A DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY OF ORAL AKST4290 IN PARTICIPANTS WITH MODERATELY SEVERE TO SEVERE DIABETIC RETINOPATHY (CAPRI)

Protocol Number:	AKST4290-231
IND Number:	154780
Clinical Phase:	2
Sponsor:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070
Study Agent:	AKST4290
Indications:	Diabetic Retinopathy (DR)
Authorized Representative:	[redacted]
Version Number:	2.0
Version Date:	19AUG2021
Replaces Version(s):	1.0

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LIST OF ABBREVIATIONS

AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMD	Age-related macular degeneration
Anti-VEGF	Anti-vascular endothelial growth factor
ASNV	Anterior-segment neovascularization
AST	Aspartate aminotransferase
AUC	Area under the curve
BCVA	Best corrected visual acuity
b.i.d.	Twice daily
bpm	Beats per minute
BRB	Blood-retinal barrier
BUN	Blood urea nitrogen
CBC	Complete blood count
[redacted]	[redacted]
CFR	Code of Federal Regulations
ChBF	Choroidal blood flow
CI-DME	Center-involved diabetic macular edema
CK-MB	Creatinine kinase-muscle/brain
CLIA	Clinical Laboratory Improvement Amendments
C_{max}	Maximum concentration
CMP	Clinical monitoring plan
CMT	Central macular thickness
COVID-19	Coronavirus Disease 2019
CRO	Contract research organization
CRT	Central retinal thickness
CSR	Clinical study report
CST	Central Subfield Thickness
[redacted]	[redacted]
DDI	Drug-drug interaction
DILI	Drug-induced liver injury
DM	Diabetes mellitus
DME	Diabetic macular edema
DNA	Deoxyribonucleic acid
DR	Diabetic retinopathy
DRSS	Diabetic Retinopathy Severity Scale
DRCRN	Diabetic Retinopathy Clinical Research Network
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form

eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
ENT	Ear, nose, and throat
EOS	End-of-study
EOT	End-of-treatment
[redacted]	[redacted]
ET	Early termination
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein angiography
FDA	Food and Drug Administration
FP	Fundus photography
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma-glutamyl transferase
HbA1c	Hemoglobin A1c
HBs	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ID	Identification
IEC	Independent ethics committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin (may be followed by an alpha-numeric designator)
INR	International normalized ratio
IOP	Intraocular pressure
IRB	Institutional review board
ISF	Investigator site file
ITT	Intent-to-treat
KM	Kaplan-Meier
LDF	Laser doppler flowmetry
LKM	Liver-kidney microsomes
LPLV	Last participant, last visit
[redacted]	[redacted]
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed-effects model for repeated measures
nAMD	Neovascular age-related macular degeneration
NPDR	Nonproliferative diabetic retinopathy
NTI	Narrow therapeutic index

NV	Neovascularization
NVD	Neovascularization of the disc
NVE	Neovascularization elsewhere
OCT	Optical coherence tomography
OCT-A	Optical coherence tomography angiography
PDR	Proliferative diabetic retinopathy
[redacted]	[redacted]
PK	Pharmacokinetic
PRP	Panretinal photocoagulation
PT	Prothrombin time
PTT	Partial thromboplastin time
QA	Quality assurance
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's correction formula
RNA	Ribonucleic acid
RPE	Retinal pigment epithelium
RSI	Reference Safety Information
SAE	Serious adverse event
SAP	Statistical analysis plan
SD-OCT	Spectral domain optical coherence tomography
SOC	Standard-of-care
SUSAR	Suspected unexpected serious adverse reaction
TDD	Total daily dose
TEAE	Treatment-emergent adverse event
Th2	T-helper 2
TNF-α	Tumor necrosis factor alpha
UACR	Urine albumin to creatinine ratio
ULN	Upper limit of normal
US	United States
VF	Visual field
WOCBP	Women of childbearing potential
WPAI-GH	Workplace Productivity and Activity Impairment General Health

PROTOCOL APPROVAL PAGE

Study Title:	A Double-Masked, Placebo-Controlled Study to Evaluate the Efficacy of Oral AKST4290 in Participants with Moderately Severe to Severe Diabetic Retinopathy (CAPRI)
Protocol Number:	AKST4290-231
Version/Date:	2.0 (19AUG2021)
Sponsor Name and Address:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070

I, the undersigned, have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practice, the Declaration of Helsinki in the latest relevant version, and applicable legal and regulatory requirements.

Approved by:

[redacted]	[redacted]	August 19, 2021
Sponsor Representative (print)	Signature	Date

STATEMENT OF COMPLIANCE

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)
Protocol Number:	AKST4290-231
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By my signature, I:

- Confirm that my staff and I have carefully read and understand this protocol or protocol amendment, have received the Investigator Brochure, and are thoroughly familiar with the appropriate use of the investigational agent described herein.

- Agree to comply with the conduct and terms of the study specified herein and with any other study conduct procedures provided by the Sponsor, Alkahest, Inc., or their designee.

- Agree to assume responsibility for the proper conduct of the study at this site, including complying with current relevant versions of the US Food and Drug Administration (FDA) regulations, European Medicines Agency (EMA), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human research participants.

- Agree not to implement deviations from or changes to the protocol or protocol amendments without agreement from the Sponsor and prior submission to and written approval (where required) from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), except when necessary to eliminate an immediate hazard to the participants, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).

- Agree to onsite monitoring of all source documents by Alkahest, Inc. or designee and to onsite inspection of source documents by appropriate regulatory authorities, including but not limited to the FDA, EMA, local governing regulatory bodies, and IRB/IEC inspectors.

Investigator's Signature Date

Print Name

PROTOCOL SUMMARY

Title:	A Double-Masked, Placebo-Controlled Study to Evaluate the Efficacy of Oral AKST4290 in Participants with Moderately Severe to Severe Diabetic Retinopathy (CAPRI)
Précis:	This study is designed to evaluate the efficacy of AKST4290 administered at a total daily dose (TDD) of 800 mg daily (400 mg twice daily [b.i.d.]) compared with placebo over a 24-week dosing period in participants with moderately severe nonproliferative diabetic retinopathy (NPDR) (Diabetic Retinopathy Severity Scale [DRSS] Level 47) to severe NPDR (DRSS Level 53) in one eye, and at least mild NPDR (DRSS Level 35) to mild proliferative diabetic retinopathy (PDR) (DRSS Level 61) in the other eye. Both eyes must not have center-involved diabetic macular edema (CI-DME) and must have good vision (defined as best corrected visual acuity [BCVA] of ≥ 69 letters using the Early Treatment Diabetic Retinopathy Study [ETDRS] method). Once enrolled, all participants will be evaluated monthly throughout the treatment period to assess efficacy and monitor overall safety, including the development of vision-threatening complications of CI-DME, potential worsening of, or progression to, proliferative diabetic retinopathy (PDR), and/or anterior-segment neovascularization (ASNV). Approximately 80 participants will be enrolled and allocated to 1 of 2 treatment arms in a 2:1 randomization scheme (AKST4290: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.
Objectives:	The primary objective is to investigate the efficacy of AKST4290. Secondary objectives also include evaluation of efficacy as well as assessment of the proportion of participants who progress to CI-DME, PDR, and/or ASNV, and time to each of these events requiring treatment. Overall safety will be assessed as well as the effect of AKST4290 on diabetic kidney disease, and the change in Workplace Productivity and Activity Impairment General Health (WPAI-GH). Exploratory objectives include the assessment of change from Baseline in BCVA (gain or loss), macular volume, central macular thickness (CMT), optical coherence tomography angiography (OCT-A) (as available at sites), and visual field (VF) as assessed by perimetry/microperimetry (as available at sites). The proportion of participants with worsening DRSS score will also be assessed as well as visual acuity area under the curve (AUC). Additional exploratory objectives include assessment of pharmacokinetics (PK), pharmacogenomics, flow cytometry, complete blood count (CBC), and the presence of selected inflammatory biomarkers in aqueous humor samples from participants who elect to participate in this optional sample collection.

Endpoints:

Primary Endpoint:

- The proportion of participants with a ≥ 3-step improvement of the DRSS score from Baseline compared with Week 24.

Secondary Endpoints:

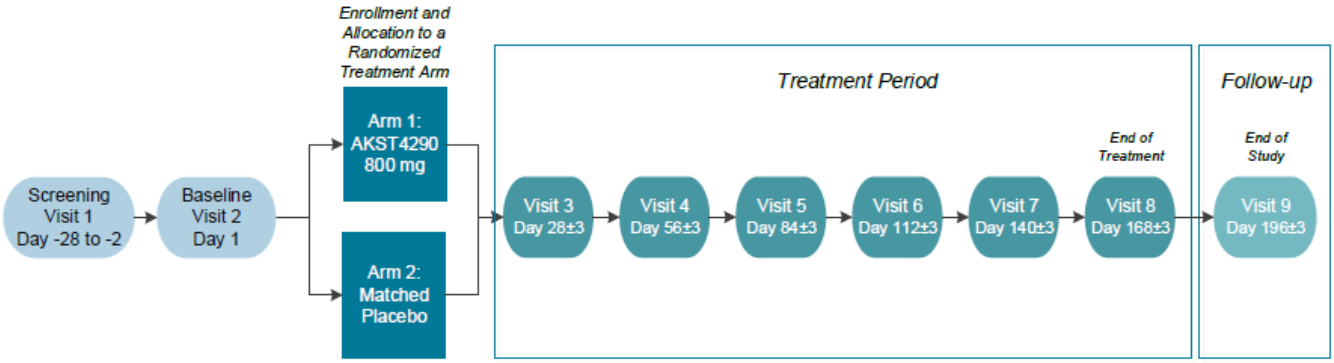
- The proportion of participants with a ≥ 2-step improvement of the DRSS score from Baseline compared with Week 24.
- The proportion of participants progressing to the following vision-threatening complications that require treatment: CI-DME, PDR, and/or ASNV as assessed by spectral domain optical coherence tomography (SD-OCT), fundus photography (FP), and fluorescein angiography (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.
- Safety as assessed by incidence and intensity of adverse events (AEs).
- Effect of AKST4290 on diabetic kidney disease as assessed by changes in clinical laboratory values over time (e.g., estimated glomerular filtration rate [eGFR], urine albumin to creatinine ratio [UACR]).
- Change from Baseline over time in the WPAI-GH.

Exploratory Endpoints:

- Change from Baseline (gain or loss) over time in BCVA using the ETDRS method.
- 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 method, 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 in participants who elect to provide these samples.

Population:	Approximately 80 participants are planned for enrollment to provide data for 69 completed participants (i.e., participants who complete the 24-week assessments).
Phase:	2
Number of Sites:	Approximately 25 sites
Description of Study Agent:	A TDD of 800 mg of AKST4290 administered orally twice daily (400mg tablets b.i.d.) or matching placebo administered orally twice daily.
Study Duration:	Approximately 14 months
Participant Participation:	Approximately 32 weeks, inclusive of a 4-week Screening period, 24-week Treatment period, and a 4-week Follow-up period.

SCHEMATIC OF STUDY DESIGN



1. KEY ROLES

1.1. Authorized Representative (Signatory) / Responsible Party

1.2. Study Organization

The name and contact information of the responsible party and individuals involved with the study (e.g., investigator(s), Sponsor's medical expert and study monitor, Sponsor's representative(s), laboratories, steering committees, and oversight committees (including IRBs/IECs, as applicable) will be maintained by the Sponsor, or their designee, and provided to the investigator.

2. INTRODUCTION

2.1. Background Information

Diabetes mellitus (DM) is a worldwide health epidemic that is expected to increase exponentially. In 2018, 34.1 million adults, or 13% of all adults in the United States (US) and 26.8% among those aged 65 years or older, had DM (CDC 2020). As of 2019, it is estimated that worldwide, 463 million people have DM and this number is projected to reach 578 million by 2030, and 700 million by 2045 (IDF 2019). Despite early intervention programs and better methods of glycemic control, DM is one of the fastest growing health problems of the 21st century (IDF 2019).

Diabetic eye disease is the most common microvascular complication of DM, and diabetic retinopathy (DR) is a leading cause of visual loss in working-age populations (Wang 2018). Among patients with DM worldwide, 35% have DR and 12% have vision-threatening diabetic retinopathy (DR) (IDF 2019). Hyperglycemia and resultant inflammation are considered key components in the pathogenesis of retinal microvascular damage (Wang 2018). The diagnosis of DR is made by clinical examination of the retina, which displays vascular abnormalities (Wang 2018). The hallmark of DR is microvascular damage to the retinal blood vessels that falls into 2 broad stages: the earlier stage of nonproliferative DR (NPDR) and the advanced, vision-threatening stages in proliferative DR (PDR) (Duh 2017). In PDR, new fragile, incompetent vessels that are susceptible to hemorrhage grow initially from the retina and/or optic disc and extend beyond the internal limiting membrane. These vessels can bleed, resulting in vitreous hemorrhage, or they can form a fibrovascular membrane and contract, resulting in tractional retinal detachment. The most common cause of vision loss in DR is due to diabetic macular edema (DME), which can occur at any DR severity level. Diabetic macular edema is caused by diabetes-induced breakdown of the blood-retinal barrier (BRB) with consequent vascular leakage of fluid and circulating proteins into the neural retina, disrupting the macular architecture and function (Duh 2017).

Without intervention, nearly half of eyes with high-risk PDR will experience profound vision loss from associated complications including vitreous hemorrhage or traction retinal detachment. Complication rates are reduced dramatically with panretinal photocoagulation (PRP) (DRS 1976). The Early Treatment Diabetic Retinopathy Study (ETDRS) demonstrated PRP reduces the risk of severe vision loss by over 90% for eyes with or approaching high risk PDR (ETDRS 1991a). Although remarkably effective at reducing risk of vision loss if applied in a timely and appropriate manner, PRP treatment destroys viable retinal tissue in the peripheral retina and is associated with potential adverse effects that may lead to transient or permanent loss of visual function, including exacerbation of existing macular edema, peripheral visual field defects, night vision loss, loss of contrast sensitivity, and potential complications from misdirected or excessive burns (Reddy 2018).

The use of intravitreal (IVT) anti-vascular endothelial growth factor (anti-VEGF) therapy in eyes with PDR led to decreased risk of DR worsening (need for PRP, vitreous hemorrhage, or vitrectomy for complications of PDR) compared with no anti-VEGF therapy in a secondary outcome reported by the Diabetic Retinopathy Clinical Research Network (DRCRN) in a trial evaluating ranibizumab for DME (DRCRN 2010). However, some eyes still worsen despite

anti-VEGF therapy, and DR severity can worsen when anti-VEGF therapy is discontinued. The inadequate response to anti-VEGF injections may be associated with the involvement of molecular pathways other than VEGF during the pathogenesis of DR (Wang 2018). Studies investigating the underlying mechanisms of DR (e.g., inflammation) are of great importance, and may provide potential targets for the development of new, alternative treatments.

Despite advances in treatment for PDR and DME, there are currently no widely used treatments for NPDR, leaving patients at risk for the development of DME and/or PDR. Worse baseline NPDR severity is strongly associated with increased risk of worsening to PDR (DRCRN 2011). Analysis of data from the ETDRS suggest that eyes with severe NPDR have a 52% risk of progressing to PDR within 1 year, and a 60% risk of worsening to PDR with high-risk characteristics within 5 years (DRCRN 2011). While IVT anti-VEGF therapies are Food and Drug Administration (FDA)-approved for severe NPDR, these treatments are not considered standard of care, as they are invasive, costly, and inconvenient for this stage of disease (AA0 2019, Solomon 2017). Treatment with PRP is performed in some select cases of severe NPDR; however, there is no clear treatment mandate generalizable to most eyes with severe NPDR that are at high risk of worsening to PDR.

3. OBJECTIVES AND PURPOSE

3.1. Primary Objective

- To investigate the efficacy of AKST4290.

3.2. Secondary Objectives

- To investigate additional measures of efficacy of AKST4290.
- To assess the proportion of participants progressing to (or worsening of) CI-DME, 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 (Section 17.5).

3.3. Exploratory Objectives

- 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 AUC.
- To investigate the impact of AKST4290 when administered at doses of 400 mg b.i.d. on 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 including [redacted] in aqueous humor collected from participants who elect to provide these samples.

4. STUDY DESIGN AND ENDPOINTS

4.1. Description of the 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 participants with moderately severe 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 ≥ 69 letters using the ETDRS method).

Eligible participants will be enrolled into 1 of 2 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 (i.e., 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 15. At specified time points, the following assessments will be performed: extended ophthalmoscopy, intraocular pressure (IOP) measurement, BCVA testing using the ETDRS method, spectral domain optical coherence tomography (SD-OCT), slit lamp examination, fluorescein angiography (FA), fundus photography (FP), and gonioscopy. At sites with the ability to perform OCT-A and VF testing, these evaluations will be conducted. The WPAI-GH questionnaire will be completed throughout the treatment period. A central reading center will be responsible for assessing all SD-OCT, OCT-A, FP and FA images, and VF evaluations, 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.

Additional safety assessments will be performed at each visit and will include the incidence and severity of adverse events (AEs), laboratory tests, vital sign measurements, concomitant medication use, and any treatment for PDR, CI-DME, and/or ASNV. A 12-lead electrocardiogram (ECG) will be performed at selected visits as shown in the schedule of assessments (Section 15). Additionally, the incidence and severity of adverse events of special interest (AESI), [redacted] will be monitored.

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 15). 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 US. The overall duration of the study, inclusive of recruitment, is approximately 14 months from study initiation (i.e., following consent of first participant) to study completion (i.e., 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.

4.2. Study Endpoints

4.2.1. Primary Endpoint

- The proportion of participants with a ≥ 3-step improvement from Baseline on the DRSS score as compared with Week 24.

4.2.2. Secondary Endpoints

- 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 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.
- Safety as assessed by incidence and intensity of AEs.
- Effect of AKST4290 on diabetic kidney disease as assessed by changes in clinical laboratory values over time (e.g., eGFR, urine albumin to creatinine ratio [UACR]).
- Change from Baseline over time in the WPAI-GH questionnaire (Section 17.5).

4.2.3. Exploratory Endpoints

- 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.

5. STUDY ENROLLMENT AND WITHDRAWAL

5.1. Inclusion Criteria

In order to be eligible for inclusion, all participants must meet the following criteria:

1. Age ≥ 18 years.

2. Type 1 or type 2 DM. Any of the following will be considered sufficient evidence that diabetes is present:

    a. Current and regular use of insulin for the treatment of diabetes.

    b. Current and regular use of oral antihyperglycemic agents for the treatment of diabetes.

    c. Documented diabetes by ADA and/or WHO criteria.

3. BCVA ETDRS visual acuity letter score ≥ 69 letters at Screening.

4. Moderately severe NPDR (DRSS Level 47) to severe NPDR (DRSS Level 53) and at least mild NPDR (DRSS Level 35) to mild PDR (DRSS Level 61) without CI-DME in either eye, as determined by the investigator and as confirmed by the central reading center.

5. Prompt PRP or anti-VEGF treatment is not required AND the investigator and potential participant are willing to wait for the development of high-risk characteristics (defined in Section 5.3) to treat PDR.

6. Media clarity, pupillary dilation, and study participant cooperation sufficient to obtain fundus photographs, FA, and OCT images.

7. The investigator and potential participant are comfortable withholding treatment for DME and PDR until protocol-defined treatment criteria are met (see Section 5.4).

5.2. Exclusion Criteria

An individual will not be eligible if any of the following exclusion criteria apply:

1. Evidence of neovascularization (NV) (including active iris or angle NV) requiring treatment, per investigator discretion. Presence of NV outside of the 7-modified ETDRS fields on ultra-widefield imaging will not be an exclusion, provided treatment is not planned. Small iris tufts are NOT an exclusion.

2. PRP or grid laser within 1000 microns of the foveal center.

3. CI-DME on clinical examination (CI is defined as DME within 1,000 microns of the foveal center) and has an OCT central subfield thickness above the following gender and OCT machine specific thresholds:

a. Zeiss Cirrus: 290 µm in women and 305 µm in men.

b. Heidelberg Spectralis: 305 µm in women and 320 µm in men.

4. Prior intraocular or periocular steroid injection within 12 months prior to enrollment and assignment to a randomized treatment.

5. Systemic anti-VEGF or pro-VEGF treatment within 4 months prior to enrollment and assignment to a randomized treatment.

6. History of vitreoretinal surgery.

7. History of major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 4 months or anticipated within the next 6 months following randomization.

8. History of DME or DR treatment with laser or intraocular injections of medication.

a. Exception: participants with a history of treatment for DME or DR that received laser or intraocular injections of medication GREATER than 12 months from screening AND have received 4 or fewer prior injections may be enrolled up to a maximum of 20 participants (i.e., approximately 25% of planned sample size).

9. Medical history or condition that, in the opinion of the investigator would preclude participation in the study, including but not limited to the following:

a. Myocardial infarction or stroke within 12 months of Screening.

b. Significant cardiac arrhythmia.

c. Active bleeding disorder.

d. Major surgery within 1 month of Screening or planned within the study period.
e. Current, active liver disease: > 3-fold elevation of liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST] over upper limit of normal [ULN]).

f. Uncontrolled high blood pressure (systolic blood pressure of 180 mm Hg or higher and/or diastolic blood pressure of 110 mm Hg or higher) despite adequate treatment during the 3 months prior to dosing.

g. Positive test result for hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV) at Screening.

h. Unstable glycemic control.

i. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to randomization or plans to do so in the next 4 months.

j. Known allergy to fluorescein dye.

10. [redacted]

[redacted]

12. [redacted]

13. [redacted]

14. Renal function as defined by eGFR < 45 mL/min/1.73 m^2, using the Modification of Diet in Renal Disease (MDRD) study equation (see Section 17.2), at Screening.

15. [redacted]

[redacted]

17. Clinically relevant abnormal laboratory value at Screening, including hematology, blood chemistry, or urinalysis (laboratory testing may be repeated once during the Screening phase).

18. Significant alcohol or drug abuse within the past 2 years.

19. Based on 12-lead ECG reading at Screening, participants with a risk of QT prolongation including:

    a. A baseline prolongation of QTc (using Fridericia's formula: ≥ 450 ms in men and ≥ 470 ms in women) with confirmation on a repeat ECG.

    b. A history of additional risk factors for Torsades de pointes arrhythmia (e.g., heart failure, hypokalemia, family history of Long QT Syndrome).

    c. The use of concomitant medications known to prolong the QT/QTc interval.

20. Significant medical conditions (as determined by medical history, examination, and clinical investigations at Screening) that may, in the opinion of the investigator, result in the any of the following:

    a. Put the participant at risk because of participation in the study.

    b. Influence the results of the study.

c. Cause concern regarding the participant's ability to participate in the study.

d. Inclusion of vulnerable persons by local regulation (e.g., imprisoned or institutionalized).

21. Malignancy for which the participant has undergone resection, radiation, or chemotherapy within the past 5 years (treated basal cell carcinoma or fully cured squamous cell carcinoma are allowed).

22. Concurrent participation in another interventional clinical trial; prior clinical trial participants must have been off study agents for at least 30 days for small molecules, 4 months for disease modifying therapies, and 1 year for vaccine or immunotherapy trials prior to Screening.

23. Female participants must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative pregnancy test at Screening. WOCBP must agree to use highly effective contraception, which includes combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, or vasectomized partner (Clinical Trial Facilitation Group 2014) prior to study entry. A woman is considered of childbearing potential following menarche and until becoming postmenopausal (no menses for at least 2 years without an alternative cause). Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in the study, she should inform her treating physician immediately. Male participants must be willing to use a barrier method contraception.

24. History of chronic renal failure requiring dialysis or kidney transplant.

5.3. Initiation of Treatment for Proliferative Diabetic Retinopathy

Treatment for PDR must not be given until one of the following criteria has been met:

1. The eye has PDR with high-risk characteristics, defined as:

   a. Neovascularization of the disc (NVD) greater than Standard photograph 10A (1/4 to 1/3 disc area), or

   b. Any NVD with pre-retinal or vitreous hemorrhage, or

   c. Neovascularization elsewhere (NVE) greater than 1/2 disc area with pre-retinal or vitreous hemorrhage.

2. The eye has vitreous hemorrhage requiring treatment that is presumed to be from PDR (either NV identified on FA or unable to assess NV due to the density of the hemorrhage, but there is no other attributable cause).

3. NV of the iris (at least 2 cumulative clock hours), definitive NV of the angle, or neovascular glaucoma development on clinical exam (photographic documentation not required)

4. The central reading center has confirmed NV is present within the 7-modified fields and approval from Sponsor medical monitor has been received to initiate treatment prior to high-risk characteristics being present.

Note: Treatment for NVE outside of the 7-modified fields without the presence of pre-retinal or vitreous hemorrhage is discouraged.

5.4. Initiation of Treatment for Center-involved Diabetic Macular Edema

Treatment for CI-DME must not be given until all the following criteria have been met:

1. At least a 10% increase in OCT central subfield thickness from baseline as confirmed by the central reading center.

2. Confirmed visual acuity loss presumed to be from DME, which is defined as either

    a. at least a 10-letter loss from baseline at a single visit, OR

    b. 5- to 9-letter losses at 2 consecutive visits that are at least 21 days apart.

5.5. Strategies for Recruitment and Retention

The Sponsor does not anticipate any specific challenges in meeting recruitment goals of enrolling and retaining a total of approximately 80 participants in this study. Participants will be recruited continuously until the planned sample size is achieved. Participants who withdraw or are withdrawn during Screening, as well as participants who discontinue, may be replaced (see Section 5.6.2).

The anticipated length of participation in the study of approximately 32 weeks is not expected to be challenging to participants. Financial support for meals and miscellaneous expenses will be available during the study, as appropriate and based on local regulations and guidelines. Use of visit transport services may also be incorporated into the trial to support the participant in maintaining study visit compliance. A description of the study will be included in local clinical trial databases, as required.

5.6. Participant Withdrawal

5.6.1. Reasons for Withdrawal

A participant may be withdrawn from study treatment for the following medical or administrative reasons:

- Occurrence of an AE that represents an unacceptable risk to the participant and when continued participation in the investigational study is not warranted, in the judgment of the

investigator, Sponsor, or medical monitor. The investigator must follow the participant until the AE resolves or is stable unless the participant is lost to follow up.

- Discontinuation of the investigational product for liver enzyme abnormalities (confirmed upon repeat testing) is required if:
  - ALT or AST ≥ 8× ULN;
  - ALT or AST ≥ 5× ULN for more than 2 weeks;
  - ALT or AST ≥ 3× ULN and (total bilirubin > 2× ULN or international normalized ratio (INR) > 1.5);
  - ALT or AST ≥ 3× ULN with new symptoms of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, dark urine, or new clinical signs of fever, rash, jaundice, eosinophilia (> 5%), or encephalopathy.

- Treatment with a prohibited concomitant medication other than the use of appropriate medications for the treatment of AEs under direction of the investigator.
- Participant noncompliance, defined as refusal or inability to adhere to the trial schedule or procedures.
- At the request of the participant (e.g., participant withdraws consent), investigator, Sponsor, or regulatory authority.
- Pregnancy.

5.6.2. Handling of Participant Withdrawals

Participants will be encouraged to complete the study and all assessments. Participants may voluntarily withdraw at any time, and the investigator may discontinue individual participants from the study at any time.

Approximately 80 participants are planned for enrollment to provide data for 69 completed participants (i.e., participants who complete the 24-week assessments). Participants who withdraw or are withdrawn may be replaced; enrollment will continue to ensure that data from 69 completed participants are available. Participants who withdraw or are withdrawn due to AEs or adverse reactions based on study procedures will not be replaced.

Participants who have received at least 1 tablet of study drug but are withdrawn or withdraw from the study will be encouraged to complete the end-of-treatment (EOT) procedures. The primary reason for study discontinuation will be documented on the electronic case report form (eCRF).

5.7. Premature Termination or Suspension of Study

The Sponsor reserves the right to terminate the study at any time. Should this be necessary, the Sponsor and/or their representatives will arrange discontinuation procedures and notify the appropriate regulatory authority(ies), IRBs, and IECs. In terminating the study, the Sponsor and the investigator will continue to protect the participant's privacy and identity as required by relevant statues and regulations.

Alkahest, Inc. has the right to terminate a study site from participating in the study at any time. Reasons for study or site termination may include, but are not limited to:

- Immediate risk to participant safety.
- Unsatisfactory participant enrollment.
- Unacceptable protocol deviations/violations as assessed by the medical monitor.
- Inaccurate or incomplete data entry and recording/fabricated data.
- Investigational site non-compliance with ICH GCP.
- Unacceptable emergent safety profile.

6. STUDY AGENTS

6.1. Study Agent and Control Description

6.1.1. Acquisition

The study agent (AKST4290) and placebo will be manufactured, labeled, packaged, and distributed by Alkahest, Inc.

6.1.2. Formulation, Appearance, Packaging, and Labeling

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. The study agent (AKST4290) and matching placebo will be delivered to the site and labeled for investigational use only according to the relevant regulatory requirements for clinical studies.

AKST4290 shows acceptable aqueous solubility and a low intrinsic permeability. Formulation development focused on protection from water due to the high hygroscopicity and sensitivity toward hydrolytic cleavage.

6.1.3. Product Storage and Stability

The study agent and matching placebo will be kept in its original packaging in a secure limited access storage area at 15° C to 30° C. A temperature log must be maintained to make certain the study agent is stored at the correct temperature. If the storage conditions are found to be outside the specified range, the site must immediately notify the sponsor or designee.

6.1.4. Dosing and Administration

Study drug will be self-administered orally b.i.d., approximately 12 hours apart. AKST4290 will be administered as a TDD 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.

6.2. Study Agent Accountability

The investigator and/or pharmacist will receive AKST4290 and placebo delivered by the Sponsor when the following requirements are fulfilled:

- Approval of the study protocol and informed consent by the IRB or IEC.
- Availability of a signed and dated clinical trial contract between the Sponsor and the investigational site.
- Approval/notification of the appropriate regulatory authority.
- Availability of the curriculum vitae of the principal investigator.
- Availability of a signed and dated clinical trial protocol or immediately imminent signing of the clinical trial protocol.

The investigator and/or pharmacist must maintain records of the delivery of the AKST4290 and placebo to the trial site, the inventory at the site, the use by each participant, and the return to the Sponsor or alternative disposition of unused AKST4290 and/or placebo.

These records will include dates, quantities, batch/serial numbers, expiry ('use by') dates, and the unique code numbers assigned to AKST4290, placebo, and study participants. The investigator/pharmacist will maintain records that document adequately that the participants were provided the doses specified by the protocol and reconcile all AKST4290 and placebo received from the Sponsor. At the time of final AKST4290 and placebo reconciliation, the investigator/pharmacist must verify that all unused or partially used portion of AKST4290 and/or placebo have been returned by the clinical trial participants and that no remaining AKST4290 and/or placebo are retained by the investigator.

Accountability records must be maintained and readily available for monitoring and auditing purposes by representatives of Alkahest, Inc., or their designee and are open to inspection by regulatory authorities at any time. The accounts of any AKST4290 and/or placebo accidentally wasted or intentionally disposed of must be maintained.

The disposal of used, partially used, or wasted AKST4290 and/or placebo must be performed in accordance with the institution's drug disposal policy or return. At study initiation, the clinical study monitor will evaluate the site's standard operating procedure for study drug disposal/destruction to ensure it complies with study requirements. At the end of the study, following final AKST4290 and placebo reconciliation by the monitor, the study site will be instructed by the sponsor to return or destroy all unused AKST4290 and placebo. A copy of the institution's drug disposal policy should be maintained or referenced in the investigator site file (ISF), if applicable.

7. STUDY PROCEDURES AND SCHEDULE

7.1. Study Procedures and Evaluations

7.1.1. Study Specific Procedures

Before any study-related procedures are performed, which includes medication washouts and restrictions, all participants must sign an informed consent that is consistent with ICH-GCP guidelines. For those participants electing to participate in the optional pharmacogenomics and/or optional aqueous humor portions of the study, separate informed consents must be obtained for deoxyribonucleic acid (DNA) banking and aqueous humor collection.

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.

7.1.1.1. Screening Procedures

During Screening, the following will be performed:

- All ophthalmological evaluations:
    - BCVA, as assessed by ETDRS (see Section 7.1.1.3.2 for a description of the procedures).
    - OCT (SD-OCT and/or OCT-A [if available at the site]) (see Section 7.1.1.3.3 for a description of the procedures).
    - Extended ophthalmoscopy (see Section 7.1.1.3.4 for a description of the procedures).
    - Slit lamp examination (see Section 7.1.1.3.4 for a description of the procedures).
    - IOP (see Section 7.1.1.3.4 for a description of the procedures).
    - Gonioscopy (see Section 7.1.1.3.4 for a description of the procedures).
    - FP evaluation and FA (see Section 7.1.1.3.5 for a description of the procedures).
- Medical history (see Section 7.1.1.1.1 for a description of the procedures).
- Demographics (see Section 7.1.1.1.2 for a description of the procedures).
- Review of medications (see Section 7.1.1.2.2 for a description of the procedures).
- Vital signs (see Section 7.1.1.2.3 for a description of the procedures).
- 12-lead ECG (see Section 7.1.1.2.4 for a description of the procedures).

- Physical examination (full) (see Section 7.1.1.2.5 for a description of the procedures).
- Blood and urine sample collection for laboratory evaluations, including pregnancy testing in WOCBP (see Section 7.1.1.2.6 for a description of the procedures).

Detailed descriptions of each of these procedures are provided in the sections immediately following. The Schedule of Events is presented in Section 15.

7.1.1.1.1. Medical History

The investigator or designee will obtain a detailed medical history by interviewing the participant during Screening. The medical history should focus on recent history, with an emphasis on the history of DR and any associated ocular procedures and/or medications. Additionally, the medical history should include:

- Current/past illnesses and conditions.
- Current symptoms of any active medical condition.
- Surgeries and procedures.
- Allergies.
- Social history (e.g., smoking, alcohol, illegal substances).

7.1.1.1.2. Demographics

Demographic information such as the participant's age, ethnicity, and race will be collected by interview with the participant at Screening.

7.1.1.2. Procedures to Assess Safety

Participants enrolled in the study will be monitored closely to assess safety and tolerability of the AKST4290 and placebo. Study-specific procedures that will be used for this purpose are summarized below. Information regarding the timing and frequency of these procedures is provided in the Schedule of Events (Section 15).

- Review of AEs (see Section 7.1.1.2.1 for a description of the procedures).
- Review of medications (see Section 7.1.1.2.2 for a description of the procedures).
- Vital signs (see Section 7.1.1.2.3 for a description of the procedures).
- 12-lead ECG (see Section 7.1.1.2.4 for a description of the procedures).
- Physical examination (targeted) (see Section 7.1.1.2.5 for a description of the procedures).

- Blood and urine sample collection for laboratory evaluations, including pregnancy testing in WOCBP (see Section 7.1.1.2.6 for a description of the procedures).

- All ophthalmological evaluations:

    - BCVA, as assessed by ETDRS (see Section 7.1.1.3.2 for a description of the procedure).

    - OCT (SD-OCT and/or OCT-A [if available at the site]) (see Section 7.1.1.3.3 for a description of the procedures).

    - Extended ophthalmoscopy (see Section 7.1.1.3.4 for a description of the procedure).

    - Slit-lamp examination (see Section 7.1.1.3.4 for a description of the procedure).

    - IOP (see Section 7.1.1.3.4 for a description of the procedure).

    - Gonioscopy (see Section 7.1.1.3.4 for a description of the procedure).

    - FP and FA (see Section 7.1.1.3.5 for a description of the procedures).

    - VF measured by perimetry/microperimetry (if available at the site (see Section 7.1.1.3.6 for a description of the procedures).

7.1.1.2.1. Review of Adverse Events

Adverse events will be reviewed, documented, and reported as required at each visit, beginning at Screening. For definitions, guidance, and additional information regarding AEs, refer to Section 8.

7.1.1.2.2. Review of Medications

At Screening, the investigator or designee should obtain a complete list of the participant's current medications, including over-the-counter drugs, herbal supplements and/or vitamins, as well as those taken by the participant in the past 3 months. Assessment of eligibility should include a review of permitted and prohibited medications. Throughout the study, any additions, discontinuations, or dosage changes in medication will be recorded. Additionally, any treatments or therapies administered for the treatment of PDR and/or CI-DME will be recorded.

7.1.1.2.3. Vital Signs

Vital sign measurements will include seated systolic and diastolic blood pressure (mm Hg), heart rate (beats per minute [bpm]), respiration rate (breaths per minute), and body temperature. Vital signs will be measured after the participant has been seated for 5 minutes.

7.1.1.2.4. 12-lead Electrocardiogram

Twelve-lead 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. In some cases, it may be appropriate to repeat abnormal ECGs to rule out technical factors contributing to ECG artifacts or abnormality. It is important that leads are placed in the same positions each time for consistency. 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. Corrected QTc will be calculated using Fridericia's correction formula (QTcF).

7.1.1.2.5. Physical Examination

At Screening, a full physical examination will be performed to assess the following organ systems: skin, ENT (ears, nose, and throat), 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 and measurement of weight as well as review of any previous abnormalities identified during the full physical examination.

7.1.1.2.6. Blood and Urine Collection for Laboratory Evaluations

Blood and urine samples will be collected according to the Schedule of Events (Section 15). Laboratory tests will include hematology, chemistry, serology, coagulation, and qualitative urinalysis, and pregnancy testing in WOCBP.

- Hematology: hemoglobin, hematocrit, red blood cell count, white blood cell count with differential [redacted] and platelets.
- Chemistry: HbA1c, glucose, sodium, potassium, calcium, inorganic phosphate, chloride, bicarbonate, magnesium, creatinine, AST, ALT, alkaline phosphatase (ALP), lactate dehydrogenase, direct and indirect bilirubin, blood urea nitrogen (BUN), total protein, albumin, and eGFR.
- Serology: HBV, HCV, and HIV.
- Coagulation: partial thromboplastin time (PTT), and prothrombin time (PT).
- Urinalysis: pH, glucose, erythrocytes, leukocytes, protein, nitrites, and 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 as detailed in the laboratory manual. Glomerular filtration rate (GFR) will be estimated by the MDRD Formula utilizing serum creatinine (see Section 17.2).

All safety laboratory measurements will be performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. Investigators will receive guidance and instructions on laboratory sampling and processing through a separate laboratory manual provided by the central laboratory.

The investigator is responsible for reviewing and documenting all laboratory results and determining if out-of-range laboratory values are clinically significant. All clinically significant values will be recorded as AEs in the eCRF and followed until resolution. Once resolved, the appropriate eCRF page(s) will be updated.

Samples may be used for re-testing, further evaluation of an AE and/or assessment, and follow-up of other exploratory endpoints. Samples that remain after study testing is complete will be stored in the event additional testing (e.g., further evaluation of an AE or assessment of effect) is required. Samples will be stored in a deidentified coded form. Participants can opt out of storage of samples for future analysis.

7.1.1.3. Procedures to Assess Efficacy

7.1.1.3.1. Diabetic Retinopathy Severity Scale

Progression of DR can be measured in discrete steps as described by the DRSS (ETDRS 1991b, Staurenghi 2018). This scale is well established for objective quantification of retinopathy severity and a validated method for quantification of DR change. When the scale is used for assessing change in overall retinopathy severity, classifying patients by retinopathy level in each eye (rather than by the worse eye, or by the right or left eye alone) retains prognostic information that would otherwise be lost (ETDRS 1991b).

The DRSS divides DR into 13 levels ranging from the absence of retinopathy to severe retinopathy (see Table 1). The DRSS can be used to describe overall DR severity as well as the change in severity over time (ETDRS 1991b). Further, DRSS scores are associated with the risk for the development of PDR (ETDRS 1991b, Staurenghi 2018). Findings from FP and FA, as provided by the central reading center, will be used to determine the DRSS score for each participant.

Table 1 Diabetic Retinopathy Severity Scale (for Individual Eyes)

Level	Severity	Definition
10	DR absent	Microaneurysms and other characteristics absent
20	Microaneurysms only	Microaneurysms definite; other characteristics absent
35	Mild NPDR	One or more of the following: • Venous loops ≥ D/1 • SE, IRMA, or VB = Q • Retinal hemorrhages present • HE ≥ D/1 • SE ≥ D/1
43	Moderate NPDR	H/Ma = 4-5 – S/1 or IRMA = D/1-3 (not both)
47	Moderately severe NPDR	Both L43 characteristics and/or 1 (only) of the following: • IRMA = D4-5 • H/Ma = S/2-3 • VB = D1
53	Severe NPDR	One or more of the following: • ≥ 2 of the 3 L47 characteristics • H/Ma ≥ S/4-5 • IRMA ≥ M/1 • VB ≥ D/2-3
61	Mild PDR	FPD or FPE present with NVD and NVE absent; or NVE = D
65	Moderate PDR	Either of the following: • NVE ≥ M/1 or NVD = D and VH or PRH = A or Q • VH or PRH = D and NVE < M/1 and NVD absent
71	High-risk PDR	Any of the following: • VH or PRH ≥ M/1 • NVE ≥ M/1 and VH or PRH ≥ D/1 • NVD = 2 and VH or PRH ≥ D/1 • NVD ≥ M
75	High-risk PDR	NVD ≥ M and VH or PRH ≥ D/1
81	Advanced PDR: fundus partially obscured, center of macula attached	NVD = cannot grade, or NVD < D and NVE = cannot grade in ≥ 1 field and absent in all others; and retinal detachment at center of macula < D
85	Advanced PDR: posterior fundus obscured, or center of macula detached	VH = VS in fields 1 and 2; or retinal detachment at center of macula = D
90	Cannot grade, even sufficiently for level 81 or 85	

Abbreviations: A = absent; D = definitely present; DR = diabetic retinopathy; DRSS = Diabetic Retinopathy Severity Scale; FPD = fibrous proliferations disc; FPE = fibrous proliferations elsewhere; HE = hard exudates; H/Ma = hemorrhages/microaneurysms; IRMA = intraretinal microvascular abnormalities; M = moderate; NPDR = nonproliferative diabetic retinopathy; NVD = neovascularization of the disc (within one disc diameter of disc margin); NVE = neovascularization elsewhere (>1 disc diameter from disc); PDR = proliferative diabetic retinopathy; PRH = preretinal hemorrhage; Q = questionable; S = severe; SE = soft exudates; VB = venous beading; VH = vitreous hemorrhage; VS = very severe.
Severity categories for characteristics graded in multiple fields are of the form ‘maximum severity/extent’, where maximum severity can be (A), (Q), (D), (M), (S), or (VS), and the extent is the number of fields at that severity level. For example, M/2–3 means that there are 2 or 3 fields from fields 3 to 7 with moderate severity, and none with higher severity.
Source: ETDRS 1991b, Staurenghi 2018

7.1.1.3.2. Best-Corrected Visual Acuity using the Early Treatment Diabetic Retinopathy Study Method

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. A detailed manual for performing refractions and measuring BCVA using the ETDRS testing method will be provided to investigators.

7.1.1.3.3. Optical Coherence Tomography

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/end of study [EOS]). Both eyes will be investigated by a trained technician using only specified SD-OCT equipment. A detailed manual for SD-OCT and OCT-A image acquisition and data transmission will be provided in the ISF. The reported central subfield thickness (CST) will be exclusive of the sub-RPE fluid.

7.1.1.3.4. Slit Lamp Examination, Extended Ophthalmoscopy, Intraocular Pressure Measurement, and 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.

7.1.1.3.5. Fundus Photography and Fluorescein Angiography

After pupillary dilation, the retina of both eyes will be imaged by FP and FA. Both eyes will be investigated by a trained technician and evaluated by the investigator. A detailed manual for FP and FA will be provided in the ISF.

7.1.1.3.6. Visual Field Testing

Evaluation of VF will be conducted using perimetry and/or microperimetry, per site availability. Evaluations of VF take approximately 10-20 minutes and are useful for assessment of some or all of the following (depending on the equipment used): peripheral vision, retinal sensitivities at specific retinal locations, and/or structural/functional analysis of the central retina.

7.1.1.3.7. Workplace Productivity and Activity Impairment Questionnaire

The WPAI-GH V2.0 is a 6-question survey used to assess the effects of a participant's health problems (i.e., physical or emotional problems or symptoms) on their ability to work and perform regular activities during the past 7 days (Section 17.5). In clinical trials, the WPAI-GH has been used to compare work impairments between treatment groups in participants with varying levels of disease severity (Zhang 2010).

7.1.1.3.8. Aqueous Humor Testing

In consenting participants, aqueous humor samples will be obtained as indicated in the Schedule of Events (Section 15) to evaluate potential biomarkers of inflammation, pathogenesis, and disease progression. Participation in aqueous humor sampling is optional.

Details of aqueous humor collection, sample processing and handling, and shipment instructions will be provided in the laboratory manual.

7.2. Laboratory Procedures and Evaluations

7.2.1. Clinical Laboratory Evaluations

Biological samples (e.g., whole blood, serum, urine) will be collected for laboratory evaluations, including pregnancy testing in WOCBP, in accordance with the Schedule of Events (Section 15). Refer to the study's laboratory manual for complete information regarding all laboratory evaluations to be performed, sample collection procedures, and related requirements.

The investigator is responsible for determining and documenting whether out-of-range laboratory values are clinically significant. All clinically significant values will be recorded as AEs in the eCRF and followed until determined to be stable or resolved unless the participant is lost to follow-up. Once resolved, the appropriate eCRF page(s) will be updated.

7.2.2. Other Tests or Procedures

7.2.2.1. Study Agent Concentration and Pharmacokinetics

Plasma concentration measurements of AKST4290 and its major metabolite will be collected to assess systemic exposure. For sampling time points and further details, please refer to Section 17.4.

For quantification of plasma concentrations of AKST4290 and its major metabolite, and for biomarker investigations (see Section 7.2.2.2), one blood sample of approximately 6 mL per sampling time point (see Section 17.4) will be taken from an antecubital or forearm vein into a potassium EDTA-anticoagulant blood drawing tube.

Details of plasma collection, sample processing and handling, and shipment instructions will be provided in the laboratory manual.

7.2.2.2. Plasma Biomarkers

Measurement of biomarkers is exploratory. Investigations might include mechanism related markers [redacted] that could identify subsets of participants who may benefit most from the treatment with AKST4290. [redacted] Biomarkers will be measured in plasma samples to investigate any change in response to treatment. After completion of the study, any remaining samples may be used for further methodological and/or other, non-genetic biomarker investigations either by the sponsor, or designee. The study samples will be discarded after completion of the additional investigations, but not later than 3 years after the final study report has been archived. The exploratory biomarker measurements will be conducted either at the sponsor's laboratories or at external contract research organizations (CROs) using appropriate methodology (e.g., immunoassays, multiplex technology).

Details of plasma collection, sample processing and handling, and shipment instructions will be provided in the laboratory manual.

7.2.2.3. Blood Biomarkers by Flow Cytometry

Characterization of immune cells using flow cytometry/CBC is exploratory. For the investigations, one blood sample of approximately 6 mL per sampling time point will be collected. Specimens must be transported for testing within a specified time period. The exploratory biomarker measurements using flow cytometry/CBC will be conducted either at the sponsor's laboratories or at external CRO's laboratory using appropriate methodology (e.g., immunoassays, multiplex technology). A Laboratory Manual/ISF will describe the handling of the samples.

7.2.2.4. Pharmacogenomic Evaluation

Pharmacogenetic analysis of prespecified genes is mandatory and a prerequisite for participation in this study. Furthermore, samples for DNA banking will be collected if participants signed a separate informed consent, but is not required for study participation.

- Prespecified genes: DNA will be extracted from 1 blood sample and genotyped for common genetic variants of the following genes: [redacted]
  - [redacted]
    - Prespecified analyses will be performed at the end of the trial and the data will be part of the report. All remaining samples will be destroyed after the end of the trial.

- DNA banking: One blood sample will be collected for retrospective, exploratory genotyping (e.g., to analyze disease or treatment-related gene variants). For information regarding future use of stored samples, see Section 12.5.

One 3-mL blood sample for prespecified pharmacogenomic testing will be obtained at Visit 2 in a potassium EDTA-anticoagulant blood drawing tube. For participants who agreed to DNA banking, one 8.5-mL blood sample will be taken at Visit 2 in a PAXgene Blood DNA sampling tube (see Section 17.4). The sample will be taken from an antecubital or forearm vein into a potassium EDTA-anticoagulant blood drawing tube.

Details of plasma collection, sample processing and handling, and shipment instructions will be provided in the laboratory manual.

7.3. Study Schedule

7.3.1. Screening

The Screening visit will occur between 2 to 28 days (Days -28 to -2) before the Baseline visit on Day 1. The assessments to be performed at Screening are detailed in the Schedule of Events in Section 15.

7.3.2. Treatment

The 24-week Treatment Period comprises Visit 2 (Baseline/Day 1) through Visit 8 (EOT/ET/Day 168). The assessments to be performed at each visit during the Treatment Period are detailed in the Schedule of Events in Section 15.

7.3.3. Early Termination or Withdrawal

In cases of early termination (ET) or withdrawal, if a participant has received at least 1 dose of AKST4290 or placebo, the site should try to perform all assessments scheduled for Visit 8 (EOT/Day 168) and for the subsequent Follow-up Visit (Visit 9 [EOS]) unless the participant has withdrawn consent (see Section 5.5).

7.3.4. Study Completion and End of Study

Study completion or EOS is defined as the end of participation for each enrolled participant. This will occur at Visit 9 unless a participant withdraws or is withdrawn (see Section 5.5 and Section 7.3.3, respectively). If a participant has withdrawn consent, study completion will be at the time of consent withdrawal and no further procedures will be performed. The EOS will occur following the last participant's last visit.

7.3.5. Schedule of Events Table

The Schedule of Events is presented in Section 15.

7.4. Concomitant Medications

All prescription, over-the-counter, and non-prescription medications (including herbal therapies and supplements) must be documented in the source documents and eCRFs. All participants should be maintained on the same medications at the same dosage and administration throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. Any changes in medications (additions, deletions, dosage changes) should be documented in the eCRF with reason for change (e.g., AEs).

7.5. Prohibited Medications, Treatments, and Procedures

Treatment with anti-VEGF and intraocular or periocular steroid injection is prohibited before the

8. ASSESSMENT OF SAFETY

Assessment of safety will be conducted by masked study personnel except in extraordinary circumstances where knowledge of whether AKST4290 or placebo was received by a participant is essential. Any instances of unmasking will be managed as indicated in Section 10.6.3.

8.1. Specification of Safety Parameters

8.1.1. Definition of Adverse Events

Per 21 Code of Federal Regulations (CFR) 312.32(a) 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.

An AE does include any:

- Exacerbation of a pre-existing illness.
- Subjective or objective symptoms spontaneously offered by the participant and/or observed by the investigator or study staff.
- Increase in frequency or intensity of a pre-existing episodic event or condition.
- Condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study (unless it can be demonstrated by medical record review that the onset of the event preceded the date/time of informed consent).
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Symptoms associated with disease not previously reported by the participant.
- Untoward medical occurrences considered by the investigator to be related to study-mandated procedures.
- Abnormal assessments (e.g., change on physical examination, ECG findings), if they represent a clinically significant finding, that were not present at baseline or worsened during the course of the study.
- Laboratory test abnormalities, if they represent a clinically significant finding, symptomatic or not, which were not present at baseline or worsened during the course of the study.

An AE DOES NOT include a/an:

- Elective medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion).
- Pre-existing diseases or conditions present or detected at the start of the study that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for cosmetic elective surgery, social and/or convenience admissions).
- Overdose of either study drug or concurrent medication without any signs or symptoms.
- Pregnancy.

8.1.2. Definition of Serious Adverse Events

Note: if either the investigator or the Sponsor believes that the event is serious, the event must be considered serious and evaluated for expedited reporting.

Note: the terms “severe” and “serious” are not synonymous. Severity (or intensity) refers to the grade of an AE. “Serious” is a regulatory definition.

A SAE (experience) or reaction is an untoward medical occurrence that, at any dose, fulfills one or more of the following criteria:

a. Results in death (i.e., the AE actually causes or leads to death).

b. Is life-threatening.

   - An AE is considered “life-threatening” if, in the view of either the investigator or Sponsor, its occurrence places the patient or participant at immediate risk of death; it does not include AEs which, had it occurred in a more severe form, might have caused death.

c. Results in inpatient hospitalization or prolongation of existing hospitalization.

   - Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an AE; hospitalization for participating in this study is not considered an AE.
   - Complications that occur during hospitalization are AEs; if a complication prolongs hospitalization, the event is an SAE.
   - “Inpatient” hospitalization means the participant has been formally admitted to a hospital for medical reasons that may or may not be overnight; it does not include presentation at a casualty or emergency room unless the event meets the definition of an Important Medical Event (in the opinion of the Investigator or Sponsor).

d. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- The term 'disability' means a substantial disruption of a person's ability to conduct normal life functions; this definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, accidental trauma (i.e., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Results in a congenital anomaly in the offspring of a participant who received drug.

f. Results in an Important Medical Event. Important Medical Events are events that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the participant 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.

- Medical and scientific judgment should be used in deciding whether prompt reporting is appropriate in this situation.

8.2. Classification of An Adverse Event

8.2.1. Severity of Event

Each AE or suspected adverse reaction must be assessed for its seriousness and severity. Severity will be assessed by the investigator or designee using the following definitions:

SEVERITY	DEFINITION
MILD	Aware of sign or symptom, but easily tolerated
MODERATE	Discomfort enough to cause interference with usual activity
SEVERE	Incapacitating with inability to work or do usual activity

Outcome will be assessed using the following categories: recovered/resolved, not recovered/ not resolved, recovered/resolved with sequelae, fatal, or unknown.

The following guidelines are to be applied to facilitate classification of the severity of AEs related to liver enzyme abnormalities (with or without bilirubin elevations):

- Elevations in ALT and/or AST ≥ 3× ULN but < 5× ULN confirmed on repeat testing are considered **mild** in severity and should be recorded separately on the AE log.

- Elevations in ALT and/or AST ≥ 5× ULN confirmed on repeat testing are considered **at least moderate** in severity and should be recorded separately on the AE log.

- Elevations in ALT and/or AST ≥ 3× ULN and a total bilirubin elevation > 2× ULN meet the protocol definition for an AESI (Section 8.4.3) of DILI, are considered **severe**, and meet the criteria for classification as an SAE per protocol definitions.

NOTE: Any of these cases can be classified as an SAE if any of the criteria defining an SAE apply.

8.2.2. Relationship to Study Agent

Investigators are required to assess the causal relationship (i.e., whether there is reasonable possibility that the study drug caused the event) using the following definitions:

- Unrelated: another cause of the AE is more plausible; a temporal sequence cannot be established with the onset of the AE and administration of the study agent; or a causal relationship is considered biologically implausible.

- Possibly Related: There is a clinically plausible time sequence between onset of the AE and administration of the study agent, but the AE could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the study agent is one or several biologically plausible AE causes.

- Definitely Related: The AE is clearly related to use of the study agent.

If either the investigator or the Sponsor considers the event related, then the event will be considered related for reporting purposes.

8.2.3. Expectedness

The Sponsor or designee will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the Reference Safety Information (RSI) described in the Investigator's Brochure (IB).

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents. "Unexpected" as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the angiotensin-converting enzyme inhibitor class and angioedema would be described in the IB as a class effect, the first case of angioedema observed with the drug under investigation should be considered unexpected for reporting purposes (FDA 2012).

This definition of "unexpected" relies entirely on the RSI in the IB as the basis for determining if newly acquired information generated from clinical trials or reported from other sources is unexpected. The suspected adverse reactions listed in the IB (i.e., "expected") are those observed with the investigational drug and for which a causal relationship between the event and the drug is suspected or confirmed.

Sponsor assessment of expectedness and relationship to study drug/causality will determine the need for expedited reporting of AEs.

8.3. Time Period/Frequency for Event Assessment/Follow-up

At every clinic visit, participants who have given informed consent will be assessed for AEs and SAEs. After the participant has had an opportunity to spontaneously mention any problems, the investigator should inquire about AEs by asking a non-leading question such as the following:

1. "How are you feeling?"
2. "Have you had any changes since your last assessment/visit?"
3. "Have you taken any new medicines since your last assessment/visit?"

8.3.1. Post-Study Adverse Event and Serious Adverse Event

The investigator is not obligated to actively seek SAE information in former study participants, but the investigator is encouraged to notify Alkahest, Inc. or their designee of any AE or SAE occurring within 30 days after a participant completes the study (or has their last visit) that the investigator judges may be reasonably related to study treatment or study participation.

8.4. Reporting Procedures

8.4.1. Adverse Event Reporting

All participants who have given informed consent will be evaluated for AEs. All AEs that occur after the time of treatment with the study drug will be considered treatment-emergent AEs (TEAEs). Participants with TEAEs must be followed until the AE is resolved or is stable, unless the participant is lost to follow up.

Each AE or suspected adverse reaction must be described as follows: the date of onset, date of resolution, severity (mild, moderate, severe), frequency of the event (single episode, intermittent, continuous), action taken with study treatment (no action taken, treatment held, treatment discontinued), outcome, causality* (unrelated, possibly related, definitely related), and seriousness criteria. Each AE or suspected adverse reaction must be recorded separately.

*Note: Causality assessment will be made only when the AE occurs after the participant has initiated at least 1 dose of the study agent. An AE occurring before the participant's exposure to study agent will always be labeled as "unrelated".

Any AE occurring during the study must be documented in the participant's medical records and as an AE in the eCRF. Any SAE occurring during the study must be documented in the participant's medical records and as an SAE in the eCRF.

A separate set of SAE pages should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same SAE page.

The investigator should attempt to establish a diagnosis of the event (that meets the definition of an AE or SAE) based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs or symptoms. The diagnosis will become the basis for the verbatim term as reported by the investigator. If no diagnosis is known and clinical signs and symptoms are not present, the abnormal finding should be recorded.

In addition to the investigator's own description of the AE, each AE will be encoded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The investigator will take all appropriate and necessary therapeutic measures required for resolution of the AE. Any medication necessary for the treatment of an AE must be recorded on the concomitant medication eCRF.

The SAE pages of the CRF should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the study CRO. It is very important that the investigator provide his/her assessment of causality to study drug as well as an applicable diagnosis at the time of the initial SAE report.

8.4.2. Serious Adverse Event Reporting

8.4.2.1. Timeframes for Reporting Serious Adverse Events

Under 21 CFR 312.32(c), the Sponsor is required to notify FDA, European Medicines Agency (EMA), and all participating investigators in a safety report of potentially serious risks from clinical trials (i.e., Suspected Unexpected Serious Adverse Reactions [SUSARS]), as soon as possible after the Sponsor receives the safety information and determines that the information qualifies for reporting:

- No later than 7 calendar days for events that are life threatening (in the opinion of the investigator or the Sponsor) or that involve death as an outcome.

- No later than 15 calendar days for all other SUSARS.

As such, prompt notification of the Sponsor, and/or the Sponsor's representatives, and promptly providing requested follow up information regarding SAEs is essential so that ethical and regulatory responsibilities and legal obligations can be satisfied. Investigators are responsible for reporting SAEs according to the following timeframes:

- All SAEs occurring during the study should be reported immediately.

- The SAE Report Form and relevant source documents, if applicable, must be completed and emailed to the drug safety representative within 24 hours of observation or learning of the event.
  NOTE: Contact information for the drug safety representative will be provided on the SAE forms and in the ISF.

- Follow up information must be sent to the CRO within 24 hours of receipt of information by the investigational site.

Serious adverse events will be followed until resolution, the condition stabilizes, the event is otherwise explained or is judged by the investigator to be no longer clinically significant, or until the participant is lost to follow up.

8.4.2.2. Serious Adverse Event Information to Report

All information available regarding an SAE must be submitted in the timeframes indicated. At a minimum, SAE reports must contain the participant identification (ID), the SAE verbatim term, onset date, relationship to study drug/causality, and a brief narrative of the event. Please note that relationship to study drug/causality as well as the reported verbatim term are very important and should be included in the initial report as it may impact expedited regulatory reporting requirements for the event. The date of SAE discovery by the site staff should be documented in the source documents.

The investigator must record all relevant information regarding an AE/SAE in the applicable sections of the eCRF. It is not acceptable for the investigator to send photocopies of the participant's medical records in lieu of completion of the appropriate AE/SAE pages. However, there may be instances when copies of medical records for certain cases are requested by the CRO and/or the Sponsor. If medical records are submitted to the CRO then all participant personal identifiers must be completely and thoroughly redacted prior to submission.

A blank SAE Report Form and instructions for SAE reporting will be provided to the site and will be maintained in the investigator's study file. The SAE Report Form must be completed and emailed to the drug safety representative according to the timeframes specified in Section 8.4.2.1. The SAE Report Form should include copies of relevant source documents, if applicable. Reconciliation of any discrepancy noted during monitoring and amending the eCRF is required.

If new information about an SAE is received or corrections to data are needed, the investigator should complete a new SAE Report Form and check the "follow-up" box on the form. This follow-up SAE Report Form should be submitted within 24 hours of learning of the information, especially if the new information concerns seriousness, relatedness, or the event term of an AE.

Sites acting under their local IRB/IEC should submit all applicable events, unanticipated problems, and safety reports to the site's local IRB/IEC, if applicable. All safety reporting deviations should also be submitted to their local IRB/IEC, if applicable.

8.4.3. Adverse Events of Special Interest

[redacted]

AESIs occurring during the study should be reported within 48 hours of observation or learning of the event, unless the event is serious, in which case the event must be reported according to the timeframes specified in Section 8.4.2.1.

8.4.4. Reporting of Pregnancy

While pregnancy itself is not considered an AE, pregnancy occurring in a clinical study must be followed to collect information regarding the experiences of gestation and pregnancy with study agent exposure. The investigator must report any pregnancy that occurs in a female study participant or female partner of a male participant subsequent to first exposure to the study agent until the EOT visit, or 3 months following a participant's last dose in the event of early withdrawal. All pregnancies will be reported to the IRB/IEC, Sponsor, and CRO. In the event of a pregnancy, treatment will be discontinued, and the participant will undergo continued safety follow up through pregnancy outcome. The study blind can be broken for safety reasons if the information is required for the management of pregnancy. Any noted intentional or unintentional breaking of the blind should be reported to the Sponsor's study team lead and quality group (see Section 10.6.3).

Any pregnancy must be followed by the investigator until delivery or to the end of pregnancy. Any anomalies, complications, abnormal outcomes, or birth defect(s) observed in the child must be reported as an SAE within 24 hours of the investigator or study personnel's first knowledge.

8.5. Study Halting Rules

The Data Safety Monitoring Board (DSMB) will make recommendations concerning the continuation, modification, or termination of the study.

8.6. Safety Oversight

Safety oversight will be provided by the Sponsor's program physician or his or her designee and the CRO's medical monitor(s) in concert with the site investigators. Additionally, a DSMB will be established. Members of the DSMB will be independent from the study conduct and free of actual or perceived conflict of interest.

The DSMB will meet at least quarterly (or more frequently as needed) to assess safety data. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to Alkahest, Inc.

In addition, the Sponsor will perform ongoing monitoring of cumulative safety data in a systematic manner to ensure that any safety signals that may impact the overall benefit/risk ratio in this specific population will be detected, assessed, and any necessary action taken. Blinded cumulative safety data (e.g., AE listings, vital sign plots, safety laboratory values, ECGs, physical examination results) will be reviewed by the CRO's Medical Monitor(s) and/or by the Sponsor's Program Physician throughout the study. If either physician detects any safety trends of concern, an ad-hoc meeting of the DSMB may be triggered.

9. CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and wellbeing of human research participants are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the study CRO in accordance with the Clinical Monitoring Plan (CMP).

- A mix of onsite and centralized risk-based monitoring will be performed to ensure the safety of clinical research participants and the accuracy and completeness of study data.

- The Sponsor will be provided with copies of monitoring reports per the timelines specified within the CMP.

- Details of clinical site monitoring tasks and scope are documented in the study's CMP. The CMP describes in detail who will conduct monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

- Independent audits may be conducted by the Sponsor's Quality Assurance (QA) function or Sponsor contracted QA consultants in accordance with a study specific QA plan to ensure monitoring practices are performed consistently across all participating sites, that monitors are following the CMP and sites conduct the study according to the protocol, GCP, and applicable regulatory requirements.

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Design Model and Analytical Plans

A Statistical Analysis Plan (SAP) with analytical details and assumptions will be developed and finalized before database lock and unmasking of the study data.

10.2. Statistical Hypotheses

The primary efficacy endpoint is the proportion of participants with a ≥ 3-step improvement in the DRSS score from baseline at Week 24. The null hypothesis to be tested is that there is no difference between AKST4290 (A) and placebo (P) in the proportion of participants with a ≥ 3-step improvement at Week 24:

H0: $pA = pP$

The alternate hypothesis is that there is a difference in the treatment group proportions:

H1: $pA \neq pP$.

10.3. Analysis Datasets

The Intent-to-Treat (ITT) Population will include all participants allocated to a randomized treatment.

The Safety Population will include all participants who receive at least 1 dose of the study drug.

10.4. Description of Statistical Methods

10.4.1. General Approach

The ITT Population will be the primary population used for efficacy analyses. For the purposes of the efficacy analysis, the treatment assignment will be according to the randomization schedule.

The Safety Population will be the primary population used for the safety analyses. For the purposes of the safety analysis, the treatment assignment will be according to the treatment the participant actually received.

10.4.2. Analysis of the Primary Endpoint

The primary estimand is the difference between treatment groups in the proportion of participants with a ≥ 3-step improvement from Baseline on the DRSS score at Week 24, among participants in the ITT Population. Participants who have missing data at the Week 24 time point will be considered non-responders. The proportion of participants with improvement at Week 24 will be compared among treatment groups using a Cochran-Mantel-Haenszel chi-square test, stratified by the DRSS category at Baseline (moderately severe or severe).

10.4.3. Analysis of the Secondary Endpoints

Analysis of the secondary endpoints will be based on the ITT Population.

The proportion of participants with a ≥ 2-step improvement from Baseline on the DRSS score at Week 24, as well as the proportion of participants progressing to CI-DME, PDR, and/or requiring ASNV requiring treatment, will be analyzed using a similar method as described for the primary endpoint.

Time to the event of CI-DME, PDR, and/or ASNV requiring treatment will be summarized using Kaplan-Meier (KM) methodology. Participants who do not experience the event will be censored at the time of their last non-missing evaluation. AKST4290 will be compared to placebo using the stratified log-rank test, stratified by the DRSS category at Baseline (moderately severe or severe). Kaplan-Meier estimates of the survival distribution function over time will be plotted by treatment group.

Clinical changes in diabetic kidney disease related laboratory values (e.g., eGFR and UACR) will be analyzed by a mixed-effects model for repeated measures (MMRM). The model will include fixed effects for treatment group, categorical visit, treatment group by visit interaction, the DRSS category at Baseline (moderately severe or severe), and the relevant Baseline value as a covariate. An appropriate covariance structure will be selected prior to database lock and the Kenward-Roger approximation will be used to calculate the denominator degrees of freedom for the test of fixed effects.

Safety will be assessed through summaries of AEs, changes in laboratory test results, and changes in vital sign measurements. In addition, all SAEs, including deaths, will be listed and summarized separately.

10.4.4. Analysis of the Exploratory Endpoints

Changes in BCVA (gain or loss) using the ETDRS, macular volume, and CMT as assessed by SD-OCT, WPAI-GH questionnaire, and OCT-A over time will be analyzed using an MMRM model similar the one described above.

The proportion of participants with a ≥ 2-step or ≥ 3-step worsening of the DRSS score from Baseline will be analyzed using a similar method as described for the primary endpoint.

The AUC between Baseline and Week 24 in BCVA using the ETDRS will be analyzed by an analysis of covariance model with a main effect for treatment group, a classification factor for the DRSS category at Baseline (moderately severe or severe), and the Baseline BCVA value as a covariate.

Other exploratory endpoints will be summarized with descriptive statistics and/or counts and percentages of participants in each category of interest.

10.4.5. Planned Interim Analyses

No interim analysis is planned.

10.4.6. Multiple Comparison/Multiplicity

The analysis of the single primary endpoint to compare AKST4290 with placebo will be evaluated at the 2-sided 5% level of significance. Analysis of secondary endpoints will be adjusted for multiple endpoint or treatment group comparisons. Analysis of any exploratory endpoints will be exploratory in nature and not based on any formal hypothesis testing. Details of all analyses will be provided in the SAP.

10.5. 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 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%.

10.6. Measures to Minimize Bias

10.6.1. Enrollment/Randomization/Masking Procedures

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 to severe NPDR). 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.

10.6.2. Evaluation of the Success of Masking

The success of masking will be assessed based on all occurrences (intentional or unintentional) of unmasking of masked study participants or study personnel (e.g., investigators, medical providers, assessors, the Sponsor, or their representatives). All intentional and unintentional unmasking will be documented and reported.

10.6.3. Breaking the Study Mask and/or Participant Code

The study mask for either AKST4290 versus placebo can be broken for safety reasons if the information is required for the management of SAEs, severe 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. In the rare event the web-based system is unavailable, [redacted] is available 24 hours/day, 7 days/week at:

Email: [redacted]

Toll-free: [redacted]

Any noted intentional or unintentional breaking of the mask should be reported to the Sponsor's study team lead and quality group. If unintentional unblinding occurs during the study, root cause analysis will be evaluated, and corrective actions implemented.

11. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this study, in compliance with ICH E6 R2 and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of regulatory agencies, the IRB/IEC, the Sponsor, or the Sponsor's representatives to examine (and when permitted by applicable law, to copy) clinical records for the purposes of QA reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participant's memory aids or evaluation checklists, pharmacy dispensing records, recorded audio tapes of counseling sessions, recorded data from automated instruments, copies or transcriptions certified after verification as being attributable, legible, accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

It is not acceptable for the eCRF to be the only record of a participant's participation in the study. This is to ensure that anyone who would access the participant's medical record has adequate knowledge that the participant is participating in a clinical trial. Source document templates will be developed for this study.

12. ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1. Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, ICH E6 R2, 21 CFR Part 320, Retention of Bioavailability and Bioequivalence Testing Samples (1993), and the Declaration of Helsinki.

12.2. Institutional Review Board

This protocol and any accompanying material to be provided to the participant (such as advertisements, participant information sheets, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IRB or IEC. Approval from the IRB/IEC must be obtained before starting the study and should be documented in a letter to the investigator specifying the protocol number, protocol version, documents reviewed, and date on which the committee met and granted the approval.

All changes to the consent form will be IRB/IEC approved; a determination will be made regarding whether previously consented participants need to be re-consented.

Any modifications or amendments to the protocol must also be submitted to the IRB/IEC for approval prior to implementation.

12.3. Informed Consent Process

12.3.1. Consent Forms

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant or healthcare power of attorney or equivalent legal representative, and written documentation of informed consent is required prior to any study-related procedures.

12.3.2. Consent Procedures and Documentation

It is the responsibility of the investigator or designee to obtain written informed consent form(s) (ICF) each participant participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures.

Participants should have the opportunity to discuss the study with their family members or other advisors and the time to consider participation in the study carefully. The participants may withdraw consent at any time throughout the course of the study. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The investigator or designee must utilize an IRB/IEC approved consent form that contains the elements required by ICH GCP and applicable regulatory requirements for documenting written informed consent. Each ICF will be appropriately signed and dated by the participant and the

person obtaining consent. A copy of the signed ICF will be provided to the participant. By signing the ICF, all parties agree they will complete the evaluations required by the study, unless they withdraw voluntarily or are terminated from the study for any reason.

Investigators will be expected to maintain a screening log of all potential study candidates that includes limited information about the potential candidate (e.g., date of screening).

All participants who provide consent will be assigned a unique study number. This number will be used to identify the participant throughout the clinical study and must be used on all study documentation related to the study participant. Once a number is assigned to a participant, that number will remain with that study participant and will not be reused.

If an individual's medical chart or results of diagnostic tests performed as part of an individual's regular medical care are going to be used for Screening, written informed consent must be obtained prior to review of that information in accordance with Health Insurance Portability and Accountability Act.

12.4. Participant and Data Confidentiality

Participant confidentiality is held in strict trust by the participating investigators, their staff, the Sponsor, and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or data will be released to any unauthorized third party without prior written approval of the Sponsor.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB/IEC, or government regulatory agencies may inspect documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The investigator must assure that participants' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. An identification code (i.e., not names) should be recorded on non-local laboratory samples, requisitions, and any documents submitted to the CRO, Sponsor, and/or IRB/IEC. The investigator must keep a participant log showing codes, names, and addresses for all participants screened and for all participants enrolled in the study. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB/IEC and institutional regulations.

12.5. Future Use of Stored Specimens

With the participant's approval and as approved by local IRB/IECs, de-identified biological samples may be stored at Alkahest, or designee, for future use. These samples could be used for research and to improve treatment. Alkahest will also be provided with a code-link that will allow linking the biological specimens with the specific data from each participant, maintaining

the masking of the identity of the study participant. Participants may choose whether the Sponsor can store and use samples for further research.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research.

When the study is completed, access to study data and/or samples will be managed by Alkahest. In the event Alkahest transfers ownership to another commercial Sponsor, ownership of the samples may be transferred as well.

13. DATA HANDLING AND RECORD KEEPING

13.1. Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, and timeliness of the data reported.

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site. Data entered in the eCRFs that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. The investigator may need to request previous medical records or transfer records, depending on the trial; also, current medical records must be available.

For each participant who receives the study agent or placebo, the eCRF must be completed in a timely manner. The investigator will review and approve the eCRF for each study participant after all data have been entered, the eCRFs have been source document verified, and all queries have been resolved. This also applies to records for those participant who fail to complete the study. If a participant withdraws from the study, the reason must be noted on the eCRF. If a participant is withdrawn from the study because of an AE, thorough efforts should be made to clearly document the outcome.

All data collection and recordkeeping procedures must be compliant with applicable ICH GCP.

13.1.1. Investigator Responsibilities

The investigator will comply with the protocol (which has been approved/given favorable opinion by an IRB/IEC), ICH GCP, and applicable regulatory requirements. The investigator is ultimately responsible for the conduct of all aspects of the study at the study site and verifies by signature the integrity of all data transmitted to the Sponsor. The term "investigator" as used in this protocol as well as in other study documents, refers to the investigator or authorized study personnel that the investigator has designated to perform certain duties. Sub-investigators as listed on Form FDA 1572 or other authorized study personnel are eligible to sign for the investigator, except where the investigator's signature is specifically required.

13.1.2. Study Files

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 separate categories (although not limited to) the following: (1) investigator's study file, and (2) participant clinical source documents.

The investigator's study file will contain the clinical study protocol/amendments, IB, eCRF, IRB/IEC approval with correspondence, ICFs, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and study specific manuals (e.g., Laboratory Manual).

Participant clinical source documents would include (although are not limited to) the following: participant hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, radiologic imaging, X-ray, pathology and special assessment reports, consultant letters, screening, and enrollment log, etc.

13.2. Study Records Retention

All clinical study documents must be retained by the investigator until 2 years after the study is discontinued and regulatory authorities have been notified. Before the investigator destroys any material related to the clinical study, he/she must obtain approval in writing from the Sponsor.

The investigator should keep a file where the full name and address of the participant and all signed informed consents are included for at least 15 years after completion of the study. Any original study-related information that permits verification of inclusion and exclusion criteria, including clinical history, a copy of all data collection logs, and documents on the use of the study agent, must be stored for as long a time period as permitted by the center.

Should the investigator wish to move study records to another location, arrangements must be made to store these in sealed containers so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the participant, appropriate copies should be made for storage outside of the site.

13.3. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or with GCP. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. When deviations occur, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3.
- 5.1 Quality Assurance and Quality Control, section 5.1.1.
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Protocol deviations will be categorized as either Major or Minor and will be defined in the study specific Protocol Deviation Plan.

Major protocol deviations are departures from the approved protocol relating to the conduct of the study that may affect the rights, safety, and/or well-being of study participants or the study outcomes or data quality. Major protocol deviations may result in data that are not deemed

evaluable for the per-protocol analysis and/or may require that participants are discontinued from the study. Major protocol deviations are Significant Clinical Issues. Note: observations categorized as Major may include those situations where there is a pattern of deviation, numerous Minor observations, or other significant deviation.

Minor protocol deviations are departures from the approved protocol relating to the conduct of a study that does not affect the rights, safety, and/or well-being of study participants or the study outcomes or data quality. Minor protocol deviations would not generally preclude participant data from the per-protocol analysis population.

Note: persistently missed or incomplete study procedures and/or study evaluations will be considered Major protocol deviations.

Coronavirus Disease 2019 (COVID-19) protocol deviations are departures from the approved protocol related to the COVID-19 pandemic. Window extensions and missed protocol assessments may be permitted to reduce the risk of COVID-19 exposure. Any deviation to the protocol to reduce the risk of COVID-19 will be captured as a "Protocol Deviation related to COVID-19" to categorize the anticipated increase in protocol deviations due to the pandemic. In addition, protocol deviations that have been prospectively identified and that can be implemented to reduce the risk of exposure while still maintaining appropriate safety monitoring and integrity of the study data. These prospective deviations (e.g., window extensions) are described in the Schedule of Events (Section 15). These measures are temporary and will be repealed as soon as the situation (e.g., governmental rules, benefit/risk assessment for the trial) allows.

All deviations will be logged and tracked by the site and CRO. Periodic review of protocol deviations will serve as an indicator of site performance.

It is the responsibility of the site to use continuous vigilance to identify and report deviations promptly to the study CRO and/or Sponsor. All deviations must be addressed in study source documents. Notification of protocol deviations must be sent to the local IRB/IEC per their guidelines. The site investigator/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

13.4. Publication and Data Sharing Policy

In compliance with The International Committee of Medical Journal Editors clinical trials registration policy and Section 801 of the FDA Amendments Act of 2007, this study will be registered by the Sponsor in ClinicalTrials.gov, a public trials registry which is sponsored by the National Library of Medicine.

Notwithstanding the Sponsor's requirements for registration and data sharing in ClinicalTrials.gov, any formal presentation or publication of data collected as a direct or indirect result of this trial will be considered as a joint publication by the investigator(s) and the Sponsor. In the case of multicenter studies, it is mandatory that the first publication be made based on the totality of data obtained from all centers, analyzed as stipulated in the protocol, and presented and interpreted as documented in the final clinical study report (CSR). The resulting publication will name investigators according to the policy of the chosen journal. Where it is not permitted

for all investigators to be included as authors, the publication will name all investigators within the publication.

Individual investigators may publish data arising from their own participants. The investigator will provide the Sponsor with copies of written publications (including abstracts and posters) at least 60 days in advance of submission. This review is to permit the Sponsor to review the communication for accuracy (thus avoiding potential discrepancies with submissions to regulatory authorities), to verify that confidential information is not inadvertently divulged (including patent protection), to allow adequate input or supplementary information that may not have been available to the investigator, and to allow establishment of co-authorship.

Investigators participating in multicenter studies must agree not to engage in presentations based on data gathered individually or by a subgroup of centers before publication of the first main publication unless this has been agreed otherwise by all other investigators and the Sponsor. However, in the event that no publication of the overall results has been submitted after approval of the CSR, investigators may publish results of one or more center's participants to the same review as outlined above. The Sponsor will circulate proposed multicenter publications to all investigators for review.

Data will be reviewed by all participating investigators prior to publication. The study Sponsor will have 90 days to review all definitive publications, such as manuscripts and book chapters, and a minimum of 30 days to review all abstracts.

14. FINANCIAL DISCLOSURE AND CONFLICT OF INTEREST POLICY

A separate financial disclosure agreement will be made between each principal investigator and Alkahest, Inc. or its authorized representative before the study agent is shipped. Each investigator will notify Alkahest, Inc. or its authorized representative of any relevant changes during the conduct of the study and for 1 year after the study has been completed. Alkahest and the study CRO will evaluate any disclosed conflicts of interest and will establish a mechanism for their management.

15. SCHEDULE OF EVENTS

Table 2 Schedule of Assessments

	Screening	Treatment							Follow-up
Visit Number	**1**	**2 (Baseline)**	**3**	**4**	**5**	**6**	**7**	**8 (EOT/ET)[b]**	**9 (EOS)**
Week		**1**	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Day	**-28 to -2**	**1**	**28**	**56**	**84**	**112**	**140**	**168**	**196**
Time Window (days)[a]			**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**
Informed consent and optional consent for aqueous humor sample collection[c]	X								
Informed consent for DNA banking[d]	X								
Demographics	X								
Medical history	X								
Inclusion/exclusion criteria[e]	X	X							
Physical examination	X	X						X	
Vital signs (seated)	X	X	X	X	X	X	X	X	
Pregnancy test (in WOCBP)[f]	X	X	X	X	X	X	X	X	
Laboratory tests[g]	X	X	X	X	X	X	X	X	X
12-lead electrocardiogram	X							X	X
HbA1c		X			X			X	
Extended ophthalmoscopy	X	X	X	X	X	X	X	X	X
Slit lamp examination	X	X	X	X	X	X	X	X	X
Intraocular pressure	X	X	X	X	X	X	X	X	X
Gonioscopy	X	X		X		X		X	X
SD-OCT	X	X	X	X	X	X	X	X	X
OCT-A (if available at the site)[h]		X						X	
Visual acuity[i]	X	X	X	X	X	X	X	X	X
Fundus photography	X	X	X	X	X	X	X	X	X
Fluorescein angiography[j]	X				X			X	
VF with perimetry and/or microperimetry (if available at the site)[h]		X						X	
Workplace Productivity and Activity Impairment General Health questionnaire		X		X		X		X	
Administration of study agent[k]		X	X	X	X	X	X	X	
Dispense study agent		X	X	X	X	X	X		
Study agent accountability		X	X	X	X	X	X	X	

	Screening	Treatment							Follow-up
Visit Number	**1**	**2 (Baseline)**	**3**	**4**	**5**	**6**	**7**	**8 (EOT/ET)[b]**	**9 (EOS)**
Week		**1**	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Day	**-28 to -2**	**1**	**28**	**56**	**84**	**112**	**140**	**168**	**196**
Time Window (days)[a]			**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**
Optional aqueous humor collection[l]		X						X	
PK blood sample collection (for plasma extraction)[m]		X	X	X	X	X	X	X	X
Biomarker plasma aliquots[m]		X						X	X
Pharmacogenomics blood sample (and optional DNA banking sample)		X[d]							
Flow cytometry/CBC blood sample collection[m]		X						X	
Prior and concomitant medication review[n]	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X
Study completion									X

Abbreviations: ASNV = anterior-segment neovascularization; BCVA = best corrected visual acuity; CBC = complete blood count; CI-DME = center-involved diabetic macular edema; EOS = end of study; EOT = end of treatment; ET = early termination; ETDRS = Early Treatment Diabetic Retinopathy Study; HbA1c = hemoglobin A1c; OCT-A = optical coherence tomography angiography; PDR = proliferative diabetic retinopathy; PK = pharmacokinetic; SD-OCT= spectral domain optical coherence tomography; VF = visual field; WOCBP = women of childbearing potential

Notes:

a. Window extensions and missed protocol assessments may be permitted to reduce the risk of COVID-19 exposure. Any deviation to the protocol to reduce the risk of COVID-19 exposure will be captured as a "Protocol Deviation related to COVID-19" to categorize the anticipated increase in protocol deviations due to the pandemic. These measures are temporary and will be repealed as soon as the situation (e.g., governmental rules, benefit/risk assessment for the trial, etc.) allows (see Section 13.3).

b. Visit 8 (EOT/ET): all study related procedures will be conducted at the conclusion of treatment on Day 168±3. Withdrawal of consent is allowed at any time. For ET participants, the EOT visit procedures should be conducted at the time of ET, in place of the next scheduled treatment visit. For participants who withdraw consent from the study, no additional study procedures will be performed.

c. All participants must sign an informed consent consistent with ICH-GCP guidelines prior to any trial related procedures, which includes medication washouts and restrictions. An optional consent for aqueous humor testing will also be provided.

d. Informed consents must be obtained for optional DNA banking. DNA banking will be optionally collected at the time of enrollment (Visit 2 [Baseline]).

e. A preliminary check of inclusion/exclusion criteria will be performed at Visit 1 (Screening) after obtaining informed consents.

f. Pregnancy testing may be performed using serum or urine. Tests should be performed at the site and results reviewed. Positive pregnancy test results should be confirmed with a serum pregnancy test performed by the central laboratory.

g. Hematology, clinical chemistry, and urinalysis. Additionally, serology testing will be performed at Visit 1 (Screening) only.

h. For sites with the capability to perform OCT-A and VF, these assessments should be performed at Visit 2 (Baseline) and Visit 8 (EOT/ET).

i. Visual acuity assessments will include BCVA as assessed using the ETDRS method.

j. Fundus photography and fluorescein angiogram must be performed once a participant has been diagnosed with CI-DME or PDR, and before rescue treatment is given.

k. 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)

	Screening	Treatment						Follow-up
Visit Number	**1**	**2 (Baseline)**	**3**	**4**	**5**	**6**	**7**	**8 (EOT/ET)[b]**	**9 (EOS)**
Week		**1**	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Day	**-28 to -2**	**1**	**28**	**56**	**84**	**112**	**140**	**168**	**196**
Time Window (days)[a]			**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**

after any pre-dose assessments (including PK sample collection), and then self-administered at home between study visits. Training on study drug administration will be conducted prior to the initial study drug administration at Visit 2.

l. If a participant requires treatment for a vision-threatening complication, the aqueous humor collection will occur prior to treatment.

m. A PK blood sample will be collected and then centrifuged for collection of plasma samples. A blood sample for biomarkeranalysis will be collected and then centrifuged for collection of plasma samples. At Visits 2 and 4, 1 post-dose PK blood sample will be collected. At Visit 2 only, 1 post-dose flow cytometry/CBC sample will be collected. The PK, biomarker, pharmacogenomic, and flow cytometry/CBC sampling schedule is presented in Table 3.

n. While unlikely to happen, it is possible that AKST4290 may reduce the plasma concentrations [redacted] therefore, clinical and/or laboratory monitoring for loss of efficacy may be required for participants taking these medications (also see Section 17.3.2).

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16.2. Unpublished References

[redacted]

[redacted]

17. APPENDICES

17.1. Clinical Evaluation of Liver Injury

17.1.1. Introduction

Clinically significant alteration of liver laboratory parameters, as described in Sections 8.2.1 and 8.4.3 (protocol-specified AESI), are to be further evaluated and followed using the procedures described in the subsequent sections.

17.1.2. Procedures

17.1.2.1. Guidelines for Evaluation and Follow-up Testing of Mild Liver Enzyme Elevations

Guidelines for evaluation and follow-up testing of mild liver enzyme elevations (ALT or AST ≥ 3× ULN but < 5× ULN without bilirubin elevations) include:

- A review of symptoms, history, and physical examination to assess for signs of liver enzyme elevation etiology, and updates to the CRF should be made as appropriate (see Section 17.1.3).
- Repeat ALT, AST, bilirubin, and ALP testing, and perform initial gamma-glutamyl transferase (GGT) testing, *within 1 week* with the central laboratory to confirm the results.
    - In cases in which the central laboratory is not immediately available (e.g., if the logistics are such that the participant´s repeat specimen would not reach the central laboratory in a reasonable timeframe), ALT, AST, and bilirubin (total and direct) will be evaluated by the local laboratory and results will be made available to the investigator and to Alkahest as soon as possible.
- If elevations are confirmed on repeat testing, follow-up testing of ALT, AST, bilirubin, ALP, and GGT should be repeated at a minimum of every 2 weeks until ALT and AST values have decreased to < 2× ULN.
- Once values reach < 2× ULN, standard per protocol laboratory assessment is adequate.

17.1.2.2. Guidelines for Evaluation and Follow-up Testing of Moderate to Severe Liver Enzyme Elevations

Guidelines for evaluation and follow-up testing of moderate (ALT or AST ≥ 5× ULN without bilirubin elevations) to severe (ALT or AST ≥ 3× ULN with an elevation of total bilirubin > 2× ULN) liver enzyme elevations include:

- A review of symptoms, history, and physical examination findings to assess for signs of liver enzyme elevation etiology, and updates to the CRF should be made as appropriate (see Section 17.1.3).

- Repeat ALT, AST, bilirubin, and ALP testing, and perform initial GGT testing, within 48-72 hours with the central laboratory to confirm the results.
    - In cases in which the central laboratory is not immediately available (e.g., if the logistics are such that the participant´s repeat specimen would not reach the central laboratory in a reasonable timeframe), ALT, AST, and bilirubin (total and direct) will be evaluated by the local laboratory and results will be made available to the investigator and to Alkahest as soon as possible.
- If confirmed on repeat testing, close observation should be initiated, follow-up testing of ALT, AST, bilirubin, ALP, and GGT should be repeated weekly until resolved, and additional one-time tests should be obtained as described in Sections 17.1.2.3 through 17.1.2.8, in consultation with the Medical Monitor.
- Once values reach < 2× ULN, standard per protocol laboratory assessment is adequate.

17.1.2.3. Clinical Chemistry

- Obtain albumin, PT or INR, creatinine kinase, creatinine kinase MB test (CK-MB), ceruloplasmin, α-1 antitrypsin, transferrin, amylase, lipase, glucose, cholesterol, and triglycerides.

17.1.2.4. Serology

- Obtain hepatitis A (anti-immunoglobulin M [IgM], anti-IGM), hepatitis B (hepatitis B antigen, anti-HBs, DNA), hepatitis C (anti- HCV), ribonucleic acid (RNA) if anti-HCV positive), hepatitis E (anti-hepatitis E virus [HEV]), anti-HEV IgM, RNA (if anti-HEV IgM positive), anti-smooth muscle antibody (titer), anti-nuclear antibody (titer), anti-liver-kidney microsomes antibody, antimitochondrial antibody, Epstein Barr virus (viral capsid antigen IgM), cytomegalovirus (IgM), herpes simplex virus (IgM), varicella (IgM), parvovirus (IgM), toxoplasmosis (IgM), total serum (IgG).

17.1.2.5. Hormones

- Thyroid-stimulating hormone.

17.1.2.6. Hematology and Coagulation

- CBC with differential.

17.1.2.7. Ultrasound

- Perform an abdominal ultrasound to assess for cirrhosis, non-perfusion, biliary tract, pancreatic, or intrahepatic pathology (e.g., bile duct stones or neoplasm).

17.1.2.8. Consultation

- Consider hepatology or general gastrointestinal consultation.

17.1.3. Case Report Form Guidelines

For all liver enzyme abnormalities (mild, moderate, severe), the following should be reported in the CRF:

- Detailed history of current symptoms, concurrent diagnoses, and medical history according to the DILI Checklist provided in the ISF.
- History of concomitant drug use (including non-prescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets according to the DILI Checklist provided in the ISF.
- History of exposure to environmental chemical agents (consider home and workplace exposure) according to the DILI Checklist provided in the ISF.

17.2. Modification of Diet in Renal Disease Formula

When serum creatinine is in mg/dL (conventional units), the GFR may be estimated based on the following MDRD formula:

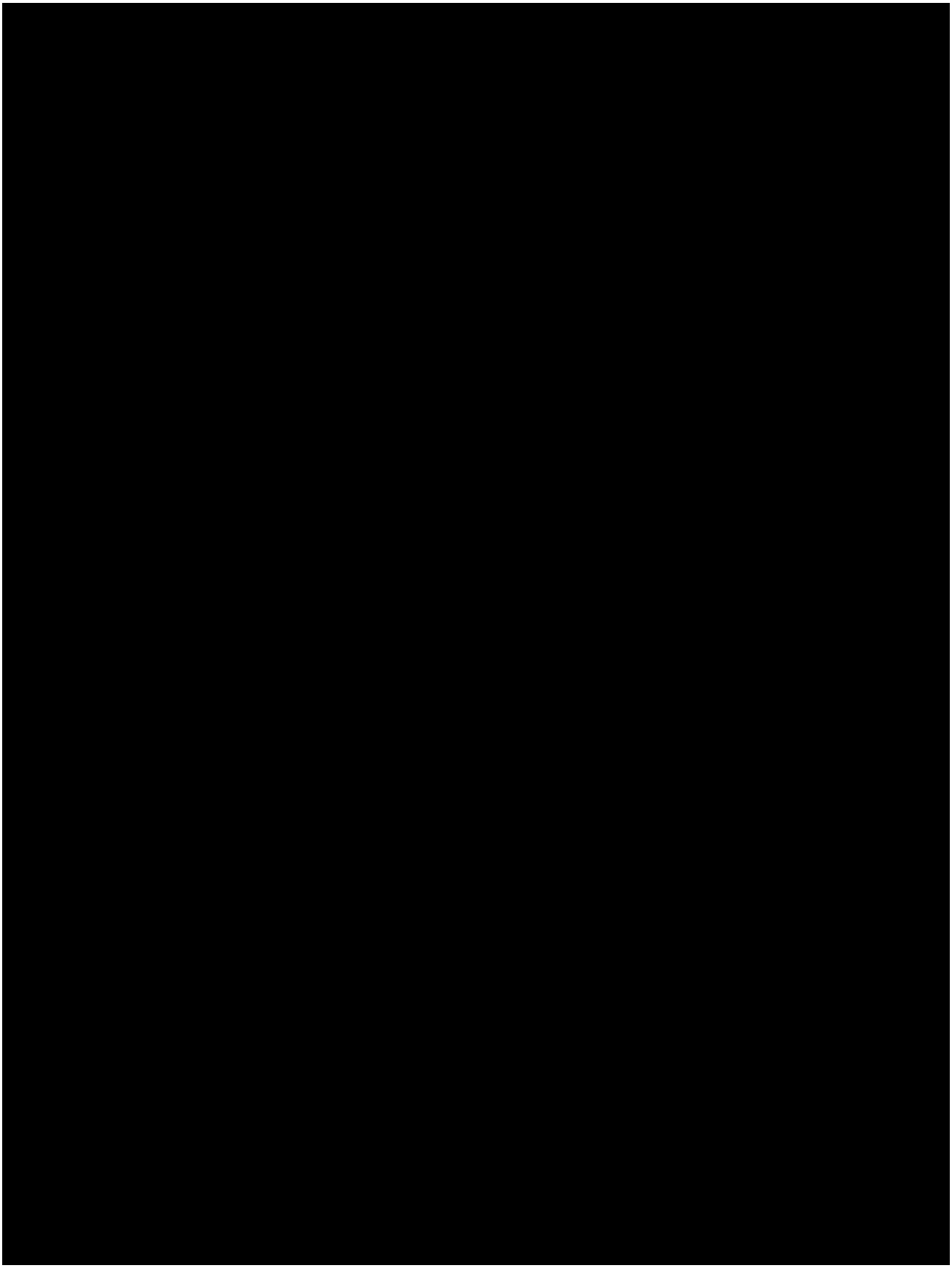
$$\text{GFR (mL/min/1.73 m}^2) = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{Age[years]})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$$

When serum creatinine is in µmol/L (SI units) the GFR may be estimated based on the following MDRD formula:

$$\text{GFR (mL/min/1.73 m}^2) = 186 \times (\text{serum creatinine}/88.4)^{-1.154} \times (\text{Age [years]})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$$

17.3. List of Prohibited Medications and Substances

[redacted]



[redacted]

17.4. Pharmacokinetic, Biomarker, Pharmacogenomic, and Flow Cytometry/CBC Sampling

The PK, biomarker, pharmacogenomic, and flow cytometry/CBC sampling schedule is presented in Table 3.

Table 3 Pharmacokinetic, Biomarker, Pharmacogenomic, and Flow Cytometry/CBC Sampling Schedule

Visit	Time Point	Time for Database Setup	PK Blood Sample	Extra Biomarker Aliquot from PK Blood Sample	Pharmacogenomic Blood Sample	Flow Cytometry/CBC
2	Prior to (i.e., within 30 min before study agent administration)	-0:15 h	X	X	X	X
	0:00 (study agent administration)	0:00 h				
	1:00 ± 30 minutes	1:00 h	X	X		X
3	-00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0:00 h	X			
4	--00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0	X			
	1:00 ± 30 minutes	1:00 h	X			
5	-00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0:00 h	X			
6	-00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0:00 h	X			
7	-00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0:00 h	X			
8	-00.45 to -00:15 min (i.e., within 30 min before study agent administration)	0:00 h	X	X		X
9	Any time during visit	0:00 h	X	X		

Abbreviations: CBC = complete blood count; h = hour(s); min = minute(s); PK = pharmacokinetic

17.5. Work Productivity and Activity Impairment Questionnaire: General Health

The WPAI-GH V2.0 is a 6-question survey used to assess the effects of a participant's health problems (i.e., physical or emotional problems or symptoms) on their ability to work and perform regular activities during the past 7 days. In clinical trials, the WPAI-GH has been used to compare work impairments between treatment groups in participants with varying levels of disease severity (Zhang 2010). The questionnaire below is provided as an EXAMPLE ONLY. The actual assessment and instructions for administration and scoring will be included in a rater reference manual or equivalent.

The following questions ask about the effect of your health problems on your ability to work and perform regular activities. By health problems we mean any physical or emotional problem or symptom. *Please fill in the blanks or circle a number, as indicated.*

1. Are you currently employed (working for pay)? ____ NO ____ YES
   If NO, check "NO" and skip to question 6.

The next questions are about the **past seven days**, not including today.

2. During the past seven days, how many hours did you miss from work because of your health problems? *Include hours you missed on sick days, times you went in late, left early, etc., because of your health problems. Do not include time you missed to participate in this study.*

   _____HOURS

3. During the past seven days, how many hours did you miss from work because of any other reason, such as vacation, holidays, time off to participate in this study?

   _____HOURS

4. During the past seven days, how many hours did you actually work?

   _____HOURS *(If "0", skip to question 6.)*

5. During the past seven days, how much did your health problems affect your productivity while you were working?

Think about days you were limited in the amount or kind of work you could do, days you accomplished less than you would like, or days you could not do your work as carefully as usual. If health problems affected your work only a little, choose a low number. Choose a high number if health problems affected your work a great deal.

Consider only how much health problems affected productivity while you were working.

Health problems had no effect on my work	0 1 2 3 4 5 6 7 8 9 10	Health problems completely prevented me from working

CIRCLE A NUMBER

6. During the past seven days, how much did your health problems affect your ability to do your regular daily activities, other than work at a job?

By regular activities, we mean the usual activities you do, such as work around the house, shopping, childcare, exercising, studying, etc. Think about times you were limited in the amount or kind of activities you could do and times you accomplished less than you would like. If health problems affected your activities only a little, choose a low number. Choose a high number if health problems affected your activities a great deal.

Consider only how much health problems affected your ability to do your regular daily activities, other than work at a job.

Health problems had no effect on my daily activities	0 1 2 3 4 5 6 7 8 9 10	Health problems completely prevented me from doing my daily activities

CIRCLE A NUMBER

18. REVISION HISTORY

18.1. Summary of Changes

Protocol Version 2.0 dated 19AUG2021
Replaces: Protocol Version 1.0 dated 08APR2021

The following table describes changes from Version 1.0 (dated 08APR2021) with justifications provided.

Section	Description	Justification
Throughout	Protocol version update. ***Previously read**:* V1.0_08APR2021 ***Now reads**:* V2.0_18AUG2021	Version control.
Throughout	Minor grammar, content, and style updates.	Minor content updates for clarity/accuracy/style of content.
Table of Contents	Minor content updates.	Minor updates required to reflect revised content.
List of Abbreviations	Minor content updates.	Minor updates required for accuracy and to reflect revised content.
Protocol Summary, 3.3, 4.2.3, 7.2.2.3, 15 (Schedule of Assessments); 17.4	Removed fluorescence-activated cell sorting (FACS) and replaced with flow cytometry.	Clarification of terminology of evaluation.
Protocol Summary, 3.3, 4.2.3, 10.4.4	Modified the exploratory objective and endpoint for assessment of change from Baseline over time in BCVA using the ETDRS method to define the change from Baseline as either gains or losses. Removed the duplicate objective and associated endpoint.	Minor update for accuracy and clarity.
Protocol Summary, 4.2.3	Modified the exploratory endpoint for change in area of ischemia or area of neovascularization from Baseline over time to allow for the assessment to be completed using OCT-A and/or FA.	Clarification of the modality for assessment of the endpoint.
4.1, 7.1.1.1, 7.1.1.2, 7.1.1.3.4, 15	Changed term from indirect ophthalmoscopy to extended ophthalmoscopy.	Administrative clarification.
5.1	[redacted]	Updated the window for previous treatment for ease of enrollment.

	[redacted]	
5.1 and 5.2	Exclusion Criterion 3, part c was modified and moved to become Inclusion Criterion 7 as follows: ***Previously read***: In addition, the investigator and potential participant are comfortable withholding treatment for DME until there is at least a 10% increase in OCT central subfield thickness with confirmed visual acuity loss (10 letter loss at a single visit or 5 to 9 letters at 2 consecutive visits) ***Now reads***: The investigator and potential participant are comfortable withholding treatment for DME and PDR until protocol-defined treatment criteria are met (see Section 5.4).	Revised for clarity.
5.2	Exclusion Criterion 4 was modified to stipulate a treatment window as follows: ***Previously read***: Prior intraocular or periocular steroid injection. ***Now reads***: Prior intraocular or periocular steroid injection within 12 months prior to enrollment and assignment to a randomized treatment.	Revised to add a window for prior treatment.
5.2	[redacted]	Content added based on regulatory feedback.
5.4	Section 5.4 added to detail the threshold for treatment of CI-DME	Content added for clarity on the treatment requirements for the occurrence of CI-DME.
6.2	***Previously read***: The disposal of used, partially used, or wasted AKST4290 and/or placebo must be performed in accordance with the institution's drug disposal policy. ***Now reads***: The disposal of used, partially used, or wasted AKST4290 and/or placebo must be performed in accordance with the institution's drug disposal policy or return.	Clarification of drug return procedures.
7.1.1.2.6	Updated the list of chemistry assessments to include eGFR and removed eGFR from the list of urinalysis assessments.	Correction as eGFR is calculated from serum creatinine and is not a urinalysis assessment.
7.5	The restriction advising participants to avoid alcoholic beverages from 48 hours before study drug administration through the EOS examination was removed.	Text was removed as alcohol is not considered a prohibited drug.
7.5, 17.3, 17.3.1, and 17.3.3	[redacted]	Content added based on regulatory feedback.

15, Schedule of Assessments	• Switched the order of assessments for Optional aqueous humor collection and PK blood sample collection for proper footnote ordering. • Corrected the footnote letter from “l” to “m” for the rows labeled as “PK blood sample collection (for plasma extraction)” and “Biomarker plasma aliquot”. • Clarified footnote “m” to indicate that PK blood samples and samples for biomarker analysis are collected in separate tubes. • Removed visual field assessment at Visit 9.	Minor changes for clarity and to remove an assessment that is not required at the End-of-Study visit.