Statistical Analysis Plan

A Multi-Center Open-label Investigation to Assess the Safety and Efficacy of Multiple Doses of DMl99 in Patients with Chronic Kidney Disease (Stage II or III)

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ABBREVIATIONS

Abbreviation	Definition
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitor
ADA	Anti-drug Antibodies
AE	Adverse Event
APOL1	Apolipoprotein L-1 gene
ARB	Angiotensin receptor blocker
ATC	Anatomical Therapeutic Chemical
BMI	Body mass index
BP	Blood Pressure
BT	Body temperature
CI	Confidence interval
CKD	Chronic kidney disease
CKD EPI	Chronic Kidney Disease Epidemiology Collaboration
CS	Abnormal clinically significant
CSR	Clinical Study Report
CV	Coefficient of Variation
DBP	Diastolic blood pressure
DM	Diabetes mellitus
ECG	Electrocardiogram
EE	Evaluable for Efficacy
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
HR	Heart Rate
ICF	Informed consent form
KLK1	Kallikrein-1
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NCS	Abnormal not clinically significant
PD	Pharmacodynamic
PE	Physical examination
PI	Principal investigator

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Abbreviation	**Definition**
PK	Pharmacokinetic
PT	Preferred Term
QTcF	Fridericia corrected QT interval
rhKLK1	Recombinant human tissue KLK1
SAE	Serious adverse event
RR	Respiratory rate
SAP	Statistical Analysis Plan
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard Deviation
SI	International System of Units
SoA	Schedule of assessments
SoC	Standard of Care
SOC	System Organ Class
TEAE	Treatment emergent adverse event
TFLs	Tables, Figures and Listings
UACR	Urinary Albumin Creatine Ratio

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1. INTRODUCTION

Chronic kidney disease (CKD) is characterized by a gradual loss of kidney function resulting from decreased filtration efficiency. Albuminuria and GFR (glomerular filtration rate) are accepted measures of kidney damage and function, respectively. Albuminuria is a measure of the permeability of the glomerular wall to large molecules and may precede a decline in kidney function. GFR represents the product of the number of functional nephrons and the average single-nephron filtration rate and is the most useful measure of overall kidney function.

Both angiotensin receptor blocker (ARB) and angiotensin converting enzyme inhibitor (ACEi) therapies have been demonstrated to reduce albuminuria and preserve renal function in patients with CKD. However, despite these treatments, patients with CKD from chronic hypertension and diabetes, continue to have ongoing albuminuria and loss of kidney function over time such that new therapeutics to prevent albuminuria and preserve kidney function are needed.

ACEi and ARB medications improve albuminuria by lowering intraglomerular pressure and reducing hyperfiltration. Where hyperfiltration results in albuminuria and eventual loss of a functioning nephron. The reduction in intraglomerular pressure is thought to occur by vasodilation of the efferent glomerular arteriole thus increasing blood flow and reducing pressure within the glomeruli. ACEi results in vasodilation by increasing bradykinin levels, a known vasodilator, and ARB indirectly by blocking AT1 receptors responsible for vasoconstriction.

Tissue kallikrein (KLK1) is normally produced by the kidneys and acts on kininogen producing lys-bradykinin to then result in vasodilation and improved blood flow. KLK1 may then act in combination with ARB medications to improve efferent glomeruli blood flow with further reduction of intraglomerular pressure, hyperfiltration and albuminuria. KLK1 isolated from porcine pancreas has been approved for use in Japan, China, and Korea to treat numerous vascular diseases including chronic kidney disease, hypertension, and retinopathy (Bayer (Kallidinogenase), Sanwa Kagaku Kenkyusho Co., Ltd (Carnaculin®), and ChangZhou Qianhong Bio-Pharma Co., Ltd (Kallidinogenase)). There are several publications of randomized controlled studies demonstrating an ARB plus orally administered porcine KLK1 compared to ARB alone demonstrated significant reductions in albuminuria in participants with CKD and diabetes (Guohong et al, 2011)

DM199 is a recombinant form of the endogenous human tissue kallikrein protein (rhKLK1) and is being developed as a subcutaneous injectable protein drug to treat CKD and ischemic diseases like acute ischemic stroke. DM199 is hypothesized to improve kidney function by restoring and stimulating the kallikrein-kinin system, restoring KLK1 level to normal range which maintains a sufficient level of bradykinin in the kidney and associated vasculature to reduce intraglomerular pressure and resultant albuminuria.

This statistical analysis plan (SAP) details the planned statistical analyses to answer the study objective(s) for Study DM199-2019-001. The proposed analyses are based on the contents of protocol v2.0 (dated 26 June 2020). Populations for analysis, data handling rules, statistical

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methods, and formats for data presentation are provided. The statistical analyses and summary tabulation described in this SAP will provide the basis for the results sections of the Clinical Study Report (CSR) for this study.

2. OVERALL STUDY DESIGN AND PLAN

This is a Phase II, multi-center, open label study in the United States (US) that is designed to evaluate the safety and efficacy of DM199, in the following 3 cohorts:

- **Cohort I:** African American participants with CKD (Stage II or III), hypertension, and non-diabetic
- **Cohort II:** Participants with IgA nephropathy diagnosis and CKD (Stage II or III)
- **Cohort III:** Participants with type 2 diabetes mellitus (DM), hypertension, and CKD (Stage II or III)

After review of screening data to determine eligibility for one of the three CKD cohorts listed above, participants will be assigned to one of two dose levels of DM199 (i.e., 2.0 or 5.0 µg/kg) SC 2x per week for 95 days and will have follow-up visits through Day 104. The first participant will receive 2.0 µg/kg and the next participant will receive 5.0 µg/kg. DiaMedica will advise investigators or designees which dose level participants will receive based on this pattern. The duration of each individual's participation in the study will consist of a screening period of 1 to 21 days (rescreening is allowed), study day 1 visit for study drug administration, and 104 days of follow-up totaling approximately 125 days from the time of consent to completion of all study activities.

3. OBJECTIVES AND ENDPOINTS

Primary objective (italicized text):

To evaluate the safety, tolerability, pharmacokinetic (PK) profile, and efficacy (measured by renal function and blood pressure [BP] change over time) of two doses of DM199 for participants in three different cohorts of Stage II or III CKD. Additionally, all African Americans in Cohorts I and III will be analyzed together and evaluated by baseline covariates for the apolipoprotein L-1 (APOL1) gene and sickle cell trait.

No secondary or exploratory objectives were defined in the protocol; however, the outcome measures were identified in Protocol Section 8 "Statistical Considerations" as either primary, secondary, or exploratory endpoints. Thus, the outcome measures were not aligned with each component of the objective. This will be corrected in this SAP and the CSR with the outcome measures associated with either safety and tolerability, efficacy, PK, or PD as shown in Table 1.

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See Section 8 for a summary of the differences in the statistical section of the protocol versus this SAP.

Table 1: Objectives and Endpoints

Objective	Endpoints
Safety and tolerability	• Incidence, severity, and causality of AEs and AESIs (i.e., injection site reactions, hypotension, orthostatic hypotension, and angioedema) • Vital signs (BP will also be analyzed as an efficacy outcome) • Electrocardiograms • Hematology and chemistry laboratory parameters (includes blood glucose) • Anti-drug antibodies
Efficacy	• Estimated glomerular filtration rate using CKD EPI equation • Urine albumin:creatinine ratio • Blood pressure
Pharmacokinetics	• Plasma PK profile • Concentration of DM199 in urine pre and post DM199 administration[1]
Pharmacodynamics	• Plasma biomarkers[2] • Urine biomarkers[3]

[1] Will include endogenous KLK1 since the assay cannot distinguish between DM199 and KLK1 in urine.
[2] Includes tumor necrosis factor receptor 1, C-reactive protein, matrix metalloproteinase-9, vascular endothelial growth factor; cystatin C, prostaglandin E2, and prostacyclin.
[3] Includes kidney injury molecule-1 and neutrophil gelatinase associated lipocalin.
CKD EPI=Chronic Kidney Disease Epidemiology Collaboration; PK=pharmacokinetics.

The primary objective of *APOL1* and sickle cell trait for African American participants in Cohort I and Cohort III (see italicized text above) were defined in Protocol Section 8.2.3 as exploratory endpoints c and d, respectively, for Cohort I only. Although protocol defined as a primary objective, these are baseline characteristics and will be analyzed and presented in the CSR as such.

4. SAMPLE SIZE

No formal sample size calculation was performed. Approximately 90 evaluable participants will be enrolled in Cohorts I, II and III (n=30 per cohort). All participants in each cohort will be assigned dosing groups centrally in a sequential fashion, where the first participant will receive 2.0 µg/kg and the next participant will receive 5.0 µg/kg and the pattern will repeat. Any early withdrawal or lost to follow-up participants who did not complete the day 39 visit will be replaced. Replaced participants will assume the same study drug dosing assignment of the participant they are replacing.

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5. RANDOMIZATION

This is a non-randomized, open label study.

Methods for dose level assignments are described in Section 4.

6. STATISTICAL ANALYSIS

6.1. Statistical Comparisons

No formal hypothesis testing will be performed as all analysis will be descriptive. Confidence intervals will be two-sided and use 95% confidence levels.

6.2. Analysis Populations

There are 2 analysis populations as described in the following sections. Participants excluded from the analysis populations and the reason for their exclusion will be listed in Appendix 16.2 of the CSR.

6.2.1. Safety Population

All participants who receive any amount of study drug will be included in the Safety Population. Participants will be summarized according to the dose they actually received. Demographic, baseline characteristics, urine concentrations of KLK1, and safety and tolerability analyses will be conducted using the Safety Population.

6.2.2. Evaluable for Efficacy Population (EE)

All participants who received at least 75% of planned study drug doses and have at least one post-baseline efficacy assessment (of all 3 of eGFR, UACR, and BP) will be included in the EE Population. Participants will be summarized according to the dose they were assigned to receive. All efficacy analyses (eGFR, UACR and BP) will be conducted using the EE Population.

6.3. Global Conventions and Definitions

6.3.1. Computing

All data listings, summaries, figures, and statistical analyses will be generated using SAS version 9.4 or higher (SAS Institute, Cary, NC).

6.3.2. General Statistical Methods

For continuous variables, the number of non-missing observations (n), mean, standard deviation (SD), median, and minimum and maximum values will be presented. The minimum and

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maximum values will be presented with the same precision as the data. The mean and median will be presented with a precision of one decimal place more than the data. The standard deviation will be presented with a precision of two decimal places more than the data.

For plasma concentrations, the coefficient of variation (CV%), geometric mean, and geometric coefficient of variation (geo CV%) values may also be presented.

For categorical variables, the number/frequency and percentage within each category of the parameter will be presented. For each parameter, all categories will be shown, and for categories with a number/frequency of zero (0), only the number, without percentage, will be presented. Percentages will be presented with one decimal place.

6.3.3. Listing Conventions

In general, listings will be sorted in the following order:

- Dose group
- Cohort
- Participant
- Parameter
- Study day

All data collected on the electronic case report forms (eCRFs) and results from external data, e.g., PK and central laboratory data will be presented in the data listings.

6.3.4. Table Conventions

In general, summaries of the data will be presented by cohort and by dose group within cohort, with the labels displayed as follows in the table outputs. All tables will additionally include an "Overall" column (i.e., all cohorts combined).

Overall (N=XX)		Cohort I (N=XX)		Cohort II (N=XX)		Cohort III (N=XX)	
DM-199 2 µg/kg (N=xx)	DM-199 5 µg/kg (N=xx)	DM-199 2 µg/kg (N=xx)	DM-199 5 µg/kg (N=xx)	DM-199 2 µg/kg (N=xx)	DM-199 5 µg/kg (N=xx)	DM-199 2 µg/kg (N=xx)	DM-199 5 µg/kg (N=xx)

6.3.5. Baseline and Study Day

Baseline values will be defined as the last available non-missing result prior to the first dose of study drug administration.

Study day will be calculated as the number of days from first study drug administration. For events/assessments on or after study drug administration:

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- Study day = date of event (or date of assessment) – date of first study drug administration + 1

For events/assessments before study drug administration:

- Study day = date of event (or date of assessment) – date of first study drug administration

6.3.6. Conventions for Missing or Partial Dates

In the rare case that an AE start date or time is missing, and it is unclear whether the AE is treatment-emergent or not, then a conservative approach will be taken, and it will be assumed that the AE occurred after study drug administration.

If a prior or concomitant medication end date or time is missing and it is unclear whether the medication is prior or concomitant, then a conservative approach will be taken, and it will be assumed that the medication was taken on or after study drug administration.

If a medical condition end date or time is missing and it is unclear whether the medical condition is previous or ongoing, then a conservative approach will be taken, and it will be assumed that the medical condition was still active on or after study drug administration.

Where the Date of Birth is missing, the following convention will be used:

- Where the day is missing and month and year are available, the day will be completed as the 15th. For example, Date of Birth specified as --JAN1980 will be completed as 15JAN1980.
- If the day and month are missing and the year is available, the day and month will be completed as 02JUL (the 183rd day of the year). For example, Date of Birth specified as -----1980 will be completed as 02JUL1980.

All dates presented in the individual participant listings will be as recorded on eCRF.

6.4. Unscheduled Visits and Visit Windows

Visit windows did not exist for this study. Additionally, since unscheduled visits did not occur, no analysis windows will be used in this study. All ‘by visit’ summaries will present nominal visits as collected.

6.5. Participant Disposition

Participant disposition will be summarized by cohort, dose group, and overall, where appropriate, for all participants. The following information will be reported:

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- Number of participants screened
- Number and percentage of participants for the following categories:
  - Enrolled
  - Treated
  - Not Treated
  - Completed the study
  - Discontinued the study
  - Reasons for study discontinuation
- Number and percentage of participants included in, and excluded from, each study population together with the reasons for exclusion from the analysis population
- Number and percentage of participants who met/did not meet all eligibility criteria, together with the criteria not met
- Number and percentage of participants at each site

All disposition data will also be listed.

6.6. Protocol Deviations

A listing of protocol deviations will be provided within Appendix 16.2 of the CSR.

6.7. Demographic and Baseline Characteristics

The comparability of cohort and dose groups with respect to participant demographics and baseline characteristics will be assessed in a descriptive manner. Continuous and categorical variable summaries will be presented by cohort and dose group for demographic and baseline characteristics.

Descriptive statistics will be presented for the demographic continuous variables including:

- Age (years)
- Height (cm)
- Weight (kg)
- Body Mass Index (kg/m^2)
- Body Temperature (degrees Celsius)
- Respiration Rate (breaths per minute)
- Systolic Blood Pressure (SBP [mmHg])

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- Diastolic Blood Pressure (DBP [mmHg])
- Heart Rate (beats per minute [bpm])
- Baseline eGFR
- Baseline UACR
- Baseline Hemoglobin

Total counts and percentages of participants will be presented for the demographic categorical variables including:

- Age group (years):
  - 18 to ≤44
  - >44 to ≤64
  - >64 to ≤75
  - >75
- Sex
- Race (and among African Americans, *APOL1* gene and sickle cell trait)
- Ethnicity

Additionally, baseline plasma KLK1 concentrations will be summarized by sex, race, and age group for each cohort and dose group. Total counts and percentages of participants falling into each plasma KLK1 quartile will also be presented.

All demographic and baseline characteristic data will also be listed.

6.8. Medical History

Previous and ongoing conditions at screening will be presented in a listing for the Safety Population. Any medical condition will be classified as previous if a stop date before dosing is recorded, otherwise, the condition will be classified as ongoing. Any clinically relevant surgeries will be included in the medical history listing.

6.9. Prior and Concomitant Medications

Medications will be coded using the World Health Organization Drug Dictionary. Concomitant medications initiated during the study will be summarized by ATC 2 class, PT, cohort, and dose group using the Safety Population. Anti-hypertension and diabetes concomitant medications will be presented in a separate table by ATC 3, PT, cohort, and dose group. Prior medications will also be summarized by ATC 2, PT, cohort and dose group using the Safety Population. Prior anti-hypertension and diabetes concomitant medications will be presented in a separate table by ATC 3, PT, cohort, and dose group.

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Prior medications are defined as all medications starting and stopping before the date of study drug administration. Concomitant medications are defined as medications taken on or after the date of study drug administration.

All prior and concomitant medications will be listed.

6.10. Exposure and Compliance

Dose amount (volume of drug administered) and number of doses administered will be summarized by cohort and dose group for the Safety and Evaluable for Efficacy (EE) Populations. Dose compliance will be calculated by summing the number of doses taken and dividing by the number of doses expected to be taken. All exposure data will be listed. Missing data will not be imputed.

6.11. Efficacy Analyses

All efficacy endpoints (primary as well as secondary) will be analyzed using the EE Population as the primary population. No hypothesis testing will be performed. Only descriptive statistics will be presented.

The primary efficacy analyses are:

1. Kidney function assessed by:
   - eGFR using CKD EPI
   - Urine albumin creatinine ratio
   - Blood pressure (vitals)
2. PK profile of DM199, assessed in plasma
3. DM199 in urine concentration of KLK1 pre- and post-study drug administration

   (The assay to be used cannot differentiate between DM199 and KLK1 in human plasma or urine.)

6.11.1. Kidney Function Analyses

eGFR will be based on CKD EPI as per the following formula:

$$GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]}$$

$\kappa = 0.7$ if female
$\kappa = 0.9$ if male
$\alpha = -0.329$ if female
$\alpha = -0.411$ if male
min = the minimum of Scr/κ or 1

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max = the maximum of Scr/κ or 1
Scr = serum creatinine (mg/dL)

eGFR will be summarized descriptively as well as plotted over time based on eGFR baseline stage according to the following categories:

- ≥ 90 mL/min (normal function)
- 60-<90 mL/min (mild impairment)
- 30-<60 mL/min (moderate impairment)
- 15-<30 mL/min (severe impairment)
- <15 mL/min (kidney failure)

Shift tables from baseline to minimum post-baseline (i.e., the lowest post-baseline eGFR values across all visits) will be presented by cohort and dose group for the eGFR categories defined above.

Additionally, scatterplots using the continuous eGFR result presenting baseline eGFR by minimum post-baseline eGFR will also be produced by cohort and dose group along with the corresponding regression equation and correlation. Decreases in eGFR from baseline will be summarized by visit, starting from the Week 6 visit until end of study, by dose group and cohort. Additionally, figures (spaghetti plots) of eGFR as well as mean decline from baseline in eGFR will be presented by dose group and then by cohort.

When there is more than one eGFR measurement per week (i.e., nominal visit and retest) we will use the worst value for that week for analysis (i.e., the smallest eGFR value).

Urine albumin creatinine ratio (UACR) values will be summarized by cohort and dose group over time in addition to change from baseline and percent change from baseline. Counts and percentages of participants will be presented by severity of UACR increase (and visit) as follows:

- <30 mg/g - normal
- ≥30-<150 mg/g - mild increase
- ≥150-<300 mg/g - moderate increase
- ≥300 mg/g - severe increase

Additionally, scatterplots using UACR result presenting baseline UACR by minimum post-baseline UACR will also be produced by cohort and dose group along with the corresponding regression equation and correlation. Additionally, figures (spaghetti plots) of UACR as well as mean change from baseline in UACR will be presented by dose group and then by cohort.

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Furthermore, a composite responder analysis will be performed using UACR and eGFR. A responder will be defined as a participant who has at least 30% reduction in UACR (over the course of the study starting from Week 6 by visit) and no worsening in eGFR (defined as a shift of at least 1 category down from baseline over the course of the study starting from Week 6 by visit). Responders will be presented by cohort, dose group, and visit as counts and percentages.

When there is more than one UACR measurements per week (i.e., nominal visit and retest), we will use the worst value for UACR for that week (i.e., the highest UACR value).

Blood Pressure: Graphs of least square mean change from baseline in sitting diastolic and sitting systolic BP over time by dose group, and then by dose group and cohort will also be presented.

Counts (percentage) of participants with both systolic BP<130 and diastolic BP<80 will be presented at baseline and end of study per dose and cohort. Using the same criteria shifts from baseline (counts and percentages) to each visit starting from Week 6 until EOS will also be presented.

6.11.2. Subgroup Efficacy Analyses

As an exploratory subgroup analysis, we will repeat the composite responder analysis using UACR and eGFR defined above for the subgroups of participants below. Subgroup analyses will be presented by dose group.

1. Participants with UACR>500 at baseline by dose group (note: UACR by visit scatter plot will also be presented)
2. Participants falling in the lowest quartile of plasma KLK1 concentration at baseline

6.11.3. Pharmacokinetics

PK analysis will be presented in a separate analysis plan.

6.11.4. Urine Concentration of KLK1 Measurements

Urine concentrations of KLK1 will be analyzed using the Safety Population. All data will be presented in listings.

6.12. Primary Safety Endpoints and Analyses

Statistical methods for safety analyses will be descriptive in nature. No formal statistical comparisons of dose groups will be made. All safety analysis will be presented by cohort and dose group for the Safety Population.

Safety endpoints include AEs, concomitant medications, clinical laboratory assessments, vital signs, physical examination, and ECGs.

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6.12.1. Adverse Events

All reported AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by System Organ Class (SOC) and Preferred Term (PT). Injection site reactions were described and the severity graded as per Table 4.4 of the protocol adapted from the FDA Guidance 2007 Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Trials. For all other AEs, Investigator's clinical judgement was the final determination to assess severity.

Analyses of AEs will be performed only for adverse events that are considered treatment emergent AEs (TEAE), where treatment emergent is defined as: any AE with onset after the administration of study medication.

An overall summary of AEs without SOC and PT will be presented with the number and percentage of participants by cohort and dose group with any of the following types of AEs:

- TEAE
- TEAE by severity (mild, moderate, severe)
- TEAE by relationship to study drug (unrelated, related; where unrelated includes none and improbable, and related includes possible, probable, definite)
- Serious TEAE
- TEAE leading to study drug discontinuation
- TEAE leading to death

In addition to the overall summary, the number and percentage of participants by cohort and dose group for each of the TEAE categories above will be summarized by SOC and PT. In these summaries, each participant will contribute only once to the incidence rate based on the most related occurrence and most severe occurrence of the AE. This is true regardless of the number of occurrences.

Summaries of TEAEs by SOC and PT will also include total number of unique AE occurrences, where unique AE occurrences include all events, regardless of whether the same participant experiences the same AE multiple times. However, if the end of a first occurrence and the start of a subsequent occurrence overlap, or the end of a first occurrence indicates "ongoing," then this would be counted as only one occurrence.

All AEs will be listed, and additional participant-level listings will be presented for deaths, SAEs, and TEAEs leading to study treatment discontinuation.

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6.12.2. Adverse Events of Special Interest (AESI)

Analyses of AESIs will be performed for those events that are considered treatment-emergent AEs (TEAE), where treatment emergent is defined as: any AE with onset after the administration of study medication.

An overall summary will be presented with the number and percentage of participants by treatment with any of the following AESIs (as detailed in the protocol):

- Facial, oral or airway angioedema
- Hypotension
- Orthostatic Hypotension
- Local Injection Site Reaction

Preferred terms of AEs that identify AESIs are defined in Appendix 1. Orthostatic Hypotension will also be checked using Blood Pressure as follows:

A symptomatic drop in blood pressure from sitting to standing (at 1 or 3 minutes) of >20 mmHg for systolic BP, or of >10 mmHg for diastolic BP, or experiencing lightheadedness or dizziness is considered abnormal and should be captured as an adverse event.

An overall summary for each AESI will be presented with the number and percentage of participants by treatment with any of the following types of AESIs:

- AESI
- AESI by severity (mild, moderate, severe)
- AESI by relationship to study drug (unrelated, related; where unrelated includes none and improbable, and related includes possible, probable, definite)
- Serious AESI
- AESI leading to study drug discontinuation
- AESI leading to death

The duration of each AESI will be summarized by treatment group with descriptive statistics.

Injection Site Adverse Reactions

Injection site adverse reaction (Pain, Tenderness, Erythema/Redness, and Induration/Swelling) will be summarized descriptively by cohort and dose group with frequencies and percentages. The severity of such reactions will also be summarized descriptively by cohort and dose group. All injection site reaction data will also be listed.

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6.12.3. Laboratory Data

For continuous parameters, descriptive statistics of the observed values at each timepoint and change from baseline values at each timepoint after study drug administration will be presented by cohort and dose group for all laboratory parameters.

All laboratory test results will be received from the central laboratories, and the results will be provided in both International System of Units (SI) and conventional units. For the TFLs, the results will be summarized or presented in SI units.

In the case where a safety laboratory parameter is recorded as "> x," "≥ x," "< x" or "≤ x," a value of x will be used for conducting analyses.

Each measurement will be classified as below, within, or above normal range based on ranges supplied by the laboratory used. Shift tables in relation to the shift in normal range from baseline will be presented at each timepoint after study drug administration.

All laboratory data will also be listed. Listings will include details of classifications as below, within, or above normal range and whether the measurements are clinically significant.

In case there are multiple laboratory tests per week (i.e., test and retest), we will use the latest value in the analysis (i.e., the retest value). This approach will be applied for all laboratory values with the exclusion of eGFR and UACR.

6.12.4. Vital Signs

Descriptive statistics of the observed values at each timepoint and change from baseline values at each timepoint after study drug administration will be presented for the following vital signs parameters (and units) by treatment:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Heart rate (beats/min)
- Respiration rate (breaths/min)
- Body temperature (degrees Celsius)
- Weight (kg)
- BMI (kg/m^2)

Additionally, the number and frequency of participants with values outside of the normal range at each timepoint will be presented by treatment group and shift tables from Baseline for all post-dose assessments.

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Table 2: Potentially Clinically Important Criteria for Vital Sign Results

Vital Sign	Lower PCI criterion	Upper PCI criterion
Pulse rate	≤50 bpm	≥130 bpm
Systolic BP	<90 mmHg	>160 mmHg
Diastolic BP	<50 mmHg	>100 mmHg
Change in systolic BP	decrease ≥30 mmHg	increase ≥20 mmHg
Change in systolic BP	decrease ≥15 mmHg	increase ≥10 mmHg
Change in diastolic BP	decrease ≥20 mmHg	increase ≥20 mmHg
Change in diastolic BP	decrease ≥10 mmHg	increase ≥10 mmHg

BP = Sitting blood pressure; PCI = potentially clinically important.

Additionally, figures (spaghetti plots) including systolic and diastolic blood pressure (mmHG) results will be presented by cohort and dose group.

All vital sign data will be listed. If multiple measurements of any of the vital sign parameters are made during the same weekly visit, we will use the average of those. Only sitting blood pressure from the vital signs CRF will be used in the blood pressure summaries in this section except for the baseline BP for which we will use pre-dose orthostatic sitting BP.

6.12.5. Electrocardiogram

Descriptive statistics of the observed values at each timepoint and change from baseline values at each timepoint after study drug administration will be presented for the following 12-lead ECG parameters by treatment:

- RR interval
- PR interval
- QT interval
- QRS interval
- QTcF (Frederica's formula, $QTcF = QT/\sqrt[3]{(RR)}$

The proportion of participants with maximum post-baseline absolute QTcF intervals will be summarized by cohort and dose group using the following thresholds:

- >450 msec
- >480 msec
- >500 msec

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The proportion of participants with maximum post-baseline increase from baseline in QTcF intervals will be summarized by cohort and dose group for the following thresholds:

- >30 msec
- >60 msec

Additionally, the overall interpretation of ECG results will be classified using frequency counts and percentages for the categories of Normal, Abnormal not clinically significant (NCS) and Abnormal clinically significant (CS) for each cohort and dose group at all scheduled time points. Shift tables from Baseline will also be summarized by cohort and dose group.

All 12-lead ECG data, including details of any abnormalities, will be listed.

6.12.6. Physical Examination

Observed values by treatment at each timepoint for physical examination data will be summarized, including Investigator assessment of clinical significance for abnormal findings. Shift tables from Baseline will also be generated.

All physical examination data, including details of clinically significant findings, will also be listed.

6.13. Pharmacodynamics

PK/PD analysis will be presented in a separate analysis plan.

6.14. Immunogenicity Analysis

Plasma DM199 anti-drug antibodies (ADA) (baseline, weeks 4, 8,12,14 and early withdrawal) will be summarized descriptively by treatment group. No formal hypothesis testing will be performed.

The incidence of ADA positive participants at each time point and at any point on study will be summarized by treatment group.

The incidence of injection site reactions (ISRs), allergic reactions, and anaphylaxis will be summarized for participants who were ADA positive and those who were not. Additionally, ADA titer levels will be summarized descriptively for the subset of participants experiencing injection site reactions, allergic reactions, and anaphylaxis.

Scatterplots and a corresponding correlation analysis between KLK1 plasma concentrations and ADA titers will be performed to assess whether ADA antibody levels are associated with lower KLK1 plasma concentrations.

PT terms for AEs to be used in above analyses (AESI as well as allergic/immune reactions) are listed in Appendix 1.

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6.15. Exploratory Analysis

Observed values and change/percent change from baseline in blood glucose will be summarized descriptively by cohort and dose group for each visit. Plots of mean change from baseline will be presented by dose group, cohort, and visit.

Counts (percentages) of participants with random blood glucose <140 or fasting blood glucose<100 will be presented by dose group, cohort, and visit. Using the same criteria shifts from baseline to each visit starting from Week 6 until EOS will also be presented.

All IgA biomarkers will be presented in listings.

7. INTERIM ANALYSIS

No formal interim analysis will be performed in this study. However, administrative analyses may be performed where there is no intention to stop the trial early for futility or efficacy or modify the sample size.

8. CHANGES TO PLANNED PROTOCOL ANALYSES

A summary of the changes to the planned protocol analyses versus the analyses in this SAP is provided in Table 3.

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Table 3: Summary of Changes to the Planned Protocol Analyses

Protocol Section	Change in this SAP
Section 5.12.1. Adverse Events	
Protocol Section 6.2.1 specifies that AEs will be graded according to the FDA Guidance 2007 Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Trials and provides an abbreviated table of AEs in Table 3 as an example rating system. In the same section the protocol specifies "The Investigator or medically qualified designee will assess severity for each AE reported during the study. The assessment will be based on the Investigator's (or medically qualified designees) clinical judgement." To clarify, the Investigator's clinical judgement was the final determination to assess severity of all AEs that were not injection site AEs in participants with CKD enrolled in this trial. Only injection site reactions were described and graded as per Table 4.4 of the protocol adapted from the FDA Guidance 2007 Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Trials.	Injection site reactions were described and severity graded as per Table 4.4 of the protocol adapted from the FDA Guidance 2007 Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Trials. For all other AEs, Investigator's clinical judgement was the final determination to assess severity.
Section 8.2.1 Primary Endpoints	
1. Safety as assessed by incidence, severity, and causality of AEs	• Will be analyzed and presented in the CSR as a safety outcome rather than a primary endpoint (see Table 1) • AESIs were not specified as an endpoint in the protocol (see Protocol section 8.2), but will be analyzed and presented in the CSR as a safety outcome (see Table 1) • Vital signs were not specified as an endpoint in the protocol, but will be analyzed and presented in the CSR as a safety outcome (see Table 1) • Hematology and chemistry laboratory assessments were not specified as an as an endpoint in the protocol, but will be analyzed and presented in the CSR as a safety outcome • ADA were not specified as an as an endpoint in the protocol, but will be analyzed and presented in the CSR as a safety outcome (see Table 1)
2. Kidney function assessed by • eGFR using CKD EPI • Urine albumin creatinine ratio • Blood pressure (vitals)	• Will be analyzed and presented in the CSR as an efficacy outcome measure rather than a primary endpoint (see Table 1)
3. Tolerability as assessed by incidence and severity of AEs	• Will be analyzed and presented in the CSR with safety outcomes rather than a primary endpoint (see Table 1)

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Protocol Section	Change in this SAP
4. PK profile of DM199 assessed in plasma	• Will be analyzed and presented in the CSR as a PK outcome rather than a primary endpoint (see Table 1)
5. DM199in urine concentrations of KLK1 pre- and post-study drug administration	• Will be analyzed and presented in the CSR as a PK outcome rather than a primary endpoint (see Table 1)
Section 8.1.3.2 Modified Intention -to-Treat (mITT) Population	Since this is a non-randomized safety study in the SAP we changed the population name to Evaluable for Efficacy (EE) Population.
Section 8.2.2 Secondary Endpoints	
1. Plasma PD biomarkers: a. TNF R1 b. C-reactive protein c. Matrix metalloproteinase d. Vascular endothelial growth factor e. Cystatin C f. PGE2 g. Prostacyclin	• Will be analyzed and presented in the CSR as a PD outcome rather than a secondary endpoint (see Table 1)
2. Urine PD biomarkers a. Kim 1 b. NGAL	• Will be analyzed and presented in the CSR as a PD outcome rather than a secondary endpoint (see Table 1)
Section 8.2.3 Exploratory Endpoints	
a. Blood glucose b. ECG	• Will be analyzed and presented in the CSR with safety outcomes rather than an exploratory endpoint
c. APOL-1 gene cohort 1 only (test for both alleles) d. Sickle cell trait cohort 1 only	• Will be analyzed and presented in the CSR as a baseline characteristic rather than an exploratory endpoint for African Americans in the 3 cohorts

ADA=antidrug antibodies; AE=adverse event; AESI=adverse event of special interest; APOL-1= apolipoprotein L-1; CSR=Clinical Study Report; ECG=electrocardiogram; KIM 1=kidney injury molecule-1; NGAL=neutrophil gelatinase associated lipocalin; PD=pharmacodynamic; PK=pharmacokinetics; SAP=statistical analysis plan.

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9. REFERENCES

Guohong et al. 2021.

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10. TABLES, FIGURES AND LISTING SHELLS

The full list of all tables, figures, and listings along with the associated prototype shells and numbering to be produced in accordance with this SAP will be maintained in a standalone TFL shell document.

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11. SCHEDULE OF ASSESSMENTS

Assessment / Study Day	Screening (-21 to Day 1)	Day 1 Pre- Dose	Day 1 Dose	Day 1, 2 Hour	Day 1, 4 Hour	Day 4	Week 2 (Day 8/11)	Week 3 (Day 15/18)	Week 5 (Day 29/32)	Week 6 (Day 36/39)	Week 7 (Day 43/46)	Week 9 (Day 57/60)	Week 10 (Day 64/67)	Week 11 (Day 71/74)	Week 13 (Day 85/88)	Weeks 4, 8 ,12, 14 (In Clinic)[1]	Week 15, 16[10]	Early Withdrawal[2]	Window (Day 1 Only)
Informed consent	X																		
Medical history/ demographics	X																		
Inclusion/exclusion criteria	X	X																	
Drug of abuse (urine)	X	X																	
Alcohol breath screen	X	X																	
HIV, HBsAg, anti- HCV[3]	X																		
Urine pregnancy test[4]	X	X														X		X	
Sequential assignment[5]		X																	
Previous and concomitant medication	X	X			X	X	2x	2x	2x	2x	2x	2x	2x	2x	2x	X		X	
Physical examination	X	X			X											X		X	1 Hour
Weight, height, and BMI calculation[6]	X	X														X		X	

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Assessment / Study Day	Screening (-21 to Day 1)	Day 1 Pre- Dose	Day 1 Dose	Day 1, 2 Hour	Day 1, 4 Hour	Day 4	Week 2 (Day 8/11)	Week 3 (Day 15/18)	Week 5 (Day 29/32)	Week 6 (Day 36/39)	Week 7 (Day 43/46)	Week 9 (Day 57/60)	Week 10 (Day 64/67)	Week 11 (Day 71/74)	Week 13 (Day 85/88)	Weeks 4, 8 ,12, 14 (In Clinic)[1]	Week 15, 16[10]	Early Withdrawal[2]	Window (Day 1 Only)
Orthostatic BP[7]	X	X			X	X*	X*												1 Hour
Vital signs[8]	X	X		X	X	X	2x	2x	2x	2x	2x	2x	2x	2x	2x	X*		X	30 Min
Blood Pressure						X*	2x	2x	2x	2x	2x	2x	2x	2x	2x	X*		X	
12-lead ECG	X	X														X		X	
Hematology Labs	X	X														X		X	
Urinalysis	X	X														X		X	
Other Labs	X[11]	X														X		X	
Serology	X																		
eGFR/UACR	X	X					X			X			X			X	X[12]	X	
Urine KLK/Plasma DM199		X		X	X		X	X								X	X	X	Blood- 15 Min Urine- 1 Hour
DM199 SC Dose[9]			X			X	2x	2x	2x	2x	2x	2x	2x	2x	2x	X*			
Adverse Event			X	X	X	X	2x	2x	2x	2x	2x	2x	2x	2x	2x	X	X	X	

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1. On weeks 4, 8, and 12, one visit must be done inside the clinic. For week 14 the clinic day should be on day 95. On home visits of these weeks, the following assessments are to be performed: vitals, dosing, blood pressure. These assessments are marked with an asterisk (*).
2. For any early Participant withdrawal, every effort should be made to complete an early withdrawal visit. Withdrawal visit must be completed in clinic.
3. Participants with positive HepC screening may be enrolled if LFT show normal liver function.
4. For women of childbearing potential only. A negative test must be confirmed prior to dose administration and monthly throughout the study.
5. A Participant must meet all inclusion criteria and none of the exclusion criteria prior to sequential assignment.
6. Weight, height, and BMI calculation required prior to dose administration. Subsequent height measurements not required, however, updated weights and BMI calculation required on each clinic visit for the remainder of the study. Use screening weight for Day 1 and unless <10kg monthly change, no change in dose.
7. Orthostatic blood pressure is to be taken at screen, pre-dose, and four hours post-dose. If the Participant has symptomatic orthostatic hypotension on Day 1, they must return to the clinic for Day 4 and be tested again. If they are symptomatic on Day 4, a nurse must perform orthostatic blood pressure at the home visit on Day 8. If symptomatic orthostatic hypotension persists, it is to the discretion of the PI whether the Participant will continue in the study. These days are marked with an asterisk (*).
8. Vitals include heart rate, breathing rate, and oral body temperature.
9. Dosing is recommended to be done within 6 hours of original dose time for duration of the study.
10. Visits completed at home on Days 99, 100, 102 and 104.
11. Only labs drawn at screening: A1c, APOL1 and Sickle cell trait.
12. Only collected on Day 104.

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12. APPENDIX 1

1. PT terms for AESI

Injection site erythema
Injection site haematoma
Injection site induration
Injection site pain
Injection site pruritus
Injection site reaction
Pain
Angioedema
Hypotension
Orthostatic hypotension
Blood pressure orthostatic

* Please note the above list is not exhaustive and it might be expanded depending on the final data.

2. PT Terms for Allergic/Immune reactions

Stomatitis
Generalised oedema
Hypersensitivity
Injection site cellulitis
Rash
Rash pruritic
Skin burning sensation
Sinusitis
Urinary tract infection
Viral infection
Leukocytosis
Pericardial effusion
Pyrexia
Arthralgia
Burning sensation
Rash pruritic
Skin burning sensation

* Please note the above list is not exhaustive and it might be expanded depending on the final data.

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