6	7	8	9	12
Study Day	-28 to -14	-14±2	-7±2	0	1	7±2	14±2	21±2	28±2	35±2	42±2	49±2	56±2	59+2	84±3
Test/Procedure															
Informed Consent[b]	X	X													
Inclusion/Exclusion Assessment		X	X[c]	X[d]	X										
Medical History	X	X													
Physical Examination		X			X								X		
Vital Signs[e]	X	X	X		X	X	X	X	X	X	X	X	X		X
AOBP[f]	X	X	X		X	X	X	X	X	X	X	X	X		X
Home BP check[g]														X	
Standing BP[h]		X			X	X							X		
24-hour ABPM[i]				X								X			
12-lead ECG[j]		X			X								X		
Pregnancy Test[k]		X			X				X				X		
PD Sample Collection[l]	X[m]	X			X				X						X
Safety Labs (hematology, chemistry, coags, cortisol, ACTH)[n]		X			X	X	X	X	X	X	X	X	X		X
Safety Labs (ACTH stimulation test)[o]		X											X		
Safety Labs (urinalysis)[p]		X			X	X	X	X	X	X	X	X	X		X
Spot Urine Collection[q]					X								X		
PK Sample Collection[r]					X	X			X				X		
Adverse Events[s]		X	X		X	X	X	X	X	X	X	X	X		X
Concomitant Medications		X	X		X	X	X	X	X	X	X	X	X		X
Study Drug Compliance		X	X		X	X	X	X	X	X	X	X	X		
IP Administration[t]		X[u]	X[u]		X	X	X	X	X	X	X	X	X		

ABPM = ambulatory blood pressure monitoring; ACTH = adrenocorticotropic hormone; AOBP = automated office blood pressure; BID = twice daily; BP = blood pressure; coags = coagulation tests; COVID-19 = coronavirus disease 2019; ECG = electrocardiogram; ICF = informed consent form; IP = investigational product; PD = pharmacodynamic; PK = pharmacokinetic; PRA = plasma renin activity

a Subjects who continue to meet all eligibility requirements will be randomized to receive MLS-101 or placebo.
b A pre-Screening ICF must be signed before entering pre-Screening period. A Screening/main study ICF must be signed before entering Screening period.
c If Screening results are available, inclusion/exclusion evaluation will be performed. If subject is not eligible based on Screening results, they will not continue to Visit 4. If Screening results are not available, subject proceeds to Visit 4.
d If Screening results are not available at Visit 4, subject should attend Visit 5 to determine final eligibility. If eligible based on Screening results, ABPM assessment can begin at Visit 5.
e Body temperature, heart rate, and respiratory rate will be measured.
f AOBP measurement is defined as the average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position.
g Blood pressure measurement to be performed at home.
h In addition to sitting measurements, standing BP will be measured at Screening/start of Placebo Run-in, Randomization, Study Week 1, and Study Week 8 with the AOBP device according to the procedure described in Appendix 12.5. In addition, standing blood pressure measurements will be measured if a subject reports symptoms of hypotension (eg, light-headedness, dizziness, presyncope, or syncope); the standing BP measurement will be assessed weekly for these subjects until symptoms resolve.
i Subjects will be given the ABPM device on the second visit of the placebo-run in period (Visit 4; Study Day -7 ± 2). The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week 7 (ie, Visit 13; Study Day 49 ± 2). Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study Week 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to COVID-19, subject exposure to COVID-19, or subject testing positive for COVID-19. Training for the ABPM procedure can be done at an office visit or via phone. If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at the end of Study Week 8.
j ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory.
k Women of childbearing potential only. A sample for serum pregnancy test will be collected at Screening/start of the single-blind, Placebo Run-in, ie, Study Day -14 ± 2, and a sample for urine pregnancy test will be collected on Study Day 1, Study Day 28 ± 2 (Study Week 4), and at the end-of-treatment visit, Study Day 56 ± 2. Follicle stimulating hormone must be used to confirm postmenopausal status in all postmenopausal women at Screening/start of Placebo Run-in.
l Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected. Cortisol assessment should occur before 10 am on the morning of the study visit.
m Pre-Screening labs include blood samples for aldosterone and PRA **only** and should be collected in the morning after at least 8 hours of fasting.
n Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (see Table 6). Cortisol assessment should occur before 10 am on the morning of the study visit.
o ACTH stimulation test should be performed as described in Appendix 12.7 at Screening/start of Placebo Run-in, Study Week 8, and whenever low cortisol levels are noted as per Section 6.5.3. ACTH stimulation test may be waived with sponsor's approval.
p Urine will be collected for urinalysis (see Table 6).
q Subjects will be given a urine collection kit on the second visit of the placebo-run in period (Visit 4; Study Day -7 ± 2). First morning urine will be collected prior to morning dose of study drug on Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels.
r Single blood samples for determination of MLS-101 will be collected at baseline (predose) and at Study Weeks 1, 4, and 8 (trough levels). Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected at baseline (predose) and at Study Weeks 1, 4, and 8. In addition, blood samples for determination of MLS-101 (and metabolites) will be collected predose, and 1, 2, 3, and 4 hours postdose on Study Days 1 (Randomization) and 28 (Study Week 4).
s Adverse events and serious adverse events will be collected from the signing of the Screening/main study ICF to 30 days post last dose of study drug.
t On site visit days, subjects should be instructed to take their morning dose of study drug at the site.
u Single-blind, 2-week run-in period of BID oral treatment with placebo.

Table 2: Schedule of Assessments for Part 2

Study Periods	Pre-Screen	Screening/Start of Placebo Run-in Week 1	Single-blind Placebo Run-in Week 2	Phone or Office Visit	Random-ization[a]	Double-blind Treatment								Phone Visit	Follow Up
Study Visit (V)	V1	V2/V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Study Week	-6 to -2	-2	-1	0	0	1	2	3	4	5	6	7	8	9	10
Study Day	-42 to -14	-14±2	-7±2	0	1	7±2	14±2	21±2	28±2	35±2	42±2	49±2	56±2	59+2	70±3
Test/Procedure															
Informed Consent[b]	X	X													
Inclusion/Exclusion Assessment		X	X[c]	X[d]	X										
Medical History	X	X													
Physical Examination		X			X								X		
Vital Signs[e]	X	X	X		X	X	X	X	X	X	X	X	X		X
AOBP[f]	X	X	X		X	X	X	X	X	X	X	X	X		X
Home BP check[g]														X	
Standing BP[h]		X			X	X							X		
24-hour ABPM[i]				X					X			X			
12-lead ECG[j]		X			X								X		
Pregnancy Test[k]		X			X				X				X		
PD Sample Collection[l]	X[m]	X			X				X						X
Safety Labs (hematology, chemistry, coags, cortisol, ACTH)[n]		X			X	X	X	X	X	X	X	X	X		X
Safety Labs (ACTH stimulation test)[o]		X											X		
Safety Labs (urinalysis)[p]		X			X	X	X	X	X	X	X	X	X		X
24-Hour Urine Collection[q]					X				X						
PK Sample Collection[r]					X	X			X				X		
Adverse Events[s]		X	X		X	X	X	X	X	X	X	X	X		X
Concomitant Medications		X	X		X	X	X	X	X	X	X	X	X		X
Study Drug Compliance		X	X		X	X	X	X	X	X	X	X	X		
IP Administration[t]		X[u]	X[u]		X	X	X	X	X	X	X	X	X		

ABPM = ambulatory blood pressure monitoring; ACTH = adrenocorticotropic hormone; AOBP = automated office blood pressure; BP = blood pressure; coags = coagulation tests; COVID-19 = coronavirus disease 2019; ECG = electrocardiogram; ICF = informed consent form; IP = investigational product; PD = pharmacodynamic; PK = pharmacokinetic; PRA = plasma renin activity; QD = once daily

a Subjects who continue to meet all eligibility requirements will be randomized to receive MLS-101 or placebo.
b A pre-Screening ICF must be signed before entering pre-Screening period. A Screening/main study ICF must be signed before entering Screening period.
c If Screening results are available, inclusion/exclusion evaluation will be performed. If subject is not eligible based on Screening results, they will not continue to Visit 4. If Screening results are not available, subject proceeds to Visit 4.
d If Screening results are not available at Visit 4, subject should attend Visit 5 to determine final eligibility. If eligible based on Screening results, ABPM assessment can begin at Visit 5.
e Body temperature, heart rate, and respiratory rate will be measured.
f AOBP measurement is defined as the average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position.
g Blood pressure measurement to be performed at home.
h In addition to sitting measurements, standing BP will be measured at Screening/start of Placebo Run-in, Randomization, Study Week 1, and Study Week 8 with the AOBP device according to the procedure described in Appendix 12.5. In addition, standing blood pressure measurements will be measured if a subject reports symptoms of hypotension (eg, light-headedness, dizziness, presyncope, or syncope); the standing BP measurement will be assessed weekly for these subjects until symptoms resolve.
i Subjects will be given the ABPM device on the second visit of the placebo-run in period (Visit 4; Study Day -7 ± 2). The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of at the end of Study Week 4 (ie, Study Day 28 ± 2) and Study Week 7 (ie, Visit 13; Study Day 49 ± 2). Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study Week 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to COVID-19, subject exposure to COVID-19, or subject testing positive for COVID-19. Training for the ABPM procedure can be done at an office visit or via phone. If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at the end of Study Week 8.
j ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory.
k Women of childbearing potential only. A sample for serum pregnancy test will be collected at Screening/start of the single-blind, Placebo Run-in, ie, Study Day -14 ± 2, and a sample for urine pregnancy test will be collected on Study Day 1, Study Day 28 ± 2 (Study Week 4), and at the end-of-treatment visit, Study Day 56 ± 2. Follicle stimulating hormone must be used to confirm postmenopausal status in all postmenopausal women at Screening/start of Placebo Run-in.
l Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected. Cortisol assessment should occur before 10 am on the morning of the study visit. Subjects should be in a fasted state unless there is a medical reason not to fast as determined by the investigator.
m Pre-Screening labs include blood samples for aldosterone and PRA **only** and should be collected in the morning after at least 8 hours of fasting.
n Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (see Table 6). Cortisol assessment should occur before 10 am on the morning of the study visit. Subjects should be in a fasted state unless there is a medical reason not to fast as determined by the investigator.
o ACTH stimulation test should be performed as described in Appendix 12.7 at Screening/start of Placebo Run-in, Study Week 8, and whenever low cortisol levels are noted as per Section 6.5.3. ACTH stimulation test may be waived with sponsor's approval.
p Urine will be collected for urinalysis (see Table 6).
q Subjects will be given a 24-hour urine collection kit on the second visit of the placebo-run in period (Visit 4; Study Day -7 ± 2). The first morning urine should be discarded before the 24-hour collection is started. Urine should be collected for 24 hours prior to V6 (Randomization) for determination of potassium, sodium, creatinine, and aldosterone levels. A second 24-hour urine collection will occur 24 hours prior to V10 (Study Week 4, Study Day 28 ± 2).
r Single blood samples for determination of MLS-101 will be collected at baseline (predose) and at Study Weeks 1, 4, and 8 (trough levels). Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected at baseline (predose) and at Study Weeks 1, 4, and 8. In addition, blood samples for determination of MLS-101 (and metabolites) will be collected predose, and 1, 2, 3, and 4 hours postdose on Study Days 1 (Randomization) and 28 (Study Week 4). Subjects should be in a fasted state unless there is a medical reason not to fast as determined by the investigator.
s Adverse events and serious adverse events will be collected from the signing of the Screening/main study ICF to 15 days post last dose of study drug.
t Subjects should administer study drug QD in the morning. On site visit days, subjects should be instructed to take their dose of study drug at the site.
u Single-blind, 2-week run-in period of QD oral treatment with placebo.

2. INTRODUCTION

2.1. Study Rationale

Mineralys is currently developing MLS-101, a selective aldosterone synthase inhibitor, for the treatment of uncontrolled hypertension in the presence of autonomous aldosterone production. This study will evaluate the safety, efficacy, and tolerability of MLS-101 in subjects with low-renin hypertension. Phase 1 data support the safety and selectivity profile of the drug with pharmacodynamically relevant changes in aldosterone and renin levels consistent with the mechanism of action of the drug. The primary objective of this Phase 2 study is to evaluate the blood pressure (BP) response in subjects with uncontrolled hypertension (hypertensive despite receiving ≥ 2 antihypertensives). Various dose levels and dose regimens will be tested to identify a dose response and a therapeutic index to inform subsequent studies to support an indication in uncontrolled hypertension, with particular emphasis on defined patient populations such as low-renin hypertension. Data from this study will be used to inform dose selection for future studies.

If successful, MLS-101 could provide the first targeted therapeutic option in patients with hypertension. By identifying patients who have hypertension associated with autonomous aldosterone production, Mineralys hopes to maximize the benefit risk profile of MLS-101 and create an avenue for the pursuit of future targeted therapeutic approaches in hypertension.

2.2. Background

Hypertension remains an important global health challenge due to its high prevalence and contribution to the development and progression of cardiovascular disease and chronic kidney disease and associated morbidity and mortality. The diagnosis and management of hypertension have not materially changed in the last 20 years, with little to no innovation in the development of novel antihypertensive therapeutics with unique mechanisms of action. With clinical practice guideline recommendations to manage BP to less than 130/80 (Whelton 2018) and recent data from the Systolic Blood Pressure Intervention Trial (SPRINT) (Wright 2015) suggesting the benefit of a lower systolic blood pressure (SBP) target in patients with underlying cardiovascular disease, additional options for clinicians to manage BP are warranted.

Hypertension has historically been considered idiopathic, and the approach for antihypertensive treatment is rarely directed toward a primary underlying mechanism. Recent evidence suggests that up to approximately 30% of patients (Baudrand 2018) who otherwise would have been classified as essential or idiopathic hypertension patients may indeed have hypertension that is mediated by an underlying mechanism - autonomous aldosterone production. As part of a physiologic negative feedback loop, these patients have suppressed systemic renin levels and fall within a category previously described as low-renin hypertension (Jose 1970, Channick 1969). Low-renin hypertension occurs predominantly in patients with autonomous nonsuppressible aldosterone production in which plasma levels of aldosterone are inappropriately high relative to the renin level. Previously, patients with low-renin hypertension had pathophysiologic changes that were classically defined as overt primary aldosteronism (PA). However, accumulating evidence indicates that a much larger and clinically relevant spectrum of renin-independent aldosterone production may exist (Vaidya 2018). While assays are readily available to identify patients with low-renin hypertension mediated by aldosterone, epidemiologic studies suggest that these patients have largely been underdiagnosed.

Data from the Prevention and Treatment of Hypertension With Algorithm based Therapy-2 (PATHWAY-2) study (Williams 2015) in patients with resistant hypertension highlights the clinical rationale for identifying patients with low-renin hypertension and the benefit of aldosterone blockade. Specifically, this trial demonstrated a greater BP lowering response of aldosterone blockade using a mineralocorticoid receptor antagonist (MRA) in patients with low renin levels. This finding points to the opportunity to use renin as a diagnostic tool to identify patients who might be best suited for aldosterone targeted therapies.

Despite these data and clinical practice guidelines (Whelton 2018) that recommend the use of MRAs in patients with resistant hypertension, the use of MRAs has been strikingly low and market data from 2019 demonstrated that fewer than 4% of treated patients were receiving MRAs when indicated (Decision Resources Group 2019). Currently, MRAs are the only available aldosterone targeted therapies and their limited use may be explained by tolerability concerns seen with spironolactone, the short acting nature of newer generation MRAs like eplerenone, which have demonstrated more modest effects on BP, and the prevalence of hyperkalemia in MRA-treated patients. Thus, novel approaches to targeting aldosterone are needed.

2.3. Benefit/Risk Assessment

First-line treatment of hypertension includes dietary and lifestyle changes, with drug therapy as second-line treatment. The most common drugs used to treat hypertension include angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, thiazide diuretics, and alpha- and beta-blockers. Despite a plethora of available treatment options, over 50% of patients currently using prescription medication to lower BP have uncontrolled hypertension (Million Hearts 2020) putting them at greater risk for all-cause mortality and cardiovascular disease outcomes. Furthermore, it is estimated that 10% to 15% of hypertensive patients have resistant hypertension defined as uncontrolled hypertension despite taking ≥ 3 antihypertensive drugs (Oparil 2015). In addition, nonadherence or intolerance to available antihypertensive agents contributes to the proportion of hypertensive patients with uncontrolled BP (Oparil 2015).

Aldosterone is a mineralocorticoid that regulates electrolyte and volume homeostasis in normal subjects, and can contribute to the development of hypertension when elevated. It is synthesized in the zona glomerulosa of the adrenal cortex from 11-deoxycorticosterone by aldosterone synthase, which is encoded by the cytochrome P450 11B2 (CYP11B2) gene (Oparil 2015). Aldosterone regulates BP mainly by acting via mineralocorticoid receptors (MRs) on organs such as renal tubules and large intestines to increase the amount of sodium reabsorbed into the bloodstream and the amount of potassium removed in the urine. Aldosterone also causes water to be reabsorbed along with sodium, which has the effect of increasing fluid volume and indirectly contributes to vascular disorders via elevated BP. Furthermore, some nonclinical studies have reported a wider distribution of MR not only in the renal tubules but also in the nonepithelial cells, including vascular smooth muscle cells (Ishikawa 2005) and mesangial cells (Nishiyama 2005), suggesting that overproduction of aldosterone may directly injure the cardiovascular system and kidneys.

Mineralocorticoid receptor antagonists have been used to inhibit the function of aldosterone in the treatment of essential and resistant hypertension, heart failure, PA, and edema. However, blockade of MR induces a counter-regulatory increase in plasma aldosterone concentration

(PAC), potentially limiting the efficacy of the MR blockade and enhancing the MR-independent effect of aldosterone, such as vasoconstriction (Romagni 2003). This suggests that decreasing PAC may be a preferable therapeutic alternative to MR blockade.

In addition to the negative effects of increasing PAC, MRAs may put patients at risk of hyperkalemia. Cortisol, like aldosterone, binds to the MRs (Arriza 1987), which enhances the excretion of potassium in urine (Mills 1960). Thus, if the action of the MR is blocked by an MRA, the excretion of potassium in urine will be reduced putting the patient at risk for hyperkalemia (Ramsay 1976). For this reason, MRAs are contraindicated in patients with hyperkalemia. In addition, MR antagonists require careful monitoring of plasma potassium, especially in patients who are at high risk for hyperkalemia such as patients with renal impairment. Given these limitations, alternative approaches to antagonizing aldosterone activity and its contribution to hypertension and end-organ damage are warranted. Inhibition of aldosterone production in the adrenal gland as opposed to blockade of aldosterone activity at the receptor represents a promising alternative approach.

Inhibition of aldosterone synthesis is not free of risks. Aldosterone synthase inhibitors may cause hyperkalemia and hyponatremia similar to mineralocorticoid receptor blockade (Hargovan 2014). Furthermore, the long-term effect of aldosterone synthase inhibitors on kidney function is not known. However, recent evidence suggests less risk for hyperkalemia when antagonizing aldosterone using nonsteroidal MRAs (Bakris 2020).

The investigational product (IP) MLS-101 is an inhibitor of CYP11B2 (aldosterone synthase), which is a rate-limiting enzyme for aldosterone production. In nonclinical studies, single oral administration of 3172473 (free-base form of MLS-101) significantly decreased PAC in a sodium-depleted monkey model. On the other hand, single oral administration of 3172473 did not affect plasma cortisol concentrations in adrenocorticotropic hormone (ACTH)-loaded monkeys even at a dose 100-fold higher than that required to reduce PAC. These results indicate that MLS-101 inhibits CYP11B2 with higher selectivity over CYP11B1, which is an enzyme for cortisol production. This high selectivity was also shown in the first-in-human study (MT-4129-E01). This study confirmed that MLS-101 decreased PAC and did not decrease serum cortisol in healthy subjects, indicating that MLS-101 may have a lower potential risk of hyperkalemia than MRAs.

Emerging evidence suggests that nonsuppressible aldosterone production can be identified in a substantial proportion of patients with hypertension who have low renin levels, and these patients can be identified using commercially available assays assessing plasma renin activity (PRA) (Brown 2020). Further, randomized controlled trials have shown that patients with low renin levels experience dramatic BP lowering when treated with aldosterone antagonists (Williams 2015). This evidence, in combination with the results from the first-in-human study with MLS-101 (Study MT-4129-E01), suggests that the identification of patients with low-renin hypertension for a novel targeted therapy using an aldosterone synthase inhibitor such as MLS-101 can maximize the benefit/risk ratio of a new therapeutic in this subpopulation of patients, directly addressing the underlying pathophysiology of their disease. Further, identification of these patients for an aldosterone-targeted therapy may not only improve blood pressure responses, but may also result in a more efficient therapeutic option that minimizes the use of relatively ineffective additional components.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Study Objectives

3.1.1. Primary Objective

- To characterize the effect of MLS-101 on BP at 5 dosing regimens versus placebo when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment

3.1.2. Secondary Objectives

- To investigate the safety and tolerability of MLS-101 at 5 dosing regimens versus placebo when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment
- To investigate the pharmacokinetic (PK) profile of MLS-101 at 5 dosing regimens when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment
- To investigate the pharmacodynamic (PD) parameters of MLS-101 at 5 dosing regimens when administered orally for the treatment of uncontrolled hypertension as add-on therapy to stable background treatment

3.2. Study Endpoints

3.2.1. Primary Efficacy Endpoint

- Change in office-measured (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) SBP from baseline to the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)

3.2.2. Secondary Efficacy Endpoints

- Change in 24-hour ambulatory blood pressure monitoring (ABPM) parameters (systolic and diastolic) from baseline to the end of Study Week 7 (ie, Study Day 49 ± 2)
- Change in office-measured (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) SBP and diastolic blood pressure (DBP) from baseline to the end of Study Weeks 1, 2, 3, 4, 5, 6, and 7 (± 2 days); change in office-measured DBP from baseline to the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)
- Proportion of subjects who achieve in office-measured BP of ≤ 130/80 mmHg by the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period)

3.2.3. Safety Endpoints

- Incidence and severity of all spontaneously reported adverse events (AEs)
- Changes in vital signs (SBP, DBP, body temperature, heart rate, and respiratory rate)
- Changes in electrocardiogram parameters (including cardiac intervals: PR, QRS, QT, and corrected QT interval using Fridericia's formula)

- Changes in clinical laboratory assessments (hematology, chemistry, coagulation, and urinalysis)
- Change in office-measured SBP from Study Week 8 (end-of-treatment period) to Study Week 12 for Part 1 and Study Week 10 for Part 2 (end of follow-up)

3.2.4. Pharmacokinetics Endpoints

- PK parameters including, if feasible, area under the plasma concentration versus time curve (AUC), maximum plasma concentration (C_{max}), time to maximum concentration (T_{max}), and half-life ($t_{1/2}$) will be summarized descriptively for Randomization (baseline) and Study Weeks 1, 4, and 8

3.2.5. Pharmacodynamics Endpoints

- Change in plasma 11-deoxycortisol and PRA from baseline to the end of Study Weeks 4 and 12 for Part 1 and end of Study Weeks 4 and 10 for Part 2 (end of follow-up)
- Change in serum aldosterone, cortisol, and 11-deoxycorticosterone concentration from baseline to the end of Study Weeks 4 and 12 for Part 1 and end of Study Weeks 4 and 10 for Part 2 (end of follow-up)

4. STUDY DESIGN

4.1. Overall Study Design

This is a 2-part Phase 2 randomized, double-blind, placebo-controlled, dose-ranging, multicenter study designed to evaluate the effect of orally administered MLS-101 on BP for the treatment of uncontrolled hypertension (hypertensive despite receiving ≥ 2 antihypertensives) when used as add-on therapy to stable background treatment in male and female subjects ≥ 18 years of age. Stable background treatment must include ≥ 2 antihypertensives (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses and stable for at least 4 weeks prior to signing the Screening/main study informed consent form (ICF). Background therapy may be adjusted at the investigator's discretion.

Subjects with a history consistent with inadequately controlled hypertension, (ie, SBP ≥ 135 mmHg on a stable treatment regimen of ≥ 2 antihypertensives), will undergo pre-Screening laboratory tests for PRA and aldosterone following their agreement to enter pre-Screening and completion of a pre-Screening ICF that has been approved by the Institutional Review Board (IRB). In Part 1 of the study, the value of PRA must be ≤ 1 ng/mL/h based on morning measurement. If the value of PRA > 1 ng/mL/h based on morning measurement, then subjects may be eligible to enter Part 2 of the study. The value for aldosterone must be ≥ 1 ng/dL based on morning measurement for both Part 1 and Part 2. Alternative target PRA and/or aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during conduct of the trial based on ongoing review of PRA and serum aldosterone values. Subjects with PRA and serum aldosterone values meeting the stipulated guidance criteria will then enter the study Screening process. If PRA and/or serum aldosterone do not meet the target guidance values during pre-Screening, the PRA and aldosterone measurements may be repeated 1 time and the pre-Screening window will reset to 2 weeks for Part 1 or 4 weeks for Part 2. If guidance criteria are revised, then previously pre-screened individuals who meet the revised criteria may be considered for entry into the Screening process with consent of the sponsor, and the pre-Screening window will reset to 2 weeks for Part 1 and 4 weeks for Part 2.

The current estimate is that approximately 1100 subjects will undergo up to 2 weeks of pre-Screening for Part 1 and 4 weeks of pre-Screening for Part 2. It is estimated that approximately 270 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in for Part 1 after completing a Screening/main study ICF that has been approved by the IRB. Assuming an approximately 40% screen failure rate, a total of approximately 160 subjects will meet all eligibility requirements to qualify for enrollment in Part 1 of the study. For Part 2, it is estimated that approximately 60 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in for Part 2 after completing a Screening/main study ICF that has been approved by the IRB. Assuming an approximately 40% screen failure rate, a total of 36 subjects will meet all eligibility requirements to qualify for enrollment in Part 2 of the study.

At the Screening/start of Placebo Run-in visit (V2/V3), subjects will complete the Screening assessments and begin a single-blind (subjects blinded to treatment allocation) run-in period (up to 2 weeks) of twice-daily (BID) oral treatment with placebo in Part 1 and once daily (QD) oral treatment with placebo in Part 2, while continuing to remain on stable doses of their background antihypertensive medications. Using an automated oscillometric sphygmomanometer device, SBP and DBP will be measured 5 times after approximately 5 minutes of rest in the seated

position according to the American Heart Association (AHA) Guidelines (Muntner 2019). The averge of the last 2 of 5 unattended measurements of SBP and DBP, respectively, will be used for the analysis. Subjects may also be provided with an automated digital oscillometric home BP device to measure BP at home at the investigator's discretion. In addition to sitting measurements, standing BP will be measured at the Screening/start of Placebo Run-in visit, Randomization, Study Week 1, and Study Week 8 with an automated office blood pressure (AOBP) device according to the procedure described in Appendix 12.5. Subjects will return to the research facility at the start of Week 2 of the run-in period (V4) for protocol-defined assessments including BP measurements.

At the second clinic visit of the Placebo Run-in period, subjects will be evaluated for eligibility based on Screening data, and if eligible, will continue Week 2 of Placebo Run-in. Subjects will be given an ABPM device and instructions on how to perform the ABPM procedure at home by the investigator. The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) in Part 2 and Study Week 7 (ie, Visit 13; Study Day 49 ± 2) in Parts 1 and 2. Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study Weeks 4 and 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to coronavirus disease 2019 (COVID-19), subject exposure to COVID-19, or subject testing positive for COVID-19. If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at Study Week 8. In addition, subjects will be given either a spot urine collection kit for Part 1 or a 24-hour urine collection kit for Part 2 on the second visit of the placebo-run in period (Visit 4) to take home for use on Study Day 1. In Part 1 of the study, first morning urine will be collected prior to morning dose of study drug on Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels. In Part 2 of the study, 24-hour urine collection will be performed on Study Day 1 and again at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone levels. After completion of the single-blind, Placebo Run-in period, subjects will participate in a telephone visit on Study Day 0 (Visit 5) in which they will be reminded of the instructions by the investigator or study coordinator on how to perform the ABPM procedure and the spot/24-hour urine collection at home. Subjects will return to the clinic on Study Day 1, and those who continue to meet all eligibility requirements and complete all baseline procedures will be randomized to MLS-101 or placebo and undergo up to 8 weeks of treatment and either 4 weeks of follow-up in Part 1 or 2 weeks of follow-up in Part 2.

A planned interim analysis (Section 9.5) was conducted for Part 1 of the study and all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) were reviewed by a Data and Safety Monitoring Board (DSMB) (Section 8.3.8). A second interim analysis at the end of Part 1 to review all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) will be conducted. This analysis will also include Part 2 subjects that have completed week 4 at the time of data cut off. An additional interim analysis may be performed to support drug development decisions.

NOTE: In the previous version of the protocol, subjects in Part 1 of the study were randomized into 6 equal treatment groups (1:1:1:1:1:1) to 12.5 mg BID, 25 mg BID, 12.5 mg QD, 50 mg QD, 100 mg QD, or placebo. After a review of the clinical data at the December 2021 interim

analysis, it was decided that the 2 lowest dose levels (12.5 mg QD and 12.5 mg BID) will be dropped due to lack of consistent meaningful reduction of blood pressure. Effective with Amendment 4, subjects will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo.

For Part 1, approximately 120 of 160 enrolled subjects ≥ 18 years of age will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo; each treatment group will consist of approximately 30 subjects stratified by BP (seated SBP ≤ 160 mmHg and seated SBP > 160 mmHg). Approximately 40 of 160 enrolled subjects were enrolled and completed the study in the 2 low dose cohorts that were discontinued with Amendment 4 as described above.

For Part 2, approximately 36 enrolled subjects ≥ 18 years of age will be randomized (5:1) to either 100 mg QD MLS-101 or placebo such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.

Subjects will orally administer the assigned study drug (MLS-101 or placebo) according to the assigned dosing regimen for 8 weeks beginning on Study Day 1. All subjects in Part 1 (regardless of dosing group) will receive BID dosing to preserve the integrity of the blind; active drug is administered as the morning dose for all QD dose groups. All subjects in Part 2 will receive QD dosing to be administered in the morning. Subjects will return to the research facility or be seen by the clinical investigator or approved home health care professional at the end of Study Weeks 1, 2, 3, 4, 5, 6, 7, and 8 (± 2 days) for protocol-defined efficacy and safety assessments and procedures, assessment of AEs, and confirmation of compliance with study drug usage. Subjects will also complete a telephone visit and BP check at home approximately 3 days post last dose of study drug. Subjects will attend up to 14 full clinic visits, including a pre-Screening visit, a Screening/start of Placebo Run-in visit, a second visit during Placebo Run-in, a clinic visit to initiate the ABPM procedure, a Randomization visit, 8 weekly visits during double-blind treatment, and an end-of-study visit scheduled 4 weeks after the last study treatment in Part 1 and 2 weeks after the last study treatment in Part 2 for final efficacy and safety assessments. Subjects should be in a fasted state for all study visits in Part 2 unless there is a medical reason not to fast as determined by the investigator.

Safety Criteria for Dose Adjustment or Stopping Treatment:

If, at any time during the study, a subject meets 1 or more of the clinical or laboratory exclusion criteria for the study (eg, seated SBP ≥ 175 mmHg or DBP ≥ 100 mmHg, serum potassium > 5.2 mEq/L, serum cortisol < 3 mcg/dL), laboratory test values will be confirmed using a local lab and BP measurements will be repeated. If laboratory exclusion criteria are met, the subject's dose of study drug may be temporarily withheld, adjusted, or stopped by the investigator in consultation with the medical monitor. The subject will receive appropriate antihypertensive treatment if clinically indicated, and will remain in the study for further safety monitoring. Guidelines for dose adjustment/stopping in the management of hyperkalemia are included in Section 6.5.1. All subjects will be given dietary counseling to avoid foods high in potassium during the study (Appendix 12.6). Guidelines for the management of hypotension are included in Section 0. In addition, serum cortisol levels will be monitored throughout the study, and all subjects will be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every study visit. Based on changes in cortisol levels, an adrenocorticotropic hormone (ACTH) stimulation test (Appendix 12.7) may be

performed and the subject's dose of study drug may be stopped. Guidelines for potential dose discontinuation in the management of adrenal insufficiency are included in Section 6.5.3.

Subjects who wish to stop treatment for any reason may stop at any time during the study. If the study treatment is permanently discontinued, the subject will remain in the study to complete all study assessments as described in the schedule of assessments (SoA) (Table 1 and Table 2) through Study Week 8 (end-of-treatment visit). Subjects should also be encouraged to return for a final follow-up visit 4 weeks after the Study Week 8 visit for Part 1 and 2 weeks after the Study Week 8 visit for Part 2, at which the end-of-study assessments will be performed.

An overview of the study design is presented in the Study Schema (Figure 1). Safety and efficacy assessments and study procedures are outlined in the SoA (Table 1 and Table 2). Detailed descriptions of study assessments are provided in Section 4.8 and Section 8.

4.2. Scientific Rationale for Study Design

The objective of Part 1 of the Phase 2 study is to evaluate both QD and BID dosing across a total daily dose of 12.5 mg to 100 mg for 8 weeks of treatment to determine at which doses substantial reductions in blood pressure occur and to determine if there is a dose response on BP with increasing doses. The dose range chosen was based on the Phase 1 study findings, and is considered to be a wide enough dose range to examine whether a dose-dependent response on BP could be observed. Further, we are interested in determining if BID dosing may be a better dosing regimen to sustain reductions in aldosterone over a 24-hour period, reduce any concomitant rise in trough aldosterone levels, and ultimately lead to a greater reduction in BP in patients. The examination of PK, PD (eg, aldosterone concentration), and BP at the doses proposed should enable a better understanding of dose requirements to be further examined in subsequent trials.

The key efficacy endpoints, both primary and secondary, were selected to assess the changes in BP from baseline with treatment. The treatment period of 8 weeks was considered sufficient to assess the maximum effect on BP for a given dose and to assess safety. The assessment of BP, assessing changes in both systolic and diastolic BP, has been standard for previous BP trials with novel therapeutics.

Data obtained from the interim analyses performed in Part 1 of the study will be used to inform Part 2.

4.3. Number of Subjects/Number of Centers

Approximately 1100 subjects will undergo up to 2 weeks of pre-Screening. For Part 1, it is estimated that approximately 270 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in, and (assuming an approximately 40% screen failure rate) a total of approximately 160 subjects will meet all eligibility requirements to qualify for enrollment in Part 1 of the study. The subjects who qualify for enrollment will be randomly divided into 4 equal treatment groups (1:1:1:1 with approximately 30 subjects per group, stratified by BP, ie, seated SBP $\leq$ 160 mmHg and seated SBP $>$ 160 mmHg) across approximately 50 study centers in the United States.

For Part 2, it is estimated that approximately 60 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in, and (assuming an approximately 40% screen failure rate) a total of 36 subjects will meet all eligibility

requirements to qualify for enrollment in Part 2 of the study. The subjects who qualify for enrollment will be randomized (5:1) to either 100 mg QD MLS-101 or placebo such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects across approximately 10 study centers in the United States.

4.4. Study Duration

It is anticipated that each subject's duration of participation in Part 1 will be approximately 16 weeks (up to 2 weeks of pre-Screening followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 4-week follow-up period). For Part 2, subject's duration of participation will be approximately 16 weeks (up to 4 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 2-week follow-up period). Total duration of the study will be approximately 21 months which includes periods for site activation, subject recruitment, treatment, and follow-up.

4.5. Justification for Dose

This Phase 2 study will evaluate both QD and BID dosing across a total daily dose of 12.5 mg to 100 mg for 8 weeks of treatment to determine if there is a dose-response on BP with increasing doses. The dose range chosen was based on the Phase 1 study findings. It is considered to be a wide enough dose range to examine whether a dose-dependent response on BP can be observed, and to identify the minimum effective and maximum tolerated doses. Further, the study will examine if BID dosing may be a better dosing regimen to sustain reductions in aldosterone over a 24-hour period, reduce any concomitant rise in trough aldosterone levels, and ultimately lead to a greater reduction in BP in patients, whereas QD dosing may provide for a break in aldosterone inhibition within a 24-hour cycle to minimize changes in serum potassium without compromising blood pressure efficacy.

The Phase 1 multiple ascending dose study demonstrated comparable physiological effects on mineralocorticoid-mediated renal tubular potassium reabsorption, a surrogate for sodium excretion and diuresis, at all dose levels tested from 40 mg QD through 360 mg QD. Based on these results, the upper end of the dose range to be tested in this study includes 50 mg QD, which is predicted to be efficacious at lowering BP, and 100 mg QD, which should result in a somewhat longer period of Cyp11B2 inhibition (10 hours versus 14 hours) while still providing a period of time during which the kidney can escape from inhibition and perform the aldosterone-mediated function of regulating potassium homeostasis. The lower end of the dose range, 12.5 mg QD, was selected based on in vitro potency and the ability to inhibit Cyp11B2 for at least 6 hours per day. This dose is anticipated to be submaximally effective in most individuals. The intermediate dosing regimens of 12.5 mg BID and 25 mg BID were selected to define the benefit/risk of BID versus QD dosing regimens with 12.5 mg BID and 25 mg BID providing temporarily more consistent inhibition of Cyp11B2. A PK/PD model was used to simulate steady state profiles and noncompartmental exposure parameters under several potential Phase 2 regimens. Details of the PK/PD model are included in the Investigator's Brochure (IB). Based on additional modeling, 100 mg BID and 300 mg QD dosing regimens are not anticipated to be necessary to achieve the PD targets.

The doses that are evaluated in this study will inform dose selection for further study in a randomized, double-blind, active-controlled Phase 2/3 study.

4.6. End of Study Definition

The end of study is defined as the date of the last visit of the last participant in the study. A participant is considered to have completed the study if he/she has completed all periods of the study including Visit 16 (end-of-study visit) at Study Week 12 and Study Week 10 for Parts 1 and 2, respectively.

At the time of study completion or early discontinuation, subjects will be queried regarding any AEs, concomitant medications, and concurrent procedures, which will be recorded on the appropriate page(s) of the source documents and electronic case report form (eCRF). At the final study visit, subjects with ongoing AEs that are considered by the investigator to be serious, study drug-related, or associated with the target area will be requested to continue to follow up with the investigator until the AE resolves, is stable, or follow-up is no longer possible/necessary. Any death that occurs while on study or within 30 days (Part 1) and 15 days (Part 2) after the subject's last dose of study drug must be reported to the sponsor/designee for the study within 24 hours after the center becomes aware of the event.

4.7. Criteria for Study Termination

Both the sponsor and the investigator reserve the right to terminate the study according to the study contract. The sponsor may issue a protocol amendment or discontinue the study entirely, based on regulatory authority or IRB/Independent Ethics Committee (IEC) recommendations, drug safety or availability concerns, discontinuation of the development program for MLS-101, or at the sponsor's discretion with at least 30 days' notice. The DSMB may also recommend study termination based on a review of the safety data.

4.8. Study Conduct

A schedule of study assessments is presented in Table 1. Details of study assessments are provided in Sections 4.8.1 to 4.8.15 and Section 8. Subjects should be in a fasted state for all study visits in Part 2 unless there is a medical reason not to fast as determined by the investigator.

4.8.1. Pre-Screening (Visit 1, Study Days -28 to -14 for Part 1 or -42 to -14 for Part 2)

Subjects with a history of hypertension, ie, SBP ≥ 135 mmHg, who meet the protocol definition for stable background treatment defined as ≥ 2 antihypertensives (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses for at least 4 weeks prior to signing the pre-Screening informed consent will enter up to 2 weeks of pre-Screening for Part 1 and 4 weeks of pre-Screening for Part 2 upon completion of the IRB-approved pre-Screening informed consent and the following procedures will be performed:

- Review medical history
- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)

- Blood draw – blood samples for aldosterone and PRA to be collected in the morning after at least 8 hours of fasting

4.8.2. Screening/Start of Placebo Run-in (Visit 2/Visit 3, Study Day -14 ± 2 Days)

All subjects with PRA and serum aldosterone values meeting the pre-Screening criteria (ie, PRA ≤ 1 ng/mL/h and aldosterone ≥ 1 ng/dL for Part 1; PRA > 1 ng/mL/h and aldosterone ≥ 1 ng/dL for Part 2; or alternative target guidance provided by the sponsor) will be eligible to enter Screening/Placebo Run-in. The investigator (or appropriate delegate at the investigator site) will obtain Screening informed consent from each subject and the following procedures will be performed:

- Inclusion/exclusion assessment
- Review medical history
- Physical examination
- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Standing BP assessment (Appendix 12.5)
- Standard 12-lead electrocardiogram (ECG); ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory
- Pregnancy test (serum; women of childbearing potential only; follicle stimulating hormone to confirm postmenopausal status in postmenopausal women)
- Blood draw (Table 6)
    - Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected.
    - Blood samples for hematology, chemistry, and coagulation tests (coags) will be collected, including hemoglobin A1c concentration and ACTH
    - Blood samples for ACTH stimulation test (Appendix 12.7)
- ACTH stimulation test (may be waived with sponsor's approval)
- Urine collection for urinalysis (Table 6)
- Review of concomitant medications

Once all Screening procedures are complete, subjects immediately enter the single-blind run-in period of BID (Part 1) or QD (Part 2) oral treatment with placebo while continuing to remain on stable doses of their background antihypertensive medications.

4.8.3. Single-blind Placebo Run-In Week 2 (Visit 4, Study Day -7 ± 2 Days)

Subjects will return to the research facility at the beginning of Week 2 of the run-in period for the following procedures:

- Inclusion/exclusion assessment (based on Screening assessment results from V2/V3). If Screening results are available, and subject is determined to be ineligible for the study, the site will review AEs, concomitant medications, and study drug compliance. Vital signs and AOBP will not be performed.
- Vital signs (body temperature, heart rate, respiratory rate) only if subject is eligible for the study or if Screening results are not yet available

- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) only if subject is eligible for the study or if Screening results are not yet available
- Review of AEs, concomitant medications, and study drug compliance for all subjects regardless of eligibility status

Subjects will be evaluated for eligibility based on Screening results and if eligible, or if Screening results are not available, subjects will continue on Week 2 of Placebo Run-in and will be given an ABPM device and instructions on how to perform the ABPM procedure by the investigator to take home for use on Study Day 0, approximately 24 hours before Randomization (Study Day 1). Note: only subjects who have Screening results and are considered eligible should be provided with the ABPM for home use. Subjects who do not have Screening results available should attend Visit 5. In addition, subjects will be given either a spot urine collection kit for Part 1 or a 24-hour urine collection kit for Part 2 to take home for use on Study Day 1. In Part 1 of the study, first morning urine will be collected prior to morning dose of study drug on Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels. In Part 2 of the study, 24-hour urine collection will be performed on Study Day 1 and again at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone levels.

4.8.4. Telephone or Office Visit (Visit 5, Study Day 0)

After completion of the single-blind, Placebo Run-in period, subjects will participate in a telephone or office visit on Study Day 0 in which they will be reminded how to perform the ABPM and spot urine collection procedures by the investigator or study coordinator. If Screening results were not available at Placebo Run-in Visit 4, subjects should participate in an office visit to review eligibility. If eligible, ABPM procedure will be initiated. The following procedures will be performed:

- Inclusion/exclusion assessment (if necessary)
- 24-hour ABPM

4.8.5. Randomization/Baseline (Visit 6, Study Day 1)

After first morning spot (Part 1) or 24-hour (Part 2) urine collection at home, subjects will return to the clinic on Study Day 1 (morning visit), and those who continue to meet all eligibility requirements will be randomized to MLS-101 or placebo and the following procedures performed prior to administration of the initial morning dose of study drug:

- Inclusion/exclusion assessment
- Physical examination
- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Standing BP assessment (Appendix 12.5)
- Spot urine collection (**Part 1 only**)
- 24-hour urine collection (**Part 2 only**)

- Standard 12-lead ECG; ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory
- Pregnancy test (urine; women of childbearing potential only)
- Blood draw (Table 6)
    - Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected
    - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration and ACTH
    - Single blood samples for determination of MLS-101 will be collected. Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected.
    - Serial PK sampling – if subject consents to additional blood draws, blood samples for determination of MLS-101 (and metabolites) are to be collected predose, and 1, 2, 3, and 4 hours postdose.
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.6. Study Week 1 (Visit 7, Study Day 7 ± 2 Days)

On Study Day 7 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Standing BP assessment (Appendix 12.5)
- Blood draw (Table 6)
    - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH
    - Single blood samples for determination of MLS-101 will be collected. Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected.
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.7. Study Week 2 (Visit 8, Study Day 14 ± 2 Days)

On Study Day 14 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Blood draw - blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (Table 6)
- Urine collection for urinalysis (Table 6)

- Review of AEs, concomitant medications, and study drug compliance

4.8.8. Study Week 3 (Visit 9, Study Day 21 ± 2 Days)

On Study Day 21 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Blood draw – blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (Table 6)
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.9. Study Week 4 (Visit 10, Study Day 28 ± 2 Days)

On Study Day 28 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Initiate 24-hour ABPM (**Part 2 only**)
- 24-hour urine collection (**Part 2 only**)
- Blood draw (Table 6)
    - Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected
    - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration and ACTH
    - Single blood samples for determination of MLS-101 will be collected. Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected.
    - Serial PK sampling – if subject consents to additional blood draws, blood samples for determination of MLS-101 (and metabolites) are to be collected predose, and 1, 2, 3, and 4 hours postdose.
- Urine collection for urinalysis (Table 6)
- Pregnancy test (urine; women of childbearing potential only)
- Review of AEs, concomitant medications, and study drug compliance

4.8.10. Study Week 5 (Visit 11, Study Day 35 ± 2 Days)

On Study Day 35 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)

- Blood draw – blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (Table 6)
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.11. Study Week 6 (Visit 12, Study Day 42 ± 2 Days)

On Study Day 42 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Blood draw – blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (Table 6)
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.12. Study Week 7 (Visit 13, Study Day 49 ± 2 Days)

On Study Day 49 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Initiate 24-hour ABPM
- Blood draw – blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH (Table 6)
- Urine will be collected for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.13. Study Week 8 (Visit 14, Study Day 56 ± 2 Days)

At the Study Week 8 visit (Visit 14), subjects will begin 24-hour ABPM procedure. On Study Day 56 ± 2 days, subjects will return to the site and the following procedures will be performed prior to site administration of the morning dose of study drug:

- Physical examination
- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Standing BP assessment (Appendix 12.5)
- Repeat 24-hour ABPM if procedure at Study Week 7 deemed a failure
- Spot urine collection (**Part 1 only**)
- Standard 12-lead ECG; ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory
- Pregnancy test (urine; women of childbearing potential only)
- Blood draw (Table 6)

  - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration, cortisol, and ACTH
  - Single blood samples for determination of MLS-101 will be collected. Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected.
  - Blood samples for ACTH stimulation test (Appendix 12.7)
- ACTH stimulation test (may be waived with sponsor's approval)
- Urine collection for urinalysis (Table 6)
- Review of AEs, concomitant medications, and study drug compliance

4.8.14. Telephone Visit (Visit 15, BP check; Study Day 59 + 2 Days)

Subjects will complete a BP check approximately 3 days post last dose of study drug. On Study Day 59 ± 2 days, subjects will participate in a telephone visit during which they will perform the following procedure at home:

- BP check

4.8.15. Study Week 10 (Part 2 Only: Visit 16, End of Study; Study Day 70 ± 3 Days)

On Study Day 70 ± 3 days, subjects will return to the site for an end-of-study visit scheduled 2 weeks after the last treatment with study drug and the following procedures will be performed:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Blood draw (Table 6)
  - Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected
  - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration and ACTH
- Urine will be collected for urinalysis (Table 6)
- Review of AEs and concomitant medications

4.8.16. Study Week 12 (Part 1 Only: Visit 16, End of Study; Study Day 84 ± 3 Days)

On Study Day 84 ± 3 days, subjects will return to the site for an end-of-study visit scheduled 4 weeks after the last treatment with study drug and the following procedures will be performed:

- Vital signs (body temperature, heart rate, respiratory rate)
- AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)
- Blood draw (Table 6)
  - Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected
  - Blood samples for hematology, chemistry, and coags will be collected, including hemoglobin A1c concentration and ACTH

- Urine will be collected for urinalysis (Table 6)
- Review of AEs and concomitant medications

4.9. Modifications to Study Conduct Due to the Coronavirus Disease 2019 (COVID-19) Pandemic

As a consequence of the COVID-19 pandemic that has had a worldwide impact, control measures in place may impact the ability to adhere to some of the study procedures described in this protocol. Due to challenges that include, but are not limited to, subject COVID-19 infections, site closures, travel restrictions, and quarantines, some modifications to study conduct during the COVID-19 pandemic may be necessary to ensure study continuity, including conducting virtual visits when on-site study visits are considered not feasible. Such modifications in study conduct always must be in accordance with local regulations/mandates.

The following are allowable, as necessary, modifications to study conduct during the COVID-19 pandemic:

- Prior to a study visit at the site, the subject may be contacted and screened for potential exposure or infection to COVID-19 per site, local, or federal requirements. If the subject is suspected to be exposed or infected with COVID-19, the on-site visit should either be re-scheduled or a virtual visit may be performed instead, as applicable.
- In the event that a subject cannot attend their regularly scheduled study visits in person due to COVID-19 necessitating a limit on in-person contact, the investigator may perform safety and efficacy assessments by phone or video. The investigator may use the technology platform that is currently available to them. Virtual assessments may include home blood pressure evaluation initiated by the subject, adverse events, and concomitant medication review. Virtual assessments will be recorded by site staff in the source documents.
- Clinical laboratory tests (chemistry and hematology) and pregnancy tests may be performed by local laboratory, if sample collection cannot be performed at the study site due to COVID-19 related limitations, including but not limited to site closure. Abnormal laboratory results should be promptly communicated to the medical monitor. Subjects' anonymity must be maintained when communicating results to the medical monitor.
- At home IP administration may continue for up to 2 weeks (at multiple times during the study if required due to COVID-19, although not consecutively) if the subject has no relevant clinically significant out of range values per previous lab reports.
- Source documentation should note that the visit was performed virtually (not face-to-face) and note the name of the local lab where laboratory tests were done, if applicable.
- If certain study procedures or assessments cannot be completed per the schedule of events, the reason for the missed assessment (ie, laboratory tests, vital signs, physical examinations, etc) must be noted in the source documentation (eg, COVID-19), captured in the protocol deviations documentation, and reported to the IRB, as applicable.

A detailed assessment of COVID-19 related risk and mitigation measures will be documented in the appropriate study plans.

5. STUDY POPULATION

5.1. Inclusion Criteria

1. Male and nonpregnant, nonlactating female subjects ≥ 18 years of age. Fertile male and female subjects and their partners must agree to use either highly effective or acceptable methods of contraception as defined in Appendix 12.8 from Screening to 90 days post last dose of study drug
2. Written informed consent Health Insurance Portability and Accountability Act authorization, and local patient privacy required documentation for this study have been obtained
3. Average of AOBP measurements taken at Screening/start of the Placebo Run-in Visit and Randomization with SBP ≥ 130 mmHg
4. Background antihypertensive treatment of ≥ 2 drugs (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses for at least 4 weeks prior to signing the Screening/main study ICF
5. For Part 1, inclusion based on morning pre-Screening visit measurement of PRA, and the value for PRA must be ≤ 1 ng/mL/h. For Part 2, inclusion based on morning pre-Screening visit measurement of PRA, and the value of PRA must be > 1 ng/mL/h. Alternative target PRA criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of PRA values.
6. Inclusion based on morning pre-Screening visit measurement of serum aldosterone for Part 1 and pre-Screening visit measurement of aldosterone for Part 2. The value for aldosterone must be ≥ 1 ng/dL based on morning measurement. Alternative target aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of serum aldosterone values.
7. Serum cortisol ≥ 18 mcg/dL (morning measurement) or serum cortisol > 3 and < 18 mcg/dL (morning measurement) at Screening/start of Placebo Run-in visit if there is no evidence of adrenal insufficiency based on normal ACTH stimulation test results. In the event that ACTH stimulation testing cannot be performed due to logistical challenges, including supply chain limitations to availability of synthetic ACTH, then subjects may be entered into the trial if morning serum cortisol is > 3 mcg/dL and they fulfill the criteria for no recent use of exogenous corticosteroid medications in dosages that could potentially cause adrenal suppression (ie, Exclusion Criteria 16a).
8. Women of childbearing potential must have a negative serum pregnancy test prior to Randomization
9. Willing and able to comply with the study instructions and attend all scheduled study visits

5.2. Exclusion Criteria

1. Concomitant use of epithelial sodium channel inhibitors or mineralocorticoid receptor antagonists, including, but not limited to amiloride, triamterene, spironolactone, eplerenone
2. Use of ACE inhibitors and ARBs in combination (ie, concomitant ACE inhibitors plus ARB therapy) as background antihypertensive treatment
3. Subjects with hypokalemia, ie, serum potassium < 3.0 mEq/L at Screening
4. Subjects with hyperkalemia, ie, serum potassium > 5.2 mEq/L at Screening for Part 1 and > 4.8 mEq/L at Screening for Part 2
5. Subjects with serum cortisol < 3 mcg/dL based on morning measurement at Screening
6. Subjects with serum sodium < 135 mEq/L at Screening
7. Subjects with estimated glomerular filtration rate < 60 mL/min/1.73m^2 at Screening
8. Subjects with type 1 or uncontrolled (hemoglobin A1c ≥ 9%) type 2 diabetes mellitus
9. Subjects with body mass index > 40 kg/m^2
10. Subjects with unstable angina who are taking short-acting oral or sublingual nitroglycerin or who have history of myocardial infarction or stroke within 6 months of Screening, sustained atrial fibrillation, ie, lasting > 12 months, or permanent atrial fibrillation; paroxysmal atrial fibrillation that terminates spontaneously or with intervention within 7 days is allowed. Chronic long-acting oral nitrates that have been stable at their prescribed doses for at least 2 weeks prior to signing the Screening/main study ICF are allowed.
11. Subjects with office SBP ≥ 175 mmHg or DBP ≥ 100 mmHg for Part 1 and SBP ≥ 160 mmHg or DBP ≥ 100 mmHg for Part 2 (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) at Pre-Screening, Screening/Start of Placebo Run-in, or Randomization
12. Subjects with a decrease in SBP ≥ 20 mmHg or DBP ≥ 10 mmHg from sitting to standing position at Screening (may be repeated during Screening at the investigator's discretion)
13. Subjects who, in the opinion of the investigator, have suspected nonadherence to antihypertensive treatment
14. Subjects who, in the opinion of the investigator, have any major medical illness or symptoms of a clinically significant illness that may limit their ability to complete the study or influence subject safety and/or the study outcome (eg, active malignancy, HIV infection)
15. Subjects who, in the opinion of the investigator, have any acute or chronic medical or psychiatric condition or laboratory abnormality that would make them unsuitable for participation in this study or that would place the subject at undue risk (eg, history of

drug, alcohol, or other substance abuse) or who have other factors limiting the ability of the subject to cooperate and to comply with this protocol

16. Subjects undergoing treatment with any of the following medications:
    a. Chronically administered oral or topical corticosteroids within 3 months of Screening or during study participation. Short-term (ie, ≤ 2 weeks) of topical corticosteroids are allowed if taken ≥ 1 month prior to Randomization.
    b. Sympathomimetic decongestants within 1 week of Screening or during study participation
    c. Theophylline unless treatment has been stable at optimum dose for at least 4 weeks prior to Screening and remains stable during study participation
    d. Regular use of phosphodiesterase type 5 inhibitors within 3 months of Screening or during study participation
    e. Chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs), other than low dose aspirin (81-325 mg), where chronic use is defined as > 3 consecutive or nonconsecutive days of treatment per week, within 2 weeks of Screening. Note: intermittent use of NSAIDs is strongly discouraged; if required, NSAIDs must not be taken more than a total of 2 days during the study (defined as the period from Randomization to the end of study period). If analgesics are required, acetaminophen is recommended.
    f. Intramuscular steroids within 3 months of Screening or during study participation
    g. Estrogen- or progesterone-containing oral contraceptives or implantable progesterone devices
    h. Strong CYP3A and CYP3A4 inhibitors (eg, clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir)
    i. Strong CYP3A and CYP3A4 inducers (eg, apalutamide, carbamazepine, enzalutamide, fosphenytoin, lumacaftor, mitotane, phenobarbital, phenytoin, primidone, rifampin, St. John's Wort)
17. Subjects with known hypersensitivity to MLS-101 or any of the excipients
18. Subjects who are night-shift workers
19. Subjects who are unable to comply with protocol requirements, including treatment compliance and instructions for ABPM
20. Subjects who are currently enrolled in an investigational drug or device study or have used an investigational drug or an investigational device treatment within 4 weeks of Screening (Note: Subjects who are enrolled in a long-term follow-up study and are not actively receiving an investigational drug or investigational device treatment may be eligible for participation in this study)

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

Dietary counseling reflecting the current standard of care will be provided to all subjects at each visit according to the instructions described in Appendix 12.6.

5.3.2. Caffeine, Alcohol, and Tobacco

- Subjects will abstain from ingesting caffeine- or xanthine-containing products (eg, coffee, tea, cola drinks, and chocolate) for 30 minutes before the start of BP measurement and 12hours before the start of dosing until after collection of the final PK (on Study Days 1, 7 ± 2, and 28 ± 2) and/or PD (at Pre-Screening, Screening/start of Placebo Run-in, and on Study Days 1, 28 ± 2, and 84 ± 3) sample.
- Subjects will abstain from alcohol for 30 minutes before the start of BP measurement and 24 hours before the start of dosing until after collection of the final PK (on Study Days 1, 7 ± 2, and 28 ± 2) and/or PD (at Pre-Screening, Screening/start of Placebo Run-in, and on Study Days 1, 28 ± 2, and 84 ± 3).
- Subjects who use tobacco products will be instructed that use of nicotine-containing products (including nicotine patches or gums and e-cigarettes) will not be permitted while they are in the clinical unit. Subjects will abstain from smoking for 30 minutes before the start of BP measurement.

5.3.3. Activity/Exercise

Subjects will abstain from strenuous exercise for 30 minutes before the start of BP measurement and for 24 hours before each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities (eg, watching television, reading).

5.4. Screen Failures

Screen failures are defined as study participants who consent to participate in the clinical study, but are not subsequently randomly assigned to treatment/entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE). Individuals who do not meet the Screening criteria for participation in this study (screen failures) may be rescreened up to 3 times.

6. STUDY DRUG ADMINISTRATION AND MANAGEMENT

6.1. Study Drug Administration

6.1.1. Description of Study Drug

The study drug product is an immediate release formulation containing standard excipients generally recognized as safe including bulking agent, disintegrant, glidant, and lubricant that are compressed into a tablet. The MLS-101 tablet contains 12.5 to 100 mg of MLS-101 drug substance (as a free-base form: [redacted]). The MLS-101 tablet and matching placebo tablet are round, white, film-coated tablets with no identifying mark. MLS-101 tablets of 12.5 mg, 25 mg, and 100 mg and placebo tablets are packaged in either 30 count 60 cc white round high density polyethylene bottles with a child-resistant cap or polyvinyl chloride/Aclar blisters with foil backing and 7 tablets per blister strip. Packaged tablets will be assigned a unique identifying number, used by the interactive web response system (IWRS) to identify the content but maintaining the blind of the subject and site. The test materials will be identified by the lot, batch numbers, retest date, and certificate of analysis.

6.1.2. Dosing and Administration

Placebo Run-In: In Part 1, upon signing the Screening/main study ICF, subjects will begin a single-blind (subjects blinded to treatment allocation), up to 2-week run-in period of BID oral treatment with placebo, while continuing to remain on stable doses of their background antihypertensive medications. Subjects will orally administer placebo morning and evening, approximately 12 hours apart. On site visit days, subjects should be instructed to take their morning dose of study drug at the site. In Part 2, upon signing the Screening/main study ICF, subjects will begin a single-blind (subjects blinded to treatment allocation), up to 2-week run-in period of QD oral treatment with placebo, while continuing to remain on stable doses of their background antihypertensive medications.

8-Week Treatment Period: In Part 1, subjects will orally administer the assigned study drug (MLS-101 [25 mg BID, 50 mg QD, 100 mg QD] or placebo) according to the assigned dosing regimen for 8 weeks beginning on Study Day 1. To maintain the integrity of the blind, all subjects (regardless of dosing group) will orally administer study drug morning and evening, approximately 12 hours apart; active drug is administered as the morning dose for all QD dose groups. Subjects will be monitored by ABPM for 24 hours before the first morning dose on Study Day 1 and for 24 hours after the morning dose on Study Day 49 ± 2 days (end of Week 7). On site visit days, subjects should be instructed to take their morning dose of study drug at the site. In Part 2, subjects will orally administer the assigned study drug (MLS-101 [100 mg QD] or placebo) according to the assigned dosing regimen for 8 weeks beginning on Study Day 1. Subjects will be monitored by ABPM for 24 hours before the first morning dose on Study Day 1, for 24 hours beginning on Study Day 28 ± 2 days (Week 4), and for 24 hours after the morning dose on Study Day 49 ± 2 days (end of Week 7).

6.2. Preparation, Handling, Storage, and Accountability

6.2.1. Study Drug Preparation

Not applicable.

6.2.2. Study Drug Packaging and Labeling

PCI Clinical Services will label, pack and release study drug in accordance with the Clinical Trials Directive 2001/20/EC and Good Manufacturing Practice Directive 2003/94/EC for Investigational Medicinal Products.

As this is a double-blind study, labeling of the tablet package will not show the treatment allocation. All other information required by regulation will appear on the label.

6.2.3. Study Drug Handling and Disposal

All study drug supplies are to be used only for this clinical study and not for any other purpose. Authorized study personnel must maintain a full record of study drug disposition (ie, a log by date received, dispensation date and subject, date of return from the subject, and detailed study drug usage by each study subject).

After reconciliation, used study drug will be returned according to instructions provided by the sponsor. At study conclusion, all remaining used supplies will be similarly returned and disposed. Authorized study personnel will record any unaccounted supplies in the final accountability records.

6.2.4. Study Drug Storage

The study drug should be stored at room temperature (59°F to 77°F [15°C to 25°C]) in an approved storage area with access limited to authorized study personnel.

6.2.5. Study Drug Accountability

Study drug (including placebo) will be maintained under adequate security by appropriate site personnel (eg, pharmacist) and in accordance with applicable regulatory requirements. Study drug remaining at the end of the study will be returned to the sponsor or their representative, or destroyed on behalf of the sponsor.

The investigator or designee must maintain adequate records of receipt and distribution of all study drug, using appropriate accountability records.

6.3. Randomization and Blinding

All subjects who qualify for Part 1 based on inclusion/exclusion criteria and complete up to a 2-week Placebo Run-in period and 24-hour ABPM procedure will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo; each treatment group will consist of approximately 30 subjects stratified by BP (seated SBP ≤ 160 mmHg and seated SBP > 160 mmHg). All subjects who qualify for Part 2 based on inclusion/exclusion criteria and complete up to a 2-week Placebo Run-in period and 24-hour ABPM procedure will be randomized (5:1) to either 100 mg QD MLS-101 or placebo such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.. The sponsor will arrange for Randomization of study subjects by study drug dosage group and treatment assignment. Randomization numbers and study drug supplies will be assigned using IWRS. Access to the Randomization codes will be controlled and documented.

The study will be conducted in a double-blind manner so that neither the center personnel nor the subject is aware of which treatment is administered. The identity of study drug assignments (MLS-101 or placebo) will be concealed from the investigator, the subject, research center staff, and all personnel involved in the conduct of the study, with the exception of the unblinded sponsor drug management staff who will oversee the study drug allocations and IWRS/dispensing compliance at the study centers.

Except when it is essential for the medical management of the subject, unblinding the treatment assignment will be considered a protocol deviation. Emergency treatment allocation information will be available by secured access to the IWRS system. If possible, the investigator should contact the sponsor before unblinding any subject's treatment assignment. In the event that this is not possible, the sponsor should be notified within 24 hours after the unblinding event. After unblinding, the subject will be discontinued from study drug treatment and will undergo Study Week 8 (end-of-treatment) assessments. Subjects will be asked to return for final follow-up approximately 4 weeks later (ie, Study Week 12/end-of-study visit). The reason for unblinding must be documented in the eCRF.

6.4. Study Drug Compliance

Compliance with study drug will be assessed at each visit. Compliance will be assessed by direct questioning and counting returned tablets during the site visits and documented in the source documents and relevant case report form. Deviation(s) from the prescribed dosage regimen should be recorded.

A record of the quantity of study drug dispensed to and administered by each subject must be maintained and reconciled with study intervention and compliance records. Study drug start and stop dates, including dates for study drug delays and/or dose reductions will also be recorded.

6.5. Dose Modification

In general, if, at any time during the study, a subject meets 1 or more of the laboratory exclusion criteria for the study (eg, seated SBP ≥ 175 mmHg or DBP ≥ 100 mmHg, serum potassium > 5.2 mEq/L, serum cortisol < 3 mcg/dL), laboratory test values will be confirmed using a local lab and BP measurements will be repeated at home. If laboratory exclusion criteria are met, the subject's dose of study drug may be temporarily withheld, adjusted, or stopped by the investigator in consultation with the medical monitor. The subject will receive appropriate antihypertensive treatment if clinically indicated, and will remain in the study for further safety monitoring. Guidelines for dose reduction/stopping in the management of hyperkalemia are included in Section 6.5.1. All subjects will be given dietary counseling to avoid foods high in potassium during the study (Appendix 12.6). Guidelines for the management of hypotension are included in Section 0. In addition, serum cortisol levels will be monitored throughout the study, and all subjects will be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every study visit. Based on changes in cortisol levels, an ACTH stimulation test (Appendix 12.7) may be performed and the subject's dose of study drug may be stopped. Guidelines for potential dose discontinuation in the management of adrenal insufficiency are included in Section 6.5.3.

6.5.1. Hyperkalemia

If serum potassium level is elevated at the central laboratory measurement, the following actions are to be taken:

- Serum potassium > 5.2 and ≤ 5.5 mEq/L: Retest at local lab to confirm. If potassium remains elevated, reduce the MLS-101 dose according to Table 3 (first dose-reduced level). Wait 2 to 7 days, then retest. If potassium remains elevated, reduce the MLS-101 dose again according to Table 3 (second dose-reduced level).
- Serum potassium > 5.5 and ≤ 6 mEq/L: Retest at local lab to confirm. If potassium remains elevated, hold dose. Wait 2 to 7 days, then retest. If potassium ≤ 5.2 mEq/L, resume dosing. If potassium > 5.2 mEq/, continue to hold dose. Wait 2 to 7 days, then retest. If potassium ≤ 5.2 mEq/L after second retest, restart at first dose-reduced level according to Table 3. If potassium > 5.2 mEq/L after second retest discontinue study drug.
- Serum potassium > 6 mEq/L: Hold study drug dose and bring subject in for an unscheduled study visit for safety ECG and serum potassium retest at local lab; subject attends next regularly scheduled visit. If potassium from local lab retest ≤ 5.2 mEq/L, resume dosing. If potassium > 5.2 mEq/L, contact the sponsor's medical monitor for further guidance.

Note: If a retest of serum potassium level falls into a higher range than the original test, follow the instructions for the higher potassium range.

Table 3: MLS-101 Dose Reduction Schedule

Assigned Dose Level	First Dose-reduced Level	Second Dose-reduced Level
25 mg BID	12.5 mg BID	0
50 mg QD	12.5 mg QD	0
100 mg QD	50 mg QD	25 mg QD

BID = twice daily; QD = once daily

6.5.2. Hypotension

6.5.2.1. Orthostatic Hypotension

Standardized assessment of standing BP will be performed at Screening/start of Placebo Run-in, Randomization, Study Week 1, and Study Week 8 as described in Appendix 12.5. In addition, assessment of standing BP will be performed if a subject reports symptoms of hypotension (eg, light-headedness, dizziness, presyncope, or syncope) during the study, and the standing BP measurement will be assessed weekly for these subjects until symptoms resolve.

Orthostatic hypotension is defined as a decrease in SBP ≥ 20 mmHg or DBP ≥ 10 mmHg from sitting to standing position within 3 minutes of standing. If subjects experience orthostatic hypotension with symptoms (eg, light-headedness, dizziness, presyncope, or syncope) during the study, it will be medically managed by the investigator; adjustment to background therapy and/or dose reduction of study drug according to Table 3 may be considered.

If subjects experience orthostatic hypotension during Screening/start of Placebo Run-in, measurement may be repeated during the Screening period to be eligible for study enrollment, and background therapy may be adjusted at the investigator's discretion.

6.5.2.2. Symptomatic Hypotension

If subjects experience symptoms of hypotension (eg, light-headedness, dizziness, presyncope, or syncope) during the study, it will be medically managed by the investigator; adjustment to background therapy and/or dose reduction of study drug according to Table 3 may be considered.

6.5.3. Adrenal Insufficiency

Serum cortisol levels will be measured at Screening/start of Placebo Run-in, Randomization, Study Weeks 1, 2, 3, 4, 5, 6, 7, 8, and at the follow up visit (Study Week 12). Cortisol assessment should occur before 10 am on the morning of the study visit. In addition, subjects should be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every study visit. If adrenal insufficiency is suspected, glucocorticoid replacement therapy should be initiated until adrenal insufficiency can be ruled out.

Based on morning cortisol level at the central laboratory measurement, the following actions are to be taken:

- Serum cortisol ≥ 18 mcg/dL: No action required.
- Serum cortisol > 3 and < 18 mcg/dL: If there are no signs or symptoms of adrenal insufficiency (see above), then no action is required. If signs or symptoms of adrenal insufficiency are observed, then an ACTH stimulation test should be performed as described in Appendix 12.7 to rule out adrenal insufficiency. If there is no evidence of adrenal insufficiency based on ACTH stimulation test results, then continue at current dose. If ACTH stimulation test is abnormal, then study drug should be discontinued. ACTH stimulation test may be waived with sponsor's approval.
- Serum cortisol < 3 mcg/dL: If at any time during the study serum cortisol < 3 mcg/dL, then measurement should be repeated. If confirmed, then an ACTH stimulation test should be performed as described in Appendix 12.7 to rule out adrenal insufficiency. If there is no evidence of adrenal insufficiency based on ACTH stimulation test results, then continue at current dose. If ACTH stimulation test is abnormal, then study drug should be discontinued. ACTH stimulation test may be waived with sponsor's approval.

Any subject who is withdrawn from the study or has low cortisol levels at the final study visit should be evaluated until full normalization of the hypothalamic-pituitary-adrenal axis occurs. In addition, all subjects should be instructed to carry a card at all times identifying the potential risk for adrenal insufficiency. The card should include the subject's information, the investigator's contact information, and the potential need for glucocorticoids in the settings of shock, surgery, etc.

6.6. Treatment of Overdose

For this study, any dose of study drug greater than 300 mg within a 24-hour time period will be considered an overdose.

In the event of an overdose, the investigator should:

- Contact the medical monitor immediately
- Evaluate the subject to determine, in consultation with the medical monitor, whether study drug should be interrupted or whether the subsequent study dose should be reduced
- Closely monitor the participant for any AE/SAE and laboratory abnormalities

6.7. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

MLS-101 may alter the clearance of drugs that rely on multidrug and toxin extrusion 1 (MATE1) transport for clearance. While the diabetic medication metformin is transported by MATE1, it can also be cleared by MATE2 and organic cation transporter; thus, significant changes in metformin bioavailability are thought to be unlikely. However, it is prudent to periodically monitor blood glucose levels as per protocol in study subjects taking metformin. Clinically significant changes in glucose control should be considered possibly related to study drug and treated accordingly with laboratory confirmation and appropriate medical interventions deemed appropriate by the site investigator and other treating healthcare providers. The medical monitor should be consulted if discontinuation of study drug is considered.

6.7.1. Prohibited Treatments

The following medications are prohibited:

- Concomitant use of epithelial sodium channel inhibitors or mineralocorticoid receptor antagonists, including, but not limited to amiloride, triamterene, spironolactone, eplerenone
- ACE inhibitors and ARBs in combination as background antihypertensive treatment
- Chronically administered oral or topical corticosteroids within 3 months of Screening or during study participation. Short-term (ie, ≤ 2 weeks) of topical corticosteroids are allowed if taken ≥ 1 month prior to Randomization.
- Sympathomimetic decongestants within 1 week of Screening or during study participation
- Theophylline unless treatment has been stable at optimum dose for at least 4 weeks prior to Screening and remains stable during study participation
- Regular use of phosphodiesterase type 5 inhibitors within 3 months of Screening or during study participation
- Chronic use of NSAIDs, other than low dose aspirin (81-325 mg), where chronic use is defined as > 3 consecutive or nonconsecutive days of treatment per week. Note: intermittent use of NSAIDs is strongly discouraged; if required, NSAIDs must not be taken more than a total of 2 days during the study (defined as the period from Randomization to the end of study period). If analgesics are required, acetaminophen is recommended.
- Intramuscular steroids within 3 months of Screening or during study participation
- Short-acting nitrates taken for angina for underlying cardiac disease. Chronic, long-acting doses of nitrates are acceptable.
- Estrogen- or progesterone-containing oral contraceptives or implantable progesterone devices

- Strong CYP3A and CYP3A4 inhibitors (eg, clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir)
- Strong CYP3A and CYP3A4 inducers (eg, apalutamide, carbamazepine, enzalutamide, fosphenytoin, lumacaftor, mitotane, phenobarbital, phenytoin, primidone, rifampin, St. John's Wort)

The decision to administer a prohibited medication/treatment during the study period is done with the safety of the subject as the primary consideration. When possible, the sponsor is to be notified before the prohibited medication/treatment is administered. If the investigator determines that any of the prohibited treatments listed above are necessary for proper care of the subject during the study, the investigator should recommend a treatment plan in consultation with the medical monitor that will allow the subject to remain in the study.

6.7.2. Permitted Treatments

Background antihypertensive treatment of ≥ 2 drugs (note: a combination pill = 2 antihypertensives) that have been stable at their prescribed doses for at least 4 weeks prior to signing the Screening/main study ICF are permitted. Any treatment other than those listed in Section 6.7.1 is allowed and should be documented in the eCRF.

7. DISCONTINUATION/WITHDRAWAL CRITERIA

7.1. Discontinuation of Study Drug

Subjects who wish to stop treatment for any reason may stop at any time during the study. If the study treatment is permanently discontinued, the subject will remain in the study to complete all assessments as described in the SoA (Table 1 and Table 2) through Study Week 8 (end-of-treatment visit). Subjects should also be encouraged to return for a final follow-up visit 4 weeks after the Study Week 8 visit for Part 1 and 2 weeks after the Study Week 8 visit for Part 2, at which the end-of-study assessments will be performed.

Details regarding subjects who discontinue study drug before Study Week 12 in Part 1 or Study Week 10 in Part 2 will be recorded on the appropriate page(s) of the eCRF. If a subject discontinues study drug because of an AE, the investigator may be requested to schedule follow-up visits until the event has resolved or stabilized (Section 8.3.3).

Reasons for discontinuing study drug may include:

- Use of prohibited/excluded medications (see Section 6.7.1)
- Administrative decision by the investigator or the sponsor
- Subject noncompliance with the study drug dosing schedule (Section 6.4)
- Development of an AE (Section 8.3)
- Unblinding of subject's study drug treatment assignment (Section 6.3)
- Subject becomes pregnant (Section 8.3.5)
- Other

7.2. Discontinuation/Withdrawal of Subjects

Subjects have the right to withdraw participation from the study at any time and for any reason and will be removed from the study upon request. However, the investigator will encourage subjects to return for subsequent observations in order to minimize the number of missing data points. If a subject is unable or unwilling to return, the reason for withdrawal will be recorded. Each subject's right to withdraw will be honored; subjects who desire no further follow-up contact will be asked to declare so in writing. Details regarding subjects who choose to discontinue study participation before Study Week 12 in Part 1 or Study Week 10 in Part 2 will be recorded on the appropriate page(s) of the eCRF.

Reasons for discontinuing/withdrawing from study may include:

- Withdrawal from participation due to subject convenience (ie, due to a change in the subject's willingness or ability to attend study visits, eg, resulting from new job, work schedule change, or move to another geographical area)
- Lost to follow-up (ie the investigator has exhausted all reasonable methods of contacting the subject, which should be documented by at least 3 unsuccessful attempts to contact the subject) (Section 7.3)
- Death
- Other

7.3. Lost to Follow Up

A subject will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, [3] telephone calls, and if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

Table 1, Table 2, and Section 4.8 outline the SoA and study conduct details, respectively, for all subjects and all study periods (Pre-Screening, Screening, Placebo Run-in, Double-blind Treatment, and Follow-up)

- Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.
- Acute safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject requires dose modification or discontinuation of study drug.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All Screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a Screening log to record details of all subjects screened and to confirm eligibility or record reasons for Screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of the Screening/main study ICF may be utilized for Screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.
- Safety results that could unblind the study will not be reported to investigative sites or other blinded personnel until the study has been unblinded.

8.1. Efficacy Assessments

8.1.1. Automated Office-Measured Blood Pressure

Accurate measurement of BP is essential to classify individuals and record study treatment effect; consequently, BP should be measured by an automatic BP device at the site that is identical for all participating sites and managed by qualified personnel. In addition, the same person should use the BP device for a given subject at each visit whenever possible, and the measurement should be performed using the same arm of the subject and the appropriate cuff size, as determined during the pre-Screening visit.

The BP must be measured at trough (ie, before study drug administration) and at approximately the same time of day (preferably between 7:00 and 10:00 am ±1 hour). The subject should be relaxed, sitting in a chair (feet on floor, back supported) for > 5 minutes. The subject should avoid caffeine, exercise, and smoking for at least 30 min before measurement, and ensure patient has emptied his/her bladder. Neither the subject nor the observer should talk during the rest period or during the measurement. Remove all clothing covering the location of cuff placement.

Details on BP procedure (measurement and transfer of data) including subject preparation (eg, arm selection, arm position, cuff size) will be provided in the BP manual and will follow the AHA guidelines.

At each timepoint indicated in the SoA, SBP and DBP will be measured 5 times using an automated oscillometric sphygmomanometer device provided by the sponsor after approximately 5 minutes of rest in the seated position according to the AHA Guidelines (Muntner 2019); the repeated measurements should be separated by 1 to 2 minutes. The last 2 of 5 unattended

measurements of SBP and DBP will be averaged for the analysis. In addition to sitting measurements, standing BP will be measured at Screening/start of Placebo Run-in, Randomization, Study Week 1, and Study Week 8 with the AOBP device to assess for hypotension according to the procedure provided in Appendix 12.5.

8.1.2. 24-hour Ambulatory Blood Pressure Monitoring

Subjects will be monitored by ABPM for 24 hours before the first dose on Study Day 1 and before the first dose on Study Day 49 ± 2 days (end of Week 7) according to the method described in the AHA scientific statement on measuring BP in humans (Muntner 2019). Blood pressure will be measured automatically every 15 to 30 minutes for a 24-hour period using an ABPM device provided to each site by the central BP laboratory or the sponsor for the duration of the study. The ABPM data will be electronically transferred to the central BP laboratory and subsequently to the contract research organization. Details on ABPM procedure (installation, recording, and transfer of data), including subject preparation, will be provided in the BP manual. At the second clinic visit of the Placebo Run-in period (Visit 4), subjects will be given an ABPM device and instructions by the investigator on how to perform the ABPM procedure at home. Subjects will participate in a telephone visit on Study Day 0 (Visit 5) in which they will be reminded of the instructions on how to perform the ABPM procedure at home by the investigator or study coordinator. The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) in Part 2 and Study Week 7 (ie, Visit 13; Study Day 49 ± 2) in Parts 1 and 2. Alternatively, sites may choose to schedule an office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure (the ABPM procedure will be initiated in the clinic at Study Weeks 4 and 7 unless a subject is not able to attend the visit due to extraordinary circumstances, such as COVID-19 restrictions including site closure, subject exposure to COVID-19, or subject testing positive for COVID-19). If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at Study Week 8. Training for the ABPM procedure can be done at an office visit or via phone.

8.2. Safety Assessments

8.2.1. Medical History

Medical history data are to be collected at the time of Pre-Screening and updated at the time of Screening.

8.2.2. Physical Examination

A complete physical examination, including height and weight, will be carried out by trained medical personnel at Screening/start of Placebo Run-in, Randomization, and at Study Week 8 (end of treatment).

8.2.3. Vital Signs

Vital signs including body temperature, heart rate, and respiratory rate are to be recorded at each study visit throughout the study. All measurements will be assessed with the subject in a seated position. Subjects should be seated for at least 5 minutes before taking the measurement. Pulse should be measured in the brachial/radial artery for at least 15 seconds.

8.2.4. Electrocardiogram

Single 12-lead ECGs will be recorded at Screening/start of Placebo Run-in, Randomization (Baseline), and at the Study Week 8 (end-of-treatment) visit. Parameters to be recorded include PR, QRS, and QT intervals, and corrected QT interval using Fridericia's formula.

Measurements will be taken after the subject has rested for at least 10 minutes in a supine position using a 12-lead (I, II, III, aVR, aVL, aVF, V1-V6) ECG system over a duration of 10 seconds. The chart speed will be set at 25 mm/sec. ECG changes consistent with hyperkalemia will require confirmation of potassium levels at a local laboratory.

8.2.5. Clinical Safety Laboratory Tests

Clinical safety laboratory tests will be conducted by a central laboratory according to the time points specified in Table 1 and Table 2. Instructions for collection, preparation, handling, and shipping clinical laboratory specimens are provided in the Laboratory Manual for the study. Analytes for clinical safety labs are listed in Table 6 (Appendix 12.9).

8.2.6. Spot Urine and 24-Hour Urine Collection

Subjects will be given either a spot urine collection kit for Part 1 or a 24-hour urine collection kit for Part 2 on the second visit of the placebo-run in period (Visit 4) to take home for use on Study Day 1. In Part 1 of the study, first morning urine will be collected prior to morning dose of study drug on Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels. In Part 2 of the study, 24-hour urine will be collected on Study Day 1 and again at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone levels.

8.2.7. Pregnancy Testing

Pregnancy tests will be performed as shown in Table 1 and Table 2. A serum pregnancy test will be performed at Screening/start of Placebo Run-in, and urine pregnancy tests will be performed at Randomization (Study Day 1) and at the Study Week 4 and Study Week 8 (end-of-treatment) visits. Pregnancy tests are required for women of childbearing potential only. Note that a woman is considered to be of childbearing potential, ie, fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone level in the postmenopausal range must be used to confirm postmenopausal status in all postmenopausal women at Screening/start of Placebo Run-in.

8.3. Adverse Events, Serious Adverse Events, and Other Safety Reporting

The definitions of AEs and SAEs are provided in Appendix 12.10. Adverse events will be reported by the subject. The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE, and remain responsible for following up on all AEs and SAEs. The method of recording, evaluating, and assessing causality of AEs and SAEs, as well as the procedures for completing and transmitting safety event report forms are provided in Appendix 12.10.

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from Screening/start of Placebo Run-in (after signed Screening/main study ICF) until 30 days post last dose of study drug in Part 1 (Study Week 12) and 15 days post last dose of study drug in Part 2 (Study Week 10) at the timepoints specified in the SoA (Table 1 and Table 2).

Adverse events that begin before the start of study drug, but after obtaining informed consent will not be considered treatment-emergent AEs. Adverse events collected during Placebo Run-in are considered treatment-emergent events.

All SAEs occurring from Screening/start of Placebo Run-in (after signed Screening/main study ICF) until 30 days post last dose of study drug in Part 1 (Study Week 12) and 15 days post last dose of study drug in Part 2 (Study Week 10) will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study drug or study participation, the investigator must promptly notify the sponsor.

In the event that study drug is interrupted, AEs and SAEs will be collected throughout the interruption. If study drug is discontinued for any reason, the reason will be recorded and the subject should be encouraged to remain in the study so that important safety information can be obtained. All AEs and SAEs leading to discontinuation of study drug or discontinuation of study will be collected. Once a subject withdraws consent to participate in the study, no further information can be collected from the subject.

8.3.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

Investigators will seek information on AEs at each subject contact. All AEs, whether reported by the subject or noted by study personnel, will be recorded in the subject's medical record and on the AE eCRF.

A consistent, nondirective questioning methodology should be adopted for eliciting AE information at all subject evaluation timepoints. Examples of nondirective questions include the following:

- "How have you felt since your last clinic visit?"
- "Have you had any new or changed health problems since you were last here?"

The method of recording, evaluating, and assessing causality of AEs and SAEs, and the procedures for completing and transmitting SAE reports are provided in Appendix 12.10.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs and AEs of special interest (as defined in Section 8.3.7) will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.3).

8.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification (within 24 hours) by the investigator to the sponsor/designee of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of subject and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/IECs, and investigators.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator Brochure and will notify the IRB/IEC, if appropriate according to local requirements.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- Any life-threatening (ie, imminent risk of death) or fatal AE that occurs while on study drug should be submitted to the medical monitor/designee with written case details on a safety event report form within 24 hours.

8.3.5. Pregnancy

- Details of all pregnancies in female subjects and female partners of male subjects will be collected after the start of the Placebo Run-in and until 30 days (Part 1) and 15 days (Part 2) after the last subject visit.
- If a pregnancy is reported, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the female subject or female partner of male subject (after obtaining the necessary signed informed consent from the female partner) pregnancy.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- The subject/pregnant female partner will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject/pregnant female partner and the neonate and the information will be forwarded to the sponsor/designee.
- Any poststudy pregnancy-related SAE considered reasonably related to the study drug by the investigator will be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study

subject/pregnant female partner, he or she may learn of an SAE through spontaneous reporting.

8.3.6. Death Events

Any AE resulting in death that occurs during the study or within 30 days (Part 1) and 15 days (Part 2) after the subject's final dose of study drug, regardless of when the final visit occurs, is considered a SAE and must be reported to the sponsor (or designee) and/or the study medical monitor within 24 hours of awareness of the event.

All deaths that occur during the protocol-specified AE reporting period (Section 8.3.1), regardless of attribution, will be recorded on an eCRF and reported in a safety event report form and expeditiously sent to the sponsor/designee. This includes death attributed to progression of disease.

When recording a death on an eCRF or safety event report form, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept whenever possible. When reporting SAEs, "death" should not be reported as an SAE term, but rather as the outcome of a specific SAE unless the event preceding the death in unknown. If an autopsy was performed, the autopsy report should be provided.

8.3.7. Adverse Events of Special Interest

Adverse events of special interest (AESI) are required to be reported by the investigator to the sponsor/designee immediately (ie, no more than 24 hours after learning of the event) regardless of their causality to the study drug treatment. The most appropriate diagnosis should be recorded, or if a diagnosis cannot be established, the abnormal laboratory values should be recorded on the safety event report form and reported to the sponsor/designee immediately, either as an AESI or and/or a SAE.

NOTE: If any of the following events were reported as SAEs then there is no need to complete a separate AESI form.

The AESIs for all study drugs (MLS-101 and placebo) include the following:

- Hyperkalemia with dose modification (dose reduction, dose hold, or permanent dose withdrawal)
- Hypotension with symptoms (eg, light-headedness, dizziness, presyncope, or syncope)
- Adrenal Insufficiency

8.3.8. Safety Monitoring Considerations

A DSMB will meet periodically to monitor the study. The primary function of this committee is safety monitoring, and the committee will not discontinue the study for a finding of positive efficacy for any of the doses being considered. As part of these reviews, the DSMB will receive summaries of study conduct measures as well as unblinded safety and efficacy data. The DSMB may recommend discontinuing enrollment for a dose for safety or lack of efficacy. The remaining sample for a discontinued dose may be shifted to 1 or more of the remaining arms subject to DSMB recommendation.

The full scope and responsibilities of the DSMB will be described in the DMSB Charter per the Guidance for Clinical Trial Sponsors: Establishment and Operation of Clinical Trial Data Monitoring Committees.

8.4. Pharmacokinetics

Single blood samples will be collected and tested for concentration of MLS-101 on Study Day 1 (predose) and at Study Weeks 1, 4, and 8 (trough levels). Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected at baseline (predose) and at Study Weeks 1, 4, and 8. At selected sites, blood samples for determination of MLS-101 (and metabolites) will be collected predose, and 1, 2, 3, and 4 hours postdose on Study Days 1 (Randomization) and 28 (Study Week 4).

Instructions for collection, preparation, handling, and shipping PK specimens are provided in the Laboratory Manual for the study.

8.5. Pharmacodynamics

Blood samples for aldosterone and PRA will be collected at Pre-Screening. Blood samples for aldosterone, cortisol, 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected at Screening/start of Placebo Run-in, Study Day 1 (baseline), Study Week 4, and Study Week 10 for Part 2 or Study Week 12 for Part 1 (end of follow up).

9. STATISTICAL CONSIDERATIONS

The statistical analysis plan (SAP) will be finalized prior to database lock and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.1. Analysis Sets

Five analysis sets defined across both parts of the study (ie, Part 1 and Part 2 data combined) are:

- **All randomized set:** all randomized subjects. This analysis set will be used for summaries of subject study disposition with subjects analyzed according to the randomized treatment group.
- **Full analysis set (FAS):** all subjects randomized and who received at least 1 dose of study treatment. All efficacy analyses will be performed using the full analysis set; subjects will be analyzed according to the randomized treatment group.
- **Per protocol set:** all subjects in the FAS who have completed the Study Week 8 visit without any major protocol deviation that could influence the validity of the data for the primary efficacy evaluations; subjects will be analyzed according to the randomized treatment group.
- **PK/PD analysis set**: subjects in the Safety analysis set who have sufficient data available for the analysis of pharmacokinetic and pharmacodynamic measurements; subjects will be analyzed according to the actual treatment received.
- **Safety analysis set:** all enrolled subjects who received at least 1 dose of study treatment. All safety analyses will be performed using the safety analysis set; subjects will be analyzed according to the actual treatment received.

9.2. Sample Size Determination

[redacted]

9.3. Statistical Methods

Unless otherwise noted, summaries will be provided on the data combined from Part 1 and Part 2.

9.3.1. Disposition, Demographics and Baseline Characteristics

The number and disposition of screened subjects, randomized subjects and treated subjects will be summarized by treatment group. Reasons for incomplete follow-up and treatment will be summarized.

Demographic and baseline characteristics will be summarized for the all randomized set, using descriptive statistics, by treatment.

9.3.2. Efficacy Analyses

The FAS will be used for the efficacy analyses. In general, descriptive statistics for continuous variables will consist of subject count, mean, standard deviation, median, and range (min, max). Descriptive statistics for categorical variables will consist of subject counts and percentages. The primary estimand is designed to answer the research question on the treatment effect of investigational therapy versus placebo in addition to standard of care on the change in office-measured blood pressure from baseline to Study Week 8. Details of the estimand components are provided in the SAP.

The primary efficacy endpoint is the Study Week 8 change from baseline in SBP. The primary efficacy analysis will be performed using a mixed model repeated measures approach with fixed effects of categorical terms for treatment, week, and treatment by week interaction and baseline SBP as a fixed continuous covariate. A least square estimate, along with the 90% CI, of the mean difference between each dose group and the placebo group will be provided for each time point, with the primary analysis inference based on the evaluation of the Week 8 estimates. P-values corresponding to the pairwise tests, not adjusted for multiplicity, of the difference between each dose group versus placebo will be provided only for the Study Week 8 time point.

For the office-measured secondary efficacy endpoints, a similar approach will be used as for the primary estimand analysis. Both primary and secondary SBP and DBP endpoints will be summarized descriptively by study week. The ABPM parameters (SBP and DBP) will also be summarized descriptively by study week.

A response to treatment assessment will be based upon whether or not the office-measured BP achieved values of ≤ 130/80 mmHg by the end of Study Week 8. Subjects without an assessment at Study Week 8 or who have received rescue medications will be considered as failures. The proportion of successes will be calculated for each treatment group. The timing of the first value of ≤ 130/80 mmHg will be summarized using a Kaplan-Meier approach within treatment group, and the relationship to baseline SBP and DBP will also be explored.

All secondary efficacy endpoints analyses will be considered supportive and any inferential statistics will be considered descriptive in nature. No adjustment for multiplicity will be performed. Further details of the estimand definition, analysis model, sensitivity and supplementary analyses, including the analyses of the data collected after Study Week 8, are given in the SAP.

Efficacy analyses will be repeated using the FAS subset of 100 mg treatment groups in order to evaluate whether there are any differences in the treatment effect depending on the levels of PRA at baseline.

9.3.3. Safety Analyses

The safety analysis set will be used for the safety analyses. Subjects will be analyzed based upon the treatment received. Select safety summaries will be repeated using the FAS subset of 100 mg treatment groups where subjects will be tabulated separately by PRA cohort at baseline (Part 1 or Part 2).

All AEs will be coded using the Medical Dictionary for Regulatory Affairs. The focus of the analyses will be on treatment emergent AEs. Results will be presented by system organ class, preferred term, and treatment group and summarized for frequency, severity, and relationship to study treatment. No formal statistical testing will be performed.

Concomitant medications will be coded using the most current World Health Organization Drug Dictionary and summarized by drug class and medication term with results presented by treatment group.

Quantitative data will be summarized by mean, standard deviation, median, and range. Laboratory abnormalities will be analyzed by summarizing frequency, severity, and changes from Baseline. Categorical data will be summarized by as counts and percentages. All results will be presented by treatment group.

9.3.4. Pharmacokinetics Analyses

Descriptive statistics (mean, standard deviation, coefficient of variation, standard error of the mean, number, minimum, maximum, and median) will be calculated for all PK parameters for MLS-101.

Pharmacokinetic data will be evaluated by noncompartmental analysis using a prespecified analysis plan. If feasible, AUC, C_{max}, T_{max}, and $t_{1/2}$ will be estimated. A population PK model may be developed to identify and characterize patient factors which may influence MLS-101 pharmacokinetic disposition.

9.3.5. Pharmacodynamics Analyses

The mean change from baseline in plasma 11-deoxycortisol and PRA will be summarized for each week recorded. The mean change will be based upon observed data. The mean change from baseline in serum aldosterone, cortisol, and 11-deoxycorticosterone concentration will be summarized for each week recorded. The mean change will be based upon observed data. A population PK-PD model may be developed to evaluate the relationship between systemic MLS-101 exposure and plasma aldosterone synthesis and disposition.

9.4. Handling of Missing Data

Given the preliminary nature of this study, no formal imputation for missing data is planned. For the office-measured efficacy endpoints, values post rescue medications will be set to missing and imputed using the last value observed before start of rescue medication. All other missing values will not be imputed.

9.5. Interim Analysis

An interim analysis of the unblinded safety and efficacy data was performed after the first 5 to 10 subjects in each dose group (30 to 60 subjects total) completed 4 weeks of treatment in Part 1. Safety summaries included tabulation of treatment emergent AEs, SAEs, and AESIs, as well as select lab and PD parameters, summarized by study visit.

At the time of the interim analysis, additional exploratory efficacy analyses using the primary efficacy endpoint was conducted within each dose group. The results of these analyses had limited, prespecified distribution and were used for internal decision making and development planning. The details of these planned analysis are provided in the final SAP.

A second interim analysis at the end of Part 1 to review all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) will be conducted. This analysis will also include Part 2 subjects that have completed week 4 at the time of data cut off.

An additional interim analysis may be performed to support drug development decisions.

10. ETHICAL AND ADMINISTRATIVE CONSIDERATIONS

10.1. Compliance Statement

This study will be conducted in accordance with the protocol and with US Food and Drug Administration (FDA) and the ICH Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and any applicable local health authority and IRB/ IEC requirements.

To the extent applicable, all references to the FDA, Federal Food, Drug, and Cosmetic Act, Code of Federal Regulations (CFR), ICH, GCP, and the like shall be interpreted as also referring to any corresponding requirements of local regulatory agencies, regulations, and laws. If there is any discrepancy between FDA, ICH, and local requirements, the most stringent standard shall apply.

10.2. Investigator Responsibilities

As required by FDA regulation (21 CFR Part 56) and ICH guidelines for GCP, the investigator at each study site must obtain IRB/IEC review and approval of the study protocol, ICFs, subject recruitment materials, and any other pertinent documents before any study related activities involving subjects are performed.

As required in 21 CFR Part 50 and ICH guidelines for GCP, the investigator or designee must comply with the informed consent process, and ensure that each subject enrolled in this clinical study understands the information presented in the IRB/IEC approved ICF and agrees voluntarily to participate in the clinical study.

The investigator or designee must submit to the IRB/IEC any written safety report or update (eg, amended IB or safety amendments and updates) provided by the sponsor or representative, according to the IEC specific reporting requirements.

The investigator must inform the IRB/IEC of the progress of the clinical study and report any non-administrative changes made to the protocol; in any case, the investigator must provide an update to the IRB/IEC at least once a year or in accordance with IRB/IEC continuing approval requirements.

The investigator must maintain a list of appropriately qualified persons to whom he/she has delegated study duties. All persons authorized to make entries and/or corrections on eCRFs or other reporting forms will be included on a Delegation of Authority form.

The clinical study report will be signed by the investigator or, in the case of multicenter studies, the coordinating investigator. The coordinating investigator, identified by the sponsor, will be any or all of the following:

- a recognized expert in the therapeutic area.
- an investigator who provided significant contributions to either the design or interpretation of the study.
- an investigator contributing a high number of eligible subjects.

10.3. Institutional Review Board/Independent Ethics Committee Review

A copy of the protocol, proposed ICF, other written subject information, and any proposed advertising material must be submitted to the IRB/IEC for written approval. A copy of the

written approval of the protocol and ICF must be received by the sponsor before recruitment of subjects into the study and shipment of investigational product.

The investigator must submit and, where necessary, obtain approval from the IRB/IEC for all subsequent protocol amendments and changes to the informed consent document. The investigator is to notify the IRB/IEC of deviations from the protocol or SAEs occurring at the site and other AE reports received from the sponsor, in accordance with local procedures.

The investigator is responsible for obtaining annual IRB/IEC approval/renewal as applicable throughout the duration of the study. Copies of the investigator's reports and the IRB/IEC continuance of approval must be sent to the sponsor.

10.4. Informed Consent and Human Subject Protection

An initial sample pre-Screening ICF and Screening/main study ICF will be provided for the investigator to prepare the informed consent documents to be used at his or her site. Updates to the template are to be communicated formally in writing from the sponsor's study monitor to the investigator. The written informed consent document is to be prepared in the language(s) of the potential subject population.

Before a subject's participation in the clinical study, the investigator is responsible for obtaining written informed consent (pre-Screening and Screening/main study) from the subject or legally acceptable representative after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol specific Screening procedures or any investigational products are administered. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical study.

The investigator is also responsible for asking the subject if the subject has a primary care physician and if the subject agrees to have his/her primary care physician informed of the subject's participation in the clinical study. If the subject agrees to such notification, the investigator is to inform the subject's primary care physician of the subject's participation in the clinical study. If the subject does not have a primary care physician and the investigator will be acting in that capacity, the investigator is to document such in the subject's medical record. The acquisition of informed consent and the subject's agreement or refusal of his/her notification of the primary care physician is to be documented in the subject's medical records, and the ICF is to be signed and personally dated by the subject, or a legally acceptable representative, and by the person who conducted the informed consent discussion. The original signed ICF is to be retained in accordance with institutional policy, and a copy of the signed consent form is to be provided to the subject or legally acceptable representative.

If a potential subject is illiterate or visually impaired and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the ICF to the subject and must allow for questions. Thereafter, both the subject and the witness must sign the ICF to attest that informed consent was freely given and understood.

10.5. Confidentiality

The investigator must ensure that the subject's confidentiality is maintained for documents submitted to the sponsor, including the following:

- Subjects are to be identified by a unique subject identification number.
- Where permitted, date of birth is to be documented and formatted in accordance with local laws and regulations.
- On the eCRF demographics page, in addition to the unique subject identification number, the subject's age at time of enrollment is to be included.
- For SAEs reported to the sponsor, any source documentation provided (eg, medical records, laboratory results) must have any subject identifier (eg, subject name, initials, medical records number) fully redacted (ie, blacked out) prior to transmission.
- Documents that are not submitted to the sponsor (eg, signed ICFs) are to be kept in confidence by the investigator, except as described below.

In compliance with the CFR/ ICH GCP Guidelines, it is required that the investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB/IEC direct access to review the subject's original medical records for verification of study related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform and obtain the consent of the subject to permit such individuals to have access to his/her study related records, including personal information.

10.6. Urgent Safety Measures

The sponsor or investigator may take appropriate urgent safety measures to protect trial participants from any immediate hazard to their health or safety. Urgent safety measures may be taken without prior authorization. The trial may continue with the urgent safety measures in place.

The investigator must inform the sponsor IMMEDIATELY if the study site initiates an urgent safety measure.

The notification must include all of the following:

- Date of the urgent safety measure
- Who made the decision
- Why the action was taken

The investigator will provide any other information that may be required to enable the sponsor to report and manage the urgent safety measure in accordance with the current regulatory and ethical requirements for expedited reporting and closeout.

10.7. Study Monitoring

The sponsor's representative(s) are responsible for contacting and visiting the investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the clinical study (eg, eCRFs and other pertinent data) provided that subject confidentiality is respected.

The sponsor's representative(s) are responsible for verifying the eCRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The sponsor's representative(s) are to have access to subject medical records and other study related records needed to verify the entries on the eCRFs.

The investigator agrees to cooperate with the sponsor's representative(s) to ensure that any problems detected in the course of these monitoring visits, including delays in completing eCRFs, are resolved.

10.8. Audits and Inspections

As stipulated by 21 CFR §312.58 and ICH guidelines for GCP, a representative of the sponsor, the FDA, or other regulatory agencies may conduct periodic site audits or inspections. The investigator or designee will provide these representatives with access to all requested materials, including eCRFs and supporting source documents. In addition, the investigator or other qualified study site personnel are to be available to answer questions, hold interviews, and provide facility tours, if requested.

10.9. Data Collection and Handling

The investigator is responsible for complying with the requirements for all assessments and data collection (including subjects not receiving protocol required therapies), as stipulated in the protocol for each subject in the study. For subjects who withdraw prior to completion of all protocol-required visits and procedures, the investigator may search publicly available records (where permitted) to ascertain survival status. This ensures that the data sets produced as an outcome of the study are as comprehensive as possible.

The investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. Data collection will involve the use of an electronic data capture system, to which only authorized personnel will have access. The investigator agrees to maintain accurate eCRFs or paper case report forms and source documentation as part of the case histories. The sponsor will supply the eCRF, which is to be completed in English.

The investigator or designee must enter all results collected during the clinical study into eCRFs. Guidelines for completion of eCRFs will be reviewed with study site personnel at the site initiation visits. Investigators are responsible for approval of the entered/corrected data. Detailed instructions may be found in the other study specific documents.

All entries made on the eCRF must be verifiable against source documents. In addition to periodic monitoring occurring within the system by study monitors, programmatic edit checks and data listings will be used to review the data for completeness, logic, and adherence to study protocol. As a result of this monitoring and these checks, queries may be electronically issued to the clinical study sites and electronically resolved by those sites.

All data collected in the context of this study will be stored and evaluated according to regulatory requirements and applicable guidance for electronic records. Also, data will be stored and evaluated in such a way as to assure subject confidentiality in accordance with the legal and regulatory requirements applying to protected health information. Study records (eg, copies of eCRFs, regulatory documents) will be retained at the study site, along with adequate source documentation. The study file and all source data must be retained for the time period required

by applicable regulatory requirements and will not be destroyed until written notification is given by the sponsor or designee for destruction.

10.10. Maintenance of Source Documents and Recordkeeping Requirements

As stipulated by 21 CFR §312.57 and ICH E6 GCP Consolidated Guidance Section 8, the investigator or designee will maintain source documentation for this clinical study that documents the treatment and study course of subjects as described in the study manual.

Source documents are original documents, data, and records from which the subject's eCRF data are obtained. These include, but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

The investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study related (essential) documentation, suitable for inspection at any time by representatives from the sponsor and/or applicable regulatory authorities.

The investigator must retain all essential documents for this clinical study until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. However, the investigator may need to retain these documents for a longer period, if required by the applicable regulatory requirements or by an agreement with the sponsor. A sponsor representative will be responsible for informing the investigator and study site regarding when they no longer need to retain these documents. Before destroying any records, the investigator must notify the sponsor and reach agreement on record destruction, or the sponsor may request an additional retention period.

10.11. Long-term Retention of Samples for Additional Future Research

Blood specimens will be collected and stored for additional analyses. These samples will be retained for long-term storage by the sponsor and described in the informed consent.

Any blood sample collected according to the SoA may be analyzed for any of the tests outlined in the protocol and for any tests necessary to minimize risks to study subjects. This includes testing to ensure that analytical methods produce reliable and valid data throughout the course of the study. It may also include, but is not limited to, investigation of unexpected results, incurred sample reanalysis, and analyses for method transfer and comparability.

All samples and associated results will be coded prior to being shipped from the site for analysis or storage. Samples will be tracked using a unique identifier that is assigned to the samples for the study. Results are stored in a secure database to ensure data integrity and control.

If permitted by local law and if informed consent is provided by the subject, the sponsor may do additional testing on remaining samples (ie, residual and back up) to investigate and better understand the disease and the dose response and/or prediction of response to the study drug. Results from this analysis are to be documented and maintained, but are not necessarily reported as part of this study. Samples may be retained for up to 20 years.

Since the evaluations are not expected to benefit the subject directly or to alter the subject's treatment course, the results of these exploratory studies are not placed in the subject's medical

record and are not to be made available to the subject, members of the subject's family, the subject's personal physician, or other third parties, except as specified in the ICF.

The subject retains the right to request that the sample material be destroyed by contacting the investigator. Following the request from the subject, the investigator is to provide the sponsor with the required study and subject number so that any remaining blood samples and any other components from the cells can be located and destroyed. Samples will be destroyed once all protocol-defined procedures have been completed.

Information collected from samples prior to the request for destruction will be retained by the sponsor. The sponsor is the exclusive owner of any data, discoveries, and derivative materials from the sample materials and is responsible for the destruction of the sample(s) at the request of the subject through the investigator, at the end of the storage period or as appropriate (eg, the scientific rationale for experimentation with a certain sample type no longer justifies keeping the sample). If a commercial product is developed from this research project, the sponsor owns the commercial product. The subject has no commercial rights to such product and has no commercial rights to the data, information, discoveries, or derivative materials gained or produced from the sample.

10.12. Publication Policy

The institution and investigator agree not to publish the results of this study without the prior written consent of the sponsor. As used herein, the term 'publish' shall include oral presentations, written abstracts, written poster presentations, and written manuscripts or reviews, etc.

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12. APPENDICES

12.1. List of Abbreviations and Definition of Terms

The following abbreviations and specialist terms are used in this study protocol.

Abbreviation/Term	Definition
ABPM	ambulatory blood pressure monitoring
ACE	angiotensin-converting enzyme
ACTH	adrenocorticotropic hormone
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
AHA	American Heart Association
AOBP	automated office blood pressure
ARB	angiotensin receptor blockers
AUC	area under the plasma concentration versus time curve
BID	twice daily
BP	blood pressure
CFR	Code of Federal Regulations
C_{max}	maximum plasma concentration
coags	coagulation tests
COVID-19	coronavirus disease 2019
CYP	cytochrome P450
DBP	diastolic blood pressure
DSMB	data safety monitoring board
ECG	electrocardiogram
eCRF	electronic case report form
FAS	full analysis set
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
HRT	hormonal replacement therapy
IB	Investigator's Brochure
IC_{50}	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IP	investigational product
IRB	Institutional Review Board
IWRS	interactive web response system
MATE	multidrug and toxin extrusion
MLS-101	a selective aldosterone synthase inhibitor
MR	mineralocorticoid receptor
MRA	mineralocorticoid receptor antagonist
NSAID	nonsteroidal anti-inflammatory drugs
PA	primary aldosteronism
PAC	plasma aldosterone concentration
PD	pharmacodynamic

Abbreviation/Term	Definition
PK	pharmacokinetic
PR	time from the onset of the P wave to the start of the QRS complex
PRA	plasma renin activity
QD	once daily
QRS	the series of deflections in an electrocardiogram that represent electrical activity generated by ventricular depolarization prior to contraction of the ventricles
QT	time from the beginning of the QRS complex to the end of the T wave
QTc	QT interval corrected by Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
SoA	schedule of assessments
T_{max}	time to maximum concentration
$t_{1/2}$	half-life

12.2. Study Contacts

Role	Study Participant	Contact Information
Medical Monitor	[redacted] IQVIA, Inc.	[redacted]
Sponsor's Medical Monitor	[redacted] Mineralys Therapeutics, Inc.	[redacted]
Sponsor's Responsible Medical Officer	[redacted] Mineralys Therapeutics, Inc.	[redacted]
Safety Event Reporting	IQVIA Biotech Safety	[redacted]

12.3. Investigator's Agreement

Study Number: MLS-101-201

Study Title: A Randomized, Double-blind, Placebo-controlled, Dose-ranging, Multicenter Phase 2 Study to Evaluate the Safety, Efficacy, and Tolerability of MLS-101 in Subjects With Uncontrolled Hypertension

Protocol Version: 7.0

Approval Date: 07 July 2022

I have read the protocol and agree to conduct the study in accordance with the protocol and all applicable laws, regulations and guidelines, including, but not limited to, the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), the United States Code of Federal Regulations, the Directives of the European Union, the ethical principles that have their origins in the Declaration of Helsinki, and applicable privacy laws.

The study will not be commenced without the prior written approval of a properly constituted institutional review board (IRB) or independent ethics committee (IEC). No changes will be made to the study protocol without the prior written approval of the sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to subjects.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____ _____

Signature Date

_____

Printed Name of Investigator

12.4. Sponsor's Signature

Study Number: MLS-101-201

Study Title: A Randomized, Double-blind, Placebo-controlled, Dose-ranging, Multicenter Phase 2 Study to Evaluate the Safety, Efficacy, and Tolerability of MLS-101 in Subjects With Uncontrolled Hypertension

Protocol Version: 7.0

Approval Date: 07 July 2022

The protocol has been reviewed and approved by me and is acceptable in its present form.

[redacted]

7/7/2022

Date

12.5. Standing Blood Pressure Measurement

Standing SBP and DBP will be measured at Screening/start of Placebo Run-in, Randomization, Study Week 1, and Study Week 8 with the AOBP device. In addition, assessment of standing BP will be performed if a subject reports symptoms of hypotension (eg, light-headedness, dizziness, presyncope, or syncope) during the study, and the standing BP measurement will be assessed weekly for these subjects until symptoms resolve.

After seated determinations, subjects will be asked to stand. Beginning when their feet touch the floor, BP will be taken approximately 1 minute later in the same arm used for the seated measurements, using the BP device. The mean of the last 2 last sitting SBP and DBP values measured by the automated device will be compared to the standing BP (within 3 minutes of standing). Subjects with a decrease in SBP $\geq$ 20 mmHg or DBP $\geq$ 10 mmHg from sitting to standing position will not be eligible for Randomization (measurement may be repeated during the Screening period to be eligible for study enrollment).

12.6. Dietary Restrictions

Subjects will be instructed to follow a healthy diet for managing blood pressure as recommended by the AHA (AHA 2016).

Aim to eat a diet that's rich in:

- Fruits (avoid fruits high in potassium, eg, bananas)
- Vegetables
- Whole-grains
- Low-fat dairy products
- Skinless poultry and fish
- Nuts and legumes
- Nontropical vegetable oils

Limit:

- Saturated and trans fats
- Sodium
- Red meat (if you do eat red meat, compare labels and select the leanest cuts available)
- Sweets and sugar-sweetened beverages

12.7. ACTH Stimulation Test

The ACTH stimulation test assesses the function of the adrenal glands and their ability to respond to ACTH. Adrenocorticotropic hormone is a hormone produced in the pituitary gland that stimulates the adrenal glands to release cortisol. Based on changes in cortisol levels, an ACTH stimulation test may be performed and the subject's dose of study drug may be withheld.

The ACTH stimulation test is recognized as the gold standard assay of adrenal insufficiency. ACTH stimulation tests may be performed in select patients based on low morning serum cortisol levels (ie, < 3 mcg/dL) and clinical evidence of adrenal insufficiency. Cosyntropin is a synthetic form of ACTH. The test consists of the following procedures:

- Obtain blood sample for pretest serum cortisol measurement (collected prior to administering synthetic ACTH)
- Administer 0.25 mg synthetic ACTH via intravenous push
- Obtain blood samples for serum cortisol measurements at 30 minutes and 60 minutes after dosing of synthetic ACTH

Post-stimulation serum cortisol levels should be greater than 18 mcg/dL. Samples for cortisol measurements will be sent to the central laboratory for analysis.

12.8. Contraceptive and Barrier Guidance

12.8.1. Definitions

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP:

1. Premenarchal

2. Premenopausal female with 1 of the following:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

12.8.2. Contraception Guidance

Investigators should counsel WOCBP and male subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy. Investigators shall advise these subjects on the use of adequate methods of contraception and will check for adherence during study visits. Subjects must agree to use adequate contraception during the study and for 90 days after the last dose of study drug.

The following methods of contraception are considered adequate for this study:

- **Highly effective methods of contraception** (Table 4): Highly effective methods of contraception have a failure rate of < 1% per year when used consistently and correctly.
- **Acceptable methods of contraception** (Table 5): Acceptable methods of contraception which may not be considered as highly effective have a failure rate of > 1% per year when used consistently and correctly.

Table 4: Highly Effective Contraceptive Methods Permitted in Study

Nonhormonal intrauterine device (eg, ParaGard®)
Bilateral tubal occlusion
Vasectomized Partner A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
Sexual Abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

Table 5: Acceptable Contraceptive Methods Permitted in Study

Combination of male condom with either cap, diaphragm, or sponge with spermicide (double-barrier method)
Male or female condom with or without spermicide
Cap, diaphragm, or sponge with spermicide

12.9. Clinical Laboratory Tests

Clinical safety laboratory tests will be conducted by a central laboratory according to the time points specified in Table 1 and Table 2. Instructions for collection, preparation, handling, and shipping clinical laboratory specimens are provided in the Laboratory Manual for the study. Analytes for clinical safety labs are listed in Table 6. The investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents. All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days (Part 1) and 15 days (Part 2) after the last dose of study drug should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor. If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.

Table 6: Clinical Laboratory Analytes

Chemistry	Hematology & Coagulation Tests	Spot/24-Hour Urine Collection & Urinalysis	Other Laboratory Measurements
Sodium Potassium Chloride Bicarbonate Total protein Albumin Calcium Magnesium Phosphorus Glucose BUN or Urea Creatinine Uric acid Phosphorus Total bilirubin Direct bilirubin LDH AST ALT Alkaline phosphatase	RBC Hemoglobin Hematocrit Platelets WBC Differential Neutrophils Eosinophils Basophils Lymphocytes Monocytes Reticulocytes PT, aPTT, and INR Hemoglobin A1c Serum cortisol Plasma ACTH	**Spot/24-Hour Urine Collection** Potassium Sodium Creatinine Aldosterone (Part 2) **Urinalysis** RBC Glucose Protein Urine pH Ketones Bilirubin Urine specific gravity Blood	**Pregnancy Testing:** Serum or Urine Pregnancy FSH (postmenopausal women only) **Pharmacokinetics:** Plasma MLS-101 and metabolites **Pharmacodynamics:** Serum aldosterone Serum cortisol Serum 11-deoxycorticosterone Plasma 11-deoxycortisol PRA

Note: Refer to the Schedule of Assessments (Table 1 and Table 2) for collection timepoints.
Abbreviations: ACTH = adrenocorticotropic hormone; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; FSH = follicle-stimulating hormone; INR = international normalized ratio; LDH = lactate dehydrogenase; PRA = plasma renin activity; PT = prothrombin time; RBC = red blood cell; WBC = white blood cells.

12.10. Definitions and Procedures for Adverse Events

12.10.1. Definition of Adverse Event

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study drug or other protocol imposed intervention, whether or not considered related to the study drug. Any medical condition or clinically significant laboratory abnormality with an onset before the first dose of study drug is considered a pre-existing condition that will be captured as part of the subject's medical history and will not be considered an AE unless the condition worsens in intensity or frequency after study enrollment.

Events meeting the AE definition include the following:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease)
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition
- New condition detected or diagnosed after study intervention administration even though it may have been present before the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected intervention-intervention interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events **not** meeting the AE definition include the following:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen

12.10.2. Definition of Serious Adverse Event

An SAE is any untoward medical occurrence that, at any dose, meets 1 or more of the following criteria:

- Is fatal (ie, the AE actually causes or leads to death)

- Is life-threatening (ie, the AE, in the view of the investigator, places the subject at immediate risk of death at the time of the event; it does not refer to an event which might hypothetically have caused death if more severe)
- Requires or prolongs inpatient hospitalization
- Results in persistent or significant disability/incapacity (ie, the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product(s)
- Is considered a significant medical event by the investigator (ie, may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above)

All AEs that do not meet any of the criteria for serious should be regarded as nonserious AEs. Elective hospitalizations for conditions that existed before administration of the study drug are not to be considered SAEs. However, prestudy conditions that worsen during the course of the study and meet the SAE criteria above would be considered SAEs.

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on subject or event outcome or action criteria usually associated with events that pose a threat to a subject's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness should be independently assessed when recording AEs and SAEs.

12.10.3. Recording and Follow-Up of AE and/or SAE

12.10.3.1. Recording of AEs and SAEs

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the sponsor in lieu of completion of the required form.
- There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

12.10.3.2. Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to one of the following categories:

- **Mild**: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- **Moderate**: Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL). Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- **Severe**: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling, limiting self care ADL. Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

12.10.3.3. Assessment of Causality

The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. The investigator will use clinical judgment to determine the relationship. The guideline below should be used to consider relatedness:

"**Unlikely**" or "**Not Related**" = there is not a reasonable possibility that the event may have been caused by study drug. The AE:

- May be judged to be due to extraneous causes such as disease or environment or toxic factors
- May be judged to be due to the subject's clinical state or other therapy being administered
- Is not biologically plausible
- Does not reappear or worsen when the study drug is readministered
- Does not follow a temporal sequence from administration of study drug

"**Possibly**" or "**Definitely**" = there is a reasonable possibility that the AE may have been caused by the study drug. The AE:

- Follows a temporal sequence from administration of study drug
- Is a known response to the study drug based on clinical or nonclinical data
- Could not be explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other therapy administered
- Disappears or decreases upon cessation or reduction of the study drug
- Reappears or worsens when the study drug is readministered

A *reasonable possibility* of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration, will be considered and investigated. The investigator will also consult the IB and/or product information, for marketed products, in his/her assessment.

For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the sponsor.

The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

12.10.3.4. Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally submitted documents.
- The investigator will submit any updated SAE data to the sponsor/designee within 24 hours of receipt of the information.

12.10.4.Reporting of AEs and SAEs

12.10.4.1. AE and SAE Reporting Period

All AEs and SAEs will be collected from Screening (after signed Screening/main study ICF) until 30 days post last dose of study drug in Part 1 (Study Week 12) and 15 days post last dose of study drug in Part 2 (Study Week 10) at the timepoints specified in the SoA (Table 1). Adverse events will be reported by the subject. The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE, and remain responsible for following up on all AEs and SAEs.

12.10.4.2. SAE Reporting to the Sponsor/Designee via Paper Safety Event Report Form

- Email transmission of the safety event report form is the preferred method to transmit this information to the sponsor/designee with facsimile transmission as the back-up method.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the safety event report form within the designated reporting timeframes.
- Contacts for safety event reporting can be found in Appendix 12.2.

12.11. Protocol Amendment Summary

DOCUMENT HISTORY	
Document	**Date**
Amendment 6	07 July 2022
Amendment 5	17 February 2022
Amendment 4	10 January 2022
Amendment 3	24 August 2021
Amendment 2	01 June 2021
Amendment 1	15 April 2021
Original Protocol	06 February 2021

12.11.1. Amendment 6 (07 July 2022)

This summary includes changes made to Protocol MLS-101-201 Amendment 5 (17 February 2022). The overall rationale for the changes implemented in this protocol amendment was include the changes made using Protocol Clarification Letters. Changes to the Protocol regarding the primary efficacy endpoint, analysis sets and addition of second interim analysis were made based on the Statistical Analysis Plan for this study.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/ Methodology and Section 4.1/Overall Study Design	For Part 2, approximately 36 enrolled subjects ≥ 18 years of age will be randomized ~~(6:1)~~**(5:1)** to either 100 mg QD MLS-101 or placebo ~~stratified by PRA levels~~ such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.	Clarified that the randomization ratio should be 5:1 not 6:1 as indicated in the protocol
Synopsis/ Methodology	Using an automated oscillometric sphygmomanometer device, SBP and DBP will be measured 5 times after approximately 5 minutes of rest in the seated position according to the American Heart Association (AHA) Guidelines (Muntner 2019). The ~~mean~~**average of the** last 2 of 5 unattended measurements of SBP and DBP, **respectively**, will be ~~averaged~~**used** for the analysis.	Updated to align with the Statistical Analysis Plan
Section 4.1/Overall Study Design	The last 2 of 5 unattended measurements of SBP and DBP will be averaged for the analysis. ~~The last 2 of 5 unattended measurements of SBP and DBP will be averaged for the analysis.~~**The averge of the last 2 of 5 unattended measurements of SBP and DBP, respectively, will be averaged used for the analysis.**	Updated to align with the Statistical Analysis Plan
Synopsis/Methodolody and Section 4.1/Overall Study Design and Section 9.5/Interim Analysis	**A second interim analysis at the end of Part 1 to review all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) will be conducted. This analysis will also include Part 2 subjects that have completed week 4 at the time of data cut off.**	Updated to align with the Statistical Analysis Plan

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Section 4.2/Scientific Rationale for Study Design	Data obtained from the interim ~~analysis~~ **analyses** performed in Part 1 of the study will be used to inform Part 2.	Updated to align with the Statistical Analysis Plan
Section 4.3/Number of Subjects/Number of Centers	The subjects who qualify for enrollment will be randomized ~~(6:1)~~**(5:1)** to either 100 mg QD MLS-101 or placebo ~~stratified by PRA levels~~ such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects across approximately 10 study centers in the United States.	Clarified that the randomization ratio should be 5:1 not 6:1 as indicated in the protocol
Section 6.3/Randomization and Blinding	All subjects who qualify for Part 2 based on inclusion/exclusion criteria and complete up to a 2-week Placebo Run-in period and 24-hour ABPM procedure will be randomized ~~(6:1)~~**(5:1)** to either 100 mg QD MLS-101 or placebo ~~stratified by PRA levels~~ such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.	Clarified that the randomization ratio should be 5:1 not 6:1 as indicated in the protocol
Synopsis/Table 2: Schedule of Assessments for Part 2 footnote q	Subjects will be given a 24-hour urine collection kit on the second visit of the placebo-run in period (Visit 4; Study Day -7 ± 2). ~~First morning urine will be collected prior to morning dose of study drug on Study Day 1 and urine collection continued for 24 hours~~ **The first morning urine should be discarded before the 24-hour collection is started. Urine should be collected for 24 hours prior to V6 (Randomization)** for determination of potassium, sodium, creatinine, and aldosterone levels. A second 24-hour urine collection will occur ~~at the end of Study Week 4 (ie, Study Day 28 ± 2).~~ **24 hours prior to V10 (Study Week 4, Study Day 28 ± 2).**	Clarified the timing of the 24-Hour Urine Collection. The 24-Hour Collection should start the day prior to V6 and V10
Synopsis/Eligibility Criteria and Section 5/Study Population Inclusion Criteria #5	For Part 1, inclusion based on morning pre-Screening visit measurement of PRA, and the value for PRA must be ≤ 1 ng/mL/h. For Part 2, inclusion based on morning **pre-**Screening visit measurement of PRA, and the value of PRA must be > 1 ng/mL/h. Alternative target PRA criteria for the study may be provided by the sponsor. These alternative targets may be adjusted	Clarified the inclusion measurement of PRA for Part 2.

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	during the conduct of the trial based on ongoing review of PRA values.	
Synopsis/Eligibility Criteria and Section 5/Study Population Inclusion Criteria #6	Inclusion based on morning pre-Screening visit measurement of serum aldosterone for Part 1 and **pre-**Screening visit measurement of aldosterone for Part 2. The value for aldosterone must be ≥ 1 ng/dL based on morning measurement. Alternative target aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of serum aldosterone values.	Clarified the inclusion measurement of Aldosterone for Part 2.
Section 9.1/Analysis Sets	PK/PD analysis set: subjects in the ~~FAS~~ **Safety analysis set** who have sufficient data available for the analysis of pharmacokinetic and pharmacodynamic measurements; subjects will be analyzed according to the actual treatment received.	Updated to align with the Statistical Analysis Plan
Section 9.3/Statistical Methods	Unless otherwise noted, summaries will be provided on the data combined from Part 1 and Part 2.~~, with all placebo subjects pooled into 1 group for tabulations.~~	Updated to align with the Statistical Analysis Plan
Section 9.3.2/Efficacy Analyses	The primary efficacy endpoint is the Study Week 8 change from baseline in SBP. ~~where the Study Week 8 value will be defined as the average of Study Week 7 and Study Week 8 measurement.~~ The primary efficacy analysis will be performed using a mixed model repeated measures approach with fixed effects of categorical terms for treatment, week, and treatment by week interaction, ~~stratification factor,~~ and baseline SBP as a fixed continuous covariate. A least square estimate, along with the 90% CI, of the mean difference between each dose group and the placebo group will be provided for each time point, with the primary analysis inference based on the evaluation of the Week 8 estimates. P-values corresponding to the pairwise tests, not adjusted for multiplicity, of the difference between each dose group versus placebo will be provided only for the Study Week 8 time point.	Updated the primary efficacy endpoint as outlined in Statistical Analysis Plan
Section 9.3.2/Efficacy Analyses	Efficacy analyses will be repeated using the FAS subset of 100 mg treatment	Updated to align with the Statistical Analysis Plan

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	groups in order to evaluate whether there are any differences in the treatment effect ~~(versus pooled placebo)~~ depending on the levels of PRA at baseline.	
General Update thoughout the protocol	**mmHg**	Updated to remove space between mm and Hg

12.11.2. Amendment 5 (17 February 2022)

This summary includes changes made to Protocol MLS-101-201 Amendment 4 (10 January 2022). The overall rationale for the changes implemented in this protocol amendment was to include a Part 2 in the study design so that subjects with high renin (ie, PRA > 1 ng/mL/h) could participate in the study. The addition of "Part 1" or "Part 2" was added throughout the document where necessary to clarify what is to be done in each part of the study; each and every instance of this addition may not be documented in the summary of changes table below.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Number of Study Centers	Approximately 50 centers in the United States **for Part** 1 **and approximately 10 centers in the United States for Part 2**	Added number of study centers for Part 2
Synopsis/Endpoints and Section 3.2.3 Safety Endpoints and Section 3.2.5 Pharmacodynamic Endpoints	*Safety* • Change in office-measured SBP from Study Week 8 (end-of-treatment period) to Study Week 12 **for Part 1 and Study Week 10 for Part 2** (end of follow-up) *Pharmacodynamics* • Change in plasma 11-deoxycortisol and plasma renin activity (PRA) from baseline to the end of Study Weeks 4 and 12 **for Part 1 and end of Study Weeks 4 and 10 for Part 2** (end of follow-up) • Change in serum aldosterone, cortisol, and 11-deoxycorticosterone concentration from baseline to the end of Study Weeks 4 and 12 **for Part 1 and end of Study Weeks 4 and 10 for Part 2** (end of follow-up)	Added appropriate Study Weeks for Part 2
Synopsis/Methodology and Section 4.1/Overall Study Design	This is a **2-part** Phase 2 randomized	Added a Part 2 of the study
Synopsis/Methodology and Section 4.1/Overall Study Design	~~The~~**In Part 1 of the study, the** value ~~for~~**of** PRA ~~will not exceed~~ **must be** ≤ 1 ng/mL**/h** based on morning measurement**. If the value of PRA > 1 ng/mL/h based on morning measurement, then subjects may be eligible to enter Part 2 of the study**. The value for aldosterone ~~will not~~**must** be ~~less than~~ ≥ 1 ng/dL based on morning measurement **for both Part 1 and Part 2.**	To allow subjects with high renin (ie, PRA > 1 ng/mL/h) to participate in the study
Synopsis/Methodology and Section 4.1/Overall Study Design	…the pre-Screening window will reset to 2 weeks **for Part 1 or 4 weeks for Part 2**. The current estimate is that approximately ~~1000~~**1100** subjects will undergo up to	• Added 4 weeks of pre-Screening for Part 2 • Adjusted number of

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	2 weeks of pre-Screening **for Part 1 and 4 weeks of pre-Screening for Part 2**.	subjects pre-screened
Synopsis/Methodology and Section 4.1/Overall Study Design	Assuming an approximately 40% ~~drop out~~**screen failure** rate, a total of approximately 160 subjects will meet all eligibility requirements to qualify for enrollment in ~~the study~~**Part 1 of the study. For Part 2, it is estimated that approximately 60 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in for Part 2 after completing a Screening/main study ICF that has been approved by the IRB. Assuming an approximately 40% screen failure rate, a total of 36 subjects will meet all eligibility requirements to qualify for enrollment in Part 2 of the study.**	To clarify how many subjects will enter Part 2 of the study
Synopsis/Methodology and Section 4.1/Overall Study Design	…run-in period (up to 2 weeks) of twice-daily (BID) oral treatment with placebo **in Part 1 and once daily (QD) oral treatment with placebo in Part 2**,…	To clarify the study treatment during the placebo run-in period of Part 2
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 8.1.2/24-hour Ambulatory Blood Pressure Monitoring	The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week **4 (ie, Visit 10, Study Day 28 ± 2) in Part 2 and Study Week** 7 (ie, Visit 13; Study Day 49 ± 2) **in Parts 1 and 2**. Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study ~~Week~~ **Weeks 4 and 7**, the ABPM procedure can be initiated at home in extraordinary circumstances…	To clarify when the ABPM procedure will be done in Parts 1 and 2
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 8.2.6/Spot Urine **and 24-Hour Urine** Collection	In addition, subjects will be given ~~a urine collection kit for a spot urine collection that will be performed on Study Day~~ **either a spot urine collection kit for Part 1 or a 24-hour urine collection kit for Part 2 on the second visit of the placebo-run in period (Visit 4) to take home for use on Study Day 1. In Part 1 of the study, first morning urine will be collected prior to morning dose of study drug on Study Day** 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine levels. **In Part 2 of the study, 24-hour urine collection will be performed on Study Day 1 and again at the end of**	To clarify that a spot urine collection will be performed for Part 1 and a 24-hour urine collection will be performed for Part 2 and to specify the weeks these collections will take place and what analytes will be tested

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone levels.	
Synopsis/Methodology and Section 4.1/Overall Study Design	…they will be reminded of the instructions by the investigator or study coordinator on how to perform the ABPM procedure and the spot/**24-hour** urine collection at home.	Added 24-hour urine instead of spot urine collection for Part 2
Synopsis/Methodology and Section 4.1/Overall Study Design	…undergo up to 8 weeks of treatment and **either** 4 weeks of follow-up **in Part 1 or 2 weeks of follow-up in Part 2**	To clarify that Part 1 has 4 weeks of follow up and Part 2 has 2 weeks of follow up
Synopsis/Methodology and Section 4.1/Overall Study Design	**For Part 2, approximately 36 enrolled subjects ≥ 18 years of age will be randomized (6:1) to either 100 mg QD MLS-101 or placebo stratified by PRA levels such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.**	To clarify how subjects enrolled in Part 2 will be randomized
Synopsis/Methodology and Section 4.1/Overall Study Design	**All subjects in Part 2 will receive QD dosing to be administered in the morning.**	To clarify that subjects in Part 2 will receive QD morning dose
Synopsis/Methodology and Section 4.1/Overall Study Design	…and an end-of-study visit scheduled 4 weeks after the last study treatment **in Part 1 and 2 weeks after the last study treatment in Part 2** for final efficacy and safety assessments	To clarify that Part 1 has 4 weeks of follow up and Part 2 has 2 weeks of follow up
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 4.8 Study Conduct	**Subjects should be in a fasted state for all study visits in Part 2 unless there is a medical reason not to fast as determined by the investigator.**	To clarify that subjects should be fasted in Part 2
Synopsis/Methodology and Section 4.1/Overall Study Design	Subjects should also be encouraged to return for a final follow-up visit 4 weeks after the Study Week 8 visit **for Part 1 and 2 weeks after the Study Week 8 visit for Part 2**, at which the ~~Study Week 12 (~~end-of-study assessments~~)~~ will be performed.	To clarify the follow-up visit for Parts 1 and 2
Synopsis/Number of Subjects	Approximately 160 subjects are planned to be enrolled **in Part 1 and approximately 36 subjects are planned to be enrolled in Part 2.**	Adding number of subjects to be enrolled in Part 2
Synopsis/Eligibility Criteria (inclusion #5 and #6) and Section 5.1/Inclusion Criteria (#5 and #6)	5. ~~Inclusion~~**For Part 1, inclusion** based on morning pre-Screening visit measurement of PRA, ~~The~~**and the** value for PRA ~~will not exceed~~**must be ≤ 1** ng/mL/**h**. **For Part 2, inclusion** based on morning **Screening visit** measurement **of PRA, and the value of PRA must be > 1 ng/mL/h…** 6. Inclusion based on morning pre-Screening visit measurement of serum	To clarify the inclusion criteria for Parts 1 and 2

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	aldosterone **for Part 1 and Screening visit measurement of aldosterone for Part 2.** The value for aldosterone ~~will not~~ **must** be ~~less than~~ ≥ 1 ng/dL based on morning measurement…	
Synopsis/Eligibility Criteria (exclusion #4 and #11) and Section 5.2/Exclusion Criteria (#4 and #11)	4. Subjects with hyperkalemia, ie, serum potassium > 5.2 mEq/L at **Screening for Part 1 and > 4.8 mEq/L at Screening for Part 2** **11.** Subjects with office SBP ≥ 175 mm Hg or DBP ≥ 100 mm Hg **for Part 1 and SBP ≥ 160 mm Hg or DBP ≥ 100 mm Hg for Part 2**	To clarify the exclusion criteria for Parts 1 and 2
Synopsis/Investigational Product, Dosage, and Mode of Administration	MLS-101 will be administered orally in the dosage 25 mg BID, 50 mg QD, or 100 mg QD **in Part 1 and orally in the dosage 100 mg QD in Part 2**.	To clarify that subjects in Part 2 will receive QD 100 mg dose
Synopsis/Duration of Subject Participation Including Follow-up	~~Up~~ **For Part 1, subjects will participate in up to** 2 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 4-week follow-up period, for a total duration of up to 16 weeks. **For Part 2, subjects will participate in up to 4 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 2-week follow-up period, for a total duration of up to 16 weeks.**	To clarify the duration of participation for Part 2
Synopsis/Statistical Methods	**The primary estimand in the study aims to answer the research question on the treatment effect of investigational therapy versus placebo in addition to standard of care on the change in office-measured blood pressure from baseline to Week 8…All analyses based on Part 2 of the study will be exploratory in nature; no formal sample size considerations will be made for Part 2.**	• To add text regarding the primary endpoint • To clarify that analyses will be exploratory in Part 2
Synopsis/Figure 1: Study Schema	Modified study schema to add Part 2 of the study	Modified study schema to add Part 2 of the study
Synopsis/Table 2 Schedule of Assessments for Part 2	Added Table 2 Schedule of Assessments for Part 2 of the study	Added Table 2 Schedule of Assessments for Part 2 of the study
Section 2.1/Study Rationale	Various dose levels and dose regimens will be tested to identify a dose response and a therapeutic index to inform subsequent studies to support an indication in **uncontrolled hypertension, with**	To modify the indication

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	particular emphasis on defined patient populations such as low-renin hypertension.	
Section 3.1.1/Primary Objective	To characterize the effect of MLS-101 on BP at 5 dosing regimens versus placebo when administered orally for the treatment of ~~low-renin~~ **uncontrolled** hypertension as add-on therapy to stable background treatment	To match primary objective in synopsis
Section 4.2/Scientific Rationale for Study Design	**Data obtained from the interim analysis performed in Part 1 of the study will be used to inform Part 2.**	To clarify purpose of interim analysis in Part 1
Section 4.3/Number of Subjects	Approximately ~~1000~~**1100** subjects will undergo up to 2 weeks of pre-Screening. ~~It~~ **For Part 1, it** is estimated that approximately 270 subjects will have qualifying PRA and serum aldosterone levels ~~during pre-Screening~~ to be eligible to enter Screening/**start of** Placebo Run-in, and (assuming ~~a~~ **an approximately** 40% ~~drop-out~~ **screen failure** rate)… **For Part 2, it is estimated that approximately 60 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in, and (assuming an approximately 40% screen failure rate) a total of 36 subjects will meet all eligibility requirements to qualify for enrollment in Part 2 of the study. The subjects who qualify for enrollment will be randomized (6:1) to either 100 mg QD MLS-101 or placebo stratified by PRA levels such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects across approximately 10 study centers in the United States.**	• To clarify number of subjects that will undergo pre-Screening • To clarify number of subjects that will enter Screening and enroll in Part 2 of the study
Section 4.4/Study Duration	**For Part 2, subject's duration of participation will be approximately 16 weeks (up to 4 weeks of pre-Screening, followed by up to 2 weeks of a Screening/single-blind Placebo Run-in period, 8 weeks of double-blind treatment with study drug, and a 2-week follow-up period).**	Added study duration for Part 2
Section 4.6/End of Study Definition	…including Visit 16 (end-of-study visit) at Study Week 12 **and Study Week 10 for Parts 1 and 2, respectively.** Any death that occurs while on study or within 30 days **(Part 1) and 15 days (Part**	• Added end-of-study visit week for Part 2 • To clarify end of data collection for Part 2

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	2) after the subject's last dose of study drug must be reported to the sponsor/designee for the study within 24 hours after the center becomes aware of the event.	
Section 4.8.1/Pre-Screening	will enter up to 2 weeks of pre-Screening **for Part 1 and 4 weeks of pre-Screening for Part 2** upon completion of the IRB-approved pre-Screening informed consent	To clarify 4 weeks of pre-Screening for Part 2
Section 4.8.2/Screening/Start of Placebo Run-in	…pre-Screening criteria (ie, PRA ≤ 1 ng/mL/**h** ~~hr~~ and aldosterone ≥ 1 ng/dL **for Part 1; PRA > 1 ng/mL/h and aldosterone ≥ 1 ng/dL for Part 2**; or alternative target guidance provided by the sponsor)…	To clarify pre-Screening criteria for Part 2 of the study
Section 4.8.2/Screening/Start of Placebo Run-in	Once all Screening procedures are complete, subjects immediately enter the single-blind run-in period of BID (Part 1) **or QD (Part 2)** oral treatment with placebo	To clarify that placebo is given QD during run-in period in Part 2 of the study
Section 4.8.3/Single-blind Placebo Run-In Week 2	In addition, subjects will be given **either** a spot urine collection **kit for Part 1 or a 24-hour urine collection kit for Part 2 to take home for use on Study Day 1. In Part 1 of** the **study, first** morning **urine will be collected prior to morning dose** of **study drug on** Study Day 1 and again at the end of Study Week 8 (ie, Study Day 56 ± 2 or end-of-treatment period) for determination of potassium, sodium, and creatinine **levels. In Part 2 of the study, 24-hour urine collection will be performed on Study Day 1 and again at the end of Study Week 4 (ie, Visit 10, Study Day 28 ± 2) for determination of potassium, sodium, creatinine, and aldosterone** levels	To clarify that a spot urine collection will be performed for Part 1 and a 24-hour urine collection will be performed for Part 2 and to specify the weeks these collections will take place and what analytes will be tested
Section 4.8.5/Randomization/Baseline	After first morning spot **(Part 1) or 24-hour (Part 2)** urine collection at home… • Spot urine collection (**Part 1 only**) • 24-hour urine collection (**Part 2 only**)	To clarify that spot urine is performed in Part 1 and 24-hour urine is performed in Part 2
Section 4.8.9/Study Week 4	• Initiate 24-hour ABPM (**Part 2 only**) • 24-hour urine collection (**Part 2 only**)	To add 24-hour ABPM and 24-hour urine at Study Week 4 for Part 2
Section 4.8.13/Study Week 8	• Spot urine collection (**Part 1 only**)	To clarify that spot urine is performed at StudyWeek 8 in Part 1 only
Section 4.8.15/Study Week 10	On Study Day ~~84~~ **70** ± 3 days, subjects will return to the site for an end-of-study visit scheduled ~~4~~ **2** weeks after the last treatment with study drug	Modified Section 4.8.15 to add the end-of-study visit at Study Week 10 for Part 2 to occur 2 weeks after last

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
		study treatment
Section 4.8.16/Study Week 12	Added Section 4.8.16 with procedures for the end-of-study visit for Part 1	To clarify that the end-of-study visit for Part 1 occurs at Study Week 12
Section 6.1.1/Description of Study Drug	MLS-101 ~~Tablets~~ **tablets** of 12.5 mg, 25 mg, and 100 mg and placebo tablets are packaged in ~~an opaque wallet card blister~~ **either 30 count 60 cc white round high density polyethylene bottles** with ~~35 film coated tablest (MLS-101~~ **a child-resistant cap or polyvinyl chloride/Aclar blisters with foil backing** and~~/or placebo~~ **7 tablets** per blister ~~(configuration of 5 x 7). Each blister pack containing a 1-week supply (35 tablets) of study drug~~ **strip. Packaged tablets** will be assigned a unique identifying number…	To update study drug packaging
Section 6.1.2/Dosing and Administration	*Placebo Run-In:* **In Part 2, upon signing the Screening/main study ICF, subjects will begin a single-blind (subjects blinded to treatment allocation), up to 2-week run-in period of QD oral treatment with placebo, while continuing to remain on stable doses of their background antihypertensive medications**. *8-week Treatment Period:* Subjects will be monitored by ABPM for 24 hours before the first morning dose on Study Day 1 and for 24 hours after the ~~last~~ morning dose on Study Day ~~56~~**49** ± 2 days (end of Week ~~8~~**7**)…**In Part 2, subjects will orally administer the assigned study drug (MLS-101 [100 mg QD] or placebo) according to the assigned dosing regimen for 8 weeks beginning on Study Day 1. Subjects will be monitored by ABPM for 24 hours before the first morning dose on Study Day 1, for 24 hours beginning on Study Day 28 ± 2 days (Week 4), and for 24 hours after the last morning dose on Study Day 49 ± 2 days (end of Week 7).**	• Added dosing for Part 2 in Placebo Run-in and 8-Week Treatment Period • Clarified that ABPM is performed at end of Study Week 7 for Part 1
Section 6.2.2/Study Drug Packaging and Labeling	…labeling of the tablet ~~blister packs~~ **package** will not show the treatment allocation. All other information required by regulation will appear on ~~blister pack~~ **the label**.	To update the study drug packaging
Section 6.3/Randomization and Blinding	**All subjects who qualify for Part 2 based on inclusion/exclusion criteria and complete up to a 2-week Placebo Run-in period and 24-hour ABPM procedure will be randomized (6:1) to**	Added the randomization and blinding instructions for Part 2 of the study

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	either 100 mg QD MLS-101 or placebo stratified by PRA levels such that the MLS-101 treatment group will consist of approximately 30 subjects and the placebo treatment group will consist of approximately 6 subjects.	
Section 6.5.1/Hyperkalemia	• Serum potassium > 5.5 and ≤ 6 mEq/L: Retest at local lab to confirm. If potassium remains elevated, hold dose. Wait 2 to 7 days, then retest. If potassium ~~<~~≤ 5.2 mEq/L~~, restart at first dose-reduced level according to Table 3~~**resume dosing**. If potassium > 5.2 mEq/L, continue to hold dose. Wait 2 to 7 days, then retest. If potassium ~~<~~ ≤ 5.2 mEq/L after second retest, restart at first dose-reduced level according to Table 3. If potassium > 5.2 mEq/L after second retest discontinue study drug. • ~~Serum potassium > 6 mEq/L: Discontinue study drug.~~ • **Serum potassium > 6 mEq/L: Hold study drug dose and bring subject in for an unscheduled study visit for safety ECG and serum potassium retest at local lab; subject attends next regularly scheduled visit. If potassium from local lab retest ≤ 5.2 mEq/L, resume dosing. If potassium > 5.2 mEq/L, contact sponsor's medical monitor for further guidance.**	To modify the actions to be taken for elevated serum potassium levels
Section 6.5.1/Hyperkalemia/Table 3	Modified the second dose-reduced level for the 100-mg dose from 12.5 mg to 25 mg QD	Modified the second dose-reduced level for the 100-mg dose from 12.5 mg to 25 mg QD
Section 7.1/Discontinuation of Study Drug	Subjects should also be encouraged to return for a final follow-up visit 4 weeks after the Study Week 8 visit for **Part 1 and 2 weeks after** the Study Week ~~12 (~~**8 visit for Part 2, at which the end-of-study**~~)~~ assessments **will be performed**.	To clarify that the follow-up visit is 2 weeks after the end-of-study visit for Part 1 and 4 weeks after the end-of-study visit for Part 2
Section 7.1/Discontinuation of Study Drug and Section 7.2/Discontinuation/Withdrawal of Subjects	…before Study Week 12 **in Part 1 or Study Week 10 in Part 2**	To clarify follow-up visit is at Study Week 12 in Part 1 and is at Study Week 10 in Part 2
Section 8.3.1/Time Period and Frequency for Collecting AE and SAE Information	All AEs and SAEs will be collected from Screening/start of Placebo Run-in (after signed Screening/main study ICF) until 30 days post last dose of study drug **in Part 1** (Study Week 12) **and 15 days post last dose of study drug in Part 2 (Study Week 10)** at the timepoints specified in the	To clarify that AEs and SAEs will be collected 30 days post last dose for Part 1 and 15 days post last dose for Part 2

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	SoA (Table 1 and Table 2). All SAEs occurring from Screening/start of Placebo Run-in (after signed Screening/main study ICF) until 30 days post last dose of study drug **in Part 1** (Study Week 12) **and 15 days post last dose of study drug in Part 2 (Study Week 10)** will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours.	
Section 8.3.5/Pregnancy	Details of all pregnancies in female subjects and female partners of male subjects will be collected after the start of the Placebo Run-in and until 30 days **(Part 1) and 15 days (Part 2)** after the last subject visit	To clarify end of data collection for Parts 1 and 2
Section 8.3.6/Death Events	Any AE resulting in death that occurs during the study or within 30 days **(Part 1) and 15 days (Part 2)** after the subject's final dose of study drug, regardless of when the final visit occurs, is considered a SAE and must be reported…	To clarify end of data collection for Parts 1 and 2
Section 8.5/Pharmacodynamics	…will be collected at Screening/start of Placebo Run-in, Study Day 1 (baseline), Study Week 4, and Study Week ~~12~~ **10 for Part 2 or Study Week 12 for Part 1** (end of follow up).	To clarify that Week 10 is end of follow up for Part 2 and Week 12 is end of follow up for Part 1
Section 9.1/Analysis Sets	~~Three~~**Five** analysis sets ~~are~~ **defined across both parts of the study (ie, Part 1 and Part 2 data combined) are:** • All randomized set: all randomized subjects. **This analysis set will be used for summaries of subject study disposition with subjects analyzed according to the randomized treatment group**. • Full analysis set **(FAS):** all subjects randomized and who received at least 1 dose of study treatment. **All efficacy analyses will be performed using the full analysis set; subjects will be analyzed according to the randomized treatment group.** • **Per protocol set: all subjects in the FAS who have completed the Study Week 8 visit without any major protocol deviation that could influence the validity of the data for the primary efficacy evaluations; subjects will be**	Added 2 analysis sets to match SAP

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	analyzed according to the randomized treatment group. • **PK/PD analysis set: subjects in the FAS who have sufficient data available for the analysis of pharmacokinetic and pharmacodynamic measurements; subjects will be analyzed according to the actual treatment received.** • Safety analysis set: all **enrolled** subjects who received at least 1 dose of study treatment. **All safety analyses will be performed using the safety analysis set; subjects will be analyzed according to the actual treatment received.**	
Section 9.2/Sample Size Determination	**All analyses based on Part 2 of the study will be exploratory in nature; no formal sample size considerations will be made for Part 2**.	To clarify that all analyses for Part 2 will be exploratory
Section 9.3/Statistical Methods	**Unless otherwise noted, summaries will be provided on the data combined from Part 1 and Part 2, with all placebo subjects pooled into 1 group for tabulations**.	To clarify that data will be combined for Parts 1 and 2
Section 9.3.2/Efficacy Analyses	In general, descriptive statistics for continuous variables will consist of subject count, mean, standard deviation, median, and range **(min, max)**…**Details of the estimand components are provided in the SAP.**	• To define the range as min, max • To specify that additional details will be provided in the SAP
Section 9.3.2/Efficacy Analyses	The primary efficacy analysis will be performed using a mixed model repeated measures approach with fixed effects of categorical terms for treatment, week, and treatment by week interaction, stratification factor ~~(Seated SBP ≤ 160 mm HG, seated SBP > 160 mm Hg)~~, and baseline SBP as a fixed continuous covariate.	Deleted stratification factor for Part 1 since Part 2 uses a different stratification factor
Section 9.3.2/Efficacy Analyses	**Efficacy analyses will be repeated using the FAS subset of 100 mg treatment groups in order to evaluate whether there are any differences in the treatment effect (versus pooled placebo) depending on the levels of PRA at baseline.**	To clarify additional efficacy analyses to be performed
Section 9.3.3/Safety Analyses	**Select safety summaries will be repeated using the FAS subset of 100 mg treatment groups where subjects will be tabulated separately by PRA cohort at baseline (Part 1 or Part 2).**	To clarify additional safety analyses to be performed
Section 9.4/Handling of Missing Data	**For the office-measured efficacy endpoints, values post rescue medications**	To clarify the handling of missing data

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	will be set to missing and imputed using the last value observed before start of rescue medication. All other missing values will not be imputed.	
Section 12.2/Study Contacts	Added sponsor's medical monitor and made minor corrections to table	Added sponsor's medical monitor and made minor corrections to table
Section 12.9/Clinical Laboratory Tests	All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days **(Part 1) and 15 days (Part 2)** after the last dose of study drug should be repeated	To clarify end of data collection for Parts 1 and 2
Section 12.9/Clinical Laboratory Tests/Table 6	Added 24-Hour Urine Collection including aldosterone for Part 2	Added 24-Hour Urine Collection including aldosterone for Part 2
Section 12.10.4.1/AE and SAE Reporting Period	All AEs and SAEs will be collected from Screening (after signed Screening/main study ICF) until 30 days post last dose of study drug **in Part 1** (Study Week 12) **and 15 days post last dose of study drug in Part 2 (Study Week 10)** at the timepoints specified in the SoA (Table 1).	To clarify the AE and SAE reporting period, specifically, they will be collected 30 days post last dose for Part 1 and 15 days post last dose for Part 2

12.11.3. Amendment 4 (10 January 2022)

This summary includes changes made to Protocol MLS-101-201 Amendment 3 (24 August 2021). The overall rationale for the changes implemented in this protocol amendment was to reduce the number of cohorts from 6 to 4 by stopping enrollment into the 2 low dose cohorts (12.5 mg QD and 12.5 mg BID). This decision was based on a review of the clinical data at the December 2021 interim analysis. In addition, the ABPM procedure is now initiated at the end of Study Week 7 instead of Study Week 8 to allow for it to be repeated at Study Week 8 if deemed a failure at Study Week 7.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Endpoints and 3.2.2/ Secondary Efficacy Endpoints	Change in 24-hour ambulatory blood pressure monitoring (ABPM) parameters (systolic and diastolic) from baseline to the end of Study Week ~~8~~**7** (ie, Study Day ~~56~~**49** ± 2 ~~or end of treatment period~~)	To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Synopsis/Methodology and Section 4.1/Overall Study Design and Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#5 and #6)	The value for PRA will not exceed ~~0.6~~**1** ng/mL based on morning measurement. The value for aldosterone will not be less than ~~6~~**1** ng/dL based on morning measurement.	To clarify the qualifying PRA and serum aldosterone levels required at prescreening for eligibility
Synopsis/Methodology and Section 4.1/Overall Study Design	The ~~initial~~**current** estimate is that approximately ~~750~~**1000** subjects will undergo up to 2 weeks of pre-Screening. It is estimated that approximately ~~225~~**270** subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter Screening/start of Placebo Run-in after completing a Screening/main study ICF that has been approved by the IRB. Assuming ~~a 20~~**an approximately 40%** drop-out rate, a total of ~~180~~**approximately 160** subjects will meet all eligibility requirements to qualify for enrollment in the study.	To update the estimate of the number of subjects that will undergo pre-Screening, screening, and enrollment based on stopping enrollment into the 2 low dose cohorts

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Methodology and Section 4.1/Overall Study Design	**The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the end of Study Week 7 (ie, Visit 13; Study Day 49 ± 2). Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. At the end of Study Week 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to coronavirus disease 2019 (COVID-19), subject exposure to COVID-19, or subject testing positive for COVID-19. If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at Study Week 8…** ~~Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure (the ABPM procedure will be initiated in the clinic at Study Week 8).~~	To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Synopsis/Methodology and Section 4.1/Overall Study Design	A **planned** interim analysis (Section 9.5) ~~is planned~~**was conducted** and all available safety and tolerability data (vital signs, BP measurements, adverse events, and safety laboratory values) ~~will be~~**were** reviewed by a Data and Safety Monitoring Board (DSMB) (Section 8.3.8). **An additional interim analysis may be performed to support drug development decisions.**	To clarify that an interim analysis was conducted and an additional interim analysis may be performed
Synopsis/Methodology and Section 4.1/Overall Study Design	**NOTE: In the previous version of the protocol**, **subjects were randomized into 6 equal treatment groups (1:1:1:1:1:1) to 12.5 mg BID, 25 mg BID, 12.5 mg once daily (QD), 50 mg QD, 100 mg QD, or placebo. After a review of the clinical data at the December 2021 interim analysis, it was decided that the 2 lowest dose levels (12.5 mg QD and 12.5 mg BID) will be dropped due to lack of consistent meaningful reduction of blood pressure. Effective with Amendment 4, subjects will be randomized into 4 equal treatment groups (1:1:1:1) to 25 mg BID, 50 mg QD, 100 mg QD, or placebo.**	To clarify that the 2 low dose cohorts will stop enrollment based on the December 2021 interim analysis

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/Methodology and Section 4.1/Overall Study Design	Approximately ~~180~~ **120 of 160** enrolled subjects ≥ 18 years of age will be randomized into ~~64~~ **64** equal treatment groups (1:1:1:1~~:1:1~~) to ~~12.5 mg BID,~~ 25 mg BID, ~~12.5 mg once daily (QD),~~ 50 mg QD, 100 mg QD, or placebo; each treatment group will consist of approximately 30 subjects stratified by BP (seated SBP ≤ 160 mm Hg and seated SBP > 160 mm Hg). ~~Based on accumulated data, when 5 to 10 subjects in all cohorts have reached the 4-week treatment visit, 2 of the 5 treatment groups may be expanded up to a maximum of 60 subjects per cohort (not to exceed a total of 210 subjects exposed to active study drug) in which the additional subjects enrolled will have morning PRA values greater than the PRA cut-off value in effect at the time of the cohort expansion. The purpose of this limited cohort expansion is to better define the predictive value of baseline PRA for treatment effect of MLS-101. A contemporaneous data safety monitoring board (DSMB) review of accumulated safety experience will be completed before a decision is made to expand cohort size.~~ **Approximately 40 of 160 enrolled subjects were enrolled and completed the study in the 2 low dose cohorts that were discontinued with Amendment 4 as described above.**	• To clarify the number of planned enrolled subjects based on stopping enrollment into the 2 low dose cohorts • To clarify that subjects will no longer be enrolled into the 2 low dose cohorts • To clarify that treatment groups will not be expanded
Synopsis/Number of Subjects (planned)	Approximately ~~180~~**160** subjects are planned to be enrolled	To clarify the number of planned enrolled subjects based on stopping enrollment into the 2 low dose cohorts
Synopsis/Eligibility Criteria and Section 5.1/Exclusion Criteria (#11)	Subjects with office SBP ≥ 175 mm Hg or DBP ≥ 100 mm Hg (average of last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) **at Pre-Screening, Screening/Start of Placebo Run-in, or Randomization**	To clarify that subjects are ineligible if SBP ≥ 175 mm Hg or DBP ≥ 100 mm Hg at Pre-screening, Screening, or Randomization
Synopsis/Eligibility Criteria and Section 5.1/Exclusion Criteria (#12)	Subjects with a decrease in SBP ≥ 20 mm Hg or DBP ≥ 10 mm Hg from sitting to standing position **at Screening** (may be repeated during Screening at the investigator's discretion)	To clarify that standing BP will be assessed at screening

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/IP, Dosage, and Mode of Administration	MLS-101 will be administered orally in the dosage ~~12.5 mg BID,~~ 25 mg BID, ~~12.5 mg QD,~~ 50 mg QD, or 100 mg QD	To remove the 2 low dose cohorts
Synopsis/Statistical Methods	With 30 subjects **planned** per arm, the mean placebo adjusted change…	To clarify that 30 subjects per arm are planned
Synopsis/Figure 1: Study Schema	Deleted 2 low dose cohorts [c] ~~Based on accumulated data, when 5 to 10 subjects in all cohorts have reached the 4-week treatment visit, 2 of the 5 treatment groups may be expanded up to a maximum of 60 subjects per cohort (not to exceed a total of 210 subjects exposed to active study drug)~~	To clarify that the 2 low dose cohorts will stop enrollment based on the December 2021 interim analysis, and to clarify that treatment groups will not be expanded.
Synopsis/SOA	Moved 24-hour ABPM from Study Week 8 to Study Week 7	To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Synopsis/Table 1: Schedule of Assessments (footnote i)	[i]Subjects will be given the ABPM device on the second visit of the placebo-run in period (Visit 4; Study Day ~~-1~~**7 ± 2**). The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again in the clinic at the **end of** Study Week **7**~~8 clinic visit~~ (ie, Visit ~~1~~**413**; Study Day ~~56~~**49** ± 2 ~~or end-of-treatment period~~). Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. **At the end of Study Week 7, the ABPM procedure can be initiated at home in extraordinary circumstances, such as site closure due to COVID-19, subject exposure to COVID-19, or subject testing positive for COVID-19…If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at the end of Study Week 8.**	• To correct the Study Day for Visit 4 • To clarify that the ABPM procedure can be initiated at home in extraordinary circumstances such as relating to COVID • To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Synopsis/Table 1: Schedule of Assessments (footnote r)	[r]Single blood…In addition, ~~at selected sites,~~ blood samples for determination of MLS-101 (and metabolites) will be collected predose, and 1, 2, 3, and 4 hours postdose on Study Days 1 (Randomization) and 28 (Study Week 4).	To clarify serial PK sampling

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Section 4.3/Number of Subjects/Number of Centers	Approximately ~~750~~**1000** subjects will undergo up to 2 weeks of pre-Screening. It is estimated that approximately ~~225~~**270** subjects will have qualifying PRA and serum aldosterone levels during pre-Screening to be eligible to enter Screening/Placebo Run-in, and (assuming a ~~20~~**40**% drop-out rate) a total of ~~180~~**approximately 160** subjects will meet all eligibility requirements to qualify for enrollment in the study. The subjects who qualify for enrollment will be randomly divided into ~~6~~**4** equal treatment groups (1:1:1:1~~:1:1~~ with approximately 30 subjects per group, stratified by BP, ie, seated SBP ≤ 160 mm Hg and seated SBP > 160 mm Hg) across approximately 50 study centers in the United States. ~~Based on accumulated data, when 5 to 10 subjects in all cohorts have reached the 4-week treatment visit, 2 of the 5 treatment groups may be expanded up to a maximum of 60 subjects per cohort (not to exceed a total of 210 subjects exposed to active study drug) in which the additional subjects enrolled will have morning PRA values greater than the PRA cut-off value in effect at the time of the cohort expansion. The purpose of this limited cohort expansion is to better define the predictive value of baseline PRA for treatment effect of MLS-101. A contemporaneous DSMB review of accumulated safety experience will be completed before a decision is made to expand cohort size.~~	• To clarify the number of planned enrolled subjects based on stopping enrollment into the 2 low dose cohorts • To clarify that subjects will no longer be enrolled into the 2 low dose cohorts • To clarify that treatment groups will not be expanded
Section 4.8.2/Screening/Start of Placebo Run-in	All subjects with PRA and serum aldosterone values meeting the pre-Screening criteria (ie, PRA ≤ ~~0.6~~**1** ng/mL/hr and aldosterone ≥ ~~6~~**1** ng/dL or alternative target guidance provided by the sponsor) will be eligible to enter Screening/Placebo Run-in	To clarify the qualifying PRA and serum aldosterone levels required at prescreening for eligibility to enter Screening/Placebo Run-in
Section 4.8.5/ Randomization/Baseline and Section 4.8.9/Study Week 4	~~In addition, at selected sites~~ **Serial PK sampling – if subject consents to additional blood draws**, blood samples for determination of MLS-101 (and metabolites) are to be collected predose, and 1, 2, 3, and 4 hours postdose	To clarify that serial PK sampling will be done if subjects consent to additional blood draws

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Section 4.8.6/Study Week 1 and Section 4.8.7/Study Week 2 and Section 4.8.8/Study Week 3 and Section 4.8.9/Study Week 4 and Section 4.8.10/Study Week 5 and Section 4.8.11/Study Week 6 and Section 4.8.12/Study Week 7 and Section 4.8.13/Study Week 8	…subjects will return to the site and the following procedures will be performed ~~after~~**prior to** site administration of the morning dose of study drug	To clarify that study procedures will be performed prior to administration of study drug
Section 4.8.12/Study Week 7	• **Initiate 24-hour ABPM**	To clarify that ABPM will be initiated at the end of Study Week 7 instead of Study Week 8
Section 4.8.13/Study Week 8	• **Repeat** 24-hour ABPM **if procedure at Study Week 7 deemed a failure**	To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Section 4.9/Modifications to Study Conduct Due to the Coronavirus Disease 2019 (COVID-19) Pandemic	**As a consequence of the COVID-19 pandemic that has had a worldwide impact, control measures in place may impact the ability to adhere to some of the study procedures described in this protocol. Due to challenges that include, but are not limited to, subject COVID-19 infections, site closures, travel restrictions, and quarantines, some modifications to study conduct during the COVID-19 pandemic may be necessary to ensure study continuity, including conducting virtual visits when on-site study visits are considered not feasible. Such modifications in study conduct always must be in accordance with local regulations/mandates.** **The following are allowable, as necessary, modifications to study conduct during the COVID-19 pandemic:** • **Prior to a study visit at the site, the subject may be contacted and screened for potential exposure or infection to COVID-19 per site, local, or federal requirements. If the subject is suspected to be exposed or infected with COVID-19, the on-site visit should either be re-scheduled or a virtual visit may be performed instead, as applicable.** • **In the event that a subject cannot attend their regularly scheduled**	To add language to account for deviations in study conduct due to COVID-19

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	study visits in person due to COVID-19 necessitating a limit on in-person contact, the investigator may perform safety and efficacy assessments by phone or video. The investigator may use the technology platform that is currently available to them. Virtual assessments may include home blood pressure evaluation initiated by the subject, adverse events, and concomitant medication review. Virtual assessments will be recorded by site staff in the source documents. • **Clinical laboratory tests (chemistry and hematology) and pregnancy tests may be performed by local laboratory, if sample collection cannot be performed at the study site due to COVID-19 related limitations, including but not limited to site closure. Abnormal laboratory results should be promptly communicated to the medical monitor. Subjects' anonymity must be maintained when communicating results to the medical monitor.** • **At home IP administration may continue for up to 2 weeks (at multiple times during the study if required due to COVID-19, although not consecutively) if the subject has no relevant clinically significant out of range values per previous lab reports.** • **Source documentation should note that the visit was performed virtually (not face-to-face) and note the name of the local lab where laboratory tests were done, if applicable.** • **If certain study procedures or assessments cannot be completed per the schedule of events, the reason for the missed assessment (ie, laboratory tests, vital signs, physical examinations, etc) must be noted in the source documentation (eg, COVID-19), captured in the protocol**	

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	deviations documentation, and reported to the IRB, as applicable. **A detailed assessment of COVID-19 related risk and mitigation measures will be documented in the appropriate study plans.**	
Section 6.1.2/Dosing and Administration	*8-Week Treatment Period*: Subjects will orally administer the assigned study drug (MLS-101 [~~12.5 mg BID,~~ 25 mg BID, ~~12.5 mg QD,~~ 50 mg QD, 100 mg QD] or placebo)	To remove the 2 low dose cohorts
Section 6.3/Randomization and Blinding	…will be randomized into ~~6~~**4** equal treatment groups (1:1:1:1~~:1:1~~) to ~~12.5 mg BID,~~ 25 mg BID, ~~12.5 mg once daily (QD),~~ 50 mg QD, 100 mg QD, or placebo;	To clarify that subjects will no longer be enrolled into the 2 low dose cohorts
Section 6.5.1/Hyperkalemia/Table 2	Modified table to remove the 2 low dose cohorts (12.5 mg BID and 12.5 mg QD)	To remove the 2 low dose cohorts
Section 8.1.2/24-hour Ambulatory Blood Pressure Monitoring	Subjects will be monitored by ABPM for 24 hours before the first dose on Study Day 1 and ~~after~~**before** the ~~last~~**first** dose on Study Day ~~5~~6**49** ± 2 days (end of Week ~~8~~**7**)… The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again at the Study Week ~~8~~**7** visit (ie, Visit ~~14~~**13**; Study Day ~~5~~6**49** ± 2 ~~or end-of-treatment period~~). Alternatively, sites may choose to schedule an office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure (the ABPM procedure will be initiated in the clinic at Study Week ~~8~~**7 unless a subject is not able to attend the visit due to extraordinary circumstances, such as COVID-19 restrictions including site closure, subject exposure to COVID-19, or subject testing positive for COVID-19). If, for any reason, the ABPM procedure is deemed a failure at the end of Study Week 7, it can be repeated at Study Week 8.**	• To clarify that ABPM will be initiated at the end of Study Week 7 instead of Study Week 8 • To add language to account for deviations in study conduct due to COVID-19 • To allow for the ABPM procedure to be repeated at Study Week 8 if deemed a failure at Study Week 7
Section 8.3.7/Adverse Events of Special Interest	Hyperkalemia **with dose modification (dose reduction, dose hold, or permanent dose withdrawal)**	To update based on new guidance that hyperkalemia will be considered an AESI if it requires dose modification

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Section 9.3.2/Efficacy Analysis	The full analysis set will be used for the efficacy analyses. ~~Subjects will be analyzed based upon the treatment received~~. In general, descriptive statistics for continuous variables will consist of subject count, mean, standard deviation, median, and range. Descriptive statistics for categorical variables will consist of subject counts and percentages. ~~As the primary intent of this study~~**The primary estimand** is **designed** to ~~provide a preliminary assessment~~**answer the research question on the treatment effect of investigational therapy versus placebo in addition to standard** of **care on** the ~~relative efficacy of the doses studied, the use of descriptive summary statistics and graphical methods rather than formal statistical tests of significance will be emphasized. Confidence intervals (90% 2-sided) will be used to guide these assessments. Given the preliminary nature of this study, no adjustment for multiplicity is anticipated~~**change in office blood pressure from baseline to Study Week 8.** The primary endpoint will be summarized by treatment group using the mean change from baseline in SPB. The mean will be based upon the last on treatment value recorded for each subject prior to the use of any antihypertensive medications prescribed as "rescue" medications (estimand based upon the hypothetical treatment strategy per International Council for Harmonisation [ICH]-E9 R1) and the 90% (2-sided) confidence interval will use the t-distribution. The mean change from baseline will also be calculated using the last recorded value (treatment policy strategy). Analyses using an analysis of covariance will also be conducted to quantify the relationship between the change from baseline and the baseline value for each treatment group. Similarly, the relationship between change from baseline and use of ACE inhibitors/ARBs at baseline will be quantified for each treatment group. The **primary efficacy endpoint is the Study Week 8 change from baseline in SBP where the Study Week 8 value will be**	To update language to match statistical analysis plan

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	defined as the average of Study Week 7 and Study Week 8 measurement. The primary efficacy analysis will be performed using a mixed model repeated measures approach with fixed effects of categorical terms for treatment, week, and treatment by week interaction, stratification factor (Seated SBP ≤ 160 mm HG, seated SBP > 160 mm Hg), and baseline SBP as a fixed continuous covariate. A least square estimate, along with the 90% CI, of the mean difference between each dose group and the placebo group will be provided for each time point, with the primary analysis inference based on the evaluation of the Week 8 estimates. P-values corresponding to the pairwise tests, not adjusted for multiplicity, of the difference between each dose group versus placebo will be provided only for the Study Week 8 time point. • For the office-measured secondary efficacy endpoints, a similar approach will be used as for the primary estimand analysis. Both primary and secondary SBP and DBP endpoints will be summarized descriptively by study week ~~based upon the subjects with data.~~ The ABPM parameters (SBP and DBP) will also be summarized descriptively by study week. • A response to treatment assessment will be based upon whether or not the office-measured BP achieved values of ≤ 130/80 mm Hg by the end of Study Week 8. ~~Each subject will be assessed as a success if the Week 8 values recorded meet this definition and will be assessed as a failure otherwise (including premature termination for any reason).~~**Subjects without an assessment at Study Week 8 or who have received rescue medications will be considered as failures.** The proportion of successes will be calculated for each treatment group. The timing of the first value of ≤ 130/80 mm Hg will be summarized using a	

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	Kaplan-Meier approach within treatment group. The relationship to baseline SBP and DBP will also be explored. **All secondary efficacy endpoints analyses will be considered supportive and any inferential statistics will be considered descriptive in nature. No adjustment for multiplicity will be performed. Further details of the estimand definition, analysis model, sensitivity and supplementary analyses, including the analyses of the data collected after Study Week 8, are given in the statistical analysis plan.**	
Section 9.5/Interim Analysis	An interim analysis of the unblinded safety and efficacy data ~~will be~~**was** performed after the first 5 to 10 subjects in each dose group (30 to 60 subjects total) completed 4 weeks of treatment. ~~As this is an exploratory, dose-finding study, no formal statistical testing will be performed, and adjustments for multiplicity will not be implemented.~~ **Safety summaries included tabulation of treatment emergent AEs, SAEs, and AESIs, as well as select lab and PD parameters, summarized by study visit.** At the time of the interim analysis, additional exploratory efficacy analyses using the primary efficacy endpoint was conducted within each dose group. The results of these analyses had limited, prespecified distribution and were used for internal decision making and development planning. The details of these planned analysis are provided in the final statistical analysis plan. **An additional interim analysis may be performed to support drug development decisions.**	To clarify what was done in the interim analysis performed in December 2021

12.11.4. Amendment 3 (24 August 2021)

This summary includes changes made to Protocol MLS-101-201 Amendment 2 (01 June 2021). The overall rationale for the changes implemented in this protocol amendment was to allow for pre-Screening PRA and aldosterone values to be adjusted during conduct of the trial based on operational experience gained through continuous review of PRA and serum aldosterone values, to allow for possible cohort expansion, and to combined Visits 2 and 3.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Study Period	~~Estimated date~~ **Date** first subject enrolled: ~~Q2~~ **30 July** 2021	Added date first subject enrolled
Synopsis/Objectives and Endpoints and Section 3.2.4/Pharmacokinetic Endpoints	PK parameters ~~of,~~ **including, if feasible,** area under the plasma concentration versus time curve…	To clarify that PK parameters will be calculated if feasible
Synopsis/Methodology and Section 4.1/Overall Study Design	Subjects with **a history consistent with inadequately controlled** hypertension ~~who meet the protocol definition for~~, **(ie, SBP ≥ 135 mm Hg on a** stable ~~background~~ treatment **regimen of ≥ 2 antihypertensives)**, will undergo…	To clarify that subjects must meet the definition of hypertension at pre-Screening as measured with AOBP
Synopsis/Methodology and Section 4.1/Overall Study Design	~~Subjects with PRA ≤ 0.6 ng/mL and serum aldosterone ≥ 6 ng/dL will then enter the study Screening process~~. **The value for PRA will not exceed 0.6 ng/mL/hr based on morning measurement. The value for aldosterone will not be less than 6 ng/dL based on morning measurement. Alternative target PRA and /or aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during conduct of the trial based on ongoing review of PRA and serum aldosterone values. Subjects with PRA and serum aldosterone values meeting the stipulated guidance criteria will then enter the study Screening process. If PRA and/or serum aldosterone do not meet the target guidance values during pre-Screening, the PRA and aldosterone measurements may be repeated 1 time and the pre-Screening window will reset to 2 weeks. If guidance criteria are revised, then previously pre-screened individuals who meet the revised criteria may be considered for entry into the Screening process with consent of the sponsor, and the pre-Screening window will reset to 2 weeks.**	To allow for pre-Screening PRA and aldosterone values to be adjusted during conduct of the trial based on operational experience gained through continuous review of PRA and serum aldosterone values

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/Methodology and Section 4.1/Overall Study Design	**The initial estimate is that** ~~A~~approximately 750 subjects will undergo up to 2 weeks of pre-Screening. It is estimated that approximately 225 subjects will have qualifying PRA and serum aldosterone levels to be eligible to enter ~~up to 4 weeks~~ **S**creening**/start of Placebo Run-in** after completing…	To allow for possible cohort expansion and to combine the Screening and start of Placebo Run-in visits (V2 and V3)
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 4.8.2/Screening	~~If PRA is > 0.6 ng/mL during Screening, the measurement should be repeated and the medical monitor should be contacted.~~	To clarify that PRA and aldosterone values must be met at Pre-Screening only for inclusion in the study
Synopsis/Methodology and Section 4.1/Overall Study Design	~~Once enrolled~~, **At the Screening/start of Placebo Run-in visit (V2/V3)**, subjects will **complete the Screening assessments and** begin a single-blind (subjects blinded to treatment allocation), ~~2 week~~ run-in period **(up to 2 weeks)**…standing BP will be measured at **the** Screening**/start of Placebo Run-in visit**	To combine the screening and start of Placebo Run-in visits (V2 and V3)
Synopsis/Methodology and Section 4.1/Overall Study Design	Subjects will return to the research facility ~~once each week~~ **at the start of Week 2** of the run-in period **(V4)** for protocol-defined assessments including BP measurements.	To clarify that subjects return at start of Week 2 of placebo run-in (Visit 4)
Synopsis/Methodology and Section 4.1/Overall Study Design	At the second clinic visit of the ~~placebo run~~**Placebo Run**-in period, subjects **will be evaluated for eligibility based on Screening data, and if eligible, will continue Week 2 of Placebo Run-in. Subjects**…	To clarify the subjects will be evaluated for eligibility based on Screening data
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 8.1.2/24-hour Ambulatory Blood Pressure Monitoring	Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure **(the ABPM procedure will be initiated in the clinic at Study Week 8).**	To clarify that the Study Week 8 ABPM is initiated in the clinic at the site visit

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 4.3/Number of Subjects/Number of Centers	**Based on accumulated data, when 5 to 10 subjects in all cohorts have reached the 4-week treatment visit, 2 of the 5 treatment groups may be expanded up to a maximum of 60 subjects per cohort (not to exceed a total of 210 subjects exposed to active study drug) in which the additional subjects enrolled will have morning PRA values greater than the PRA cut-off value in effect at the time of the cohort expansion. The purpose of this limited cohort expansion is to better define the predictive value of baseline PRA for treatment effect of MLS-101. A contemporaneous data safety monitoring board (DSMB) review of accumulated safety experience will be completed before a decision is made to expand cohort size.**	To allow for possible cohort expansion
Synopsis/Methodology and Section 4.1/Overall Study Design	Subjects will attend up to 14 full clinic visits, including a pre-**S**~~s~~creening visit, a **S**~~s~~creening/**start of Placebo Run-in visit**, ~~2 visits~~**a second visit** during **P**~~p~~lacebo **R**~~r~~un-in, **a clinic visit to initiate the ABPM procedure,** a Randomization visit…	To clarify subject clinic visits
Synopsis/Methodology and Section 4.1/Overall Study Design	~~In general, i~~**I**f, at any time during the study, a subject meets 1 or more of the **clinical or** laboratory exclusion criteria for the study…and BP measurements will be repeated ~~at home~~.	To clarify that clinical and laboratory data are used for exclusion criteria, and that the BP measurement is done in the clinic
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#3)	3. ~~Automated office blood pressure (~~**Average of** AOBP~~) with SBP ≥ 135 mm Hg (average of last 2 of 5 unattended~~ measurements **taken** ~~using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position)~~ at Screening~~, the~~ /start of the ~~2 week~~ Placebo Run-in **Visit** ~~period~~, and ~~on the day of~~ **R**~~r~~andomization **with SBP ≥ 130 mm Hg**	To clarify that subjects must have average AOBP measurements at Screening/start of Placebo Run-in and Randomization of SBP ≥ 130 mm Hg
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#5)	5. ~~PRA ≤ 0.6 ng/mL based on morning measurement~~ **Inclusion based on morning pre-Screening visit measurement of PRA. The value for PRA will not exceed 0.6 ng/mL/hr based on morning measurement. Alternative target PRA criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of PRA values.**	To allow for pre-Screening PRA values to be adjusted during conduct of the trial based on ongoing review of PRA values

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#6)	6. ~~Serum aldosterone ≥ 6 ng/dL based on morning measurement~~ **Inclusion based on morning pre-Screening visit measurement of serum aldosterone. The value for aldosterone will not be less than 6 ng/dL based on morning measurement. Alternative target aldosterone criteria for the study may be provided by the sponsor. These alternative targets may be adjusted during the conduct of the trial based on ongoing review of serum aldosterone values.**	To allow for pre-Screening aldosterone values to be adjusted during conduct of the trial based on ongoing review of serum aldosterone values
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#7)	7. Serum cortisol ≥ 18 mcg/dL (morning measurement) or serum cortisol > 3 and < 18 mcg/dL (morning measurement) **at Screening/start of Placebo Run-in visit** if there is no evidence of adrenal insufficiency based on normal ACTH stimulation test results. **In the event that ACTH stimulation testing cannot be performed due to logistical challenges, including supply chain limitations to availability of synthetic ACTH, then subjects may be entered into the trial if morning serum cortisol is > 3 mcg/dL and they fulfill the criteria for no recent use of exogenous corticosteroid medications in dosages that could potentially cause adrenal suppression (ie, Exclusion Criteria 16a).**	To clarify the action to be taken if ACTH stimulation test cannot be performed due to logistical challenges
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#8)	Women of childbearing potential must have a negative serum pregnancy test ~~within 7 days of the start of the single-blind, placebo run-in, ie, between Study Days -21 and -14~~**7 prior to Randomization**	To clarify that negative serum pregnancy test is required before Randomization
Synopsis/Eligibility Criteria and Section 5.1/Exclusion Criteria (#2)	2. Use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) in combination **(ie, concomitant ACE inhibitors plus ARB therapy)** as background antihypertensive treatment	To clarify definition of using ACE inhibitors and ARBs in combination
Synopsis/Eligibility Criteria and Section 5.1/Exclusion Criteria (#3, #4, #5, #6, #7)	…**at Screening**	To clarify that these measurements are taken at the Screening Visit
Synopsis/Duration of Subject Participation Including Follow-up	Up to 2 weeks of pre-**S**~~s~~creening ~~and up to 4 weeks of~~ , followed by **up to** 2 weeks of **a Screening**/single-blind **P**~~p~~lacebo **R**~~r~~un-in period, 8 weeks of double-blind treatment with study drug and a 4-week follow-up period, for a total duration of up to ~~20~~**16** weeks.	To clarify duration of subject participation

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/Figure 1: Study Schema	• Deleted Screening box and combined Screening with Placebo Run-in • ~~AOPB~~**BP** = ~~automated office~~ blood pressure • Added footnotes: **[a] If Screening results are available, inclusion/exclusion evaluation will be performed. If subject is not eligible based on Screening results, they will not continue to Visit 4. If Screening results are not available, subject proceeds to Visit 4. If Screening results are not available at Visit 4, subject should attend Visit 5 to determine final eligibility. If eligible based on Screening results, ABPM assessment can begin at Visit 5.** **[b] The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1). Alternatively, sites may choose to schedule an office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure. Training for the ABPM procedure can be done at an office visit or via phone.** **[c] Based on accumulated data, when 5 to 10 subjects in all cohorts have reached the 4-week treatment visit, 2 of the 5 treatment groups may be expanded up to a maximum of 60 subjects per cohort (not to exceed a total of 210 subjects exposed to active study drug)**	• To combine the screening and start of Placebo Run-in visits (V2 and V3) • To clarify that the BP check at Visit 15 is performed at home and not in the clinic • Added footnotes to clarify which visits subjects should attend, when ABPM procedure should be initiated, and to allow for possible cohort expansion
Synopsis/Table 1: Schedule of Assessments	• Combined Visit 2 and Visit 3 • Deleted AOBP at Study Week 9 from table and replaced with **Home BP check**. • Modified footnote **a.** ~~All data collected at Randomization (predose) are considered the baseline values for the purposes of data analyses.~~ **Subjects who continue to meet all eligibility requirements will be randomized to receive MLS-101 or placebo.** • Added footnote **c. If Screening results are available, inclusion/exclusion evaluation will be performed. If subject is not eligible based on Screening results, they will not continue to Visit 4. If Screening results are not available, subject proceeds to Visit 4.**	• To combine the screening and start of Placebo Run-in visits (V2 and V3) • To clarify that the BP check at Visit 15 is performed at home and not in clinic • To delete definition of baseline values • To clarify eligibility based on availability of Screening results at Visit 4 • To clarify eligibility based on availability of Screening results at Visit 5 • To clarify that the BP check at Visit 15 is performed at home and

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	• Added footnote **d. If Screening results are not available at Visit 4, subject should attend Visit 5 to determine final eligibility. If eligible based on Screening results, ABPM assessment can begin at Visit 5.** • Added footnote g**. Blood pressure measurement to be performed at home.** • Modified footnote i. The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again **in the clinic** at the Study Week 8 **clinic** visit • Modified footnote k. A sample for serum pregnancy test will be collected at Screening ~~(within 7 days of the~~ /start of the single-blind, **P~~p~~**lacebo **R~~r~~**un-in, ie, ~~between~~ Study ~~Days -21 and~~**Day** -14 **± 2** • Modified footnote l. ~~If PRA > 0.6 ng/mL, measurement should be repeated and medical monitor should be contacted.~~ • Modified footnote t. On site visit days, subjects should be instructed to take their morning dose of study drug at ~~home prior to arriving at~~ the site	not in clinic • To clarify that the Study Week 8 ABPM procedure will be initiated in the clinic • To clarify serum pregnancy test • To clarify that PRA and aldosterone values must be met at Pre-Screening only for inclusion in the study • To clarify that subjects should take morning dose of study drug at site on site visit days
Section 4.3/Number of Subjects/Number of Centers	… to be eligible to enter ~~up to 4 weeks of screening~~ **Screening/Placebo Run-in**…	To combine the screening and start of Placebo Run-in visits (V2 and V3)
Section 4.4/Study Duration	It is anticipated that each subject's duration of participation will be approximately ~~20~~**16** weeks (up to 2 weeks of pre-**S~~s~~**creening ~~and up to 4 weeks of screening~~, followed by **up to** 2 weeks of **Screening**/single-blind **P~~p~~**lacebo **R~~r~~**un-in period, 8 weeks of double-blind treatment with study drug, and a 4-week follow-up period). Total duration of the study will be approximately ~~22~~**21** months	To clarify duration of subject participation and total study duration
Section 4.8.1/Pre-Screening	Subjects with **a history of** hypertension, **ie, SBP ≥ 135 mm Hg,** who meet the protocol definition…	To clarify criteria subjects must meet to enter pre-Screening

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Section 4.8.2/Screening/**Start of Placebo Run-in (Visit 2/Visit 3, Study Day -14 ± 2 Days**	• All subjects with PRA ~~≤ 0.6 ng/mL~~ and serum aldosterone **values meeting the pre-Screening criteria (ie, PRA ≤ 0.6 ng/mL/hr and aldosterone** ≥ 6 ng/dL **or alternative target guidance provided by the sponsor)** will be eligible to enter ~~up to 4 weeks of~~ Screening/**Placebo Run-in.** • ~~If PRA > 0.6 ng/mL, measurement should be repeated and medical monitor should be contacted~~. • **Once all Screening procedures are complete, subjects immediately enter the single-blind run-in period of BID oral treatment with placebo while continuing to remain on stable doses of their background antihypertensive medications.**	• To clarify criteria necessary to enter Screening/Placebo Run-in • To clarify that Pre-Screening measurements of PRA and aldosterone determine ability to enter Screening
Section 4.8.3/Single-blind Placebo Run-in **Week 2 (Visit 4, Study Day -7 ± 2 Days)**	Subjects will return to the research facility at the beginning of Week 2 of the run-in period for the following procedures: • Inclusion/exclusion assessment (based on Screening assessment results from V2/V3). If Screening results are available, and subject is determined to be ineligible for the study, the site will review AEs, concomitant medications, and study drug compliance. Vital signs and AOBP will not be performed. • Vital signs (body temperature, heart rate, respiratory rate) only if subject is eligible for the study or if Screening results are not yet available • AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) **only if subject is eligible for the study or if Screening results are not yet available** • Review of AEs, concomitant medications, and study drug compliance ~~On the second visit of the placebo run-in period (Visit 4),~~ **for all** subjects **regardless of eligibility status** **Subjects will be evaluated for eligibility based on Screening results and if eligible, or if Screening results are not available, subjects will continue on Week 2 of Placebo Run-in and** will be	To clarify the study procedures to be done at the beginning of Week 2 of the run-in period

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	given an ABPM device and instructions on how to perform the ABPM procedure by the investigator to take home for use on Study Day 0, approximately 24 hours before Randomization (Study Day 1). **Note: only subjects who have Screening results and are considered eligible should be provided with the ABPM for home use. Subjects who do not have Screening results available should attend Visit 5.**	
Section 4.8.4/Telephone or Office Visit	After completion of the single-blind, **P**~~p~~lacebo **R**~~r~~un-in period, subjects will participate in a telephone **or office** visit on Study Day 0 in which they will be reminded how to perform the ABPM and spot urine collection procedures by the investigator or study coordinator. ~~Alternatively, sites may choose to schedule~~ **If Screening results were not available at Placebo Run-in Visit 4, subjects should participate in an** ~~abbreviated~~ office visit ~~on Study Day 0 to initiate the~~**to review eligibility. If eligible,** ABPM procedure **will be initiated**. The following procedures will be performed: • **Inclusion/exclusion assessment (if necessary)**	To clarify the study procedures to be done at the beginning of Week 2 of the run-in period
Section 4.8.6/Study Week 1 and Section 4.8.7/Study Week 2 and Section 4.8.8/Study Week 3 and Section 4.8.9/Study Week 4 and Section 4.8.10/Study Week 5 and Section 4.8.11/Study Week 6 and Section 4.8.12/Study Week 7 and Section 4.8.13/Study Week 8	…subjects will return to the site and the following procedures will be performed after ~~home~~ **site** administration of the morning dose of study drug:	To clarify that subjects should take morning dose of study drug at site on clinic days
Section 4.8.13/Study Week 8	Added 24-hour ABPM in list of procedures	To clarify that the Study Week 8 ABPM procedure will be initiated in the clinic
Section 4.8.14/Telephone Visit	…subjects will participate in a telephone visit during which they will perform the following procedure **at home**: • AOBP (average of the last 2 of 5 unattended measurements using an automated oscillometric sphygmomanometer device after approximately 5 minutes of rest in the seated position) • **BP check**	To clarify that the BP check at Visit 15 is performed at home and not in clinic

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Section 5.3.2/Caffeine Alcohol, and Tobacco and throughout the protocol for consistency (eg, Sections 6.5.2.1, 6.5.3, 8.1.1, 8.2.2, 8.2.4, 8.2.7, 8.3.1, 8.5)	Screening**/start of Placebo Run-in**…	To clarify Screening and start of Placebo Run-in are combined in the same visit
Section 5.4/Screen Failures	Individuals who do not meet the Screening criteria for participation in this study (screen failures) may be rescreened up to 3 times ~~during the 4-week Screening period. Details on reScreening are provided in the study manual.~~	To clarify that subjects may be rescreened up to 3 times with no further details provided
Section 6.1.2/Dosing and Administration	• *Placebo Run-In:* ~~Once enrolled~~ **Upon signing the Screening/main study ICF** • On site visit days, subjects should be instructed to take their morning dose of study drug at ~~home prior to arriving at~~ the site	• To clarify that subjects begin placebo run-in after signing ICF • To clarify that subjects should take morning dose of study drug at site on site visit days
Section 6.3/Randomization and Blinding	All subjects **who qualify based on inclusion/exclusion** criteria and complete ~~the~~ **up to a** 2-week **P~~p~~**lacebo **R~~r~~**un-in period…	To clarify subject eligibility to enter Randomization
Section 9.3.2/Efficacy Analyses	The mean will be based upon the last on treatment value recorded for each subject prior to the use of any **antihypertensive medications prescribed as** "rescue" medications…	To clarify that rescue medications are defined as prescribed antihypertensive medications

12.11.5. Amendment 2 (01 June 2021)

This summary includes changes made to Protocol MLS-101-201 Amendment 1 (15 April 2021). The overall rationale for the changes implemented in this protocol amendment was to modify the dose groups.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Objectives and Endpoints and Section 3.1/Study Objectives	at ~~6~~**5** dosing regimens versus placebo	Changed from 6 to 5 dosing regimens versus placebo
Synopsis/Methodology and Section 4.1/Overall Study Design	Stable background treatment must include ≥ 2 antihypertensives (note: a combination pill = 2 antihypertensives) that have been stable at their ~~maximum tolerated~~ **prescribed** doses and stable for at least 4 weeks prior to signing the Screening/main study informed consent form (ICF). **Background therapy may be adjusted at the investigator's discretion.**	Modified language describing background therapy
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 4.3 Number of Subjects/Number of Study Centers	Approximately ~~875~~ **750** subjects will undergo up to 2 weeks of pre-Screening. It is estimated that approximately ~~263~~ **225** subjects will have qualifying PRA…Assuming a 20% drop-out rate, a total of ~~210~~ **180** subjects will meet all eligibility requirements…	Adjusted numbers due to dropping a dose group
Synopsis/Methodology and Section 4.1/Overall Study Design	…SBP and DBP will be measured 5 times after approximately 5 minutes of rest in the seated position…**Subjects may also be provided with an automated digital oscillometric home BP device to measure BP at home at the investigator's discretion**.	Added the option of allowing subjects to measure BP at home
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 6.3/Randomization and Blinding	Approximately ~~210~~ **180** enrolled subjects ≥ 18 years of age will be randomized into ~~7~~ **6** equal treatment groups (1:1:1:1:1:1~~:1~~) to 12.5 mg BID, 25 mg BID, ~~100~~ **12.5** mg ~~BID 50 mg~~ once daily (QD), ~~100~~ **50** mg QD, ~~300~~ **100** mg QD, or placebo	Modified dose groups
Synopsis/Number of Subjects (planned)	Approximately **180** ~~210~~ subjects are planned to be enrolled	Adjusted numbers due to dropping a dose group
Synopsis/Eligibility Criteria and Section 5.1/Inclusion Criteria (#4) and Section 6.7.2/Permitted Treatments	Background antihypertensive treatment of ≥ 2 drugs…that have been stable at their ~~maximum tolerated~~ **prescribed** doses	Modified language describing background therapy

Synopsis/Eligibility Criteria and Section 5.2/Exclusion Criteria (#3)	Subjects with hypokalemia, ie, serum potassium < 3.0 mEq/L ~~despite oral administration of potassium chloride (6 g/day)~~	Modified hypokalemia exclusion criteria
Synopsis/Eligibility Criteria and Section 5.2/Exclusion Criteria (#10)	Chronic long-acting oral nitrates that have been stable at their ~~maximum tolerated~~ **prescribed** doses	Modified language describing use of nitrates
Synopsis/Eligibility Criteria and Section 5.2/Exclusion Criteria (#12)	Subjects with a decrease in SBP ≥ 20 mm Hg or DBP ≥ 10 mm Hg from sitting to standing position (may be ~~rescreened up to 3 times~~ **repeated during Screening at the investigator's discretion**)	Clarified that standing BP measurement may be repeated during Screening
Synopsis/Eligibility Criteria and Section 5.2/Exclusion Criteria (#16) and Section 6.7.1/Prohibited Treatments	a. Chronically administered oral or topical corticosteroids… Short-term (ie, ≤ 2 weeks) of topical corticosteroids are allowed if taken ≥ 1 month prior to ~~Screening~~ **Randomization.** d. **Regular use of p~~P~~**hosphodiesterase type 5 inhibitors within 3 months of Screening or during study participation	Clarified short-term corticosteroids allowed ≥ 1 month prior to Randomization and that regular use of phosphodiesterase type 5 inhibitors are prohibited
Synopsis/Investigational Product, Dosage, and Mode of Administration	MLS-101 will be administered orally in the dosage 12.5 mg BID, 25 mg BID, ~~100~~ **12.5** mg ~~BID~~ **QD**, 50 mg QD, **or** 100 mg QD, ~~or 300 mg QD~~	Modified dose groups
Synopsis/Figure 1: Study Schema	Modified dose groups	Modified dose groups
Synopsis/Table 1: Schedule of Assessments, footnote f and Section 8.1.2/24-hour Ambulatory Blood Pressure Monitoring	The ABPM procedure will be initiated at home approximately 24 hours before Randomization (Study Day 1) and again ~~approximately 24 hours before~~ **at** the Study Week 8 visit	Clarified timing of second ABPM procedure
Synopsis/Table 1: Schedule of Assessments, footnote l and Section 6.5.3/Adrenal Insufficiency	**ACTH stimulation test may be waived with sponsor's approval**	Added option to waive ACTH test
Synopsis/Table 1: Schedule of Assessments, footnote q and Section 6.1.2/Dosing and Administration	**On site visit days, subjects should be instructed to take their morning dose of study drug at home prior to arriving at the site.**	Clarified that morning dose should occur at home on site visit days
Section 4.2/Scientific Rationale for Study Drugs and Section 4.5/Justification for Dose	…evaluate both QD and BID dosing across a total daily dose of ~~25~~ **12.5** mg to ~~300~~ **100** mg for 8 weeks…	Modified dose range
Section 4.3/Number of Subjects/Number of Centers	The subjects who qualify for enrollment will be randomly divided into ~~7~~ **6** equal treatment groups (1:1:1:1:1:1~~:1~~ with approximately 30 subjects per group	Changed from 7 to 6 equal treatment groups
Section 4.5/Justification for Dose	~~The proposed doses in this study provide 2 cohorts (100 mg BID and 300 mg QD) projected to provide systemic MLS-101 exposure above the aldosterone synthase inhibition half maximal inhibitory concentration (IC_{50}) for an average of 24 hours and 22 hours, respectively, and 4 cohorts (12.5 mg BID, 25 mg BID, 50 mg QD, and 100 mg QD) projected to provide exposures above the IC_{50} for an~~	Clarified rationale behind new dose selection

	~~average of 6, 13, 10, and 14 hours, respectively, which allows for testing the value of aldosterone break. A 25 mg QD dose would be below the minimally effective dose given the limited time above the current IC_{50} and was therefore not considered. Similarly, doses above 100 mg BID would provide 24-hour coverage beyond the current IC_{50}.~~ **The Phase 1 multiple ascending dose study demonstrated comparable physiological effects on mineralocorticoid-mediated renal tubular potassium reabsorption, a surrogate for sodium excretion and diuresis, at all dose levels tested from 40 mg QD through 360 mg QD. Based on these results, the upper end of the dose range to be tested in this study includes 50 mg QD, which is predicted to be efficacious at lowering BP, and 100 mg QD, which should result in a somewhat longer period of Cyp11B2 inhibition (10 hours versus 14 hours) while still providing a period of time during which the kidney can escape from inhibition and perform the aldosterone-mediated function of regulating potassium homeostasis. The lower end of the dose range, 12.5 mg QD, was selected based on in vitro potency and the ability to inhibit Cyp11B2 for at least 6 hours per day. This dose is anticipated to be submaximally effective in most individuals. The intermediate dosing regimens of 12.5 mg BID and 25 mg BID were selected to define the benefit/risk of BID versus QD dosing regimens with 12.5 mg BID and 25 mg BID providing temporarily more consistent inhibition of Cyp11B2.** A PK/PD model was used to simulate steady state profiles and noncompartmental exposure parameters under several potential Phase 2 regimens. Details of the PK/PD model are included in the Investigator's Brochure (IB). **Based on additional modeling, 100 mg BID and 300 mg QD dosing regimens are not anticipated to be necessary to achieve the PD targets.**	

Section 4.8.1/Pre-Screening	…stable background treatment defined as ≥ 2 antihypertensives (note: a combination pill = 2 antihypertensives) that have been stable at their ~~maximum tolerated~~ **prescribed** doses…	Modified language describing background therapy
Section 4.8.2/Screening and Section 4.8.13/Study Week 8	• **ACTH stimulation test (may be waived with sponsor's approval)**	Added ACTH stimulation test
Section 4.8.6/Study Week 1 and Section 4.8.7/Study Week 2 and Section 4.8.8/Study Week 3 and Section 4.8.9/Study Week 4 and Section 4.8.10/Study Week 5 and Section 4.8.11/Study Week 6 and Section 4.8.12/Study Week 7	…the following procedures will be performed ~~prior to~~ **after home** administration of the morning dose of study drug:	Clarified that morning dose should occur at home on site visit days
Section 4.8.13/Study Week 8	~~Approximately 24 hours before~~ **At** the Study Week 8 visit…	Clarified timing of second ABPM procedure
Section 6.1.2/Dosing and Administration	Subjects will orally administer the assigned study drug (MLS-101 [12.5 mg BID, 25 mg BID, ~~100~~ **12.5** mg ~~BID~~ **QD**, 50 mg QD, 100 mg QD, ~~300 mg QD~~]	Modified dose groups
Section 6.1.2/Dosing and Administration	Subjects will be monitored by ABPM for 24 hours before the first morning dose on Study Day 1 and for 24 hours ~~before~~ **after** the last morning…	Clarified timing of second ABPM procedure
Section 6.5.1/Hyperkalemia, Table 2	Modified table to reflect new dosing regimens	Modified table to reflect new dosing regimens
Section 6.5.2.1/Orthostatic Hypotension	~~In the case of~~ **If subjects experience** orthostatic hypotension during Screening, ~~subjects~~ **measurement** may be ~~rescreened up to 3 times~~ **repeated during the Screening period** to be eligible for study enrollment…	Clarified that orthostatic hypotension measurement may be repeated during Screening
Section 8.1.2/24-hour Ambulatory Blood Pressure Monitoring	Subjects will be monitored by ABPM for 24 hours before the first dose on Study Day 1 and ~~before~~ **after** the last dose…	Clarified timing of second ABPM procedure
Section 9.5/Interim Analysis	…will be performed after the first **5 to** 10 subjects in each dose group (~~70~~ **30 to 60** subjects total) complete 4 weeks of treatment	Modified number of total subjects required to complete 4 weeks of treatment before interim analysis
Section 12.2/Study Contacts	Corrected Sponsor's email address	Corrected Sponsor's email address
Section 12.5 Standing Blood Pressure Measurement	…(**measurement** may be ~~rescreened up~~ **repeated during the Screening period** to **be eligible for study enrollment**).	Clarified that orthostatic hypotension measurement may be repeated during Screening

12.11.6. Amendment 1 (15 April 2021)

This summary includes changes made to Protocol MLS-101-201 (06 February 2021). The overall rationale for the changes implemented in this protocol amendment was to clarify specific procedures.

The following is a summary of content-oriented changes that were made to each section of the protocol, and a brief rationale for these changes. Minor editorial and document formatting revisions have not been summarized.

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Title of Study and Appendix 12.3 and Appendix 12.4	A Randomized, Double-blind, Placebo-Controlled, Dose-Ranging, Multicenter Phase 2 Study to Evaluate the Safety, Efficacy, and Tolerability of MLS-101 in Subjects With ~~Low Renin~~ **Uncontrolled** Hypertension	Modified definition of hypertension
Synopsis/Objectives and Endpoints and Section 3.1.2/Secondary Objectives	…for the treatment of ~~low renin~~ **uncontrolled** hypertension…	Modified definition of hypertension
Synopsis/Pharmacokinetics and Table 1: SoA and Section 3.2.4/Pharmacokinetic Endpoints	Added Study Week 8 pharmacokinetic assessment	Additional pharmacokinetic measurement
Synopsis/Pharmacodynamics and Section 3.2.5/Pharmacodynamic Endpoints	Change in plasma ~~aldosterone, 11-deoxycorticosterone,~~ 11-deoxycortisol and plasma renin activity (PRA) from baseline to the end of Study Weeks 4 and 12 (end of follow-up) Change in serum **aldosterone**, cortisol, and **11-deoxycorticosterone** concentration from baseline to the end of Study Weeks 4 and 12 (end of follow-up)	Clarified that serum aldosterone and 11-deoxycorticosterone will be measured (not plasma)
Synopsis/Methodology and Section 2.1/Study Rationale and Section 4.1/Overall Study Design	… ~~low renin~~ **uncontrolled** hypertension (~~PRA ≤ 0.6 ng/mL~~ **hypertensive despite receiving ≥ 2 antihypertensives**)…	Modified definition of hypertension
Synopsis/Methodology and Section 4.1/Overall Study Design and Section 6.1.2/Dosing and Administration	…a single-blind **(subjects blinded to treatment allocation)**…	Clarified who is blinded in Placebo Run-in phase
Synopsis/Methodology and Table 1: SoA and Figure 1 and Section 4.1/Overall Study Design and Section 4.8.4/Telephone or Office Visit and Section 8.1.2/ 24-Hour ABPM	**Alternatively, sites may choose to schedule an abbreviated office visit on Study Day 0 (Visit 5) to initiate the ABPM procedure.**	Added ability to schedule an abbreviated office visit to initiate ABPM

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
Synopsis/Safety Criteria for Dose Adjustment or Stopping Treatment and Section 4.1/Safety Criteria for Dose Adjustment or Stopping Treatment and Section 6.5 Dose Adjustment	a subject meets 1 or more of the laboratory exclusion criteria for the study (eg, seated SBP ≥ 175 mm Hg or DBP ≥ 100 mm Hg, serum potassium > 5.2 mEq/L, **serum cortisol < 3 mcg/dL**)	Added serum cortisol level as exclusion criteria
Synopsis/ Safety Criteria for Dose Adjustment or Stopping Treatment and Section 4.1/Safety Criteria for Dose Adjustment or Stopping Treatment and Section 6.5/Dose Modification	… and ~~the subject's dose of study drug may be adjusted or temporarily withheld at the discretion of the investigator in consultation with the medical monitor~~. **all subjects will be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every study visit. Based on changes in cortisol levels, an adrenocorticotropic hormone (ACTH) stimulation test (Appendix 12.7) may be performed and the subject's dose of study drug may be stopped. Guidelines for dose potential dose discontinuation in the management of adrenal insufficiency are included in Section 6.5.3.**	Added more specific guidelines regarding adrenal insufficiency
Synopsis/Eligibility Criteria and Section 5.1/ Inclusion Criteria	1…female subjects and their partners must agree to use ~~1 of the following~~ **either highly effective or acceptable** methods of contraception **as defined in Appendix 12.8** from Screening to 90 days post last dose of study drug: ~~a. Double barrier method (eg, condom with spermicide in conjunction with the use of an intrauterine device or diaphragm)~~ b. ~~Surgical sterilization, ie, tubal ligation or vasectomy~~	Added more detailed contraceptive language in Appendix 12.8
Synopsis/Eligibility Criteria and Section 5.1/ Inclusion Criteria	**7. Serum cortisol ≥ 18 mcg/dL (morning measurement) or serum cortisol > 3 and < 18 mcg/dL (morning measurement) if there is no evidence of adrenal insufficiency based on normal ACTH stimulation test results**	Added inclusion criteria regarding cortisol
Synopsis/Eligibility Criteria and Section 5.2/ Exclusion Criteria	**5. Subjects with serum cortisol < 3 mcg/dL based on morning measurement** **6. Subjects with serum sodium < 135 mEq/L**	Added exclusion criteria regarding cortisol and sodium

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Synopsis/Eligibility Criteria and Section 5.2/ Exclusion Criteria and Section 6.7.1/Prohibited Treatments	16a. Chronically administered oral or topical corticosteroids within 3 months of Screening or during study participation. **Short-term (ie, ≤ 2 weeks) of topical corticosteroids are allowed if taken ≥ 1 month prior to Screening.** 16e. Chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs), other than low dose aspirin **(81-325 mg)**,…	Modified exclusion criteria 16a and 16e
Table 1: Schedule of Assessments	• Added pregnancy test at Week 4 • Added ACTH stimulation test at Screening and Week 8 • Added PK collection at Week 8 • Modified footnotes	Addressed safety concerns regarding pregnancy and cortisol levels
Table 1: Schedule of Assessments (footnotes i, j) and Section 4.8/Study Conduct and Section 8.5/Pharmacodynamics	Blood **samples** ~~plasma~~ for aldosterone and…	Replaced blood plasma with blood samples since both serum and plasma collected
Table 1: Schedule of Assessments (footnote p)	**All** ~~A~~**a**dverse events ~~collected during Placebo Run-in are not considered treatment-emergent events for the purposes of data analyses.~~ **S and s**erious adverse events will be collected from the signing…	Clarified timing of adverse event collection
Section 2.1/Study Rationale	By ~~using a biomarker to~~ identify**ing** patients **who have** ~~low-renin~~ hypertension **associated with** ~~from~~ autonomous aldosterone…	Modified definition of hypertension
Section 4.8.2/Screening and Section 4.8.13/Study Week 8	• **Blood samples for ACTH stimulation test (Appendix 12.7)**	Added ACTH stimulation test at Screening and Week 8
Section 4.8.9/Study Week 4 and Section 8.2.7/Pregnancy Testing	• **Pregnancy test (urine; women of childbearing potential only)**	Added pregnancy test at Week 4
Section 4.8.13/Study Week 8	**Single blood samples for determination of MLS-101 will be collected. Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, or other exploratory analyses will also be collected**	Added PK sample collection at Week 8
Section 6.5.3/Adrenal Insufficiency	**Serum cortisol levels will be measured at Screening, Randomization, Study Weeks 1, 2, 3, 4, 5, 6, 7, 8, and at the follow up visit (Study Week 12). Cortisol assessment should occur before 10 am on the morning of the study visit. In addition, subjects should be monitored for signs and symptoms of adrenal insufficiency (ie, nausea, vomiting, light-headedness, low BP, or electrolyte abnormalities) at every**	Added Section 6.5.3 on adrenal insufficiency and instructions of management of adrenal insufficiency

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	study visit. If adrenal insufficiency is suspected, glucocorticoid replacement therapy should be initiated until adrenal insufficiency can be ruled out. **Based on morning cortisol level at the central laboratory measurement, the following actions are to be taken:** • **Serum cortisol ≥ 18 mcg/dL: No action required.** • **Serum cortisol > 3 and < 18 mcg/dL: If there are no signs or symptoms of adrenal insufficiency (see above), then no action is required. If signs or symptoms of adrenal insufficiency are observed, then an ACTH stimulation test should be performed as described in Appendix 12.7 to rule out adrenal insufficiency. If there is no evidence of adrenal insufficiency based on ACTH stimulation test results, then continue at current dose. If ACTH stimulation test is abnormal, then study drug should be discontinued.** • **Serum cortisol < 3 mcg/dL: If at any time during the study serum cortisol < 3 mcg/dL, then measurement should be repeated. If confirmed, then an ACTH stimulation test should be performed as described in Appendix 12.7 to rule out adrenal insufficiency. If there is no evidence of adrenal insufficiency based on ACTH stimulation test results, then continue at current dose. If ACTH stimulation test is abnormal, then study drug should be discontinued.** **Any subject who is withdrawn from the study or has low cortisol levels at the final study visit should be evaluated until full normalization of the hypothalamic-pituitary-adrenal axis occurs. In addition, all subjects should be instructed to carry a card at all times identifying the potential risk for adrenal insufficiency. The card should include the subject's information, the investigator's contact**	

Section/Section Title	Description of Change (strikeout denotes a deletion; bold denotes an addition)	Brief Rationale for Change
	information, and the potential need for glucocorticoids in the settings of shock, surgery, etc.	
Section 6.7.1/Prohibited Treatments	**ACE inhibitors and ARBs in combination as background antihypertensive treatment**	Added prohibited treatment to be consistent with Exclusion Criteria
Section 8.2.4/Electrocardiogram	~~These ECG readings will be sent to a core ECG laboratory for central review. Any abnormalities observed at Screening will be reported to the investigator to assist in Inclusion/Exclusion evaluations.~~	Deleted ECG central lab review
Section 8.3.1/Time Period and Frequency for Collecting AE and SAE Information	~~Medical occurrences~~ **Adverse events** that begin before the start of study drug, but after obtaining informed consent will **not** be recorded as ~~medical history/current medical conditions, not as~~ treatment-emergent AEs. Adverse events collected during Placebo Run-in are ~~not~~ considered treatment-emergent events ~~for the purposes of data analyses~~.	Clarified timing of collection of adverse events
Section 8.3.7/Adverse Events of Special Interest	The AESIs for all study drugs **(MLS-101 and placebo)** include the following: • Hyperkalemia • Hypotension with symptoms (eg, light-headedness, dizziness, presyncope, or syncope) • **Adrenal Insufficiency**	Clarified definition of study drugs and added adrenal insufficiency as an AESI
Section 8.4/Pharmacokinetics	…on Study Day 1 (predose), and at Study Weeks 1, 4, and **8** (trough levels). Additional blood samples for long-term storage for metabolites, drug-drug interaction profiles of background therapy, **or other exploratory analyses** will also be collected **at baseline (predose) and at Study Weeks 1, 4, and 8.**	Added additional PK sample collection at Study Week 8 and clarified that additional blood samples will also be collected at these time points
Section 8.5/Pharmacodynamics	Blood ~~plasma~~ **samples** for aldosterone, **cortisol,** 11-deoxycorticosterone, 11-deoxycortisol, and PRA will be collected at Screening, Study Day 1 (baseline), Study Week 4, and Study Week 12 (end of follow up).	Added cortisol to pharmacodynamic measurements
Section 9.3.3/Safety Analyses	The safety analysis set will be used for the ~~efficacy~~**safety** analyses.	Corrected typo

Section/Section Title	**Description of Change** (strikeout denotes a deletion; bold denotes an addition)	**Brief Rationale for Change**
Section 9.3.5/Pharmacodynamic Analyses	The mean change from baseline in plasma ~~aldosterone, 11-deoxycorticosterone,~~ 11-deoxycortisol, and PRA will be summarized… The mean change from baseline in serum **aldosterone**, cortisol, and **11-deoxycorticosterone** concentration will be summarized for each week recorded	Clarified that serum aldosterone and 11-deoxycorticosterone will be measured (not plasma)
Section 12.7/ACTH Stimulation Test	Added Appendix 12.7	Added instructions on performing ACTH stimulation test
Section 12.8/Contraceptive and Barrier Guidance	Added Appendix 12.8	Added contraceptive and barrier guidance
Section 12.9/Clinical Laboratory Tests	Pharmacodynamics: ~~Plasma~~ **Serum** aldosterone ~~Plasma~~ **Serum cortisol** **Serum** 11-deoxycorticosterone	Clarified that serum aldosterone and 11-deoxycorticosterone will be measured (not plasma) and that cortisol will be collected as part of pharmacodynamics