A Randomized, Double-Blind, Phase 2a Study to Evaluate the Tolerability, Feasibility, and Efficacy of AKST1210 in Patients on Hemodialysis with Cognitive Impairment Associated with End-Stage Renal Disease

Protocol Number:	AKST1210-201
Clinical Phase:	2a
Sponsor:	Alkahest, Inc. 125 Shoreway Road, Suite D San Carlos, CA 94070
Study Device:	AKST1210
Indications:	End-stage renal disease with cognitive impairment (ESRD-CI) in patients on hemodialysis (HD)
Authorized Representative:	[redacted]
Version Number:	V2.1
Version Date:	14FEB2020
Replaces Version(s):	V2.0

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADE	Adverse device effect
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
b2M	Beta 2-microglobulin, β_2-microglobulin
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
BRINK	Brain in Kidney Disease
BUN	Blood urea nitrogen
CBC	Complete blood count
CFR	Code of Federal Regulations
CK	Creatine kinase
CMP	Clinical Monitoring Plan
CRA	Clinical research associate
CRF	Case report form
CRO	Contract Research Organization
DBP	Diastolic blood pressure
[redacted]	[redacted]
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EOS	End of Study
ESA	Erythropoiesis-stimulating agents
ESRD	End-stage renal disease
ESRD-CI	End-stage renal disease with cognitive impairment
eGFR	Estimated glomerular filtration rate
ENT	Ear, nose, throat
FACIT	Functional Assessments of Chronic Illness Therapy
FDA	Food and Drug Administration
GCP	Good Clinical Practice
[redacted]	[redacted]
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCV Ab	Hepatitis C antibody
HD	Hemodialysis
HDL	High-density lipoprotein
HF	High-flux
HgB	Hemoglobin

HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HIV Ab	Human immunodeficiency virus antibody
ICH	International Conference on Harmonization
ICH E6 R2	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guidance for Industry, Good Clinical Practice: Consolidated Guidance, Revision 2
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IDH	Intradialytic hypotension/hypotensive event
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intent-to-treat
IV	Intravenous
LAR	Legally authorized representative
[redacted]	[redacted]
[redacted]	[redacted]
MAP	Mean arterial pressure
MDRD	Modification in diet in renal disease
MedDRA	Medical Dictionary for Regulatory Activities
MHC-1	Major histocompatibility class 1
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
PHQ-9	Patient Health Questionnaire-9
PSQI	Pittsburgh Sleep Quality Index
PT	Preferred Term; prothrombin time
PT/INR	Prothrombin time/international normalized ratio
PVG	Pharmacovigilance
QTc	QT interval corrected for heart rate
qPCR	Quantitative polymerase chain reaction
SADE	Serious adverse device effect
SAE	Serious adverse event
SAP	Statistical Analytical Plan
SBP	Systolic blood pressure
SF-36	Short-Form Health Survey
SOC	System Organ Class
SOP	Standard operating procedure
TEAE	Treatment emergent adverse event
TSH	Thyroid-stimulating hormone
UADE	Unanticipated adverse device effect
US	United States
WOCBP	Women of childbearing potential

LIST OF DEFINITIONS

Intradialytic hypotension/hypotensive event (IDH)	A decrease in systolic blood pressure (SBP) ≥ 20 mm Hg or a decrease in mean arterial pressure (MAP) ≥10 mm Hg associated with symptoms that include: abdominal discomfort; yawning; sighing; nausea; vomiting; muscle cramps; restlessness; dizziness or fainting; and anxiety (K/DOQI Workgroup 2005).
Expected IDH Rate	The expected number of occurrences of IDH per 4-week treatment period based on the Historical IDH Rate. This is used to assess whether an increase in IDH events has occurred and guide decisions about escalation, de-escalation, and discontinuation.
Historical IDH Rate	The number of occurrences of IDH as documented in a subject's medical record during a recent, consecutive 8-week interval prior to randomization. This 8-week period may include the 2-week Run-in. Subjects with 5 or more IDH events during this 8-week interval will be excluded.

PROTOCOL APPROVAL PAGE

Study Title:	A Randomized, Double-Blind, Phase 2a Study to Evaluate the Tolerability, Feasibility, and Efficacy of AKST1210 in Patients on Hemodialysis with Cognitive Impairment Associated with End-Stage Renal Disease
Protocol Number:	AKST1210-201
Version/Date:	V2.1, 14FEB2020
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, 21 Code of Federal Regulations (CFR) 812, and International Organization for Standardization (ISO) 14155:2011, and applicable legal and regulatory requirements.

Approved by:

[redacted]

[redacted]

STATEMENT OF COMPLIANCE

Protocol Title:	A Randomized, Double-Blind, Phase 2a Study to Evaluate the Tolerability, Feasibility, and Efficacy of AKST1210 in Patients on Hemodialysis with Cognitive Impairment Associated with End-Stage Renal Disease
Protocol Number:	AKST1210-201
Version/Date:	V2.1, 14FEB2020

By my signature, I:

- Confirm that my staff and I have carefully read and understand this protocol or protocol amendment and are thoroughly familiar with the appropriate use of the investigational device 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 United States (US) Food and Drug Administration (FDA) regulations, 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 subjects.
- 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 subjects, 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, local governing regulatory bodies, and IRB/IEC inspectors.

Investigator's Signature Date

Print Name

PROTOCOL SUMMARY

Title:	A Randomized, Double-Blind, Phase 2a Study to Evaluate the Tolerability, Feasibility, and Efficacy of AKST1210 in Patients on Hemodialysis with Cognitive Impairment Associated with End-Stage Renal Disease
Précis:	This is a randomized, double-blind study, to be conducted at up to 5 hemodialysis (HD) centers in approximately 20 evaluable subjects randomized in a 1:1 ratio (n=10 per arm) to either the AKST1210 column (the investigational treatment) or to the control (no column), to assess the tolerability, feasibility, and efficacy of AKST1210 (a beta 2-microglobulin [b2M] apheresis column) in subjects with end-stage renal disease with cognitive impairment (ESRD-CI) undergoing HD 3 times per week. Subjects will undergo a Screening visit followed by a 2-week Run-in period. During the Run-in period, subjects' HD flow rate will be adjusted to a maximum of 250 mL/min. Subjects that meet all inclusion criteria and none of the exclusion criteria will then be randomized to either receive HD with the AKST1210 column or control (i.e., no column). The AKST1210 column will be connected in series before (upstream of) the HD cartridge for the duration of each HD session. The control will consist of a covered surrogate object of similar size/shape as the AKST1210 column which will be set up in such a way to mimic the appearance of an in-line AKST1210 column. The subject, investigator, and outcomes assessor will be blinded as to treatment. There will be three (3) 4-week treatment periods, and all subjects will receive 3 HD treatments per week for 12 weeks. Subjects who may occasionally require an additional HD session per week would not receive the investigational intervention on that additional day. Subjects randomized to the AKST1210 column will start with a 150 mL column (S-15) used during Weeks 1 to 4 (Treatment Period 1). Subjects who meet specific criteria will undergo escalation of the column size to a 250 mL column (S-25) during Weeks 5 to 8 (Treatment Period 2) and then to a 350 mL column (S-35) during Weeks 9 to 12 (Treatment Period 3). Subjects randomized to control (no column) will receive identical escalation evaluations to maintain the treatment assignment blind. The rules specifying the criteria for escalation, de-escalation, and discontinuation are provided in Appendix 17.1. Following Treatment Period 3, subjects should resume HD in accordance with standard of care. Subjects will return for an End of Study (EOS) Visit during Week 14.
Objectives:	The primary objective is evaluation of the safety and tolerability of AKST1210 for the treatment of ESRD-CI in subjects receiving HD. The secondary objectives are to evaluate the feasibility of conducting expanded studies of AKST1210 in subjects with ESRD-CI who are undergoing HD 3 times per week as well as the efficacy of the column.

The exploratory objectives include serial compositional analysis of plasma to identify specific biomarkers possibly associated with cognitive function and/or indicators of disease progression by proteomic analyses, magnitude of b2M removal, and possible relationship to changes in cognition.

Endpoints:

Primary Endpoint:

- Incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) coded by the Medical Dictionary for Regulatory Activities (MedDRA) preferred term (PT) and MedDRA System Organ Class (SOC).

Secondary Endpoints:

- Feasibility of all procedures, including blinding.
- Tolerability of each column size escalation, as measured by subject compliance with study visit schedule/completion, visit procedures, and subject retention.
- The change in IDH Rate above each subject's Historical IDH Rate for each column size.
- The incidence of IDH leading to de-escalation or discontinuation.
- The incidence of worsening anemia by column size.
- The incidence of anemia leading to de-escalation or discontinuation.
- Change from baseline in levels of b2M at Weeks 4, 8, 12 and EOS and evaluation of any differences in the magnitude of change based on column size and duration of treatment with a specific column size.
- Change in Montreal Cognitive Assessment (MoCA) from baseline to EOS.
- Change in cognitive domains as measured with the Cogstate battery (i.e., verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall).
- Change in quality of life per the Short-Form Health Survey (SF-36).
- Change in the Patient Health Questionnaire-9 (PHQ-9).
- Change in sleep quality as measured by the Pittsburgh Sleep Quality Index (PSQI).
- Change in fatigue as measured by the Fatigue Questionnaire – Functional Assessments of Chronic Illness Therapy (FACIT).
- Changes from baseline in laboratory test data, vital sign measurements, and electrocardiograms (ECGs).

Exploratory Endpoints:

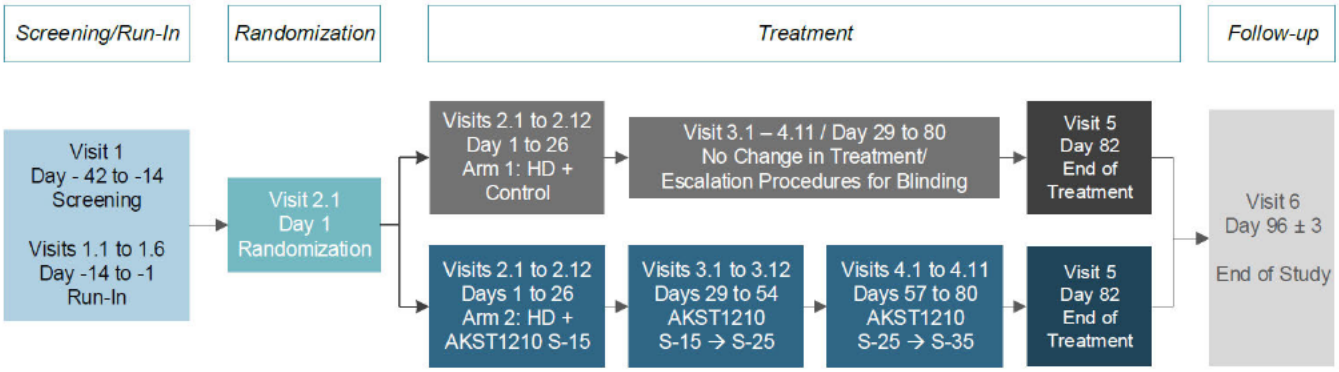
- Serial compositional analysis of plasma to identify specific biomarkers associated with cognitive function and/or indicators of disease progression by proteomic analyses.
- Magnitude of b2M removal and possible relationship to changes in cognition.

Population:

Approximately 26 male and female subjects, ≥ 40 years of age on HD with ESRD-CI, will be enrolled. Assuming a drop-out rate of 20%, enrollment at

	this level will yield approximately 20 evaluable subjects.
Sample Size:	Twenty (20) evaluable subjects randomized in a 1:1 ratio (n=10 per arm) to either the AKST1210 column (the investigational treatment) or to control (no column).
Phase:	2a
Number of Sites:	Up to 5 sites in the US.
Description of Study device:	[redacted]
Description of Study Control:	A covered surrogate object of similar size/shape as the AKST1210 column that will be set up in a manner that mimics the appearance of an in-line column.
Study Duration:	Approximately 12 months
Subject Duration:	Approximately 16-20 weeks

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

1.1 AUTHORIZED REPRESENTATIVE (SIGNATORY) / RESPONSIBLE PARTY

[redacted]

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 IECs and IRBs, as applicable) will be maintained by the Sponsor, or their designee, and provided to the investigator.

2 INTRODUCTION

[redacted]

[redacted]

[redacted]

3 OBJECTIVES AND PURPOSE

The primary objectives of this study are to assess the safety and tolerability of AKST1210 in subjects with ESRD-CI who are receiving HD. Secondarily, this study aims to assess the feasibility of device size escalation as well as the efficacy of AKST1210. Exploratory objectives include blood and plasma collection to identify specific biomarkers associated with cognitive function and/or indicators of ESRD-CI and b2M progression.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This will be a randomized, double-blind study conducted at up to 5 sites in the US. Approximately 26 male and female subjects, ≥ 40 years of age on HD with ESRD-CI, will be enrolled. Assuming a drop-out rate of 20%, enrollment at this level will yield approximately 20 evaluable subjects.

During Screening (Visit 1, Day -42 through Day -14), subjects and/or their legally authorized representative (LAR) will provide informed consent, and subjects will undergo screening assessments including provision of demographics and medical history, physical examination, 12-lead ECG, laboratory testing (including pregnancy testing if applicable), as well as tests to assess cognitive function and depression. A blood sample will also be obtained and sent to the site's local laboratory to determine baseline b2M concentration.

If the Visit 1 Screening eligibility criteria are met, subjects will commence the Run-in Period (Visits 1.1-1.6, Day -14 through Day -1). During Run-in, subjects will undergo HD at a reduced flow rate; have safety assessments; complete tests to assess cognitive function, quality of life, quality of sleep, and fatigue; and have blood samples collected for b2M and plasma proteomics. All subjects will have their HD flow rate adjusted to a maximum flow rate of 250 mL/minute, thus necessitating an increase in the overall length of dialysis time to approximately 4 hours.

Subjects who continue to meet eligibility requirements will be randomized at Visit 2 (Day 1) to one of two treatment arms: AKST1210 (the investigational treatment) or control (no column). The AKST1210 column will be connected in series before (upstream of) the HD dialyzer for the duration of each HD session, and an appropriate covering of either the AKST1210 column or control will be in place for blinding purposes. All randomized subjects will continue to undergo dialysis at the adjusted, maximum flow rate of 250 mL/minute at the extended dialysis time, similar to the Run-in Period. The subject, investigator, and outcomes assessor(s) will be blinded as to treatment (i.e., double-blind study).

There will be three (3) 4-week treatment periods, and subjects will receive 3 HD treatments with AKST1210 or control per week for 12 weeks. Subjects who may occasionally require an additional HD session per week would not receive the intervention on that additional day. Subjects randomized to the AKST1210 column will receive a 150 mL column (AKST1210 S-15) during Weeks 1 to 4 (Treatment Period 1). Subjects will then be evaluated for escalation to the 250 mL column (AKST1210 S-25) during Weeks 5 to 8 (Treatment Period 2) followed by evaluation for escalation to the 350 mL column (AKST1210 S-35) for Weeks 9 to 12 (Treatment Period 3). Subjects will be evaluated for escalation, de-escalation, or discontinuation based on specific criteria as specified in Appendix 17.1. Subjects will complete an EOS Visit during Week 14.

Subjects randomized to control will also receive 3 HD treatments per week for 12 weeks for blinding purposes with similar escalation, de-escalation, and discontinuation procedures (see Appendix 17.1).

During each Treatment Period, subjects will undergo tests to assess cognitive function, quality of life, quality of sleep, and fatigue, as well as collection of blood samples for b2M and plasma proteomics. AE assessments; review of concomitant medications; targeted physical examinations; monitoring of vital signs; and assessment of fluid status will occur at every treatment visit.

In the event of early termination of a subject who has been randomized and received at least 1 HD treatment, the EOS procedures will be performed unless the subject has withdrawn consent.

The overall duration of the study/recruitment period is approximately 12 months from study initiation (i.e., following consent of first subject) to study completion (i.e., last subject, last visit). Subject participation is expected to be approximately 16 to 20 weeks, unless prematurely discontinued.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

- Incidence of TEAEs and SAEs coded by MedDRA PT and MedDRA SOC.

4.2.2 SECONDARY ENDPOINTS

- Feasibility of all procedures, including blinding.
- Tolerability of each column size escalation, as measured by subject compliance with study visit schedule/completion, visit procedures, and subject retention.
- The change in IDH Rate above each subject's Historical IDH Rate for each column size.
- The incidence of IDH leading to de-escalation or discontinuation.
- The incidence of worsening anemia by column size.
- The incidence of anemia leading to de-escalation or discontinuation.
- Change from baseline in levels of b2M at Weeks 4, 8, 12 and EOS and evaluation of any differences in the magnitude of change based on column size and duration of treatment with a specific column size.
- Change in MoCA from baseline to EOS (Nasreddine 2005).
- Change in cognitive domains as measured with the Cogstate battery (i.e., verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall).
- Change in quality of life per the SF-36 (Laucis 2015).
- Change in sleep quality as measured by the PSQI (Buysse 1989).
- Change in fatigue as measured by the FACIT (Webster 2003).
- Changes from baseline in laboratory test data, vital sign measurements, and ECGs.

4.2.3 EXPLORATORY ENDPOINTS

- Serial compositional analysis of plasma to identify specific biomarkers associated with cognitive function and/or indicators of disease progression by proteomic analyses.
- Magnitude of b2M removal and possible relationship to changes in cognition.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 INCLUSION CRITERIA

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

1. Male and female subjects, ≥ 40 years of age.
2. b2M level > 20 mg/L.
3. On chronic HD due to ESRD for ≥ 12 months.
4. HD performed using standard HF dialyzers (medium cut-off dialyzers are not acceptable).
5. Score on the MoCA ≥ 16 and ≤ 23.
6. Life expectancy > 6 months (as determined by the investigator).
7. Body mass index (BMI) ≥ 20 and ≤ 36.
8. If on medications for cognition (e.g., rivastigmine, galantamine, donepezil, memantine), must be on stable regimen for at least 8 weeks prior to Screening and remain on stable dose for the duration of the study.
9. If on antidepressant medications, must be on stable regimen for at least 8 weeks prior to Screening and remain on a stable dose for the duration of the study.
10. Must be on stable regimens (> 4 weeks) of all treatments for concomitant diseases (e.g., diabetes, hypertension, congestive heart failure).
11. The subject must be able to follow the study protocol, receive the treatment in the established timeframe, and continue during the follow-up interval.
12. The subject must have sufficient visual and auditory acuity to reliably complete all study assessments.
13. Provided a signed and dated informed consent form.

5.2 EXCLUSION CRITERIA

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

1. [redacted]
2. During Run-in, subjects who are not able to comply with or tolerate extended HD duration of approximately 4 hours at a maximum flow rate of 250 mL/min as assessed by the investigator.
3. Subjects who experience 3 or more IDH events during the Run-in period.
4. [redacted]
5. Subjects for whom extracorporeal circulation therapy is contraindicated, such as those with severe cardiac insufficiency, acute myocardial infarction, severe cardiac arrhythmia, acute seizure disorder, or severe uncontrolled hypertension.
6. History of hypersensitivity to heparin or to the AKST1210 column or to materials in the column.
7. Subjects who are at a higher risk of IDH including:
    - Subjects with medical records indicating the occurrence of ≥ 5 events of IDH during a recent, consecutive 8-week interval prior to randomization;
    - Subjects requiring or expected to require extensive fluid management;
    - Presence of pre-dialysis hypotension, defined as a SBP < 90 mm Hg and/or a diastolic blood pressure (DBP) < 50 mm Hg, before any of the last 3 dialysis sessions prior to screening;
    - A diagnosis of interdialytic hypotension;

- A diagnosis of IDH; and/or
- A diagnosis of autonomic dysfunction.

8. Pregnant or breast-feeding subjects or subjects who are planning to become pregnant. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative serum/urine pregnancy test at Screening, prior to treatment, and at EOS. WOCBP and men must agree to use highly effective contraception (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.
[redacted]
10. Clinically significant abnormalities on Screening ECG including QT interval corrected for heart rate (QTc) (using Fridericia's correction formula) of ≥ 500 ms in men and ≥ 520 ms in women.
11. Delirium (encephalopathy).
12. Subjects on anti-seizure medications for a seizure disorder.
[redacted]
14. Subjects with a hemoglobin level < 9.0 g/dL.
15. Concurrent or recent participation in another investigational clinical trial. Prior clinical trial subjects must have discontinued investigational agents at least 30 days prior to Screening.
16. History of severe depression/suicidality requiring hospitalization in the last 6 months.
17. Significant alcohol or drug abuse within the past 2 years.
18. Subjects planning to receive renal transplantation during the study.
19. Subjects unable to understand or answer the questionnaires even with proper assistance.
20. Any other condition and/or situation that the investigator believes may interfere with the safety of the subject, study conduct, or interpretation of study data.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

The Sponsor does not anticipate any specific challenges in meeting recruitment goals of enrolling and retaining a total of 20 evaluable subjects in this study. Approximately 26 male and female subjects will be enrolled. Assuming a drop-out rate of 20%, enrollment at this level will yield approximately 20 evaluable subjects. Subjects will be recruited continuously until the planned sample size is achieved. Subjects who withdraw or are withdrawn during Screening/Run-in, as well as subjects who discontinue or are unblinded prior to Week 8, may be replaced (see Section 5.4.2 Handling of Participant Withdrawals or Termination).

The estimated duration of subject participation in the study of 16 to 20 weeks is not expected to be challenging to subjects. Subjects will be compensated for the additional time required for study participation; details will be provided in the informed consent form. A description of the study will be included in local clinical trial databases, as required.

5.4 SUBJECT WITHDRAWAL/DISCONTINUATION

5.4.1 REASONS FOR WITHDRAWAL/DISCONTINUATION

A subject will be withdrawn from the study for the following medical or administrative reasons:

- Occurrence of an AE that represents an unacceptable risk to the subject 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 subject until the AE resolves or is stable, unless the subject is lost to follow up.
- Treatment with a prohibited concomitant medication other than the use of appropriate medications for the treatment of AEs under direction of the investigator.
- Subject noncompliance, defined as refusal or inability to adhere to the trial schedule or procedures.
- At the request of the subject or the subject's LAR (e.g., subject withdraws consent), investigator, Sponsor, or regulatory authority.
- Pregnancy.
- A subject will be discontinued if the number of IDH events during a treatment period exceeds a predetermined threshold above the subject's Historical IDH Rate after de-escalation to the lowest column size (see Appendix 17.1).
- A subject will be discontinued if Hgb levels remain < 9 g/dL despite optimized iron and ESA treatment and after de-escalation to the lowest column size (see Appendix 17.1).

In the event of early withdrawal/discontinuation of a subject who has been randomized and received at least 1 HD treatment, the EOS procedures will be performed unless the subject has withdrawn consent.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS/DISCONTINUATION

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

Approximately 26 subjects will be enrolled with the intent of obtaining 20 evaluable subjects randomized in a 1:1 ratio to either active (AKST1210 column) or control. Subjects who discontinue or are unblinded prior to Week 8 may be replaced.

Subjects who have received at least 1 HD with AKST1210/control but are withdrawn or withdraw from the study will be encouraged to complete the EOS procedures within 4 to 6 weeks of their last visit. The primary reason for study discontinuation will be documented on the case report form (CRF).

5.5 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 investigators, appropriate regulatory authority(ies), and IRB(s). In terminating the study, the Sponsor and the investigators will continue to protect the subjects' privacy and identity as required by relevant statutes 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 subject safety.
- Unsatisfactory subject enrollment.
- Unacceptable Protocol Deviations as assessed by the Sponsor's Program Physician.
- Inaccurate or incomplete data entry and recording/fabricated data.
- Investigational site non-compliance with ICH/Good Clinical Practice (GCP).
- Unacceptable emergent safety profile.

If Alkahest were to receive withdrawal of IRB or FDA approval, or other action on the part of the IRB or FDA that

affects the study, the Sponsor will notify the FDA and/or all reviewing IRBs, and all participating investigators, within 5 days of the IRB or FDA decision.

6 STUDY DEVICE

6.1 STUDY DEVICE AND CONTROL DESCRIPTION

[redacted]

All columns will be shipped to the clinical sites by [redacted]. Information regarding the initial supply of AKST1210, ordering of resupply, and instructions for column receipt and accountability are provided in the Device Procedures Manual.

6.1.2 DESCRIPTION, APPEARANCE, PACKAGING, AND LABELING

6.1.2.1 Device/Control Description and Appearance

The AKST1210 b2M apheresis column is an investigational device and may only be used for subjects who have been enrolled in the AKST1210-201 study and who are under the supervision of an appropriately qualified investigator.

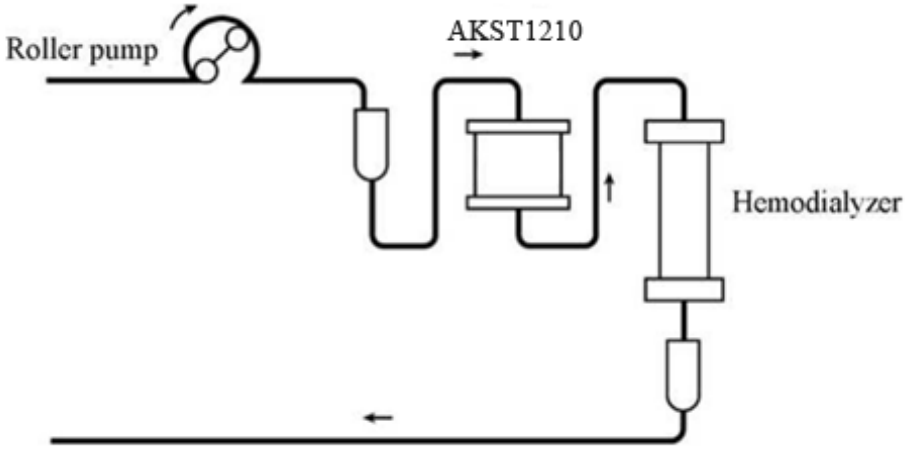
[redacted]

Table 2 Description of the AKST1210 Model Sizes

Model	Column Capacity (mL)	Extracorporeal Volume (mL)
S-15	150	65
S-25	250	105
S-35	350	177

The AKST1210 column is an extracorporeal column for adsorption of b2M from circulating blood, and it is placed in series with a hemodialyzer in an HD circuit. Figure 3 depicts the connection of the AKST1210 column in the HD blood circuit.

Figure 3 AKST1210 Column in the Hemodialysis Blood Circuit



The control will consist of a covered surrogate object of similar size/shape as the AKST1210 column. The control will not in any way be in contact with circulating blood, as it sits external to the blood circuit. The control will be set up and draped in the same fashion as the AKST1210 column. Additional details can be found in the Device Procedures Manual.

6.1.2.2 Packaging and Labeling

The AKST1210 columns will be individually packaged in a plastic bag bearing an investigational label. Six columns will be packaged in each white kit and each carton will contain 2 kits for a total of 12 columns per carton.

The [redacted] devices will be used and will not be modified in any way except for the addition of an Investigational Use label on the immediate package of the device with the appropriate investigational statement per 21 CFR 812.5(a): “CAUTION - Investigational device. Limited by Federal (or United States) law to investigational use."

[redacted]

6.1.3 PRODUCT STORAGE

The AKST1210 column should be handled with care during transportation and storage. The AKST1210 column should be kept in a clean place between 5 °C and 30 °C. Direct sunlight, high humidity, excessive vibration, and freezing conditions should be avoided. Do not drop the AKST1210 column or strike it with a hard instrument (e.g., forceps). Do not use any AKST1210 column that has been damaged or frozen.

The fluid pathway of the AKST1210 column is sterile and nonpyrogenic. Careful aseptic handling techniques are necessary to maintain this condition. Do not use the device if the package, the bag, or the product is damaged. Remove the device from the bag just before use.

6.1.4 PREPARATION

The AKST1210 column will be connected in series before (upstream of) the dialyzer for the duration of each HD session. The control will be set up such that it appears to be connected in series before (upstream of) the dialyzer. The subject, investigator, and outcomes assessor must remain blinded to treatment assignment throughout. Specifics of column/control preparation (rinsing, priming, and disposal) and blinding procedures are specified in the Device Procedures Manual.

.

6.1.5 COLUMN SIZE ESCALATION AND ADMINISTRATION

The initial AKST1210 size used during Weeks 1 to 4 (Treatment Period 1) will be the 150 mL column

(AKST1210 S-15). Following evaluation for IDH Rate and anemia (see Appendix 17.1), subjects may be escalated to the 250 mL column (AKST1210 S-25) for Weeks 5 to 8, followed by the 350 mL column (AKST1210 S-35) for Weeks 9 to 12. The subject, investigator, and outcomes assessor must remain blinded to treatment assignment at all times. Careful attention should be given to ensure that all subjects, regardless of treatment assignment, undergo the same evaluations for escalation, de-escalation, and discontinuation.

6.1.6 DURATION OF THERAPY

The treatment period for each subject is 12 weeks. The total duration of subject participation is expected to be approximately 16 to 20 weeks.

6.2 STUDY DEVICE AND CONTROL ACCOUNTABILITY

Under the **blinded supervision** of the investigator, the unblinded study coordinator or other qualified personnel is responsible for ensuring adequate accountability of all used and unused AKST1210 columns and controls. This includes acknowledgment of receipt of each shipment of AKST1210 (quantity and condition), subject dispensing records, and destruction of used study devices. Study device dispensing records will document quantities received and quantities dispensed to subjects including the date dispensed, size of column (S-15, S-25, or S-35), lot number, subject's study identifier, initials of the individual responsible for dispensing, and initials of the unblinded dialysis nurse, or other qualified medical professional, administering the study device. When subjects receive a control rather than an AKST1210 column, key information will also be captured for each treatment. Device/control accountability will be monitored by an unblinded clinical research associate (CRA).

Accountability records must be maintained and readily available for inspection by representatives of Alkahest, Inc., or their designee, and are open to inspection by regulatory authorities at any time. Accounts of any study device accidentally wasted or intentionally disposed of must be maintained. As these records contain unblinding information, they must be stored in a securely locked, controlled-access location.

All unused AKST1210 study devices should be kept securely at the site until otherwise instructed. All unused containers of study devices at the site should not be returned or destroyed without prior written approval from the Sponsor, and authorized destruction must be performed in accordance with the site's or hospital's administrative policy (or equivalent), and/or relevant national regulations. The site's procedure(s) for study device disposal/destruction will be evaluated by the Sponsor or an authorized representative in order to ensure that it complies with study requirements. A copy of the site's device disposal policy should be maintained or referenced in the investigator's study file.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

7.1.1.1 Screening/Run-In Procedures

During the Screening Visit(s)*, the following procedures will be performed:

- Informed consent
- Evaluation of inclusion/exclusion criteria
- Demographics
- Medical history and review of medications
- Physical examination, including measurements of height and weight
- 12-lead ECG
- Screening laboratory tests (including pregnancy test for WOCBP only)
- b2M laboratory test (assayed at a local laboratory)
- MoCA
- PHQ-9

*Note: The Screening Visit may be split to allow for sufficient time to complete all required procedures. Review AEs and concomitant medications during split visits, as applicable.

During the Run-in period, the following procedures will be performed:

- HD at an adjusted maximum flow rate of 250 mL/min
- Vital signs
- Targeted physical examination, including weight measurement
- b2M/proteomic samples collected
- Laboratory tests (e.g., Prothrombin Time [PT], Blood Urea Nitrogen [BUN], Creatinine, and serum pregnancy test for WOCBP)
- Concomitant medication review
- AE evaluation/collection
- Verification of eligibility

Detailed descriptions of each of these procedures are provided in the sections immediately following. Information pertaining to the schedule of events during Screening/Run-In is provided in Section 15.1.1.

7.1.1.1.1 Demographics
Demographic information such as the subject's education level, ethnicity, and race will be collected by interview with the subject.

7.1.1.1.2 Medical History and Review of Medications
The investigator or designee will obtain a detailed medical history through interview with the subject during Screening. The medical history should focus on recent history, with an emphasis on the history of cognitive symptoms related to ESRD-CI. Additionally, the medical history should include:

- Current/past illnesses and conditions
- Current symptoms of any active medical condition

- Surgeries and procedures
- Allergies

The investigator or designee should obtain a complete list of the subject's current medications, including over-the-counter drugs, herbal supplements, and/or vitamins, as well as those taken by the subject in the past 3 months and any regimen changes in the last 3 months. Any additions, discontinuation, or dosage changes in medication during the course of the study will be recorded.

7.1.1.1.3 Physical Examination
A complete physical examination will be performed at Screening to assess the following organ systems: skin, ENT (ears, nose, and throat), head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, and neurologic and lymphatic systems. The neurological exam will include cranial nerves (fundoscopic exam, pupillary light reflex, extraocular muscles, facial sensation and symmetry, palate and tongue, and head turning and shoulder shrug); muscle strength; reflexes (biceps, triceps, knees, ankles, and plantar); coordination (finger-to-nose, heel-knee-shin); sensory function (light touch and pinprick); and gait. Physical and neurological examination assessments performed within 3 months of the Screening visit need not be repeated. Height will be measured at Screening; weight will be measured at Screening and throughout the study.

7.1.1.1.4 12-Lead ECG
A 12-lead ECG will be performed after the subject 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 overall conclusion with the interpretation of the ECGs will be recorded on the appropriate CRF. The interpretation of the ECGs will be recorded as normal, abnormal but not clinically significant, or abnormal and clinically significant. QTc will be calculated using Fridericia's correction formula.

7.1.1.1.5 Screening and Run-In Laboratory Evaluations
Biological samples will be collected and analyzed at Screening and Run-In. Blood will be drawn by a qualified medical provider. Labs will be completed by the site's local laboratory. Samples for Screening labs should be collected either on a non-dialysis day or prior to HD. For additional information, refer to Section 15.2 Schedule of Laboratory Tests.

7.1.1.1.6 Hemodialysis
Beginning at Run-in, HD will be conducted 3 times weekly at a modified maximum flow rate of 250 mL/min over a period of approximately 4 hours. The following key dialysis parameters will be captured: post-pump perfusion pressure (mm Hg), blood flow rate (mL/min), dialysis time (min), and type of dialyzer. Adequate safety precautions should be employed prior to and throughout HD, particularly for subjects who are at risk for developing IDH. Refer to Table 1 in Section 2.3.1 for risks associated with the AKST1210 column as well as proposed mitigation strategies.

7.1.1.1.6.1 Heparinization
Subjects will undergo heparinization according to institutional practices at the start of, and throughout, each HD session. The schedule below is a guideline for proposed heparin bolus and continuous heparin treatment during the Treatment Periods. The investigator may modify dose and schedule according to the subject's condition(s) or measured coagulation parameters e.g. activated partial thromboplastin time (aPTT) values.

- S-15: 1,000 IU bolus, followed by 1,000 IU per hr (continuous infusion until the end of the procedure)
- S-25: 1,500 IU bolus followed by 1,500 IU per hr (continuous infusion until the end of the procedure)
- S-35: 2,000 IU bolus followed by 2,000 IU per hr (continuous infusion until the end of the procedure)

At the discretion of the investigator, heparin de-escalation of 250 IU/hr can be instituted with the S-25 or S-35 column beginning with the second hour of dialysis, with the heparin dose not to fall below 1,000 IU/hr.

7.1.1.1.7 Montreal Cognitive Assessment
The MoCA (Nasreddine 2005) is a commonly used screening test easily administered by non-specialist staff. It assesses the domains of attention and concentration, executive functions, memory, language, visuo-constructional skills, conceptual thinking, calculations, and orientation.

7.1.1.1.8 Patient Health Questionnaire-9
The PHQ-9 is a multipurpose instrument for screening, diagnosing, monitoring, and measuring the severity of depression. The 9 items of the PHQ-9 are based directly on the 9 diagnostic criteria for major depressive disorder. Symptoms are rated from 0 (not at all) to 3 (nearly every day) (Kroenke 2001). It takes about 5 to 7 minutes to complete.

7.1.1.1.9 Biospecimen Collection for Beta 2-Microglobulin (b2M) and Proteomics
Blood levels of b2M are required to be collected at Screening and results obtained from the site's local laboratory to evaluate eligibility (Section 5). Samples for Screening labs should be collected either on a non-dialysis day or prior to HD.

Blood and plasma samples obtained for b2M and proteomics for all other study visits will be collected, processed, stored, and shipped to a designated biorepository in accordance with the AKST1210 b2M and Proteomics Lab Manual.

7.1.1.2 Procedures to Assess Safety
Subjects enrolled in the trial will be monitored closely to assess safety and tolerability of the investigational device and intervention. 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 Section 15 Schedule of Events.

- Review of AEs
- Vital signs
- Assessment of fluid status
- Targeted physical exam, weight measurements
- Laboratory assessments

7.1.1.2.1 Review of Adverse Events
Adverse events (AEs) 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 Monitoring Vital Signs
Vital signs monitoring will occur at every visit. Seated/reclined BP will be recorded before each dialysis session, every 30 minutes during dialysis, and at the end of the session. Other vital signs will be captured at the beginning and end of each visit; these include heart rate (beats per minute [bpm]), respiratory rate (breaths per minute), and body temperature. Vital signs will be measured after the subject has been seated/reclined for ~5 minutes.

Subjects will be monitored for IDH (see Definitions). During Run-in if a subject experiences 3 or more IDH events then they will be excluded from further participation (see Section 5.2). After randomization, should the number of occurrences of IDH exceed a predetermined threshold based on the subject's Expected IDH Rate, then the subject will be de-escalated to a lower column size or discontinued as appropriate (Appendix 17.1).

In subjects who experience IDH, the following questions must be asked to assess whether the IDH is accompanied by cognitive impairment (confusion, disorientation):

- What is your name?
- Where are you?
- What is the day of the week?
- What is today's date and what is the month?
- Who is the President?
- Who is the Governor?

Regardless of whether the subject shows signs of cognitive impairment, it is important that the IDH be addressed expeditiously as per standard procedures at the site. These procedures may include changes to the ultrafiltration rate, Trendelenburg position, and administration of saline. Once the IDH has been addressed and the dialysis session has concluded, the same questions should be asked again to assess whether the transient cognitive impairment (if present) resolved with the resolution of the episode of IDH.

7.1.1.2.3 Assessment of Fluid Status
Fluid status will be assessed during the Treatment Periods according to institutional standards of care. This could include the following: pre and post HD bioimpedance, lung ultrasound monitoring of Kerley B-lines (Lichtenstein 2017), and continuous hematocrit monitoring during HD as deemed appropriate by the investigator/HD staff (Rodriguez 2005). Data will be collected concerning the performance of these procedures, as appropriate, and the outcomes assessed.

7.1.1.2.4 Targeted Physical Examination
A targeted physical examination (i.e., a re-examination of any abnormalities noted previously or new signs and symptoms that warrant clinical assessment) will be performed according to the Schedule of Events (Section 15). Weight will be monitored throughout the study.

7.1.1.2.5 Laboratory Evaluations
Biological samples will be collected and analyzed according to the Schedule of Laboratory Tests (Section 15.2). Blood will be drawn by a qualified medical provider. Clinical labs will be processed and resulted by the site's local laboratory.

7.1.1.2.5.1 Pregnancy Testing
Serum pregnancy testing will be performed in WOCBP in accordance with the Schedule of Events (Section 15).

7.1.1.3 Procedures to Assess Efficacy

Cognitive function, depression, sleep quality, quality of life, and fatigue will be assessed using validated instruments. To limit subject fatigue, all testing will be conducted prior to HD. All testing will be performed by qualified evaluators who have undergone standardized rater training and certification, as appropriate. The same evaluator should be used for the duration of each subject's participation unless a change in rater is unavoidable. The following scales will be administered to subjects enrolled in the trial in accordance with the Schedule of Events (Section 15):

1. MoCA
2. PHQ-9
3. Cogstate test battery
4. SF-36 Short Form
5. FACIT
6. PSQI

Descriptions of each assessment are provided below.

7.1.1.3.1 Montreal Cognitive Assessment
See Section 7.1.1.1.7.

7.1.1.3.2 Patient Health Questionnaire-9
See Section 7.1.1.1.8.

7.1.1.3.3 Cogstate Test Battery
The Cogstate test battery is intended to assess cognitive function in several areas including verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall. To maintain quality and interrater reliability, all raters will be trained on each assessment before study start, and assessments will be administered in a quiet environment by trained personnel.

To avoid any confusion while testing, it is important that subjects take 2 familiarization assessments to become acquainted with the device and requirements of the tests. This helps achieve a stable baseline to more accurately reflect subjects' true cognitive function. The familiarization assessments can be administered at any time during Run-in but prior to randomization.

7.1.1.3.4 Short Form-36
The SF-36 is a set of generic, coherent, and easily administered quality of life measures. These measures rely on patient self-reporting and are now widely utilized by managed care organizations and Medicare for routine monitoring and assessment of care outcomes in adult patients.

7.1.1.3.5 Fatigue Questionnaire - Functional Assessment of Chronic Illness Therapy
The FACIT Fatigue Scale (Webster 2003) is a short, 13-item tool that is easy to administer and measures an individual's level of fatigue during their usual daily activities over the past week. The level of fatigue is measured on a 4-point Likert scale (4 = not at all fatigued to 0 = very much fatigued). The score range 0 to 52. A score of less than 30 indicates severe fatigue. The higher the score, the better the quality of life.

7.1.1.3.6 Pittsburgh Sleep Quality Index
The PSQI (Buysse 1989) is an effective instrument used to measure the quality and patterns of sleep in older adults. It differentiates "poor" from "good" sleep by measuring 7 domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime

dysfunction over the last month. The subject self-rates each of these 7 areas of sleep. Scoring of the answers is based on a 0 to 3 scale, whereby 3 reflects the negative extreme on the Likert Scale. A global sum of "5" or greater indicates a "poor" sleeper.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Biological samples will be collected for laboratory evaluations in accordance with Section 15.2 Schedule of Laboratory Tests. Screening and clinical labs, including the screening measurement of b2M for inclusion, will be processed by each site's local laboratory and results entered in the CRF.

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 CRF and followed until determined to be stable or resolved, unless the subject is lost to follow up. Once resolved, the appropriate CRF page(s) will be updated.

7.2.2 OTHER TESTS OR PROCEDURES

7.2.2.1 Plasma Proteomics

For more information regarding the timing and procedures for sample collection and related requirements, refer to the AKST1210 b2M and Proteomics Laboratory Manual as well as Section 15.1 Schedule of Events Tables and Section 15.2 Schedule Of Laboratory Tests.

Serial compositional analysis of plasma will be conducted to identify specific biomarkers associated with cognitive function and/or indicators of disease progression by proteomic analyses. Plasma will be analyzed by proteomics using mass spectrometry and targeted approaches to assess the specific signature of proteins in subjects at baseline and to assess the changes in the proteome with repeated AKST1210 treatment. These methodologies will provide a broad overview of the proteins that are present in the plasma sample. From this research, it is hoped that key proteins that are drivers of cognitive function and/or indicators of disease progression can be identified. By understanding the composition and function of plasma samples from the trial, the goal is to identify potential biomarkers relevant to further optimizing treatment in ESRD-CI.

7.2.3 SPECIMEN PREPARATION, HANDLING, STORAGE, AND SHIPPING

Each site should follow their local laboratory's standard procedures for specimen preparation, handling, storage, and shipping of Screening and clinical labs, including the screening sample for b2M.

Site personnel should refer to the AKST1210 b2M and Proteomics Laboratory Manual for instructions pertaining to the preparation, handling, storage, and shipping of specimens for b2M and proteomics.

7.3 STUDY SCHEDULE

7.3.1 SCREENING/RUN-IN

For a complete list of Screening/Run-In procedures and assessments, please see Section 15.1.1.

7.3.2 RANDOMIZATION/TREATMENT

Subjects will be randomized after eligibility has been confirmed at Visit 2.1.

For a complete list of Treatment Period 1 (Visit 2/Month 1) procedures and assessments, please see Section 15.1.2.

For a complete list of Treatment Period 2 (Visit 3/Month 2) procedures and assessments, please see Section 15.1.3.

For a complete list of Treatment Period 3 (Visit 4/Month 3) procedures and assessments, please see Section 15.1.4.

7.3.3 END OF TREATMENT/END OF STUDY VISITS

For a complete list of the End of Treatment (Visit 5) and EOS (Visit 6) procedures and assessments, please see Section 15.1.5.

7.3.4 EARLY WITHDRAWAL

In the event of early withdrawal or discontinuation of a subject who has been randomized and received at least 1 HD treatment, the EOS procedures will be performed unless the subject has withdrawn consent (also see Section 5.4).

7.4 CONCOMITANT MEDICATIONS

Concomitant medications taken by the subject during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications, and non-prescription medications.

8 ASSESSMENT OF SAFETY

Assessment of safety will be conducted by blinded study personnel who are medically qualified by training and experience (e.g., study investigators) except in extraordinary circumstances where knowledge of whether AKST1210 was received by a subject is essential. When assessing relatedness, it should be assumed the subject was randomized to the AKST1210 column unless unblinding is required. Any instances of unblinding will be managed as indicated in Section 10.6.3 Breaking the Study Blind/Subject Code.

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENT AND ADVERSE DEVICE EFFECT

Per ISO 14155:2011 an adverse event (AE) is any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including laboratory findings) in subjects, users, or other persons, whether or not related to the investigational medical device.

- Note 1: This definition includes events related to the investigational medical device or control.
- Note 2: This definition includes events related to the procedures involved.
- Note 3: For users or other persons, this definition is restricted to events related to investigational medical devices.

Per ISO 14155:2011, an adverse device effect (ADE) is an AE that is assessed as related to the use of an investigational medical device:

- Note 1: This definition includes AEs resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.
- Note 2: This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENT AND SERIOUS ADVERSE DEVICE EFFECT

Per ISO 14155:2011 a serious adverse event (SAE) is an AE that:

a. Leads to death.

b. Leads to serious deterioration in the health of the subject, that either results in:
   - A life-threatening illness or injury, or
   - A permanent impairment of a body structure or a body function, or

- Inpatient or prolonged hospitalization, or
- Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or body function.

c. Leads to fetal distress, fetal death, or a congenital abnormality or birth defect.

Per ISO 14155:2011 a serious adverse device effect (SADE) is an ADE that results in any of the consequences characteristic of a SAE.

All SAEs should be reported in the timeframe outlined in Section 8.4

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

Each AE/ADE 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.

8.2.2 RELATIONSHIP TO STUDY DEVICE

Investigators are required to assess relatedness (i.e., whether there is reasonable possibility that the study device 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 device; 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 device, but the AE could also be attributed to concurrent or underlying disease, or the use of other devices or procedures. Possibly related should be used when the study device is one or several biologically plausible AE causes.
- Definitely Related: The AE is clearly related to use of the study device.

If either the investigator or the Sponsor considers the event related, then the event will be considered related for reporting purposes and will therefore be an ADE or SADE.

8.2.3 EXPECTEDNESS OF ADVERSE DEVICE EFFECTS

8.2.3.1 Unanticipated Adverse Device Effect

Per 21 CFR 812.3(s) an unanticipated adverse device effect (UADE), "means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects."

The investigator is required to make an initial assessment of whether an SADE is considered anticipated according to the known risks and associated device effects identified in Section 2.3.1 and the AKST1210 Investigator's Brochure. If an SADE is considered unanticipated by the investigator, it must be reported to the Sponsor or designee according to the timeframes outlined in Section 8.4.2.1. Once received, the Sponsor or designee will be responsible for making the final determination of whether the device effect is unanticipated for subsequent reporting to FDA.

8.3 TIME PERIOD/FREQUENCY FOR EVENT ASSESSMENT/FOLLOW-UP

At every clinic visit, subjects will be assessed for AEs and SAEs. After the subject 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 SAFETY ASSESSMENT

The investigator is not obligated to actively seek safety information in former study subjects, but the investigator is encouraged to notify the Sponsor or their designee of any AE/ADE or SAE/SADE occurring within 30 days after a subject 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/EFFECT REPORTING

All subjects who have given informed consent will be evaluated for AEs/ADEs. All AEs/ADEs that occur after initiation of treatment with the study device/control will be considered treatment emergent (TEAEs). Subjects with TEAEs must be followed until the AE/ADE is resolved or is stable, unless the subject is lost to follow-up.

Each AE/ADE 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 device (no action taken, device discontinued), outcome, relationship to study device* (unrelated, possibly related, definitely related), and seriousness criteria. Each AE/ADE must be recorded separately in the eCRF.

***Note**: Assessment of the relationship to study device will be made only when the AE occurs after the subject has initiated at least one (1) treatment with the study device. An AE occurring before the subject's exposure to study device will always be labeled as "unrelated."

Any AE/ADE or SAE/SADE occurring during the study must be documented in the subject's source documentation and as an AE/ADE in the CRF. All SAE/SADEs should be reported to the Sponsor according to the reporting timeframes specified in Section 8.4.2.1.

The investigator should attempt to establish a diagnosis of the event (that meets the definition of an AE/ADE or SAE/SADE) based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/ADE and/or SAE/SADE 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/ADE, each AE/ADE will be encoded according to the MedDRA. The investigator will take all appropriate and necessary therapeutic measures required for resolution of and AE/ADE. Any medication necessary for the treatment of an AE/ADE must be recorded on the concomitant medication CRF.

8.4.2 SERIOUS AND UNANTICIPATED ADVERSE EVENTS/EFFECTS REPORTING

8.4.2.1 Timeframes for Reporting

As per 21 CFR 812.150 an investigator shall submit to the Sponsor and to the reviewing IRB a report of any UADE (see Section 8.2.3.1) occurring during an investigation as soon as possible after the investigator first learns of the effect. Prompt notification of the Sponsor, and/or the Sponsor's representatives, and promptly providing requested follow-up information regarding such events is essential so that ethical and regulatory responsibilities and legal obligations can be satisfied. Investigators are responsible for reporting all SAEs (including SADEs and UADEs) according to the following timeframes:

- Initial notification (e.g., a completed SAE Report Form) and relevant source documents, if applicable, must be completed and faxed or emailed to [redacted] **within 24 hours** of observation or learning of the event.
  [redacted]
- Follow-up information must be sent to the [redacted] **within 24 hours** of receipt of information by the investigational site.

All SAEs (including SADEs and UADEs) must be reported to the IRB as soon as possible but in no event later than 10 working days after becoming aware of the event.

In addition to the IRB, the Sponsor will notify the FDA and all participating investigators in a safety report of potentially serious, unanticipated risks from clinical trials as soon as possible after the Sponsor receives the safety information and determines that the information qualifies for reporting:

- No later than 7 working 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 10 working days for all other UADEs.

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

8.4.2.2 SAE/SADE/UADE Information to Report

The SAE Report Form must be completed and faxed or emailed according to the timeframes specified above. The submission should include copies of relevant source documents/medical records, if applicable. At a minimum, the SAE Report Form must contain the subject number, verbatim term, onset date, relationship to study device, and a brief narrative of the event. Please note that **relationship to study device 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 discovery by the site staff should be documented in the source documents.

A separate SAE Report Form should be used to report 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 Report Form.

The investigator must record all relevant information regarding an SAE/SADE/UADE in the applicable sections of the report form, and the form must be signed by the investigator or his/her designee before transmittal to the Contract Research Organization (CRO) (see Section 8.4.2.1). It is very important that the investigator provide his/her assessment of relationship to the study device as well as an applicable diagnosis at the time of the initial SAE report. It is not acceptable for the investigator to send photocopies of the subject's medical records in lieu of completion of the appropriate SAE Report Form. 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 subject personal identifiers must be completely and thoroughly redacted prior to submission.

A blank SAE Report Form and instructions for reporting will be provided to the site and will be maintained in the investigator's study file.

If new information about an SAE/SADE/UADE 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.

The SAE/SADE/UADE pages of the CRF should also be completed as thoroughly as possible.

8.4.3 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 device exposure. The investigator must report any pregnancy that occurs in a female study subject or female partner of a male subject subsequent to first exposure to the study device until EOS, or within 3 months following a subject's last use of the device in the event of early termination. All pregnancies will be reported to the IRB, Sponsor,

and CRO. In the event of a pregnancy, treatment will be discontinued, and the subject will undergo continued safety follow-up through pregnancy outcome.

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 and Safety Monitoring Board (DSMB) will make recommendations concerning the continuation, modification, or termination of the trial.

8.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise, including ESRD and hemodialysis. 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 well-being of human subjects are protected; that the reported trial data are accurate, complete, and verifiable; and that the conduct of the trial 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).
- Monitoring will be performed to ensure the safety of clinical subjects 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.
- Co-monitoring visits and/or independent audits may be conducted by the Sponsor in accordance with a quality oversight plan or equivalent to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

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 unblinding of the study data.

10.2 STATISTICAL HYPOTHESES

Because the primary objective of the study is safety and tolerability, the study is not designed to detect statistically significant differences between the AKST1210 column and control treatment on the efficacy endpoints. The statistical approach toward secondary efficacy endpoints will be primarily descriptive; within-subject changes from baseline for each treatment group and among-group differences will be evaluated.

10.3 ANALYSIS DATASETS

Three (3) analysis datasets are possible; however, analyses may not necessarily be conducted with all 3:

- **Intention-to-Treat (ITT) Dataset**: all randomized subjects.
- **Safety Dataset**: all subjects who received HD after randomization.
- **Evaluable Dataset:** all subjects who complete through Week 8.
    - **Per Protocol Dataset:** a subset of the Evaluable Dataset. A detailed description of the reasons for exclusion from the Per Protocol population will be included in the SAP.

The presentation of baseline characteristics will be conducted on the ITT dataset. All safety analyses will be performed for the Safety Dataset. Analyses of the secondary endpoints will focus on the Evaluable Dataset.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

Using the Evaluable Dataset, all secondary endpoints will be summarized serially over time using descriptive statistics to assess the within-subject changes and between-group differences. Overall baseline and demographic data will be summarized using descriptive statistics.

For analysis of the primary and secondary endpoints, the following will be considered:

- For endpoints that are continuous in nature:
    - Number of observations, mean, median, minimum and maximum, and standard deviation (SD) values will be presented as descriptive summary
- For endpoints that are categorical in nature:
    - Frequency counts and percentages will be presented as descriptive summary

Subject disposition (e.g., the number of subjects randomized, completed, and discontinued) will be summarized, and medical history data will be listed. Prior and concomitant medications taken from Screening and during the study will be categorized by World Health Organization classification for therapeutic class and medication name, listed and summarized by number and percentage of subjects.

Final analyses are not limited to the summaries described herein. As noted above, analytical details and assumptions will be fully presented in the SAP.

10.4.2 ANALYSIS OF THE PRIMARY ENDPOINT

Safety and tolerability will be evaluated by examining the occurrence of TEAEs and AEs leading to de-escalation and/or discontinuation from the study.

Summary tabulations of the reported AEs will be presented by arm and column size after the verbatim terms have been coded to PTs and SOCs using the MedDRA Version 21.0 coding dictionary. The summaries will include severity and attribution to the study device. Multiple reports of the same AE by the same subject will be counted only once at the highest severity and strongest attribution to the study device.

Additional details are presented in Section 10.4.4.

10.4.3 ANALYSIS OF THE SECONDARY EFFICACY ENDPOINTS

The study is not powered to detect significant changes in cognition, function, etc.; however, using available data from analysis of the secondary efficacy endpoints, including changes in scores from baseline, descriptive summaries will be developed. Of particular interest will be the within-subject changes from baseline and their distribution around a null value of zero and a comparison between groups to evaluate any trends in differences between subjects randomized to active vs. control.

10.4.4 ANALYSIS OF THE SECONDARY SAFETY ENDPOINTS

Actual values and changes from baseline in clinical laboratory measurements, vital signs, and body weight, will also be assessed and summarized. Laboratory shift tables or graphics displaying the change (number of subjects) relative to the reference range from baseline to each study visit may also be presented for each test. The investigator should exercise his or her medical and scientific judgment in deciding and documenting whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

For secondary safety endpoints that are continuous in nature (e.g., clinical laboratory parameters, systolic and diastolic BP, heart rate, respiratory rate, body temperature, and body weight) the mean, median, minimum, maximum, and standard deviation will be summarized.

For secondary safety endpoints that are categorical in nature (e.g., physical examination or ECG abnormalities), the frequency counts and percentages will be presented as a descriptive summary.

Per-subject extent of exposure will be listed.

10.4.5 ADHERENCE AND RETENTION ANALYSES

Subject adherence with the study visits schedule, visit procedures, HD procedures, and subject retention will be assessed. Subject adherence may vary across the treatment arms. Reasons for study discontinuation will be compared across treatment arms and across other subgroups of interest, as appropriate.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

See Section 10.4.1.

10.4.7 PLANNED INTERIM ANALYSES

Not applicable.

10.4.8 ADDITIONAL SUBGROUP ANALYSES

Not applicable.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

No adjustments for multiplicity will be employed.

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

This will be further defined in the SAP.

10.4.11 EXPLORATORY ANALYSES

Not applicable.

10.5 SAMPLE SIZE

Approximately 26 male and female subjects, ≥ 40 years of age on HD with ESRD-CI, will be enrolled. Assuming a drop-out rate of 20%, enrollment at this level will yield approximately 20 evaluable subjects (evaluable is defined as completed through Week 8). Subjects will be randomized in a 1:1 ratio consisting of 1 treatment arm (AKST1210) and 1 non-active, control arm; n=10 per group. The sample size was chosen based on clinical considerations of an exploratory study designed to test safety and tolerability (Thabane 2010).

While the study is not statistically powered to detect differences in measures of clinical efficacy or biomarker endpoints, the proposed sample size may be sufficient to identify trends in efficacy endpoints that will be used to determine the appropriate sample size for subsequent studies.

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-blinded (the subject, investigator, and outcomes assessor will be blinded as to treatment assignment and randomized in a 1:1 ratio [AKST1210: control]). The randomization codes will be generated by a statistician that has no involvement in the study other than generation and maintenance of the randomization codes. Blinding and randomization procedures are described in the Device Procedures Manual.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

Success of blinding will be assessed based on all occurrences (intentional or unintentional) of unblinding of blinded study subjects, or study personnel (e.g., investigators, medical providers, outcomes assessors, the

Sponsor or their representatives). All intentional and unintentional unblinding will be documented and reported.

10.6.3 BREAKING THE STUDY BLIND/SUBJECT CODE

This is a double-blind study. Blinding procedures are described in the Device Procedures Manual.

If unintentional unblinding occurs during the study, root cause analysis will be evaluated, and corrective actions implemented as applicable.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 R2 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of regulatory agencies, the IRB, the Sponsor, or the Sponsor's representatives to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance 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 trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subject'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 accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject 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 CRF to be the only record of a subject's participation in the study. This is to ensure that anyone who would access the subject's medical record has adequate knowledge that the subject 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 this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in, 21 CFR Parts 11,50, 54, 56, 812, ICH E6 R2, and the Declaration of Helsinki.

12.2 INSTITUTIONAL REVIEW BOARD

This protocol and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IRB. Approval from the IRB 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 approved; a determination will be made regarding whether

previously consented subjects need to be re-consented.

Any amendments to the protocol must also be submitted to the IRB for approval prior to implementation.

If the investigator were to receive withdrawal of IRB approval, or other action on the part of the IRB that affects the study, the investigator will provide written notification within 5 days of the IRB decision to the Sponsor. If Alkahest were to receive withdrawal of IRB approval, or other action on the part of the IRB that affects the study, the Sponsor will provide written notification within 5 days of the IRB decision to the FDA and investigators.

12.3 INFORMED CONSENT PROCESS

12.3.1 CONSENT FORMS

Consent forms describing in detail the study device, study procedures, and risks are given to the subject or subject's LAR 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 from each subject participating in this study or their legally authorized representative after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures.

Subjects should have the opportunity to discuss the study with their family members or other advisors and the time to consider participation in the trial carefully. The subjects may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subjects 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-approved consent form that contains the elements required by ICH GCP and applicable regulatory requirements for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject and/or their legally authorized representative and the person obtaining consent. A copy of the signed consent form will be provided to the subject and/or their legally authorized representative. By signing the informed consent form, 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 subjects who provide consent will be assigned a unique study number. This number will be used to identify the subject throughout the clinical study and must be used on all study documentation related to the study subject. Once a number is assigned to a subject, that number will remain with that study subject 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 the Health Insurance Portability and Accountability Act (HIPAA).

12.4 PARTICIPANT AND DATA CONFIDENTIALITY

Subject 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 in addition to the clinical information relating to subjects. 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, 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 subjects in this study. The clinical study site will permit access to such records.

The study subject'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, Institutional regulations or Clinical Trial Agreement, whichever is longest. Subject data that is transmitted to the Sponsor, CRO, and/or IRB will not include contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study number. This unique study number should be recorded on non-local lab samples, requisitions, and any documents submitted to the CRO, Sponsor, and/or IRB. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected.

12.5 FUTURE USE OF STORED SPECIMENS

With the subject's approval and as approved by IRBs, biological samples may be stored at Alkahest, or designee, for future use. These samples could be used for future research designed to improve methods for diagnosis, prevention, and/or treatment. Alkahest will also be provided with a code-link that will allow linking the biological specimens with the specific data from each subject, maintaining blinding of the identity of the study subject. Subjects may choose whether the Sponsor can store and use samples for further research.

During the conduct of the study, an individual subject can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent for biospecimen storage will not be possible after the study is completed.

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 subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site. Data entered in the CRFs 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.

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

13.1.1 INVESTIGATOR RESPONSIBILITIES

The investigator will comply with the protocol (which has been approved/given favorable opinion by an IRB), 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 or other authorized study personnel are eligible to sign for the investigator, except where the investigator's signature is specifically required.

13.1.1.1 Reporting Responsibilities

Investigators are required to:

- Submit AEs/ADEs/SAEs/SADEs/UADEs to the study Sponsor or designee, as per the reporting requirements outlined in Section 8.4.
- Submit to their IRB a report of any UADEs, or events of subject death, occurring during an investigation as soon as possible but in no event later than 10 working days after the investigator first learns of the event.
- Notify the study Sponsor of withdrawal of IRB approval or other action on the part of the IRB that affects the study as soon as possible but in no event later than 5 working days.
- Notify the Sponsor, Monitor, and IRB of study progress at regular intervals but in no event less often than yearly.
- Notify the Sponsor/IRB of any significant deviations from the investigational plan for emergency purposes, as soon as possible but in no event later than 5 working days after deviation occurs to protect the life or physical well-being of a subject in an emergency.
  - For non-emergency purposes, obtain prior approval by Sponsor and, if deviation may affect scientific soundness of the trial or the rights, safety, or welfare of subject, also obtain approval by the IRB and FDA as an Investigational Device Exemption (IDE) supplement.
- Notify the Sponsor and IRB if a subject is treated with an investigational device without first obtaining informed consent, within 5 working days of use of the investigational device.
- Notify the Sponsor and IRB with a final study report within 3 months after termination or completion of study or termination of site's participation.

13.1.2 STUDY FILES

Investigators are required to maintain on file the following accurate, complete, and current records relating to this study:

- All correspondence relating to the study with another investigator, an IRB involved in the study, Alkahest, Inc., a monitor, or FDA, including required reports.
- Records of receipt, use, or disposition of a device.
- All clinical forms and documentation, including:
  - Records of each subject's case history and exposure to the device.
  - A copy of the signed subject consent form.
  - Date and time of exposure to the investigational device.
  - All procedure and follow-up report forms, including supporting documents.
  - Records of any adverse event or device effect, including supporting documentation.
  - Records pertaining to subject deaths during the study.
  - The study protocol/amendments including documentation and rationale for any deviations from the clinical protocol.
  - Any other records required by Alkahest, Inc. or FDA.

The investigator's study file will contain all of these documents, as well as staff curriculum vitae and authorization forms, and other appropriate documents and study-specific manuals (e.g., Device Procedures Manual).

13.2 STUDY RECORDS RETENTION

Per 21 CFR 812.140(d), an investigator or Sponsor shall maintain the records required by this subpart during the investigation and for a period of 2 years after the latter of the following 2 dates: the date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application, a notice of completion of a product development protocol, a humanitarian device exemption application, a premarket notification submission, or a request for De Novo classification.

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 subject and all signed informed consents are included for at least 15 years after completion of the trial. 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 device, 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 subject, 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 subject, 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 which may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect the rights, safety, and/or wellbeing of study participants. Examples of Major Protocol Deviations include, but are not limited to:

- Failure to obtain informed consent (i.e., no evidence of informed consent)
- Enrolling subjects in violation of key eligibility criteria designed to ensure a specific subject population
- A device dispensing or related error that could have affected the safety of the subject
- Failure to collect data necessary to interpret the primary endpoint(s), as this may compromise the scientific value of the trial.

Major Protocol Deviations may result in data that are not deemed evaluable for the *per protocol* analysis and/or may require that subjects are discontinued from the study.

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 wellbeing of study participants or the study outcomes or data quality. Examples of Minor Protocol Deviations include, but are not limited to:

- A protocol visit date outside of a visit window
- An isolated case of a missed or incomplete study procedure
- An isolated incident of a missed or incomplete study evaluation

Minor Protocol Deviations would not generally preclude subject data from the *per protocol* analysis population. Observations categorized as Minor may become Major if not corrected.

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

Per 21 CFR 812.150(a)(4), it is the responsibility of the site to use continuous vigilance to identify and report deviations promptly (within 5 working days) 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 per their guidelines. The site investigator/study staff is responsible for knowing and adhering to their IRB requirements.

13.4 PUBLICATION AND DATA SHARING POLICY

In compliance with The International Committee of Medical Journal Editors (ICMJE) clinical trials registration policy and Section 801 of the Food and Drug Administration 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. 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 subjects. 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 Clinical Study Report, investigators may publish results of one or more center's subjects 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 investigator and Alkahest, Inc. or its authorized representative before the study device 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

15.1 SCHEDULE OF STUDY EVENTS

SECTION 15.1.1 SCREENING AND RUN-IN

Procedures							
Visit	Visit 1: Screening[1]	Visits 1.1 – 1.6: Run-In					
Visit Number	V1	V1.1	V1.2	V1.3	V1.4	V1.5	V1.6
Study Week	Week -6 to -2	Week -2 to -1					
Study Day(s)	Days -42 to -14	Days -14 to -1					
Informed consent	X						
Demographics	X						
Medical history	X						
Physical examination	X						
12-lead ECG	X						
Height	X						
Weight	X	X	X	X	X	X	X
Targeted physical exam		X	X	X	X	X	X
Vital signs[2]	X	X	X	X	X	X	X
Laboratory tests[3]	X		X[4]				X[4]
Serum pregnancy test (WOCBP only)	X					X (V1.5 or V1.6)	
Hemodialysis @ maximum flow rate of 250 mL/min		X	X	X	X	X	X
MoCA, PHQ-9[5]	X						
Cogstate test battery[6]		Complete a total of two familiarization assessments prior to V2.1					
Blood sample for b2M[7]	X (local lab)					X	
Blood sample for plasma proteomics[7]						X	
Adverse events and concomitant medications	X	X	X	X	X	X	X

1. The Screening Visit may be split to allow for sufficient time to complete all procedures. AEs and concomitant medications should be reviewed during split visits, as applicable.
2. For additional information, see Section 7.1.1.2.2.
3. For additional information, see Section 7.1.1.1.5 and Section 15.2 Schedule of Laboratory Tests.
4. Laboratory testing for BUN, creatinine, and aPTT only. Samples should be taken prior to HD and again mid-way through HD (~2 hrs into treatment). Samples for aPTT may be taken anytime blood clots are suspected in the circuit or at the discretion of the investigator.
5. For additional information, see Section 7.1.1.1.7 and Section 7.1.1.1.8.
6. For additional information, see Section 7.1.1.3.3.
7. For additional information, see Section 7.1.1.1.9. The V1 b2M sample is to be assayed at a local lab for screening purposes. This sample should be collected prior to HD or on a non-dialysis day.
8. For additional information, see Section 7.2.2.1 and Section 15.2 Schedule of Laboratory Tests.

SECTION 15.1.2 TREATMENT PERIOD 1 (VISIT 2/MONTH 1)

Procedures												
Visit	Visit 2: Treatment Period 1											
Visit Number	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	2.10	2.11	2.12
Study Week	Week 1			Week 2			Week 3			Week 4		
Study Day (± 1 day)	Day 1	Day 3	Day 5	Day 8	Day 10	Day 12	Day 15	Day 17	Day 19	Day 22	Day 24	Day 26
Verify eligibility[1]	X											
Randomization[2]	X											
HD (250 mL/min for ~4 h)	X	X	X	X	X	X	X	X	X	X	X	X
AKST1210 S-15 or control	X	X	X	X	X	X	X	X	X	X	X	X
Vitals signs[3]	X	X	X	X	X	X	X	X	X	X	X	X
Targeted physical exam	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory tests (local lab)[4]	X	X			X		X	X				X
Cogstate test battery, FACIT, PSQI, SF-36	X											
Blood samples for b2M[5]											X	
Blood samples for plasma proteomics[6]											X	
Assessment of fluid status[7]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events and concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Monitor tolerability per Appendix 17.1	X	X	X	X	X	X	X	X	X	X	X	X[8]

1. Assess tolerability of Run-in period prior to randomization (see Section 5.2).
2. Refer to the Device Procedures Manual for guidance.
3. Vital signs to be collected at multiple timepoints, see Section 7.1.1.2.2.
4. For additional information, see Section 7.1.1.2.5 and Section 15.2 Schedule of Laboratory Tests.
5. For additional information, see Section 7.1.1.1.9 and Section 15.2 Schedule of Laboratory Tests.
6. For additional informaiton, see Section 7.2.2.1 and Section 15.2 Schedule of Laboratory Tests.
7. For additional information, see Section 7.1.1.2.3.
8. Post HD, if subject has not already been discontinued, evaluate for escalation to S-25 per Appendix 17.1.

SECTION 15.1.3 TREATMENT PERIOD 2 (VISIT 3/MONTH 2)

Procedures												
Visit	Visit 3: Treatment Period 2											
Visit Number	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	3.10	3.11	3.12
Study Week	Week 5			Week 6			Week 7			Week 8		
Study Day (± 1 day)	29	31	33	36	38	40	43	45	47	50	52	54
HD (250 mL/min for ~4 h)	X	X	X	X	X	X	X	X	X	X	X	X
AKST1210 S-25 or control	X	X	X	X	X	X	X	X	X	X	X	X
Vitals signs[1]	X	X	X	X	X	X	X	X	X	X	X	X
Targeted physical exam	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory tests (local lab)[2]	X	X			X		X	X				X
Cogstate test battery, FACIT, PSQI, SF-36	X											
Blood samples for b2M[3]											X	
Blood samples for plasma proteomics[4]											X	
Assessment of fluid status[5]	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events and concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Monitor for de-escalation or discontinuation per Appendix 17.1	X	X	X	X	X	X	X	X	X	X	X	X[6]

1. Vital signs to be collected at multiple timepoints, see Section 7.1.1.2.2.
2. For additional information, see Section 7.1.1.2.5 and Section 15.2 Schedule of Laboratory Tests.
3. For additional information, see Section 7.1.1.1.9 and Section 15.2 Schedule of Laboratory Tests.
4. For additional information, see Section 7.2.2.1 and Section 15.2 Schedule of Laboratory Tests.
5. For additional information, see Section 7.1.1.2.3.
6. Post HD, if subject has not already been de-escalated or discontinued, evaluate for escalation to S-35 per Appendix 17.1.

SECTION 15.1.4 TREATMENT PERIOD 3 (VISIT 4/MONTH 3)

Procedures												
Visit	Visit 4: Treatment Period 3											End of Tx
Visit Number	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	4.10	4.11	4.12
Study Week	Week 9			Week 10			Week 11			Week 12		
Study Day (± 1 day)	57	59	61	64	66	68	71	73	75	78	80	82
HD (250 mL/min for ~4 h)	X	X	X	X	X	X	X	X	X	X	X	Refer to Section 15.1.5
AKST1210 S-35 or control	X	X	X	X	X	X	X	X	X	X	X	
Vitals signs[1]	X	X	X	X	X	X	X	X	X	X	X	
Targeted physical exam	X	X	X	X	X	X	X	X	X	X	X	
Weight	X	X	X	X	X	X	X	X	X	X	X	
Laboratory tests[2]	X	X			X		X	X		X		
Cogstate test battery, FACIT, PSQI, SF-36	X											
Blood samples for b2M[3]											X	
Blood samples for plasma proteomics[4]											X	
Assessment of fluid status[5]	X	X	X	X	X	X	X	X	X	X	X	
Adverse events and concomitant medications	X	X	X	X	X	X	X	X	X	X	X	
Monitor for de-escalation or discontinuation per Appendix 17.1	X	X	X	X	X	X	X	X	X	X	X	

1. Vital signs to be collected at multiple timepoints, see Section 7.1.1.2.2.
2. For additional information, see Section 7.1.1.2.5 and Section 15.2 Schedule of Laboratory Tests.
3. For additional information, see Section 7.1.1.1.9 and Section 15.2 Schedule of Laboratory Tests.
4. For additional information, see Section 7.2.2.1 and Section 15.2 Schedule of Laboratory Tests.
5. For additional information, see Section 7.1.1.2.3.

SECTION 15.1.5 END OF TREATMENT AND END OF STUDY VISITS

Procedures		
Visit	Visit 5: End of Treatment	Visit 6: End of Study (EOS)
Study Week	Week 12 (continued)	Week 14
Study Day	Day 82 ± 1	Day 96 ± 3
Targeted physical examination	X	X
Weight	X	X
12-lead ECG		X
Vital signs[1]	X	X
Laboratory tests[2]	X[2]	X[3]
Serum pregnancy test (WOCBP only)		X
HD	250 mL/min flow rate for ~4h	X (flow rate per standard of care)
AKST1210 or Control	X	
MoCA, PHQ-9		X
Cogstate test battery, FACIT, PSQI, SF-36	X	X
Blood sample for b2M[4]		X
Blood sample for plasma proteomics[5]		X
Assessment of fluid status[6]	X	X
Adverse events and concomitant medications	X	X

1. For additional information, see Section 7.1.1.2.2.
2. Refer to Section 15.2 Schedule of Laboratory Tests.
3. Refer to Section 7.1.1.2.5 and Section 15.2 Schedule of Laboratory Tests.
4. For additional information, see Section 7.1.1.1.9 and Section 15.2 Schedule of Laboratory Tests.
5. For additional information, see Section 7.2.2.1 and Section 15.2 Schedule of Laboratory Tests.
6. For additional information, see Section 7.1.1.2.3.

15.2 SCHEDULE OF LABORATORY TESTS

		Schedule of Laboratory Tests								
		Screening	Run-In		Treatment Period V2 [Period 1] / V3 [Period 2] / V4 [Period 3]				End-of-Treatment V5	End-of-Study V6
Study Day(s) (± 1 day)		**V1**	**V1.5**	**V1.2, V1.6**	**D1, D29, D57**	**D3, D10, D17, D26, D31, D38, D45, D54, D59, D66, D73, D78**	**D15, D43, D71**	**D24, D52, D80**	**D82**	**D96±3**
Proteomics and b2M										
	Blood sample for b2M (local lab for screening only)	X								
	Blood sample for plasma proteomics (research sample)		X					X		X
	b2M (research sample)		X					X		X
Serum Pregnancy		X	X (V1.5 or V1.6)							X
Hematology										
	Complete blood count (CBC)	X			X				X	X
	Neutrophils	X			X				X	X
	Total lymphocytes	X			X				X	X
	Monocytes	X			X				X	X
	Eosinophils	X			X				X	X
	Basophils	X			X				X	X
	Iron	X			X				X	X
	Hemoglobin (HgB)	X			X		X		X	X

		Schedule of Laboratory Tests								
		Screening	Run-In		Treatment Period V2 [Period 1] / V3 [Period 2] / V4 [Period 3]				End-of-Treatment V5	End-of-Study V6
Study Day(s) (± 1 day)		**V1**	**V1.5**	**V1.2, V1.6**	**D1, D29, D57**	**D3, D10, D17, D26, D31, D38, D45, D54, D59, D66, D73, D78**	**D15, D43, D71**	**D24, D52, D80**	**D82**	**D96±3**
Chemistry										
	Sodium	X			X					X
	Calcium (Total)	X			X					X
	Potassium	X			X					X
	Chloride	X			X					X
	Bicarbonate	X			X					X
	Blood urea nitrogen (BUN)	X		X	X	X			X	X
	Creatinine	X		X	X	X			X	X
	Protein (total)	X			X					X
	Albumin	X			X					X
	Bilirubin (total)	X			X					X
	Aspartate transaminase (AST)	X			X					X
	Alanine aminotransferase (ALT)	X			X					X
	Gamma-glutamyl transpeptidase (GGT)	X								X
	Alkaline phosphatase (ALP)	X								X
	Amylase	X								X
	Lipase	X								X
	Lactate dehydrogenase (LDH)	X								X
	Creatine kinase (CK)	X								X
	Glucose	X			X					X
	Hemoglobin A1c (HbA1c)	X								X
	Phosphate	X			X					X

		Schedule of Laboratory Tests								
		Screening	Run-In		Treatment Period V2 [Period 1] / V3 [Period 2] / V4 [Period 3]				End-of-Treatment	End-of-Study
									V5	V6
Study Day(s) (± 1 day)		**V1**	**V1.5**	**V1.2, V1.6**	**D1, D29, D57**	**D3, D10, D17, D26, D31, D38, D45, D54, D59, D66, D73, D78**	**D15, D43, D71**	**D24, D52, D80**	**D82**	**D96±3**
	Magnesium	X								X
	Cholesterol	X								X
	Triglycerides	X								X
	High-density lipoprotein (HDL)	X								X
	Low-density lipoprotein (LDL)	X								X
Coagulation										
	Prothrombin time/ international normalized ratio (PT/INR)	X			X					X
	Activated partial thromboplastin time (aPTT)	X			X	X			X	X
Serology										
	Hepatitis B surface/core antigen (HBsAg)	X								
	Hepatitis C antibody (HCV Ab)	X								
	Human immunodeficiency virus antibody (HIV Ab, HIV1/HIV-2)	X								
Other Clinical Labs										
	Thyroid-stimulating hormone (TSH ultrasensitive)	X								X

16 REFERENCES

16.1 PUBLISHED REFERENCES

[redacted]

Buysse DJ, Reynolds CF III, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. J Psych Res. 1989;28(2):193-213.

Chu NM, Deng A, Ying H, Haugen CE, Wang JMG, Segev DL, et al. Dynamic frailty before kidney transplantation: time of measurement matters. Transplantation. 2019;103:1700-1704.

Clinical Trial Facilitation Group. Recommendations related to contraception and pregnancy testing in clinical trials. Version 2014-09-15:1-13.

Griva K, Thompson D, Jayasena D, Davenport A, Stanton MH, Newman P. Cognitive functioning pre- to post-kidney transplantation—a prospective study. Nephrol Dial Transpl. 2006;21(11):3275–3282.

Gupta A, Rebecca J, Lepping RJ, Yua ASL, Perea RD, Honea RA, et al. Cognitive function and white matter changes associated with renal transplantation. Am J Nephrol. 2016;43:50–57.

[redacted]

K/DOQI Workgroup. K/DOQI Clinical Practice Guidelines. K/DOQI clinical practice guidelines for cardiovascular disease in dialysis patients. Am J Kidney Dis. 2005;45(4 Suppl 3): S1–153.

Kessler M, Moureau F, Nguyen P. Anticoagulation in chronic hemodialysis: progress toward an optimal approach. Sem Dialysis. 2015;28:474-489.

Kroenke K, Spitzer RL, Williams JBW. Validity of a brief depression severity measure. J Gen Intern Med. 2001;16:606-613.

Kurella-Tamura M, Wadley V, Yaffe K, et al. Kidney function and cognitive impairment in US adults: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Kidney Dis. 2008; 52(2): 227-234.

Laucis NC, Hays RD, Bhattacharyya T. Scoring the SF-36 in orthopaedics: a brief guide. J Bone Joint Surg Am. 2015;97:1628-1634.

Lichtenstein D: Novel approaches to ultrasonography of the lung and pleural space: where are we now? Breathe. 2017;13:100–111.

Murray AM, Bell EJ, Tupper DE, Davey CS, Pederson SL, Amiot EM, et al: The brain in kidney disease (BRINK) cohort study: design and baseline cognitive function. Am J Kidney Dis. 2016;67(4):593–600.

Nasreddine ZS, Phillips NA, Bedirian V,Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Am Geriatr Soc. 2005;53:695-699.

Rodriguez HJ, Domenici R, Diroll A, Goykhman I. Assessment of dry weight by monitoring changes in blood volume during hemodialysis using Crit-Line. Kidney Intl. 2005;68:854-861.

Roumelioti ME, Trietley G, Nolin TD, Ng YH, Zu Z, Alaini A, et al. Beta-2 microglobulin clearance in high-flux dialysis and convective dialysis modalities: a meta-analysis of published studies. Nephrol Dial Transplant. 2018;33(3):542-552.

Sarnak M, Tighiouart H, Scott T, Lou KV, Sorensen EP, Giang LM, et al. Frequency and risk factors for poor cognitive performance in hemodialysis patients. Neurology. 2013;80:471–480.

Smith LK, He Y, Park JS, Bieri G, Snethlage CE, Lin K, et al: β_2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. Nat Med. 2015;21:932-937.

Tamura MK, Vittinghoff E, Hsu C, Tam K, Seliger SL, Sozio S,et al: Loss of executive function after dialysis initiation in adults with chronic kidney disease. Kidney Int. 2017;91:948–953

Thabane L, Ma J, Chu R, Cheng J, Ismaila A, Rios LP, et al. A tutorial on pilot studies: the what, why and how. BMC Med Res Methodol. 2010;10:1.

Webster K, Cella D, Yost K. The functional assessment of chronic illness therapy (FACIT) measurement system: properties, applications and interpretation. Health Qual Life Outcomes. 2003;1(79):1-7.

[redacted]

16.2 UNPUBLISHED REFERENCES

AKST1210 Investigator's Brochure. August, 2019.

ALK-PC-030. A study of beta 2-microglobulin removal for therapeutic utility in cognitive processes. June 02, 2017.

[redacted]

17 APPENDICES

APPENDIX 17.1 ESCALATION, DE-ESCALATION, AND DISCONTINUATION RULES FOR TREATMENT WITH AKST1210 OR CONTROL

The following definitions are relevant to evaluating whether to escalate, de-escalate, or discontinue a subject:

- ***Intradialytic hypotension/hypotensive event (IDH)***: A decrease in SBP ≥ 20 mm Hg or a decrease in MAP ≥10 mm Hg associated with symptoms that include: abdominal discomfort; yawning; sighing; nausea; vomiting; muscle cramps; restlessness; dizziness or fainting; and anxiety (K/DOQI Workgroup 2005).
- ***Expected IDH Rate***: The expected number of occurrences of IDH per 4-week treatment period used to assess escalation, de-escalation, and discontinuation. Expected IDH is derived from a subject's Historical IDH Rate (see table below).
- ***Historical IDH Rate***: The number of occurrences of IDH as documented in a subject's medical record during a recent, consecutive 8-week interval prior to randomization. This 8-week period may include the 2-week Run-in. Subjects with 5 or more IDH events during this 8-week interval will be excluded.

The table below provides the corresponding Expected IDH Rate based on the Historical IDH Rate and summarizes the rules for escalation, de-escalation, or discontinuation based on the number of occurrences of IDH.

Historical IDH Rate over 8 weeks	Expected IDH Rate over 4 weeks	Escalation Rule	De-escalation or Discontinuation Rule
0	0	1 or less	2 in 4 weeks
1	1	2 or less	3 in 4 weeks
2	1		
3	2	3 or less	4 in 4 weeks
4	2		

Escalation Rules: A subject may be escalated to the next larger column size if they experience no more than 1 IDH event above their Expected IDH Rate in a 4-week treatment period and has an Hgb level ≥ 9 g/dL.

De-escalation Rules: A subject should be de-escalated to a previously tolerated column size if at any time during a 4-week treatment period they experience 2 or more IDH events above the Expected IDH Rate and/or has a Hgb level < 9 g/dL despite optimized ESA treatment.

Discontinuation Rules: A subject should be discontinued if they experience 2 or more IDH events above their Expected IDH Rate in a 4-week treatment period after de-escalation to the lowest column size (AKST1210 S-15) and/or has an Hgb level < 9 g/dL despite optimized ESA treatment after de-escalation to the lowest column size (AKST1210 S-15). Subjects may also be discontinued at any time at the discretion of the investigator.

As a reminder, all occurrences of IDH after the time of consent should be recorded as AEs. Refer to Section 8 for additional information concerning safety reporting.

18 REVISION HISTORY

18.1 SUMMARY OF CHANGES

Protocol Version 2.1 dated 14FEB2020
Replaces: Protocol Version 2.0 dated 24JAN2020

The following table describes changes from Version 2.0 (dated 24JAN2020) with justifications provided.

Section(s)	Description	Justification
Throughout	Protocol version update. ***Previously read***: V2.0_24JAN2020 ***Now reads***: V2.1 14FEB2020	Version control.
5.2	***Previously read:*** Pregnant or breast-feeding subjects or subjects who are planning to become pregnant. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative serum pregnancy test at Screening, prior to treatment, and at EOS. WOCBP must agree to use highly effective contraception (Clinical Trial Facilitation Group 2014) prior to study entry. A WOCBP is defined as a woman who can become pregnant. 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 ***Now reads***: Pregnant or breast-feeding subjects or subjects who are planning to become pregnant. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative serum/urine pregnancy test at Screening, prior to treatment, and at EOS. WOCBP and men must agree to use highly effective contraception (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.	Reverted to the original language to ensure safety, including the need for male subjects to use highly effective contraception for safety reasons.
15.1.2, 15.1.3, 15.1.4	Updated the Schedule of Events to indicate that local laboratory testing will be performed on Days 15, 43, and 71, as specified in Section 15.2.	For protocol clarity and consistency with the Schedule of Laboratory Tests.

Protocol Version 2.0 dated 24JAN2020
Replaces: Protocol Version 1.2 dated 13NOV2019

The following table describes changes from Version 1.2 (dated 13NOV2019) with justifications provided.

Section(s)	Description	Justification
Throughout	Protocol version update. ***Previously read:*** V1.2 13NOV2019 ***Now reads:*** V2.0_24JAN2020	Version control.
Throughout	Minor grammar and content updates.	Minor grammar/content updates for clarity/accuracy of content.
Table of Contents	Updated to reflect content changes.	Minor updates required to reflect revised content.
List of Abbreviations	Minor content updates.	Minor updates required to reflect revised content.
List of Definitions	Added List of Definitions.	To clarify important definitions relevant throughout the protocol for evaluation of eligibility and safety monitoring.
[redacted]	[redacted]	[redacted]
Protocol Summary (Précis)	Updated to account for all study visits, including Screening and End of Study.	Revised for clarity and consistency.
Protocol Summary (Précis), 4.1	***Added***: Subjects who may occasionally require an additional HD session per week would not receive the intervention on that additional day.	Incorporated for clarity to ensure that the column is not used as part of HD more than 3 times per week in subjects who may occasionally require an additional HD session.
Protocol Summary (Endpoints), 4.2.2	Updates to the list of secondary endpoints: ***Previously read:*** Feasibility of each column size escalation as measured by subject compliance with study visit schedule/completion, visit procedures, and subject retention. ***Now reads:*** • Feasibility of all procedures, including blinding. • Tolerability of each column size escalation as measured by subject compliance with study visit schedule/completion, visit procedures, and subject retention.	Revised for clarity and inclusion of additional endpoints to enable quantification of the occurrence of IDH and anemia.

	Added: • The change in IDH Rate above each subject's Historical IDH Rate for each column size. • The incidence of IDH leading to de-escalation or discontinuation. • The incidence of worsening anemia by column size. • The incidence of anemia leading to de-escalation or discontinuation.	Revised for clarity and to enable quantification of the occurrence of IDH and anemia.
	Previously read: Change from baseline in b2M concentration in post-HD circuit and post-AKST1210 levels by column size and duration of treatment at Week 4, Week 8, and Week 12, and Week 14 at End of Study (EOS) (2 weeks after treatment conclusion). ***Now reads:*** Change from baseline in levels of b2M at Weeks 4, 8, 12 and EOS and evaluation of any differences in the magnitude of change based on column size and duration of treatment with a specific column size.	Revised for clarity.
	Previously read: Change in cognition per the Cogstate test battery (i.e., verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall). ***Now reads***: Change in cognitive domains as measured with the Cogstate battery (i.e., verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall).	Revised for clarity.
Protocol Summary (Subject Duration), Schematic of Study Design	Screening period extended from -4 to -2 weeks to -6 to -2 weeks (-42 to -14 days); thus, duration for subjects may be extended an additional 2 weeks (also see changes to Schedule of Study Events [15.1.1] detailed below).	Screening period extended to enhance study feasibility.
[redacted]	[redacted]	[redacted]
2.3.1, Table 1	Modified Table 1 to provide more explicit mitigation strategies/preventative measures for IDH and anemia.	Updated to provide clarity regarding mitigation strategies that serve to minimize the risk of IDH and anemia from occurring in study subjects.
2.3.1	***Added***: […] because data on the potential effects of	Updated to provide guidance

	AKST1210 on plasma levels of medications are limited, it is recommended that all IV and oral medications be given after each dialysis session, unless clinically needed before or during dialysis.	as to the timing of IV medications given that data regarding potential clearance of medications is limited.
4.1, Schedule of Events	Extended Screening Visit window from 2 weeks (Day -28 to -14) to 4 weeks (Day -42 to -14).	To enhance feasibility of performing required screening assessments within window.
4.1	Added language to reflect that informed consent may be obtained by a subject's legally authorized representative (LAR) when applicable.	Updated for clarity and consistency with Section 12.
4.1, Schedule of Events	Incorporated language to clarify that the blood sample to assess b2M levels at Screening should be sent to a site's local laboratory.	Updated for clarity and consistency.
4.1, 5.2, Schedule of Events	Incorporated language to clarify that eligibility will be assessed during Run-in.	Updated for clarity and consistency.
4.1	Modified to include a more comprehensive overview of study visit procedures (e.g., targeted physical examinations, monitoring of vital signs).	Updated for clarity and consistency with Section 7 and the Schedule of Events.
5.1	***Previously read***: 5. Medical records indicating occurrence of < 5 intradialytic hypotensive events related to HD in 8 weeks prior to randomization. ***This has been revised to be an exclusion criterion (see Section 5.2, new #7, bullet 1) and Now reads:*** Subjects with medical records indicating the occurrence of ≥ 5 events of IDH during a recent, consecutive 8-week interval prior to randomization. Modified the window in which subjects should be on a stable regimen of either medications for cognition and/or antidepressants to be 8 weeks prior to Screening.	Updated for clarity and to enhance protocol feasibility in obtaining sufficient medical records to assess a subject's Historical IDH Rate. Updated for clarity and to help ensure consistency in results obtained from the scales conducted during Screening.
5.2	Updated exclusion criterion #2 as follows: ***Previously read*:** Subjects who are not able to tolerate extended duration of > 4 hours at a maximum flow rate of 250 mL/min. ***Now reads*:** During Run-in, subjects who are not able to comply with or tolerate extended HD duration of approximately 4 hours at a maximum flow rate of 250 mL/min as assessed by the investigator. ***Added*** exclusion criterion #3 as follows: Subjects who experience 3 or more IDH events	Clarification of evaluation period for tolerability of flow rate. Incorporated to clarify

	during the Run-in period. [redacted] [redacted] Updated exclusion criterion #5 (previously #4) as follows: ***Previously read*:** Subjects for whom extracorporeal circulation therapy is contraindicated, such as those with severe cardiac insufficiency, acute myocardial infarction, severe cardiac arrhythmia, acute seizure disorder, severe uncontrolled hypertension, or hypotension requiring or expected to require extensive fluid management. ***Now reads*:** Subjects for whom extracorporeal circulation therapy is contraindicated, such as those with severe cardiac insufficiency, acute myocardial infarction, severe cardiac arrhythmia, acute seizure disorder, or severe uncontrolled hypertension. Updated exclusion criterion #7 (previously #6) as follows: ***Previously read*:** Subjects who are at a high risk of hypotension from their HD history based on investigator assessment. ***Now reads:*** Subjects who are at a higher risk of IDH including: • Subjects with medical records indicating the occurrence of ≥ 5 events of IDH during a recent, consecutive 8-week interval prior to the Run-in period; • Subjects requiring or expected to require	evaluation of IDH events during Run-in and define parameters for exclusion [redacted] Moved content regarding hypotension and more clearly defined this in exclusion criterion #7. Incorporated additional exclusion criteria to clarify exclusion criteria for subjects at a higher risk of IDH; all exclusion criteria pertaining to hypotension have been grouped together for clarity.

	extensive fluid management; • Presence of pre-dialysis hypotension, defined as a systolic blood pressure (SBP) < 90 mm Hg and/or a diastolic blood pressure (DBP) < 50 mm Hg, before any of the last 3 dialysis sessions prior to screening; • A diagnosis of interdialytic hypotension; • A diagnosis of IDH; and/or • A diagnosis of autonomic dysfunction.	
	Updated exclusion criterion #8 (previously #7) as follows: ***Previously read*:** Pregnant or breast-feeding subjects or subjects who are planning to become pregnant. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative serum/urine pregnancy test at Screening, prior to treatment, and at EOS. WOCBP and men must agree to use highly effective contraception (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. ***Now reads*:** Pregnant or breast-feeding subjects or subjects who are planning to become pregnant. Female subjects must not be pregnant or breastfeeding. Women of childbearing potential (WOCBP) must have a negative serum pregnancy test at Screening, prior to treatment, and at EOS. WOCBP must agree to use highly effective contraception (Clinical Trial Facilitation Group 2014) prior to study entry. A WOCBP is defined as a woman who can become pregnant. 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.	Revised to clarify that women who have undergone sterilization are not considered WOCBP; removed urine pregnancy test as only a serum pregnancy test will be used; eliminated the need for men to use highly effective contraception based on safety information from previous trials.
	Added exclusion criterion #12 as follows: Subjects on anti-seizure medications for a seizure disorder.	Incorporated to exclude subjects requiring anti-seizure medications.

	Added exclusion criterion #14 as follows: Subjects with a hemoglobin level < 9 g/dL.	Incorporated to exclude subjects with anemia.
	Updated exclusion criterion #15 (previously #12) as follows: ***Previously read*:** Concurrent or recent participation in another investigational clinical trial. • Prior clinical trial subjects must have discontinued investigational agents at least 30 days prior to Screening for small molecules, and 1 year prior to Screening for vaccine or immunotherapy agents. ***Now reads*:** Concurrent or recent participation in another investigational clinical trial. Prior clinical trial subjects must have discontinued investigational agents at least 30 days prior to Screening.	Clarification of timing of use for investigational agents prior to Screening.
	Updated exclusion criterion #16 (previously #13) as follows: ***Previously read*:** History of severe depression/suicidality requiring hospitalization. ***Now reads*:** History of severe depression/suicidality requiring hospitalization in the last 6 months.	Clarification of timing of hospitalization for severe depression/suicidality.
5.3, 5.4.2	Content revised to indicate that subjects who discontinue or are unblinded prior to Week 8 may be replaced (previously indicated prior to Week 5).	Revised to provide clarity regarding timing for replacement of subjects.
5.4.1	***Added***: • A subject will be discontinued if the number of IDH events during a treatment period exceeds a predetermined threshold above the subject's Historical IDH Rate after de-escalation to the lowest column size (see Appendix 17.1). • A subject will be discontinued if hemoglobin levels remain < 9 g/dL despite optimized iron and ESA treatment and after de-escalation to the lowest column size (see Appendix 17.1).	Clarification to align with revised Definitions, study procedures, and Appendix 17.1.
6.1.2.1	Clarified that the control will consist of a covered surrogate object of similar size/shape as the AKST1210 column and incorporated a reference to the Device Procedures Manual.	Revised for clarity and consistency.
6.1.5	***Added***: Careful attention should be given to ensure that all subjects, regardless of treatment assignment, undergo the same evaluations for escalation, de-escalation, and discontinuation.	Added for clarity and to ensure adequacy of blinding procedures.
6.1.6	Removed Section 6.1.6 Route of Administration	Route of administration is not

		applicable, and information regarding how the device is connected to the circuit is described elsewhere in the protocol (6.1.4) and Device Procedures Manual.
7.1.1.1, Schedule of Study Events (15.1.1-15.1.5)	Updated to account for all procedures performed at Screening and during Run-in. ***Added***: The Screening Visit may be split to allow for sufficient time to complete all required procedures.	To provide clarity and consistency. A split visit at Screening is intended to aid feasibility.
7.1.1.1.5, 7.1.1.1.9, 7.1.1.2.5, 7.2.1, 7.2.3, Schedule of Study Events (15.1.1-15.1.5)	Modified to more clearly specify laboratory tests that should be conducted at Screening and Run-in and which laboratory tests should be performed locally versus those required to be stored and shipped to a repository. Samples for screening labs should be collected either on a non-dialysis day or prior to HD. In addition, urine testing will be eliminated.	Updated to provide enhanced clarity; urine testing removed.
7.1.1.1.6	Added a reference to Table 1 in Section 2.3.1 to highlight proposed mitigations for known safety risks, such as IDH and anemia.	Updated to provide cross-reference to additional assessments for enhanced safety.
7.1.1.1.6.1	Created a subsection to 7.1.1.1.6 specific to heparinization procedures and restructured the content to more clearly specify the guidance for de-escalation of heparinization.	Updated for clarity.
7.1.1.1.9	Content revised to indicate that detailed procedures for b2M collection will be included in the AKST1210 b2M and Proteomics Lab Manual.	Updated for clarification of location of details for procedures related to collection of b2M and proteomics.
7.1.1.2	Updated to provide a more comprehensive list of procedures performed to assess safety (e.g., targeted physical exams, weight measurements).	Updated for clarity.
7.1.1.2.2	Added an assessment for cognitive dysfunction (confusion, alteration of consciousness) in the context of IDH. Added that BP can be obtained in a seated or reclined position. Added procedures related to evaluation of IDH.	Updated to enable prompt identification and intervention in the event of decreased cerebral blood flow in the context of IDH and/or hypovolemia Clarification for positioning options for BP. Clarification of monitoring for IDH.
7.1.1.2.4	Updated to describe a “targeted” physical examination versus a (complete) physical examination as described in Section 7.1.1.1.3	Revised to differentiate between a “targeted” physical examination and (complete)

		physical examination.
7.3.4	Updates/removal of duplicative content related to withdrawal and evaluation.	Clarification of content.
7.5	Updated the list of prohibited concomitant medications, treatments, and procedures.	Revised for clarity and consistency with the Inclusion/Exclusion Criteria.
8	***Previously read:*** Assessment of safety will be conducted by blinded study personnel except in extraordinary circumstances where knowledge of whether AKST1210 was received by a subject is essential. ***Now reads:*** Assessment of safety will be conducted by blinded study personnel who are medically qualified by training and experience (e.g., study investigators) except in extraordinary circumstances where knowledge of whether AKST1210 was received by a subject is essential.	Added to clarify assessment of safety.
8.5, 8.6	Modified to indicate that safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB).	Added to clarify safety oversight.
10.3, 10.5	Defined the evaluable dataset as all subjects who complete through Week 8.	The definition of Evaluable subjects for analysis had not been included previously. Further details on the statistical populations will be included in the Statistical Analysis Plan.
13.3	Updated the definitions of Major and Minor Protocol Deviations and included examples of each for clarity.	To align with the definitions in Alkahest's SOP-CL-007 on Protocol Deviations Oversight and Reporting.
Schedule of Study Events (15.1.1 – 15.1.5), Schedule of Laboratory Tests	Revisions to study events/timing and laboratory testing. Key changes relate to extension of Screening period from -4 to -2 weeks to -6 to -2 weeks (now -42 to -14 days [previously -28 to -14 days]), removal of laboratory samples that are not relevant to the subject population, and additional testing of Hgb to support monitoring for anemia.	Revised to align with changes described in the protocol, specifically Section 7.
Appendix 17.1	Updated the escalation, de-escalation, and discontinuation rules to specify criteria related to the occurrence of worsening anemia. Under the revised criteria, subjects with a hemoglobin (HgB) < 9 g/dL will not be escalated to the next column size and subjects whose HgB decreases to < 9 g/dL at a given column size despite optimized ESA treatment will be de-escalated or discontinued as applicable.	Updated to account for monitoring of anemia and to provide enhanced clarity concerning escalation, de-escalation, and discontinuation rules.
Appendix 17.2	Appendix was eliminated.	Duplicative content was removed for clarity.

Protocol Version 1.2 dated 13NOV2019
Replaces: Protocol Version 1.0 dated 11OCT2019

The following table describes changes from Version 1.1 (dated 11OCT2019) with justifications provided.

Section(s)	Description	Justification
Throughout	Protocol version update. ***Previously read**:* V1.1_11OCT2019 ***Now reads**:* V1.2_13NOV2019	Version control.
Throughout	Minor grammar and content updates.	Minor grammar/content updates for clarity/accuracy of content.
Table of Contents	Minor content updates.	Minor updates required to reflect revised content.
List of Abbreviations	Minor content updates.	Minor updates required to reflect revised content.
6.1.2.1	Content revised to remove term "control device" and replace with "control" only.	Revised to prevent confusion regarding control.
6.2, 15.1.2, 15.1.3, 15.1.4, 15.1.5	Control accountability added to heading (6.2) and content of sections.	Revised to include accountability for control.
8	The following sentence was added, "When assessing relatedness, it should be assumed the subject was randomized to the AKST1210 column unless unblinding is required."	Revisions related to prevention of inadvertent unblinding.
8.1.2	Section removed and Section 8.1.3 renumbered to Section 8.1.2.	Device deficiencies and user errors will now be reported as technical errors.
8.2.1	Term "ADE" added to section.	Provide clarity regarding adverse event classification.
8.2.3	Heading changed to, "Expectedness of Adverse Device Effects."	Provide clarity regarding section content.
8.2.3.1	Second paragraph revised to information regarding assessment and reporting of a UADE.	Provide clarity regarding UADE assessment and reporting.
8.3.1	Heading changed to "Post-Study Safety Assessment."	Avoid use of abbreviations in heading per style guidelines.
8.4.1, 8.4.2	Extensive content revisions related to reporting procedures.	Revisions related to compliance with standard device protocols.
8.5	Refinement of content related to description of the "Safety Evaluation Meeting."	Minor updates for clarity/accuracy of content.
12.2	Content updates related to written notification IRB/IEC withdrawal of approval.	Minor updates for clarity/accuracy of content.
12.3.2	The following sentence was removed, "It is required that	Duplication of content now

	written informed consent be obtained within 5 working days of the use of the investigational device.	provided in (new) Section 13.1.1.1.
13.1.1	Content updates related to investigator reporting responsibilities.	Revisions related to compliance with standard device protocols.

Protocol Version 1.1 dated 11OCT2019
Replaces: Protocol Version 1.0 dated 06SEP2019

The following table describes changes from Version 1.0 (dated 06SEP2019) with justifications provided.

Section(s)	Description	Justification
Throughout	Protocol version update. ***Previously read**:* V1.0_06SEP2019 ***Now reads**:* V1.1 11OCT2019	Version control.
Throughout	Minor grammar and content updates.	Minor grammar/content updates for clarity/accuracy of content.
Table of Contents	Minor content updates.	Minor updates required to reflect revised content.
List of Abbreviations	Added abbreviations related to device events.	Revisions related to compliance with standard device protocols.
Protocol Approval Page	Addition of 21 Code of Federal Regulations (CFR) 812, and International Organization for Standardization (ISO) 14155:2011.	Revisions related to compliance with standard device protocols.
Protocol Summary, 4.1, 5.3, 5.4.2, 10.5	Content added to indicate there will be approximately 26 subjects enrolled with the intent of obtaining 20 evaluable subjects (assuming a drop-out rate of 20%).	Revised subject enrollment to obtain appropriate number of evaluable subjects.
2.3.1	Content revised and extended to include detailed description of known potential risks for device (previously only included a cross-reference to the Investigator's Brochure.	Revisions related to compliance with standard device protocols
4.1	Clarification of maximum flow rate of 250 mL/minute, which increased overall length of dialysis time to approximately 4 hours.	Provide clarity regarding flow rate and extended length of dialysis.
5.5	Content added regarding Sponsor/investigator notification of IRB withdrawal.	Revisions related to compliance with standard device protocols.
6	Extensive content additions to fully describe device labeling, storage, escalation, and administration. A brief description of the control was also added.	Revisions related to compliance with standard device protocols.
7.1.1.1.6	Content revised for clarification of heparin regimen during Treatment Periods.	Provide clarity regarding heparin regimen.
7.1.1.1.8	Content revised for clarification of PHQ-9 administration.	Provide clarity regarding PHQ-9 administration.

8	Extensive content revisions related to safety parameters as well as device event descriptions, classifications, expectedness, reporting procedures, and study halting rules.	Revisions related to compliance with standard device protocols.
12, 13	Content updates related to reporting procedures, data handling, and record keeping that are specific to device trials.	Revisions related to compliance with standard device protocols.
15.2	Added Calcium (Total/Free) to serum chemistry.	Added for monitoring of calcium levels.