STATISTICAL ANALYSIS PLAN

Alkahest, Inc.

AKST4290-231

Protocol Title:	A Double-Masked, Placebo-Controlled Study to Evaluate the Efficacy of Oral AKST4290 in Participants with Moderately Severe to Severe Diabetic Retinopathy (CAPRI)
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Sponsor:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070
Prepared By:	[redacted]
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STATISTICAL ANALYSIS PLAN APPROVAL

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ABBREVIATIONS

Table 1 List of Abbreviations

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ASNV	Anterior-segment neovascularization
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under the curve
BUN	Blood urea nitrogen
CBC	Complete blood count
CI-DME	Center-involved diabetic macular edema
CMT	Central macular thickness
CSR	Clinical Study Report
DILI	Drug-induced liver injury
DM	Diabetes mellitus
DR	Diabetic retinopathy
DRSS	Diabetic Retinopathy Severity Scale
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
ENT	Ears, nose, and throat
EOT	End of Treatment
EOS	End of Study
ET	Early Termination
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein angiography
FP-4W	Fundus Photography 4 Wide
FP-7M	Fundus Photography Modified 7 Field
FP	Fundus photography
HbA1c	Hemoglobin A1c
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICH	International Council for Harmonisation
IOP	Intraocular pressure
IL	Interleukin
LPLV	Last participant, last visit

Abbreviation	Definition
MedDRA	Medical Dictionary for Regulatory Activities
NPDR	Nonproliferative diabetic retinopathy
OCT-A	Optical coherence tomography angiography
OD	Oculus dexter
OS	Oculus sinister
PDR	Proliferative diabetic retinopathy
PK	Pharmacokinetics
PT	Prothrombin time
PTT	Partial thromboplastin time
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's correction formula
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD-OCT	Spectral domain optical coherence tomography
SI	Système International
TEAE	Treatment-emergent adverse event
TNF-α	Tumor necrosis factor-alpha
UACR	Urine albumin to creatinine ratio
ULN	Upper limit of normal
VF	Visual field
WHODDE	World Health Organization Drug Dictionary Enhanced
WPAI-GH	Workplace Productivity and Activity Impairment General Health

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide comprehensive and detailed descriptions of the methods and presentation of data analyses proposed for Alkahest, Inc. Protocol AKST4290-231 (A Double-Masked, Placebo-Controlled Study to Evaluate the Efficacy of Oral AKST4290 in Participants with Moderately Severe to Severe Diabetic Retinopathy [CAPRI]). Descriptions of planned analyses are provided in order to avoid post hoc decisions that may affect the interpretation of the statistical analysis. The statistical methods applied in the design and planned analyses of this study are consistent with the International Council for Harmonisation (ICH) guidelines Statistical Principles for Clinical Trials (E9) (1998) and Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials (E9[R1]) (2017).

This SAP will be finalized prior to data analysis and before treatment unmasking and database lock to provide comprehensive details of the listings to be presented in the Clinical Study Report (CSR). Any changes between the statistical methods provided in the clinical study protocol and this SAP will be explained herein; any changes or deviations from this SAP relative to the final analysis will be fully documented in the CSR. Minor changes or deviations from the templates for provided listings need not be documented in the CSR.

On 09Dec2021, study investigators were notified the AKST4290-231 study was being terminated. Safety findings related to liver enzyme elevations were discovered in other AKST4290 studies. Out of an abundance of caution, active study participants were required to discontinue study drug and new enrollment was discontinued. Three study participants were randomized and taking study drug at the time of this notification. This SAP has been modified to accommodate the limited number participants enrolled in this prematurely concluded study.

2. STUDY OBJECTIVES

2.1 Primary Study Objective

The primary objective of this study is to investigate the efficacy of AKST4290.

2.2 Secondary Study Objectives

The secondary objectives of this study are:

- To investigate additional measures of efficacy of AKST4290;
- To assess the proportion of participants progressing to (or worsening of) center-involved diabetic macular edema (CI-DME), proliferative diabetic retinopathy (PDR), and/or anterior-segment neovascularization (ASNV);
- To assess the time to event of CI-DME, PDR, and/or ASNV requiring treatment;
- To assess the overall safety of AKST4290;
- To assess the effect of AKST4290 on diabetic kidney disease;

- To evaluate the changes from Baseline in the Workplace Productivity and Activity Impairment General Health (WPAI-GH) questionnaire.

2.3 Exploratory Study Objectives

The exploratory objectives of this study are:

- To assess the mean change from Baseline (gain or loss) in BCVA;
- To assess the mean change from Baseline in macular volume and central macular thickness (CMT);
- To assess the proportion of participants with worsening of Diabetic Retinopathy Severity Scale (DRSS) score;
- To assess visual acuity area under the curve (AUC);
- To investigate the impact of AKST4290 when administered at doses of 400 mg b.i.d. on pharmacokinetics (PK), pharmacogenomic, and flow cytometry/complete blood count (CBC) evaluations of interest conducted on blood and plasma samples;
- To evaluate changes from Baseline in optical coherence tomography angiography (OCT-A) (as available at sites);
- To evaluate changes from Baseline in visual field (VF) as assessed by perimetry/microperimetry evaluations (as available at sites);
- To evaluate the change from Baseline over time and the presence of inflammatory biomarkers [redacted] n aqueous humor collected from participants who elect to provide these samples.

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design

This is a Phase 2, double-masked, placebo-controlled multicenter study to evaluate the efficacy of AKST4290 administered at an 800 mg daily dose (400 mg b.i.d.) as compared with placebo over a 24-week dosing period in participants with moderately severe nonproliferative diabetic retinopathy (NPDR) (DRSS Level 47) to severe NPDR (DRSS Level 53) in one eye, and at least mild NPDR (DRSS Level 35) to mild PDR (DRSS Level 61) in the other eye. Both eyes must not have CI-DME, and must have good vision (defined as BCVA of $\geq$ 69 letters using the Early Treatment Diabetic Retinopathy Study [ETDRS] method).

Eligible participants will be enrolled into one of two randomized treatment arms (active and placebo) in a 2:1 fashion of active to placebo. Participants will receive treatment for a total of 24 weeks with either AKST4290 800 mg daily (400 mg b.i.d.) in Arm 1 or placebo (matching tablets) in Arm 2.

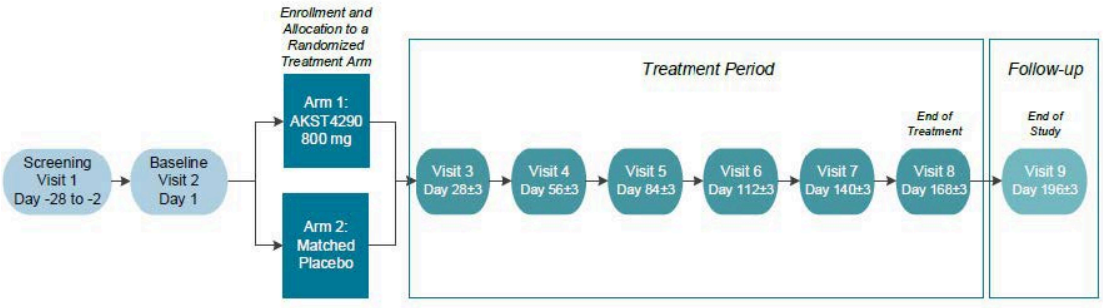
Once enrolled and allocated to a randomized treatment, all participants will be evaluated monthly throughout the treatment period (ie, at Visits 2, 3, 4, 5, 6, 7, and 8) to assess efficacy and monitor safety for the development of the vision-threatening complications of CI-DME, PDR, and/or ASNV. Participants who develop CI-DME (as confirmed by the central reading center), PDR (as confirmed by the central reading center), or ASNV (as confirmed by the investigator) that requires treatment will continue participation in the study. The central reading center must confirm progression to CI-DME or PDR before the investigator initiates treatment.

Specific safety, tolerability, and efficacy assessments will occur at every visit as presented in the schedule of assessments in Section 3.2. Blood samples for the assessment of PK, biomarkers, and pharmacogenomics will be obtained at various time points during the study as shown in the schedule of assessments (Section 3.2). Participation in the pharmacogenomic and aqueous humor sampling is optional and is not required for participation in the study; for those participants electing to participate in either of these optional portions, blood samples for the assessment of pharmacogenomics and aqueous humor samples will be collected.

The study will be conducted at approximately 25 sites in the United States. Approximately 80 participants are planned for enrollment to provide data for 69 completed participants (ie, participants who complete the 24-week assessments). The overall duration of the study, inclusive of recruitment, is approximately 14 months from study initiation (ie, following consent of first participant) to study completion (ie, last participant, last visit [LPLV]). The participation period for each participant is approximately 32 weeks, inclusive of a 4-week Screening period, 24-week Treatment period, and a 4-week Follow-up period.

A study flow diagram is provided in Figure 1.

Figure 1 Study Flow Diagram



3.2 Schedule of Assessments

For the complete schedule of assessments, refer to Section 15 (Schedule of Events) and Section 17.4 (Pharmacokinetic, Biomarker, Pharmacogenomic, and Flow Cytometry/CBC Sampling) of the clinical study protocol.

3.3 Treatment

3.3.1 Treatment Administered

Study treatment consists of AKST4290 or matched placebo. AKST4290 is a film-coated pink, oblong tablet manufactured by Alkahest, Inc., with a unit strength of 400 mg and is packaged in "foil-foil" blister packets. Each packet will include 14 tablets.

Study drug will be self-administered orally b.i.d., approximately 12 hours apart. AKST4290 will be administered as a total daily dose of 800 mg (400 mg b.i.d.). Matching placebo will be self-administered orally b.i.d. following the same dosing instructions as AKST4290.

Participants will receive training on study drug administration prior to initial study drug administration at Visit 2. Study drug will be self-administered in the clinic under the supervision of study personnel during every in-clinic visit of the Treatment Period (Visits 2-8) after any pre-dose assessments (including PK sample collection), and then self-administered at home between study visits.

When study drug is dispensed, participants will receive a total of 5 packets, which represents a 5-week supply (4 weeks of treatment plus an additional week). Study drug accountability will be assessed by packet.

3.3.2 Method of Assigning Subjects to Treatment Groups

To minimize the potential bias at the time of randomization, the study will be double-masked and randomized in a 2:1 ratio (active 400 mg b.i.d.: placebo b.i.d.) based on a block randomization schema. The randomization will be stratified by DRSS category at baseline (moderately severe NPDR [DRSS level 47] to severe NPDR [DRSS level 53]). The randomization codes will be generated by a statistician who is not involved in the study other than generation and maintenance of the randomization codes.

3.3.3 Masking Procedures

Maintenance and assignment of the randomization codes will be by a web-based randomization system. The study mask for either AKST4290 versus placebo can be broken for safety reasons if the information is required for the management of serious adverse events (SAEs), severe adverse events (AEs), or pregnancies. Before breaking the mask, every attempt should be made to discuss the need with the Sponsor program physician, or designee. When some degree of unmasking must occur, this should be limited to the fewest number of people on a need-to-know basis.

The investigator can obtain the AKST4290 or placebo treatment allocation for their participant through the web-based randomization system or by contacting the interactive response technology vendor.

3.4 Efficacy and Safety Variables

3.4.1 Efficacy Variables

3.4.1.1 Primary Efficacy Variable

The primary efficacy endpoint is the proportion of participants with a ≥ 3-step improvement from baseline on the DRSS score as compared with Week 24.

Progression of diabetic retinopathy (DR) is measured in discrete steps as described by the DRSS (ETDRS 1991b, Staurenghi 2018). The DRSS divides DR into 13 levels ranging from the absence of retinopathy to severe retinopathy (see Table 1 of the clinical study protocol) and is used to describe overall DR severity as well as the change in severity over time.

After pupillary dilation, the retina of both eyes will be imaged by fundus photography (FP) and fluorescein angiography (FA). Both eyes will be investigated by a trained technician and evaluated by the investigator. Findings from FP and FA, as provided by the central reading center, will be used to determine the DRSS score for each participant. Fundus photography will be performed at Visit 1 (Screening), each of the in-clinic study visits in the Treatment Period, Visit 2 (Baseline) through Visit 8 (End of Treatment [EOT]/Early Termination [ET]), and Visit 9 (End of Study [EOS]); fluorescein angiography will be completed at Visits 1 (Screening), Visit 5, and Visit 8 (EOT/ ET). Fundus photography and FA must be performed once a participant has been diagnosed with CI-DME or PDR, and before rescue treatment is given. Given the abbreviated scope of the SAP due to the study status, the DRSS level determined for each eye will be presented as assigned by the central reading center and a single bilateral severity level will not be derived to determine step-wise improvements.

3.4.1.2 Secondary Efficacy Variables

Secondary efficacy endpoints include the following:

- The proportion of participants with a ≥ 2-step improvement from Baseline on the DRSS score as compared with Week 24.
- The proportion of participants progressing to the following vision-threatening complications that require treatment: CI-DME, PDR, and/or ASNV as assessed by spectral domain optical coherence tomography (SD-OCT), FP, and FA, as appropriate. The central reading center must confirm progression to CI-DME and PDR before treatment is initiated; progression to ASNV, and subsequent treatment, does not require photo documentation.
- Time to the following vision-threatening event(s) that require treatment: CI-DME, PDR, and/or ASNV.
- Effect of AKST4290 on diabetic kidney disease as assessed by changes in clinical laboratory values over time (eg, estimated glomerular filtration rate [eGFR], urine albumin to creatinine ratio [UACR]).
- Change from Baseline over time in the WPAI-GH questionnaire.

The DRSS score as derived from FP and FA findings, is described in Section 3.4.1.1.

Assessment of vision-threatening complications including CI-DME, PDR and ASNV is based on SD-OCT, FP, and FA as appropriate. Evaluation of FP and FA are described in Section 3.4.1.1. The retinal layers of both eyes will be visualized, and thickness measured by SD-OCT at Visit 1 (Screening), each in-clinic study visit of the Treatment Period, and Visit 9 (EOS). Both eyes will be investigated by a trained technician using only specified SD-OCT equipment. A central reading center will be responsible for assessing all SD-OCT, OCT-A, FP, VF, and FA images to confirm study eligibility and progression to PDR and/or CI-DME and will provide the imaging data for inclusion in the database for the evaluation of efficacy and safety. Progression to vision-threatening complications is collected directly on the electronic case report form (eCRF) including whether or not the complication required treatment. Subjects who progress to a vision-threatening complication and treatment is indicated by a "Yes" response to the question "Was the vision-threatening complication treated?" will be considered to have a vision-threatening complication that required treatment.

Collection of laboratory parameters, including eGFR and UACR, is described in Section 3.4.2.2.

The WPAI-GH V2.0 is a 6-question survey used to assess the effects of a participant's health problems (ie, physical or emotional problems or symptoms) on their ability to work and perform regular activities during the past seven days. The WPAI-GH will be performed at Visit 2 (Baseline), Visit 4, Visit 6, and Visit 8 (EOT/ET).

The WPAI-GH questions will be analyzed as impairment percentages, in which higher percentages indicate greater impairment and less productivity (Reilly 1993). The following parameters will be calculated (multiply scores by 100 to express in percentages):

- Percent of work time missed due to health: Q2 divided by (Q2 plus Q4)
- Percent of impairment while working due to health: Q5 divided by 10
- Percent of overall work impairment due to health: Q2 divided by (Q2 plus Q4) plus [(1 - (Q2 divided by (Q2 plus Q4))) multiplied by (Q5 divided by 10)]
- Percent of activity impairment due to health: Q6 divided by 10

A subject must have a response provided for all questions used in the percentage for the impairment percentage parameter to be calculated.

3.4.1.3 Exploratory Efficacy Variables

Exploratory efficacy endpoints include the following:

- Change from Baseline (gain or loss) over time in BCVA using the ETDRS.
- Change from Baseline over time in macular volume and CMT as assessed by SD-OCT.
- Proportion of participants with a ≥ 2-step or ≥ 3-step worsening of DRSS score from Baseline over time.

- BCVA, using the ETDRS, AUC between Baseline and Week 24.
- PK, pharmacogenomic, and flow cytometry/CBC evaluations of interest.
- Change in area of ischemia or area of neovascularization (if PDR develops) from Baseline over time using OCT-A and/or FA (if available at the site).
- Change from Baseline over time in VF as evaluated by perimetry/microperimetry (if available at the site).
- Evaluation of the presence of markers of inflammation [redacted] in aqueous humor collected in a single eye in participants who elect to provide these samples.

Best-corrected visual acuity will be assessed using ETDRS charts at 4 meters initial testing distance and assessed in both eyes. The trained technician measuring the BCVA using ETDRS should be the same throughout the study period. Assessments of BCVA will be performed at Visit 1 (Screening), each in-clinic study visit of the Treatment Period, and Visit 9 (EOS). Given the abbreviated scope of the SAP due to the study status, AUC intervals will not be calculated.

Assessments of SD-OCT are described in Section 3.4.1.2. The retinal layers of both eyes will be visualized, and thickness measured by SD-OCT and, if available at the site, additional scans using OCT-A will be obtained. For sites with the capability to perform OCT-A, these assessments should be performed at Visit 2 (Baseline) and Visit 8 (EOT/ET).

The DRSS score as derived from FP and FA findings, is described in Section 3.4.1.1.

Evaluation of VF will be conducted using perimetry and/or microperimetry, per site availability. Evaluations of VF takes approximately 10-20 minutes and will be performed at Visit 2 (Baseline) and Visit 8 (EOT/ET) for sites with the capability to perform the assessment.

Sampling of biomarker, pharmacogenomic, and flow cytometry/CBC will be performed as described Section 17.4 of the clinical study protocol. A PK blood sample will be collected and then centrifuged for collection of plasma samples. Biomarker plasma aliquots will be obtained from the PK samples. Details on aqueous humor collection, plasma biomarkers, blood biomarkers by flow cytometry, and pharmacogenomic evaluation are provided in Section 7.1.1.3.8, 7.2.2.2, 7.2.2.3 and 7.2.2.4 of the clinical study protocol, respectively.

3.4.2 Safety Variables

3.4.2.1 Adverse Events

An AE is any untoward (unfavorable, harmful, or pathologic) medical occurrence in a participant administered a pharmaceutical (investigational) product even if the event does not necessarily have a causal relationship with this treatment.

An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding that is deemed clinically significant), symptom, or disease

temporally associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product.

All participants who have given informed consent will be evaluated for AEs.

3.4.2.2 Laboratory Parameters

Blood and urine samples will be collected at each study visit defined in the schedule of events (Section 15 of the clinical study protocol). Laboratory tests will include:

- Hematology: hemoglobin, hematocrit, red blood cell count, white blood cell count with differential[redacted], platelets.
- Chemistry: hemoglobin A1c (HbA1c), glucose, sodium, potassium, calcium, inorganic phosphate, chloride, bicarbonate, magnesium, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase, direct and indirect bilirubin, blood urea nitrogen (BUN), total protein, albumin, and eGFR.
- Serology: hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).
- Coagulation: partial thromboplastin time (PTT), prothrombin time (PT).
- Urinalysis: pH, glucose, erythrocytes, leukocytes, protein, nitrites, UACR.
- Pregnancy: pregnancy testing will be performed at the site in either urine or serum; a positive test result must be confirmed by a serum pregnancy test performed by the central laboratory.

Additional laboratory parameters may be reported. Clinical evaluation of liver injury, including additional testing, may be performed as defined in Section 17.1 of the clinical study protocol. Samples may be used for re-testing, further evaluation of an AE and/or assessment, and follow-up of other exploratory endpoints.

3.4.2.3 Vital Signs

Vital sign measurements will include seated systolic and diastolic blood pressure (mmHg), heart rate (beats per minute), respiration rate (breaths per minute), and body temperature. Vital signs will be measured after the participant has been seated for 5 minutes. Vital signs will be performed at Screening and every visit in the Treatment Period, Visit 2 (Baseline) through 8 (EOT/ET).

3.4.2.4 12-lead Electrocardiogram

Twelve-lead electrocardiograms (ECGs) (I, II, III, aVR, aVL, aVF, V1 - V6) will be recorded using a computerized electrocardiograph. A 12-lead ECG will be performed after the participant has rested quietly for at least 5 minutes in a supine position at Visit 1 (Screening), Visit 8 (EOT/ET), and Visit 9 (EOS). In some cases, it may be appropriate to repeat abnormal ECGs to rule out technical factors contributing to ECG artifacts or abnormality. The final interpretation of the ECGs will be recorded on the

appropriate eCRF. The interpretation of the ECGs will be recorded as normal, abnormal but not clinically significant, or abnormal and clinically significant. Heart rate, QT and QT interval corrected for heart rate using Fridericia's correction formula (QTcF) will also be reported.

3.4.2.5 Physical Examination

At Visit 1 (Screening), a full physical examination will be performed to assess the following organ systems: skin, ears, nose, and throat (ENT), head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, and lymphatic systems. Height and weight will be measured.

During the study period, a targeted physical examination, including auscultation of the heart, measurement of weight, and review of any previous abnormalities identified during the full physical examination will be performed.

3.4.2.6 Additional Ophthalmological Assessments

Additional ophthalmological assessments performed include:

- Slit lamp examination (dilated and undilated)
- Extended Ophthalmoscopy
- Intraocular pressure measurements (IOP)
- Gonioscopy

The slit lamp examination, IOP measurement, gonioscopy, and extended ophthalmoscopy are to be performed in both eyes. The anterior and posterior segments of the eye should be assessed. Extended ophthalmoscopy is an assessment of the peripheral retina, usually performed with a 20 diopter lens and an indirect ophthalmoscope, to examine for retinal abnormalities such as retinal tears, detachment, hemorrhage, retinal artery perfusion, etc. Intraocular pressure will be measured using applanation Goldmann tonometry in both eyes during each visit. A gonioscope, together with a slit lamp or operating microscope, will be used to view the iridocorneal angle. Extended ophthalmoscopy is an assessment of the peripheral retina after pupillary dilation, usually performed with a 20 diopter lens and an indirect ophthalmoscope, to examine for retinal abnormalities such as neovascularization, retinal tears, detachment, retinal hemorrhage, etc. Assessments will be performed at each study visit defined in the schedule of events (Section 15 of the clinical study protocol).

3.4.3 Pharmacokinetic Variables

Plasma concentration measurements of AKST4290 and its major metabolites will be collected to assess systemic exposure. Sampling of PK will be performed as described Section 17.4 of the clinical study protocol.

3.5 Data Quality Assurance

Report summaries will be generated using validated Base SAS® software, version 9.4 or higher, on a PC or server-based platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a review of the program log, review of output or dataset format and structure, and independent confirmatory programming to verify output results or dataset content. Additionally, all outputs will undergo a review by a senior level team member before finalization.

The content of the source data will be reviewed on an ongoing basis by project statistical programmers and statisticians. Data will be checked for missing values, invalid records, and extreme outliers through defensive programming applications, analysis-based edit checks, and other programmatic testing procedures. All findings will be forwarded to the project data manager for appropriate action and resolution.

4. STATISTICAL METHODS

4.1 General Methodology

Data will be summarized by Emanate biostatistics personnel. Given the paucity of study participants, data collected during the study and follow-up period will be reported with listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all listings will be in conformance with guidelines specified by the ICH in Appendix 7 of the Electronic Common Technical Document Specification (Apr 2003).

4.1.1 Reporting Conventions

In general, all data collected and any derived data will be presented in subject data listings for all enrolled subjects. Listings will be ordered by site, subject number, treatment group, and assessment or event date. The treatment group presented in listings will be based upon the actual treatment received.

4.1.2 Summarization by Visit

All data, including unscheduled visits, will be included in subject listings.

4.1.3 Data Handling Rules

Values reported as greater than or less than some quantifiable limit (eg, "<1.0") will be presented as collected in the subject listings.

4.1.4 Standard Calculations

Where appropriate, the calculated study day of each assessment or event will be presented with the assessment or event date on subject data listings, where study day will be determined as:

- The assessment/event date minus the date of first dose of study drug, if the assessment/event date is prior to the date of first dose; and

- The assessment/event date minus the date of first dose of study drug, plus one, if the assessment/event date is on or after the date of first dose.

Other variables requiring calculations will be derived using the following formulas:

- **Days:** A duration between two dates expressed in days will be calculated using the following conventions:

    - Later date – earlier date + 1, if the earlier date is on or after the date of first dose of study drug; or

    - Later date – earlier date, if the earlier date is prior to the date of first dose of study drug.

- **Years:** A duration expressed in years will be calculated by dividing the duration in days by 365.25.

4.2 Study Subjects

4.2.1 Disposition of Subjects

Subject disposition will be presented in a listing to include the date of first dose, study exit date, subject status and reason for early termination (when applicable). Whether or not the subject was unmasked including the date/reason when applicable will also be reported. A listing of screen failures, to include the date of study exit and reason for screen failure, will also be presented.

4.2.2 Protocol Deviations

Major and minor protocol violations will be presented in a listing. Major protocol violations are protocol deviations captured on-study that are deemed by the Sponsor to potentially impact the efficacy or safety conclusions of the study.

All major protocol violations will be determined and appropriately categorized prior to database lock and prior to breaking the mask of the treatment group assignments.

4.2.3 Plasma Concentrations

Raw plasma concentration values for AKST4290 and the corresponding metabolites, M737 and M227, will be provided in listings by sampling time point for participants receiving AKST4290.

4.2.4 Pharmacokinetic Analysis

Pharmacokinetic parameters will not be derived due to the limited number of sampling timepoints.

4.3 Efficacy Evaluations

Given the abbreviated scope of the SAP due to the study status, efficacy endpoint analysis will be presented in subject data listings, generally relying on the data as collected in the electronic data capture (EDC) system or from the central reading center.

4.3.1 Demographic and Other Baseline Characteristics

Demographic variables including age, sex, ethnicity, race and childbearing potential will be presented in a listing. Baseline disease characteristics include diabetes mellitus (DM) type category (Type 1 or Type 2), time since date of DM diagnosis, time since DR diagnosis for oculus dexter (OD) and oculus sinister (OS) eyes, and baseline DRSS levels for OD and OS eyes. Time since DM or DR diagnosis (in years) is calculated as the informed consent date – the date of diagnosis (DM or DR) divided by 365.25.

Medical history will be presented in a listing. Verbatim terms on case report forms will be mapped to preferred terms and system organ classes using the Medical Dictionary for Regulatory Activities (MedDRA, version 24.0).

4.3.2 Measurements of Treatment Compliance

Compliance to study treatment regimen will be reported as collected on the Study Drug Administration and Accountability eCRF page. The number of tablets dispensed and returned for each visit will be presented in a listing. A separate listing will be provided to present any administration information reported on the Missed/Additional Doses eCRF page.

4.3.3 Efficacy Endpoint Analysis

The DRSS level determined for each eye, OD and OS, will be presented as reported by the central reading center in the fundus photography (Fundus Photography Modified 7 Field [FP-7M] or Fundus Photography 4 Wide [FP-4W]) assessment; a single bilateral severity level will not be derived to determine step-wise improvements/worsening over time. Assessments of FA will also be reported in a listing.

Subjects that progress to any vision-threatening complication that requires treatment (CI-DME, PDR, and/or ANSV) will be reported in a listing to include the vision-threatening complication type, confirmation details associated with the progression, and any corresponding treatment information. Additionally, the treatment of PDR review criteria collected by the center reading center will also be presented in a listing, as applicable.

Effect of AKST4290 on diabetic kidney disease as assessed by eGFR and UACR will be presented in the corresponding laboratory listings as described in Section 4.4.4.

Responses to WPAI-GH questionnaire, as well as the corresponding derived impairment percentages described in Section 3.4.1.2, will be presented in a listing.

Results for assessments of BCVA using ETDRS, SD-OCT, OCT-A (as available at the site), and VF (as available at the site) will be presented in a listing.

Presentation of pharmacogenomics data will generally be conducted by a separate vendor and is outside the scope of this analysis plan. [redacted]

[redacted] These biomarkers will be measured in plasma samples to investigate any change in response to treatment. Blood biomarkers using flow cytometry/CBC will be measured to characterize immune cells. Flow cytometry results will be presented in a listing. Additional summary and/or presentation of biomarkers data will be conducted by a separate vendor and are outside the scope of this analysis plan.

4.4 Safety Evaluation

Safety listings will include all subjects who receive at least one dose of study drug. Subjects who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis.

4.4.1 Extent of Exposure

Extent of exposure to study treatment will provided in listings. The duration of exposure will be presented in days and calculated as the date of last dose of study drug minus the date of first dose of study drug, plus one. Total dose received (mg) will be calculated as the total number of tablets dispensed – total number of tablets returned, multiplied by 400.

4.4.2 Adverse Events

Treatment-emergent adverse events (TEAEs) are defined as those AEs with onset after the first dose of study drug or existing events that worsened after the first dose during the study. Events reported with a partial onset date (eg, month and year are reported but the day is missing) will be classified as treatment-emergent if it cannot be confirmed that the event onset was prior to the first dose of study drug based on the available date entries.

Verbatim terms on case report forms will be mapped to preferred terms and system organ classes using the MedDRA, version 24.0.

Adverse event data will be presented in data listings by subject, treatment group, and event. Serious AEs and AEs leading to discontinuation of the study drug will be presented in separate data listings.

4.4.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

The following AEs will be defined as adverse events of special interest (AESI):

- [redacted]

- [redacted]

All deaths during the study, including the post treatment follow-up period, will be listed by subject, to include any specifying details of the death. Serious AEs and other significant AEs, including those that led to discontinuation or interruption of the study drug and AESIs, will be provided in separate subject data listings.

4.4.4 Clinical Laboratory Evaluation

All presentation of laboratory results will be based on data analyzed by the central laboratory and presented in Système International (SI) units, as suggested by the Center for Biologics Evaluation and Research and the Center for Drug Evaluation and Research *Position on Use of SI Units for Lab Tests* (Oct 2013). All data will be included in by-subject data listings. Laboratory measurements identified as abnormal (ie, outside the normal range) will also be listed separately by subject, laboratory test, and unit. In addition, normal ranges provided by the central laboratory will be presented in a separate listing.

4.4.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

4.4.5.1 Vital Signs

Vital sign parameter measurements will be provided in a listing.

4.4.5.2 12-Lead Electrocardiogram

Twelve-Lead ECG interval parameters will be provided in a listing. Twelve-lead ECG will be classified by the investigator as “normal,” “abnormal, not clinically significant,” or “abnormal, clinically significant.”

Prolonged QTc intervals will be flagged in the ECG listing as QTcF measurements (msec) that are >450 at each visit where ECG is routinely collected per the clinical study protocol.

4.4.5.3 Physical Examination

Results of the physical examination will be presented in subject data listings by subject, study visit, and body system.

4.4.5.4 Slit Lamp Examination (Dilated and Undilated) and Gonioscopy

Results of slit lamp examination and gonioscopy assessments will be presented in a listing.

4.4.5.5 Extended Ophthalmoscopy

Extended ophthalmoscopy assessments of the peripheral retina are categorized as absent (or no), present (or yes), or questionable (when available) and will be presented in a listing.

4.4.5.6 Intraocular Pressure

Intraocular pressure (mmHg) will be presented in a listing.

4.4.5.7 Prior and Concomitant Medications and Procedures

Medications will be coded using the World Health Organization Drug Dictionary Enhanced (WHODDE), version B3 March 2021. Medications entered on the eCRF will be mapped to Anatomical Therapeutic Chemical (ATC) drug class (level 1) and drug name.

The study phase (prior and/or concomitant status) of each medication will be determined programmatically based on medication start and end dates. A prior medication is defined as any medication administered prior to the date of the first dose of study drug. A concomitant medication is defined as any medication administered on or after the date of the first dose of study drug. A medication may be defined as both prior and concomitant. If it cannot be determined whether a medication was received prior to the start of study drug dosing due to partial or missing medication start and/or end dates, it will be considered a prior medication. Likewise, if it cannot be determined whether a medication was received after the start of study drug dosing, it will be considered concomitant.

The study phase during which each medication was received (eg, prior, concomitant, or both) will be presented on the listing of prior and concomitant medications.

Prior and concomitant procedures will be provided in a listing. The study phase during which each procedure was performed (eg, prior or concomitant) will be determined based on the reported date of the procedure using the similar logic as described for medications; if partial dates are reported for a procedure date, a procedure may be defined as both prior and concomitant. If it cannot be determined whether a procedure was received prior to the start of study drug dosing due to a partial or missing procedure date, it will be considered a prior procedure. Likewise, if it cannot be determined whether a procedure was received after the start of study drug dosing, it will be considered concomitant.

4.5 Determination of Sample Size

Due to a dearth of studies with a 3-step improvement, as well as potential variabilities between each eye, the sample size is powered on the primary hypothesis comparing the difference between treatment arms (AKST4290 and placebo) in the proportion of participants with a ≥ 2-step improvement of DR severity level on DRSS score in a single eye from baseline as compared with Week 24. All calculations were based on the following fixed assumptions:

- Target 80% power.
- 2-sided alpha = 0.046.
    - It should be noted that to account for Data and Safety Monitoring Board (DSMB) interim data reviews, the alpha spending approach has been adopted using an alpha reduction of 0.001 for each review

(assuming a total of 4 reviews). The required sample size does not change if only three DSMB meetings are necessary.

- Test of 2 independent proportions based on the normal approximation.
- Based calculation on PANORAMA (NCT02718326, EYLEA Full Prescribing Information), RISE/RIDE (Nguyen 2012, Wykoff 2018), and VIVID/VISTA (Korobelnik 2014, Brown 2015) study data.

The total sample size using the assumptions stipulated is approximately 80 enrolled participants to allow for a total of 69 completed participants. The randomization schema is 2:1 (AKST4290:placebo). Group sample sizes of 46 in the AKST4290 group and 23 in the placebo group will achieve approximately 80% power to detect a difference in group proportions of 24%. The proportion in the AKST4290 group is assumed to be 6% under the null hypothesis and 30% under the alternate hypothesis. The proportion in the placebo group is assumed to be 6%.

4.6 Changes in the Conduct of the Study or Planned Analyses

This SAP has been modified to accommodate the limited number participants enrolled in this prematurely concluded study. Three study participants were randomized and taking study drug at the time Investigators were requested to discontinue the study. Given the limited data, all data will be presented in the form of listings (as described in the Data Listings Layouts document).

5. REFERENCE LIST

[redacted]