AbViro LLC

AV1-PPD-0005

A Phase 1a, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Determine the Safety and Pharmacokinetics of AV-1 in Healthy Male and Female Adult Subjects

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Statistical Analysis Plan

Version 1.0

Prepared by:

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[redacted]

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List of Abbreviations

λz	apparent terminal elimination rate constant
AE	adverse event
$AUC_{0\text{-}tlast}$	area under the curve from time 0 to the last quantifiable concentration, calculated using the linear trapezoidal method
$AUC_{0\text{-}48}$	area under the serum concentration-time curve from time 0 to 48 hours postdose, calculated using the linear trapezoidal method
$AUC_{0\text{-}\infty}$	extrapolation of the area under the curve from time 0 to infinity
% AUC_{extrap}	percentage of the area extrapolated for calculation of $AUC_{0\text{-}\infty}$
BLQ	below the limit of quantification
CI	confidence interval
CL	total serum clearance
C_{max}	maximum (peak) serum drug concentration
CSR	clinical study report
ECG	electrocardiogram
ECL	electrochemiluminescence
eCRF	electronic case report form
ELISA	enzyme-linked immunosorbent assay
EOS	end of study
ET	early termination
IP	investigational product
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
PK	pharmacokinetic(s)
PT	preferred term
QTcF	QT interval corrected for heart rate using Fridericia's formula
SAE	serious adverse event
SD	standard deviation
SOC	system organ class
$t_{1/2}$	apparent terminal half life
TEAE	treatment-emergent adverse event
T_{max}	time to reach maximum observed serum concentration
ULN	upper limit of normal
V_z	volume of distribution during the terminal phase

1. Introduction

This document outlines the statistical methods to be implemented during the analysis of data collected within the scope of AbViro, Protocol AV1-PPD-0005 (A Phase 1a, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Determine the Safety and Pharmacokinetics of AV-1 in Healthy Male and Female Adult Subjects), Protocol Amendment 2, 07 May 2020.

This is a Phase 1a, first-in-human study with AV-1 and is designed to provide initial safety, pharmacokinetics (PK), and incidence of anti-AV-1 antibody responses data regarding AV-1 to help guide further development of AV-1 as a potential treatment against dengue.

The purpose of this statistical analysis plan is to define the planned statistical analysis of the study data consistent with the study objectives.

2. Objectives

2.1. Primary Objective

The primary objective of this study is to determine the safety of a single intravenous (IV) dose of AV-1 in healthy adult subjects.

2.2. Secondary Objectives

The secondary objectives of this study are as follows:

- To characterize the PK of AV-1 following a single IV dose.
- To evaluate if subjects develop anti-AV-1 antibodies following a single IV dose of AV-1.

3. Investigational Plan

3.1. Overall Study Design and Plan

This is a Phase 1a, first-in-human, single-center, double-blind, randomized, placebo-controlled, sequential-group, single ascending dose study to determine the safety of AV-1 in healthy male and female adult subjects when administered as a single IV infusion. Additionally, the PK and the incidence of anti-AV-1 antibody responses will be studied.

Five sequential cohorts of 8 subjects (6 active and 2 placebo) each (Cohorts 1 to 5) are planned for this study. The starting dose of AV-1 will be 30 mg:

The study will consist of a screening period, check-in, a treatment period, and 8 follow-up visits for each cohort.

Potential subjects will be screened to assess their eligibility to enter the study within 35 days prior to investigational product (IP) administration. Eligible subjects will check in to the clinical site on Day -1 (the day before dosing). In each cohort, subjects will fast overnight (nothing to eat or drink except water) for at least 10 hours before the start of the IP infusion. Subjects will remain fasted for 4 hours after dosing with the IP. Subjects will be randomly assigned to receive AV-1 or placebo as follows:

- Cohort 1: AV-1 30 mg (n = 6) or placebo (n = 2) administered as an IV dose
- Cohort 2: AV-1 90 mg (n = 6) or placebo (n = 2) administered as an IV dose
- Cohort 3: AV-1 250 mg (n = 6) or placebo (n = 2) administered as an IV dose
- Cohort 4: AV-1 500 mg (n = 6) or placebo (n = 2) administered as an IV dose
- Cohort 5: AV-1 1000 mg (n = 6) or placebo (n = 2) administered as an IV dose

Sentinel dosing will be used in each cohort as discussed in Protocol Section 8.2.3. In each cohort, the Investigator will review safety data for the sentinel subjects and notify the Sponsor of intent to proceed with dosing the rest of the cohort with a minimum of 72 hours between dosing of the sentinel subjects and dosing the rest of the cohort. Safety data through Day 8 will be reviewed in a blinded fashion by the Safety Review Committee for each dose cohort before escalating to the next dose cohort. Based on the review of safety data, the Sponsor and the Investigator may choose to repeat a dose level, administer a dose less than the previous dose, escalate to a dose lower than the next planned dose, or prolong the duration of the infusion.

Subjects will be confined to the clinical unit from Day -1 until discharge on Day 5. On Day 5, subjects will be discharged after all protocol-specified assessments have been completed. Subjects will return for 8 follow-up visits on Days 8 (+2), 15 (±2), 22 (±2), 29 (±2), 43 (±3), 57 (±3), 85 (±5), and 120 (±5). Subjects will be asked to attend an early termination (ET) visit if they withdraw or are withdrawn from the study prior to the final follow-up visit on Day 120 (±5).

The planned duration for each cohort (screening to final follow-up visit) is approximately 22 weeks.

Safety, PK, and anti-AV-1 antibody endpoints will be evaluated in the study. Clinical laboratory evaluations, physical examination findings, vital sign measurements, safety 12-lead electrocardiograms (ECGs), incidence and severity of adverse events (AEs), and incidence of serious AEs (SAEs) will be monitored to assess safety as specified in protocol Sections 7 and 8.

3.2. Study Endpoints

3.2.1. Safety Endpoints

The primary safety endpoints are clinical laboratory evaluations (hematology, chemistry, urinalysis), physical examination findings, vital sign measurements, safety 12-lead ECG parameters, incidence and severity of AEs, and incidence of SAEs.

3.2.2. Pharmacokinetic Endpoints

The secondary PK samples will be assessed for AV-1 concentrations. The following PK parameters for AV-1 will be calculated as endpoints:

- C_{max}: Maximum observed serum drug concentration
- T_{max}: Time to reach maximum serum concentration following drug administration
- $AUC_{0\text{-}tlast}$: Area under the serum concentration versus time curve from time 0 to the last quantifiable concentration in serum
- $AUC_{0\text{-}48}$: Area under the serum concentration versus time curve from time 0 to 48 hours postdose
- $AUC_{0\text{-}\infty}$: Extrapolation of the area under the curve from time 0 to infinity
- $t_{1/2}$: Terminal half-life
- CL: Total serum clearance
- V_z: Volume of distribution during the terminal phase

3.2.3. Immunogenicity Endpoints

Incidence of anti-AV-1 antibodies as measured by the proportion of subjects with detectable anti-AV-1 antibody signal in serum, including treatment-emergent anti-AV-antibodies, using an electrochemiluminescence (ECL) enzyme-linked immunosorbent assay (ELISA).

4. General Statistical Considerations

All data collected will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics.

For categorical variables, frequencies and percentages will be presented. Continuous variables will be summarized using descriptive statistics (number of subjects, mean, median, standard deviation [SD], minimum, and maximum).

Unless specified otherwise, baseline will be defined as the last non-missing assessment prior to the study drug administration. Unscheduled visits will be used in determining baseline.

Unless otherwise indicated, outputs which are summarized by treatment will be summarized for each dose level for subjects on active study drug; subjects who receive placebo will be pooled into a single group.

The study day of dosing will be considered as Day 1. Study day will be calculated relative to the first dose date as follows:

- If assessment date is on or after the first dose of study drug administration, then Study Day = Assessment Date – Dose Date + 1
- Otherwise, Study Day = Assessment Date – Dose Date

4.1. Sample Size

This study will enroll a total of 40 subjects in 5 cohorts of 8 subjects each and up to 8 subjects for replacements as necessary. Each cohort will consist of 6 AV-1 treated and 2 placebo treated subjects. The sample size (N = 40) for this study is based on clinical and practical considerations and not on a formal statistical power calculation. The sample size is considered sufficient to evaluate the objectives of the study.

4.2. Analysis Populations

The Safety population will include all subjects who receive any amount of the IP. The safety population will also be used for anti-AV-1 antibody assessment.

The PK population will include subjects who receive the full dose of AV-1 and have at minimum all samples from predose through 1.5 hours from the start of infusion to have sufficient concentration data to support accurate estimation of at least 1 PK parameter.

5. Subject Disposition, Protocol Deviations, and Follow-up

5.1. Disposition

Subject disposition will be summarized by treatment and total for all randomized subjects.

The number of subjects who are randomized in the study and the number and percentage of subjects who complete the study will be presented. Frequency and percentage of subjects who withdraw or discontinue from the study, and the reason for withdrawal or discontinuation, will also be summarized.

Subject disposition data, analysis populations, and randomization data will be presented in data listings.

5.2. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. An important deviation (sometimes referred to as a major or significant deviation) is a subset of protocol deviations that leads to a subject being discontinued from the study, or significantly affects the subject's rights, safety, or well-being or the completeness, accuracy, and reliability of the study data. An important deviation can include nonadherence to inclusion or exclusion criteria or nonadherence to Food and Drug Administration regulations or International Council for Harmonisation (ICH) E6(R2) guidelines.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The Investigator will be notified in writing by the monitor of deviations. The Institutional Review Board should be notified of all protocol deviations, if appropriate, in a timely manner.

Important protocol deviations will be summarized for all randomized subjects.

All protocol deviations will be presented in a data listing, including the categorization of the deviation as important or not. Details of admission criteria deviations will be presented in a separate data listing.

6. Demographics and Baseline Characteristics

6.1. Demographics

Demographic and baseline characteristics will be summarized by treatment and total for Safety population.

The demographic characteristics consist of age (years), sex, race, and ethnicity. The baseline characteristics consist of baseline weight (kg), height (cm), and body mass index (kg/m^2). Percentages will be based on the total number of subjects in the Safety population.

Subject demographic and baseline characteristics will be presented in a data listing.

6.1.1. Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version to be delineated in the Clinical Study Report [CSR]).

Medical history will be presented in a data listing for safety population.

7. Treatments, Medications, and Meals

7.1. Prior and Concomitant Medications

Information regarding prior medications taken by the subject within the 30 days before signing the informed consent form will be recorded in the subject's electronic case report form (eCRF).

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given at the discretion of the investigator. If a concomitant medication is taken, except for those specified in the protocol, a joint decision will be made by the investigator and the Sponsor to continue or discontinue the subject based on the time the medication was administered, its pharmacology and PK, and whether the use of the medication will compromise the safety of the subject or the interpretation of the data. The investigator is responsible for ensuring that details regarding the medication are adequately recorded in the eCRF.

Prior and concomitant medications and therapies will be coded using the latest version of the World Health Organization Drug Dictionary.

All prior and concomitant medications will be presented in a data listing for the safety population.

7.2. Medical and Surgical Treatment Procedures

All medical and surgical treatment procedures will be coded using MedDRA (version to be delineated in the CSR). All medical and surgical treatment procedures will be presented in a data listing for safety population.

7.3. Study Drug Administration

All doses of study drug will be administered at the study site under direct observation of site personnel and recorded in the eCRF. Study site personnel will confirm that the subject has received the dose of study drug. The start and stop date/time, and amount of study drug dosing will be recorded on the appropriate page of the eCRF. If a subject does not receive study drug, the reason for the missed dose will be recorded.

All study drug administration data will be presented in a data listing.

8. Pharmacokinetic Analysis

8.1. Data Handling

Serum concentrations that are below the limit of quantification (BLQ) will be treated as zero for calculation of descriptive statistics. Mean BLQ concentrations will be presented as BLQ, and the SD and CV will be reported as not applicable.

For PK analysis, BLQ values will be treated as zero with the exception that a BLQ value between 2 quantifiable concentrations will be set as missing. If consecutive BLQ concentrations are followed by quantifiable concentrations in the terminal phase, those concentrations after BLQ concentrations will be treated as missing.

Missing concentrations will be excluded from all calculations and PK analyses.

8.2. Serum Concentrations

Blood samples for the determination of serum concentrations of AV-1 will be collected on Day 1 at the following time points: predose (within 15 minutes), and at 0.5, 1 (end of infusion), 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after the start of the infusion and on Day 5 prior to discharge, and at the follow-up visits on Days 8 (+2), 15 (±2), 22 (±2), 29 (±2),85 (±5), and 120 (±5) (or ET). AV-1 serum concentrations will be determined using a validated ECL ELISA method based on the MesoScale Discovery platform.

AV-1 serum concentration data will be summarized using descriptive statistics (N, n [non-missing values within the population], arithmetic mean, SD, CV%, median, minimum, and maximum) by dose group and scheduled sampling time. Individual and mean AV-1 serum concentration versus time data will be plotted by dose group. For ease of presentation, mean serum concentrations of AV-1 will be plotted by nominal time by dose group on both linear and semi-logarithmic scales.

Serum PK concentrations of AV-1 will be reported to the precision of the raw data in listing presentations; summary statistics for arithmetic mean, median, minimum, maximum, and SD will be reported to 3 significant figures; and CV% will be reported to 1 decimal place.

8.3. Serum Pharmacokinetic Parameters

The serum concentration-time data for AV-1 will be analyzed by non-compartmental analysis using Phoenix® WinNonlin® (Certara USA, Inc., Princeton, NJ) Version 8.0 or higher. Actual sampling times will be used for the estimation of all serum PK parameters.

PK parameters of AV-1 will be summarized using descriptive statistics by dose group. If data allow, the following PK parameters will be calculated:

Parameter	**Description**
C_{max}	Maximum observed serum drug concentration
T_{max}	Time to reach maximum observed serum concentration
AUC_{0-48}	Area under the serum concentration-time curve from time 0 to 48 hours postdose, calculated using the linear trapezoidal method
$AUC_{0-tlast}$	Area under the curve from time 0 to the last quantifiable concentration, calculated using the linear trapezoidal method
$AUC_{0-\infty}$	Area under the serum concentration-time curve from time 0 extrapolated to infinity, calculated as [$AUC_{0-tlast}$ + (C_t/λ_z)] where C_t is the last observed serum drug concentration. $\%AUC_{exp}$ must be <0.20 to retain $AUC_{0-\infty}$ and associated parameters in the summary and statistical tables
$t_{1/2}$	Apparent terminal half-life, calculated as:ln(2)/ λ_z
CL	Total serum clearance, calculated as Dose/$AUC_{0-\infty}$
V_z	Volume of distribution during the terminal phase, calculated as Dose/$AUC_{0-\infty}$* λ_z

In addition to the above PK parameters, which will be listed and summarized, the following parameters will also be listed to document the selection of data points used to estimate $t_{1/2}$ using non-compartmental procedures:

Parameter	**Description**
$\%AUC_{exp}$	Percentage of the area extrapolated for calculation of $AUC_{0-\infty}$.
λ_z	Apparent terminal elimination rate constant, where λ_z is the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase.
Number of points	Number of data points used to estimate λ_z; a minimum of 3 data points must be used, and C_{max} must not be included.
λ_z lower	Lower bound used for the estimation of λ_z.
λ_z upper	Upper bound used for the estimation of λ_z.
Rsq	r^2, the coefficient of determination (goodness of fit statistic); visual inspection of the terminal slope will be performed. In general, λ_z may only be retained if $r^2 \geq 0.80$

Serum PK parameters for AV-1 will be presented in data listings and summarized using descriptive statistics (n, arithmetic mean, SD, CV%, median, minimum, and maximum) by dose group. Geometric means and geometric CV will be reported for $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max}. T_{max} will be summarized using the descriptive statistics median, minimum, and maximum only.

Serum PK parameters of AV-1 will be displayed to 3 significant figures in all data listings and summary tables, with exception of time variables (T_{max}, λz lower, and λz upper) which will be displayed to 2 decimal places.

8.4. Pharmacokinetic Statistical Analysis

Dose-proportionality for AV-1 will be evaluated for C_{max}, $AUC_{0\text{-}tlast}$, and $AUC_{0\text{-}\infty}$. A power model will be fitted to describe the relationship between Y (C_{max}, $AUC_{0\text{-}tlast}$ and $AUC_{0\text{-}\infty}$) and X (dose) using the least-squares linear regression model [$\ln(Y) = \ln(\beta_0) + \beta_1 \ln(X)$].

From each model, the intercept of regression line [$\ln(\beta_0)$] and the slope of the regression line β_1 will be presented along with the 90% confidence interval (CI) of the slope. Dose-proportionality will be concluded if the 90% CI of the slope β_1 lies entirely within [$1+\ln(0.8)/\ln(r)$, $1+\ln(1.25)/\ln(r)$], where r is a ratio that describes the dose range and is defined as the ratio of highest dose/lowest dose. If dose proportionality is not observed, then the lowest dose followed by the highest dose in succession will be removed, and the model will be fit again after the removal of a dose until dose proportionality is achieved or less than 3 dose groups remain.

The statistical analyses will be based on the PK population.

9. Anti-AV-1 Antibody Analysis

Blood samples for immunogenicity analysis will be collected at Check-in (Day –1) and on Days 29 (±2), 85 (±5), and 120 (±5) (or ET). A validated ECL ELISA method will be used for detection and confirmation of preexisting and treatment-emergent anti-AV-1 antibodies in serum samples based on the MesoScale Discovery platform that utilizes labeled AV-1 for detection of anti-AV-1 antibodies and treatment emergent antibodies.

Frequency and percentage of subjects who show detectable anti-AV-1 antibody signal in serum, including treatment-emergent anti-AV- antibodies, will be summarized for the safety population. Pharmacokinetic parameters may be displayed based on anti-AV-1 antibody status as applicable.

Anti-AV-1 antibody data will be presented in a data listing for the safety population.

10. Safety Analysis

All safety summaries and analyses will be conducted for the safety population.

10.1. Adverse Events

An AE is defined by ICH E6(R2) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor. All AEs, including solicited local (infusion site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and in the eCRF.

A treatment-emergent AE (TEAE) is defined as any event that does not present before exposure to study drug or any event already present that worsens in intensity or frequency after exposure.

Only TEAEs will be included in summary tables.

Adverse events will be coded by preferred term (PT) and system organ class (SOC) using MedDRA (version to be delineated in the CSR).

A subject may have more than 1 AE for an SOC or PT. A subject with 2 or more AEs within the same level of summarization will be counted only once in that summarization level. In addition to the summarization of subjects with AE at each level, the frequencies of AE occurrences will also be summarized at each level. Percentages will be based upon the number of subjects in the safety population for each treatment group.

All AEs will be presented in a data listing.

10.1.1. Incidence of Adverse Events

An overview of AEs will be presented by treatment and total, including number and percentage of subjects with any:

- Treatment-emergent AE
- Treatment-related TEAE
- Moderate TEAE

- Treatment-related moderate TEAE
- Severe TEAE
- Treatment-related severe TEAE
- Serious TEAE
- Treatment-related Serious TEAE
- Treatment-emergent AE leading to early discontinuation
- Death

All TEAEs will be presented in a summary table by treatment and total for each SOC and PT. Percentages will be calculated out of the number of subjects in the safety population.

10.1.2. Relationship of Adverse Events to Study Drug

The relationship or association of the study drug in causing or contributing to the AE will be characterized by the investigator as "Related" or "Not Related".

All TEAEs will be presented in a summary table for each treatment group and total by relationship to study drug. If a subject has 2 or more TEAEs in the same SOC (or with the same PT) with a different relationship to study drug, then a "Related" event will be used for that subject. If the relationship information is missing, the AE will be considered related in the summary but will be presented as missing in the data listings.

10.1.3. Severity of Adverse Event

All AEs (laboratory and clinical symptoms) will be graded for severity. The severity will be classified as mild, moderate, or severe using the following criteria:

- Mild (Grade 1): Events that are transient and may require only minimal or no treatment or therapeutic intervention and do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

All TEAEs will be summarized for each treatment group and total by maximum severity. If the severity information is missing, the AE will be considered severe in the summary but will be presented as missing in the data listings.

10.1.4. Serious Adverse Events

An AE or suspected adverse reaction is considered an SAE/suspected unexpected serious adverse reaction if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be presented in a data listing.

10.1.5. Adverse Events Leading to Study Drug Discontinuation

An AE where the answer to "What action was taken with study treatment?" is "Drug Withdrawn" will be considered an AE leading to study drug discontinuation.

All AEs leading to study drug discontinuation will be presented in a data listing.

10.2. Clinical Laboratory Evaluations

Clinical laboratory testing will occur at Screening, Check-in, predose (within 60 minutes) and 48 hours after the start of the IP infusion on Day 1; and on Days 8, 15, 22, 43, 85, and 120. A complete list of assessments is provided in Appendix 2.

Actual results and changes from baseline values for hematology, coagulation, serum chemistry, and urinalysis tests will be summarized by visit and treatment for subjects in the safety population. The key parameters will be evaluated based on toxicity grading scale in Appendix 3, and the toxicity grades will be summarized in tables for subjects in safety population.

Shift from baseline in hematology, coagulation, serum chemistry, and urinalysis test results relative to the reference range will be summarized by visit and treatment using the frequency count and percentage of subjects in each category.

Serology, urine drug screen, Flavivirus screening, and pregnancy test data will not be summarized.

All laboratory data will be presented in data listings.

10.3. Vital Sign Measurements and Weight

Vital signs will be measured at Screening and Check-in; within 60 minutes prior to start of the IP infusion; at 0.5, 1 (±10 minutes), 2, 4, 8, 24, and 48 hours after the start of the IP infusion; and on Days 5, 8, 15, 22, 43, 85, and 120 (or ET). Vital signs will include systolic and diastolic blood pressure, heart rate, and oral body temperature. The subject will have rested comfortably in a seated or supine position for at least 5 minutes before all measurements are taken.

Actual values and changes from baseline for vital sign data will be summarized by visit and treatment for subjects in the safety population. Changes from baseline to each scheduled post-baseline visit will be presented. Only systolic and diastolic blood pressure, heart rate, respiratory rate and temperature will be included in the summary tables. These key parameters will also be graded based on toxicity grading scale in Appendix 3, and the grades will be summarized in a table by visit and treatment for subjects in the safety population.

All vital sign, height, and weight measurements will be presented in a data listing.

10.4. Electrocardiograms

Single 12-lead ECG recordings will be made at Screening, within 60 minutes prior to the start of the IP infusion; at 1 (±10 minutes), 2, 4, 8, and 24 hours after the start of the IP infusion; and on Days 8, 43, 85, and 120 (or ET). Electrocardiograms will be recorded after the subject has been in the supine position for at least 10 minutes. A single repeat measurement is permitted at Screening for eligibility determination. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF. Assessments should include comments on whether the tracings are normal or abnormal; rhythm; presence of arrhythmia or conduction defects; morphology; any evidence of myocardial infarction; or ST-segment, T-wave, and U-wave abnormalities.

Actual values and changes from baseline for numeric ECG data will be summarized by visit and treatment for subjects in the safety population. Key parameters including QTcF and PR interval will be graded based on toxicity grading scale in Appendix 3, and the

toxicity grades will be summarized in a table by visit and treatment for subjects in the safety population.

Shift from baseline in interpretation of ECG results will be summarized by visit and treatment using the frequency count and percentage of subjects in each category.

All ECG data will be presented in a data listing.

10.5. Physical Examination

A full physical examination will be performed at Screening (skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities). Focused examination (lungs, cardiovascular, abdomen, and skin) will be performed on Days 5, 8, and 15. Targeted, symptom-directed physical examinations will be performed at all other follow-up visits.
Physical examination results will be presented in a data listing for the safety population.

11. Changes from the Planned Analysis

Different from the Protocol, the safety population is defined as all subjects who receive any amount of IP in this SAP.

12. Interim Analysis

No formal interim analysis is anticipated for this study. The blinded safety data will be reviewed by the investigators, Medical Monitor and Sponsor's representative to ensure that it is safe to proceed with the planned dose escalation of IP.

13. References

International Council for Harmonisation (ICH) E6 (R2) GCP: Integrated Addendum to ICH E6 (R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 [2018])
https://www.fda.gov/media/93884/download.

Protocol AV1-PPD-0005: A Phase 1a, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Determine the Safety and Pharmacokinetics of AV-1 in Healthy Male and Female Adult Subjects, Protocol Amendment 2, 07 May 2020.

14. Appendices

Appendix 1 Schedule of Events

Phase	Screening	Check-in	Treatment Period											EOS/ ET
Procedure[a] / Day	–35 to –2	–1	1	2	3	5	8 (+2)	15 (±2)	22 (±2)	29 (±2)	43 (±3)	57 (±3)	85 (±5)	120 (±5)
Admission to clinic		X												
Discharge from clinic[b]						X								
Outpatient visit							X	X	X	X	X	X	X	X[c]
Informed consent	X													
Demographics	X													
Serology[d]	X													
Flavivirus serology test[e]	X													
Serum FSH[f]	X													
Inclusion/exclusion criteria	X	X												
Medical history[g]	X	X				X	X	X	X	X	X	X	X	X
Height, weight, and BMI[h]	X	X											X	X
Physical examination[i]	X	X				X	X	X	X	X	X	X	X	X
Vital sign measurements[j]	X	X	X	X	X	X	X	X	X		X		X	X
12-lead ECG[k]	X		X	X			X				X		X	X
Clinical laboratory testing[l]	X	X	X		X		X	X	X		X		X	X
Urine drug[m]	X	X							X		X		X	
Urine cotinine testing		X												
Pregnancy test[n]	X	X												
Randomization[o]			X											
AV-1 or placebo administration[p]			X											
PK blood sample collection[q]			X	X	X	X	X	X	X	X	X	X	X	X
Anti-AV-1 antibody blood sample collection		X								X			X	X
Hypersensitivity panel collection[r]		X	X	X										
Fasting period[s]		X	X											
Non-fasting period[t]				X	X	X								

Phase	Screening	Check-in	Treatment Period											EOS/ ET
Procedure[a] Day	**–35 to –2**	**–1**	**1**	**2**	**3**	**5**	**8 (+2)**	**15 (±2)**	**22 (±2)**	**29 (±2)**	**43 (±3)**	**57 (±3)**	**85 (±5)**	**120 (±5)**
Solicited AEs, Unsolicited AEs, and SAEs[u]			◄	―	―	―	―	―	X	―	―	―	―	►
Prior/concomitant medications		◄	―	―	―	―	―	―	X	―	―	―	―	►

Abbreviations: AEs, adverse events; BMI, body mass index; ECG, electrocardiogram; EOS, end of study, ET, early termination; FSH, follicle-stimulating hormone; IP, investigational product; PK, pharmacokinetic; QTcF, QT interval corrected for heart rate using Fridericia's formula; SAE, serious adverse event.

Notes:

[a] When procedures overlap or occur at the same time point, they should be performed in the following order: ECG, vital signs, then PK sampling which should be timed to occur last and as close to the scheduled time window as possible.

[b] Discharge from the clinical site on Day 5 after all study related activities are completed.

[c] The final follow-up visit will occur on Day 120 (approximately Week 17).

[d] Serology testing will include hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody types 1 and 2.

[e] Flavivirus testing will include dengue, Zika, and West Nile viruses.

[f] Females only. A serum FSH test may be performed at Screening to confirm postmenopausal status along with at least 12 months of amenorrhea.

[g] Interim medical history will be done after initial thorough medical and medication history taken at Screening. Any new disorders after dosing on Day 1 will be recorded as an AE.

[h] Height and weight will be measured, and BMI will be calculated at Screening only. Only weight will be measured at Check-in (Day –1) and on Days 85 and 120.

[i] A full physical examination will be performed at Screening (skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities). Focused examination (lungs, cardiovascular, abdomen, skin) will be performed on Days 5, 8, and 15. Targeted, symptom-directed physical examination will be performed at all other time points.

[j] Vital signs will be measured at Screening and Check-in; within 60 minutes prior to start of the IP infusion; at 0.5, 1 (±10 minutes), 2, 4, 8, 24, and 48 hours after the start of the IP infusion; and on Days 5, 8, 15, 22, 43, 85, and 120. Vital signs will be measured after the subject has been in the seated or supine position for at least 5 minutes. Vital signs will include systolic and diastolic blood pressure, heart rate, and oral body temperature.

[k] Single 12-lead ECG recordings will be made at Screening, within 60 minutes prior to the start of the IP infusion; at 1 (±10 minutes), 2, 4, 8, and 24 hours after the start of the IP infusion; and on Days 8, 43, 85, and 120. Electrocardiograms will be recorded after the subject has been in the supine position for at least 10 minutes. A single repeat measurement is permitted at Screening for eligibility determination. Measurements of the following intervals will be reported: RR interval, PR interval, QRS width, QT interval, and QTcF. Assessments should include comments on whether the tracings are normal or abnormal; rhythm; presence of arrhythmia or conduction defects; morphology; any evidence of myocardial infarction; or ST-segment, T-wave, and U-wave abnormalities.

(l) Clinical laboratory testing will occur at Screening, Check-in, predose (within 60 minutes) and 48 hours after the start of the IP infusion on Day 1; and on Days 8, 15, 22, 43, 85, and 120. A complete list of assessments is provided in protocol Section 7.2 Blood and urine samples will be collected under fasted conditions and prepared per the clinic's standard procedures.

(m) Urine drug screen will occur at Screening and Check-in and on Days 22, 43, and 85 per the clinic's standard procedures and per Section 7.2.

(n) Women of childbearing potential only. A serum pregnancy test will be performed at Screening and Check-in, and tests must be negative prior to enrollment and dosing, respectively.

(o) After verification of eligibility, subjects will be randomly assigned before dosing on Day 1.

(p) The start of the IP infusion will be called "0" hour and is denoted with grey shading. All doses of the IP will be administered intravenously over a period of 60 minutes. Subjects will maintain an upright (ie, seated or standing) position for at least 4 hours after the end of infusion.

(q) Blood samples for PK analysis of AV-1 in serum will be collected predose (within 15 minutes); at 0.5, 1 (end of infusion), 1.25, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after the start of infusion, Day 5 prior to discharge, and at the follow-up visits on Days 8, 15, 22, 29, 85, and 120 (or ET).

(r) A blood sample will be drawn on Day –1 and tested only if a suspected hypersensitivity reaction occurs during or following infusion on Day 1 at the discretion of the Investigator in consultation with the Sponsor. At the discretion of the Investigator, an additional blood sample will be drawn as soon as possible after the event occurs (Day 1) and again the following day (Day 2).

(s) During fasting periods, subjects should have nothing to eat or drink except water from 10 hours prior to start of the IP infusion until 4 hours after the end of infusion. Water is permitted as desired except for the period of 1 hour prior to the start of the IP infusion until 1 hour after the end of the infusion.

(t) During non-fasting periods when subjects are at the clinical site, subjects should receive standardized meals per the clinic's standard procedures that are scheduled at the same time in each treatment period during the study.

(u) Solicited AEs will be assessed from the start of IP infusion until Day 8 and unsolicited AEs will be assessed from the start of IP infusion through the final follow-up visit on Day 120. All AEs should be followed until they are resolved, stable, or judged by the Investigator to be not clinically significant.

Appendix 2 Clinical Laboratory Assessments

The following clinical laboratory assessments will be performed:

Hematology	Absolute neutrophil count, hemoglobin, platelet count, and white blood cell count
Coagulation[a]	Prothrombin time and activated partial thromboplastin time
Serum Chemistry	Alanine aminotransferase, albumin[a], alkaline phosphatase, aspartate aminotransferase, bicarbonate[b], blood urea nitrogen, calcium[a], chloride[b], creatinine[c], glucose (fasted), potassium, sodium, and total bilirubin
Urinalysis	Blood, glucose[a], and protein
Serology (IgG)[a]	Hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antibody types 1 and 2
Flavivirus Screening (IgM and IgG)[a]	DENV antibody, Zika virus antibody (IgM only), and West Nile virus antibody
Urine Drug Screen[d]	Amphetamines (includes methamphetamines and ecstasy/methylenedioxymethamphetamine), barbiturates, benzodiazepines, cannabinoids, cocaine metabolites, cotinine, ethyl alcohol, methadone, methamphetamines, opiates (including heroin, codeine, and oxycodone/oxymorphone)
Pregnancy Test (female subjects only)[e]	Follicle-stimulating hormone, serum pregnancy test (human chorionic gonadotropin)

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.
Note: Results of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen creatinine, and total bilirubin that are below the reference ranges are acceptable and will not exclude participation.
[a] Clinical laboratory assessments will be performed at Screening and Check-in on Day -1 only, except serology and flavivirus screening will occur only at Screening.
[b] Bicarbonate and chloride will only be used for calculation of anion gap ([sodium + potassium] - [chloride + bicarbonate)]. Volunteers with an anion gap greater than the upper limit of normal at Screening and Check-in on Day -1 will be excluded from participation in the study.
[c] Volunteers with an estimated creatinine clearance (calculated using CKD-EPI method) <90 mL/min/1.73 cm2 at Screening will be excluded from participation in the study.
[d] Urine drug screening will be performed at Screening, Check-in on Day –1, on Day 22 (±2), and on Day 43 (±3), except cotinine screening will occur only on Day –1.
[e] For females in menses, screening urinalysis may be postponed or repeated, but a result should be available prior to Day 1.

Appendix 3 Toxicity Tables

Table 17-1 Toxicity Grading Scales for Clinical Adverse Events and Reactions

Cardiovascular	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Arrhythmia	N/A	Asymptomatic; transient signs; no medical intervention required	Recurrent or persistent symptoms; medical intervention required
Hemorrhage, Blood Loss*	Estimated blood loss is <100 mL	Estimated blood loss is ≥100 mL; no transfusion required	Transfusion required
QTc (Fridericia's correction)	Asymptomatic, QT interval 450 to 479 msec (male) and 460 to 479 msec (female) OR increase in interval <30 msec above baseline	Asymptomatic, QTc interval 480 to 499 msec OR increase in interval 30 to 50 msec above baseline	Asymptomatic, QTc interval ≥500 msec OR increase in interval ≥60 msec above baseline
PR Interval (prolonged)	PR interval 0.21 to 0.25 sec	PR interval >0.25 sec	Type II 2nd degree AV block OR ventricular pause >3 sec
Respiratory	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Cough	Transient; no treatment	Persistent	Interferes with daily activities
Bronchospasm, Acute	Transient; no treatment; forced expiratory volume 71 to 80% of predicted peak flow	Requires medical intervention; normalizes with bronchodilator; forced expiratory volume 60 to 70% of predicted peak flow	No normalization with bronchodilator; forced expiratory volume <60% of predicted peak flow
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities; no treatment	Prevents daily and usual social activity OR requires treatment
Gastrointestinal	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Nausea	No interference with activity	Some interference with activity	Prevents daily activity
Vomiting	No interference with activity OR 1 to 2 episodes/24 hours	Some interference with activity OR >2 episodes/24 hours	Prevents daily activity OR requires IV hydration OR requires medical intervention
Diarrhea	2 to 3 loose or watery stools OR <400 grams/24 hours	4 to 5 loose or watery stools OR 400 to 800 grams/24 hours	6 or more loose or watery stools OR >800 grams/24 hours OR requires IV hydration OR requires medical intervention
Local Reactions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours OR interferes with activity	Any use of narcotic pain reliever OR prevents daily activity

Tenderness	Discomfort only to touch	Discomfort with movement or mild discomfort at rest	Significant discomfort at rest
Erythema/Redness**	2.5 to 5 cm	5.1 to 10 cm	>10 cm
Induration/Swelling***	2.5 to 5 cm AND does not interfere with activity	5.1 to 10 cm OR interferes with activity	>10 cm OR prevents daily activity
Systemic Reactions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Allergic Reaction	Pruritus without rash	Localized urticaria OR requires oral therapy	Generalized urticaria; angioedema OR anaphylaxis OR requires epinephrine
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours OR some interference with activity	Significant, any use of narcotic pain reliever OR prevents daily activity OR requires triptans
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity
Systemic Illness	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention

*Does not include blood loss from study.
**The local reaction should be measured at the greatest single diameter and recorded as a continuous variable in addition to grading.
***Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Table 17-2 Vital Signs Toxicity Grading

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C) ** (°F) **	38.0 to 38.4 100.4 to 101.1	38.5 to 38.9 101.2 to 102.0	39.0 to 40 102.1 to 104
Tachycardia - beats per minute	101 to 115	116 to 130	>130
Bradycardia - beats per minute***	50 to 54	45 to 49	<45
Hypertension (systolic) - mm Hg	141 to 150	151 to 155	>155
Hypertension (diastolic) - mm Hg	91 to 95	96 to 100	>100
Hypotension (systolic) – mm Hg	85 to 89	80 to 84	<80
Respiratory rate – breaths per minute	17 to 20	21 to 25	>25

*Subject should be at rest for all vital sign measurements.
**Oral temperature; no recent hot or cold beverages or smoking.
***When resting heart rate is between 60 to 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Table 17-3 Laboratory Toxicity Grading

Laboratory Tests	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Liver Function Tests – ALT increase by factor	1.1 – 2.5 × ULN	2.6 – 5.0 × ULN	>5.0 × ULN
Liver Function Tests – AST increase by factor	1.1 – 2.5 × ULN	2.6 – 5.0 × ULN	>5.0 × ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	<2.5
Alkaline Phosphatase – ALP increase by factor	1.1 – 2.0 × ULN	>2.0 – 3.0 × ULN	>3.0 × ULN
Anion Gap* – mEq/L	N/A	N/A	N/A
Bilirubin (total) – mg/dL	1.1 – <1.6 × ULN	1.6 – < 2.6 × ULN	≥ 2.6 × ULN
Blood Urea Nitrogen mg/dL	27 – 30	31 – 35	> 35
Calcium – Hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4
Calcium – Hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0
Creatinine – mg/dL	1.2 – 1.3 × ULN	>1.3 – 1.8 × ULN	>1.8 × ULN
Glucose (fasted) – Hypoglycemia mg/dL	50 – 54	40 – 49	30 – 39
Glucose (fasted) – Hyperglycemia Fasting – mg/dL	117 – 127	128 – 142	>143
Potassium – Hypokalemia mEq/L	3.3 – 3.4	3.1 – 3.2	2.9 – 3.0
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6
Sodium – Hyponatremia mEq/L	129 – 131	127 – 128	122 – 126
Sodium – Hypernatremia mEq/L	146 – 147	148 – 149	150 – 152
Hematology			
WBC increase - cell/mm^3	9,800 – 14,000	14,001 – 19,000	19,001 – 24,000
WBC decrease - cell/mm^3	2,000 – 3,099	1,000 – 1,999	500 – 999
Absolute Neutrophil Count - cell/mm^3	1,000 – 1.499	500 – 999	0 – 499
Hemoglobin Decrease (female) - gm/dL	9.7 – 10.7	8.2 – 9.6	6.7 – 8.1
Hemoglobin Decrease (male) - gm/dL	11.7 – 12.7	9.76 – 11.6	7.6 – 9.6
Platelets Decrease - cell/mm^3	125,000 – 139,000	100,000 – 124,000	<100,000
Coagulation			
Prothrombin Time – increase by factor	>1.0 – 1.2 × ULN	1.2 – 1.4 × ULN	>1.4 × ULN
Activated Partial thromboplastin time – increase by factor	>1.0 – 1.2 × ULN	1.2 – 1.4 × ULN	>1.4 × ULN

Laboratory Tests	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Urine			
Blood**	1+	2+	>2+
Glucose	1+	2+	>2+
Protein	1+	2+	>2+

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transferase; AST, aspartate aminotransferase; ULN, upper limit of the normal range, WBC, white blood cell.

*Only values above the ULN and below the LLN are considered abnormal.

**For females: In case of menstruation, screening urinalysis must be postponed, but a result should be available prior to Day 1. Hematuria is acceptable for females in menses for the purposes of evaluating study halting criteria and dose escalation.

Source: DHHS 2007.

Table 17-4 Infusion-related Reactions and Toxicity Grading

Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (eg, antihistamines, nonsteroidal anti-inflammatory drugs, narcotics [opioids], intravenous fluids); prophylactic medication indicated for less than or equal to 24 hours.	Prolonged (eg, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae.

Source: Adapted from the National Cancer Institute Common Terminology Criteria for Adverse Events (Version 5.0) for infusion-related reactions.