STATISTICAL ANALYSIS PLAN

Based on:
Protocol Version 2.1, Dated 14Feb2020

SPONSOR:	Alkahest, Inc.
PROTOCOL NUMBER:	AKST1210-201
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
ORIGINAL PROTOCOL DATE:	06SEP2019
PROTOCOL AMENDMENTS:	24JAN2020, Version 2.0

[redacted]

SAP Approval

By signing the following, I agree to the contents in the Statistical Analysis Plan (SAP) and its associated attachments. Once the SAP has been signed, the analyses and programming of the tables, figures, and listings (TFLs) based upon this document can proceed. Any modifications to the SAP and TFLs made after signing may result in a change order.

Approved by:

Name	Title	Signature	Date
[redacted]	[redacted]	[redacted]	8/25/2021
[redacted]	[redacted]		[redacted]

Abbreviations

Abbreviation	Definition
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ADE	Adverse device effect
AE	Adverse event
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
b2M	Beta 2-microglobulin, β2-microglobulin
BMI	Body mass index
BP	Blood pressure/pain (for SF-36)
BUN	Blood urea nitrogen
CBC	Complete blood count
C	Control
CI	Confidence interval
CK	Creatinine kinase
COVID-19	Coronavirus disease 2019
CSR	Clinical study report
DET	Detection Test
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EDC	Electronic data capture
EOD	End of dialysis
EOS	End of study
EOT	End of treatment
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
GGT	Gamma-glutamyl transferase
GML TM	Modified Groton Maze Learning Test
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HD	Hemodialysis
HDL	High-density lipoprotein
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
HR	Heart rate
IDH	Intradialytic hypotension/hypotensive event
IDN	Identification Test
IgA	Immunoglobulin A
ISLT	International Shipping List Test-Immediate Recall
ISRL	International Shipping List Test-Delayed Recall
ITT	Intent-to-treat

IV	Intravenous
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LM	Least-squares
MAP	Mean arterial pressure
MedDRA	Medical Dictionary for Regulatory Activities
MH	Emotional well-being
MMRM	Mixed-effect model for repeated measures
MoCA	Montreal Cognitive Assessment
ONB	One-back Test
PF	Physical functioning
PHQ-9	Patient Health Questionnaire-9
PSQI	Sleep Quality Index
PT	Preferred Term
PTD	Prior to dialysis
PT/INR	Prothrombin time/international normalized ratio
QTc	QT interval corrected for heart rate
RBC	Red blood cell
RE	Emotional problems
RP	Role limitations due to physical health
RR	Respiration rate
SADE	Serious adverse device effect
SAE	Serious adverse event
SAP	Statistical Analytical Plan
SD	Standard deviation
SE	Standard error
SF	Social functioning
SF-36	Short Form-36
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
TFL	Tables, figures, and listings
TSH	Thyroid-stimulating hormone
UADE	Unanticipated adverse device effect
VIT	Energy/fatigue
WOCBP	Women of Childbearing Potential

Table of Contents

1 Introduction

This Statistical Analysis Plan (SAP) describes the planned statistical analyses to be performed for data from Protocol AKST1210-201, "A Randomized, Double-Blind, Phase 2a Placebo-Controlled Study to Evaluate the Tolerability, Feasibility, and Efficacy of AKST1210 in Patients on Hemodialysis with Cognitive Impairment Associated with End-Stage Renal Disease". This SAP was created using Clinical Protocol AKST1210-201 Version 2.1 dated 14FEB2020. Reference materials for this statistical plan include the protocol and the accompanying sample data collection documents. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the analysis.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Conference on Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials (1998). All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association (2016) and the Royal Statistical Society (2014) for statistical practice.

The planned analyses identified in this SAP may be included in clinical study reports (CSRs), regulatory submissions, or future manuscripts. In addition, post-hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc or unplanned exploratory analysis performed will be clearly identified as such in the final CSR.

The statistical plan described hereafter is an *a priori* plan. It will be submitted to file prior to any unblinded inferential or descriptive analysis of data pertaining to Alkahest's study AKST1210-201.

The table and listing shells will be provided in separate files as attachments to this SAP.

2 Study Objectives

2.1 Primary Objective

The primary objective is to assess the safety and tolerability of AKST1210 for the treatment of ESRD-CI in subjects receiving HD.

2.2 Secondary Objectives

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.

2.3 Exploratory Objectives

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.

3 Study Endpoints

3.1 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 grouped by MedDRA System Organ Class (SOC).

3.2 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 end of study (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).

3.3 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.

4 Study Overview

4.1 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 Protocol Appendix 17.1. Subjects will complete an EOS Visit during Week 14.

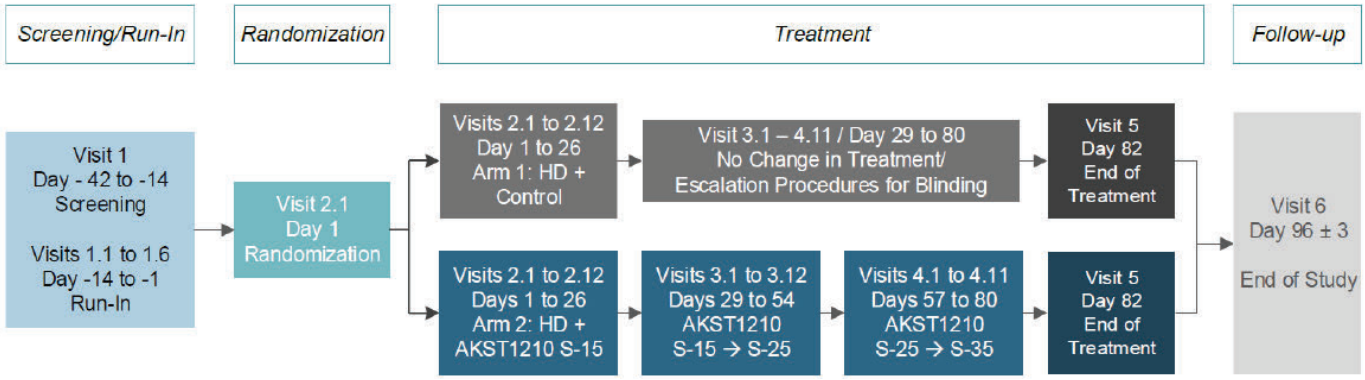


Figure 1. Study Schema

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 Protocol 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 Statistical Hypothesis

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 will be evaluated by Wilcoxon signed-rank test. Hypothesis testing will be performed for the B2M data analysis and cognitive change based on Cogstate test battery, MoCA and PHQ-9. Please refer to the relevant sections below for details.

4.3 Sample Size Justification

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. 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 et al., 2010).

The study is not statistically powered to detect differences in measures of clinical efficacy or biomarker endpoints. In addition, 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.

4.4 Device/Control Used

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. 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.

4.5. Randomization/Blinding and Unblinding

This is a randomized and double-blind study.

4.5.1 Randomization

Subjects will be randomized in a 1:1 ratio to either the AKST1210 column (the investigational treatment) or to control (no column). The study will be performed using a manual randomization process. Subjects will be randomized at Visit 2.1, after eligibility has been confirmed (including Day –14 to –1 Run-In). Subjects who discontinue or are unblinded may be replaced. Subjects who withdraw or are withdrawn during screening will be replaced. Replacement subjects will receive the same treatment as the original subjects. Subjects who have been unblinded will not be replaced.

4.5.2 Blinding and Unblinding

Unblinded site staff should prepare the dispensed AKST1210 column for the hemodialysis treatment according to the instructions for AKST1210 column operation. The subject and any blinded study personnel should not be permitted in the area during set up. After the column has been rinsed, primed, and prepared for treatment within the subject's hemodialysis circuit, the column should be held in place with a clamp. It should then be draped or wrapped with an opaque covering in order to ensure that the subject and blinded site staff remain blinded to the treatment assignment. After the AKST1210 column has been set up and draped appropriately, the subject should be brought to the treatment area to begin hemodialysis.

Subjects randomized to the control arm will not have an AKST1210 column used throughout their study participation. In place of an AKST1210 column, a control will be used to occupy the same space as an AKST1210 column and be held in place by an identical clamp used for the active treatment group. It will be draped or

wrapped with an opaque covering in order to ensure that the subject and blinded site staff remain blinded to the treatment assignment. The unblinded dialysis nurse/technician should set up the hemodialysis circuit as per their standard procedure and ensure that the control is attached to the hemodialysis machine in the same approximate location that an AKST1210 column would be placed and then route the hemodialysis lines through the control to simulate an in line AKST1210 column. Once the control has been set up and draped appropriately, the subject should be brought to the treatment area to begin hemodialysis. To maintain the blind, the control should be examined as frequently as the AKST1210 column during subjects' dialysis sessions.

To maintain the overall quality and legitimacy of the clinical trial, intentional unblinding of blinded staff prior to Database Lock at the site-level should not occur. If an exceptional circumstance requires blinded staff to be unblinded for adequate management of a subject, the investigator should discuss this with the unblinded Medical Monitor first. If unblinding is necessary, the subject's treatment assignment must NOT be disclosed to the subject and/or other blinded study personnel, blinded clinical research associates, or the Sponsor to the absolute extent possible.

All intentional and unintentional unblinding should be reported promptly to the unblinded clinical research associate, ideally within 24 hours of discovery. If accidental unblinding occurs, a root cause analysis may be performed, and preventative measures may be put in place to prevent further instances of unintentional, accidental unblinding.

4.6 Interim Analyses

Not applicable.

4.7 Study Assessment Time Points

The study consists of 6 protocol-specified visits which will be assessed as nominal visits from an analysis perspective:

1) Visit 1 Screening/Run-In: screening will be conducted at Days -42 to -14 and run-in will be conducted at Days -14 to -1.
2) Visit 2 Treatment Period 1 (Weeks 1- 4): randomization occurs on the same day as Day 1 and the first use of the device/control. Tolerability is monitored during each hemodialysis session. Evaluation for escalation, or discontinuation will be performed on Day 26.
3) Visit 3 Treatment Period 2 (Weeks 5- 8): Evaluation for de-escalation, escalation, or discontinuation will be performed on Day 54. Monitoring for de-escalation or discontinuation is assessed during each hemodialysis session.
4) Visit 4 Treatment Period 3 (Weeks 9- 12). Monitoring for de-escalation or discontinuation is assessed during each hemodialysis session.
5) Visit 5 End of Treatment (EOT) (Week 12, Day 82)

6) Visit 6 EOS (Week 14): In the event of early termination of a subject who has received at least 1 treatment, the end of study procedures will be performed unless the subject has withdrawn consent.

4.8 Schedule of Event

Refer to Protocol section 15.

4.9 Data Safety Monitoring Board (DSMB)

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.

5 Statistical Methodology

5.1 General Considerations

This section presents the statistical approaches that are anticipated for the analysis of the study data. These approaches may at times require modifications due to unanticipated features of the data. Deviations from analyses summarized in this document will be noted in the CSR.

All statistical analyses will be performed using SAS® Version 9.4 or higher, unless otherwise noted. Data will be summarized using descriptive statistics, unless otherwise noted. Number of subjects (n) with non-missing values mean, median, standard deviation, minimum, and maximum will be summarized for continuous (quantitative) variables. Categorical (qualitative) variables will be summarized using frequencies and percentages of subjects who are in a particular category or each possible value. In general, the denominator for the percentage calculation will be based upon the total number of subjects in the study population for each treatment group, unless otherwise specified. The denominator for by-visit displays will be the number of subjects in the relevant study population with non-missing data at each visit. Presentations will be by treatment group (AKST1210 and Control) and Total, unless otherwise noted.

The minimum and maximum will be reported with the same degree of precision (i.e., the same number of decimal places) as the observed data. Measures of location (mean and median) will be reported to 1 degree precision more than the observed data and measures of spread (SD) will be reported to 2 degrees of precision more than the observed data.

Percentages will be presented to 1 decimal place, unless otherwise specified. Percentages will not be displayed when the numerator for a cell is 0.

Unless otherwise indicated, all statistical tests will be conducted at 0.05 significance level using 2-tailed tests, and *P* values will be reported. Corresponding 95% CIs will be presented for statistical tests.

In general, all summary tables will be supported by a relevant subject data listing including all subjects who are randomized. The listings will include all data collected, and will be sorted by subject ID, and actual visit date, as applicable, unless otherwise noted. The listing will also include the column size for each of the three treatment periods as applicable.

Study day 1 is the first day of HD treatment with AKST1210 or control. Study day is the day relative to the study day 1.

Baseline is the last non-missing value prior to the start of study hemodialysis during the run-in period V1.1, unless otherwise specified. Specifically:

- All the clinical labs, PE, and ECG will use Screening as the Baseline.
- Vital signs will use V1.1 prior to first study hemodialysis as the Baseline.
- PHQ-9 and MoCA will use the Screening Visit for Baseline.
- All the other questionnaires will use Visit 2.1 as the baseline

If values are missing at the defined baseline visit above an unscheduled value will be used, provided that the unscheduled assessment occurs prior to the start of the first hemodialysis during the Run-in period V1.1.

Change from baseline is the post-treatment value minus baseline value.

5.2 Study Populations for Analysis

5.2.1 Intention-to-Treat (ITT) Population

The ITT population includes all randomized subjects. Analysis will be done according to the treatment subjects were randomized to. All baseline characteristics will be summarized for the ITT population.

5.2.2 Safety Population

The Safety population will consist of all subjects who received at least 1 HD treatment with AKST1210/Control after randomization. All safety endpoints will be summarized for the Safety population. Analysis will be done according to the actual treatment subjects received.

5.2.3 Evaluable Population

The Evaluable population will consist of all subjects who completed through Week 8, defined as having a week 8 visit. All secondary endpoints will be summarized for the Evaluable population. Analysis will be done according to the treatment subjects were randomized to.

5.2.4 Per-Protocol (PP) Population

The PP population is a subset of the Evaluable population who completed all of the three (3), 4-week HD treatment periods, and completed the study without any protocol deviations that impact analyses. Such 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 or wellbeing of study participants. A listing of study subjects with protocol deviations, which would preclude inclusion in the PP population will be provided by the Sponsor prior to database lock. Deviations related to coronavirus disease 2019 (COVID-19) will also be evaluated and provided prior to database lock.

The PP population will be used to support the secondary efficacy endpoint analysis. Analysis will be done according to the treatment that subjects were randomized to.

5.2.5 Subgroup Analyses

Not applicable.

5.3 Subject Disposition and Withdrawals

All subjects who signed the informed consent will be accounted for in Subject Disposition. The following disposition information will be summarized by the number and percentages of subjects:

- Subjects who signed the informed consent, and who comprise the ITT, Safety, Evaluable and PP populations
- Subjects who complete the study or withdraw prematurely along with the reason for discontinuation

A listing of subject disposition and subjects with failed eligibility criteria will be provided.

A listing of Safety subjects excluded from the Evaluable/PP population with the reason for exclusion will be provided.

5.4 Demographics and other Baseline Characteristics

5.4.1 Demographics

Sex, women of childbearing potential (WOCBP), age (in years), race, ethnicity, baseline BMI (kg/m^2) and subjects' highest education level will be summarized by treatment group and Total for the ITT population. BMI (kg/m^2) will be calculated as: 10,000*weight (kg) / the square of height (cm). If height is collected in inches, it will be converted to cm by dividing the value by 2.54. If weight is collected in pounds, it will be converted to kg by dividing the value by 2.205.

The means of continuous demographic variables will be tested using Wilcoxon rank-sum test and the proportions of categorical demographic variables will be

tested using Fisher's Exact test in order to evaluate the effectiveness of the randomization in producing homogeneous pre-treatment groups.

5.4.2 Baseline Disease Characteristics

Baseline disease characteristics will be summarized by treatment group and Total for the ITT population for the following:

- Baseline Historical IDH Rate (0,1,2,3,4)
- Dialysis Vintage
- Baseline b2M Level (V1.5)
- Baseline MoCA
- Baseline PHQ-9

5.4.3 Medical History

The number and percent of subjects reporting various medical histories grouped by system organ class (SOC) and preferred terms (PT) using MedDRA dictionary version 23.0 will be tabulated, as collected in the EDC. Subject medical history as well as allergies, surgeries and procedures will be listed.

5.4.4 Serology

Serology, including hepatitis B surface/core antigen (HBsAg), hepatitis C antibody (HCV Ab), and human immunodeficiency virus antibody (HIV Ab, HIV1/HIV-2), will be collected at Visit 1. Serology will not be summarized but will be provided as a listing.

5.4.5 Baseline Efficacy and Safety Assessment

The Baseline efficacy and safety data needed for the CSR will be extracted from corresponding tables described in the corresponding Efficacy Analysis and Safety Analysis section.

5.5 Protocol Deviations

Protocol deviations will be summarized by treatment group and Total with each deviation category broken down into major and minor for the ITT and Safety populations separately.

5.6 Methods for Handling Missing Data

No imputation of missing data will be performed.

5.7 Efficacy Analyses

Efficacy analyses will evaluate the feasibility of conducting expanded studies of AKST1210 in subjects with ESRD-CI who are undergoing HD 3 times per week, and its efficacy. The efficacy analyses will be descriptive only. Except where indicated, efficacy parameters will be summarized using the Evaluable and PP populations. All efficacy data, regardless of population, will be presented in data listings.

5.7.1 Feasibility of all Procedures

Feasibility of all procedures will be determined by the number of assessments that can be completed by the subject, visits that are completed, dialysis sessions that are completed with the longer dialysis time and the number of times the dialysis session becomes unblinded to treatment. A less than 75% completion by subjects would determine the procedure non-feasible calculated by treatment period and study overall. The percentage of subjects who are missing at each procedure will also be summarized.

5.7.2 Tolerability of Column Size, including Subject Compliance with Visit Completion, Procedures, Subject Retention

The number and percentage of subjects will be summarized by column size (AKST1210 S-15, S-25, and S-35), overall AKST1210 and control for the following:

- Subjects entered into each treatment period
- Subjects who de-escalated to a lower column size in treatment period 3 categorized by reason: IDH only, Anemia only, IDH and Anemia. Anemia is defined as having a hemoglobin (Hgb) lab value of < 9g/dL, Subjects who were discontinued due to treatment or HD
- Subjects who completed all the visits

5.7.3 Change in IDH Rate Above Historical IDH Rate for Column Size

The number and percentage of subjects will be summarized by column size (AKST1210 S-15, S-25, and S-35), overall AKST1210 and control for the following:

- Historical IDH Rate (0,1,2,3,4)
- Number of IDH (0,1,2,3,4,5, 6)
- Change in IDH Rate above the Historical IDH Rate (0,1,2, 3)

A listing of IDH Rate, including each subject's historical IDH rate and number of hypotensive events, will be provided.

5.7.4 Incidence of IDH Leading to De-escalation or Discontinuation

The number and percentage of subjects with any IDH leading to de-escalation or discontinuation from AKST1210/control will be summarized by treatment group and Total.

5.7.5 Incidence of Worsening Anemia by Column Size

The number and percentage of subjects with any worsening anemia will be summarized by column size (AKST1210 S-15, S-25, and S-35), overall AKST1210 and control. Worsening anemia is defined as subjects with a Hgb < 9 g/dL, which decreased from baseline (or the prior measurement) of no less than 1 g/dL.

5.7.6 Incidence of Anemia Leading to De-escalation or Discontinuation

The number and percentage of subjects with any anemia leading to de-escalation or discontinuation from AKST1210/control will be summarized by treatment group and Total.

5.7.7 Change from Baseline in Levels of b2M, Evaluation of Change Based on Column Size

Blood sampling for b2M concentration will occur at Run-in, during the treatment period, and at EOS. During the treatment period, blood samples will be collected at 3 sample locations (A, B, C, Figure 2) and at multiple time points prior to dialysis (PTD), at 1 hour, 2 hours, and at the end of dialysis (EOD) at the end of each 4-week treatment (V2.11, V3.11 and V4.11). Detailed collection schedule could be found in Figure 2 and Table 1.

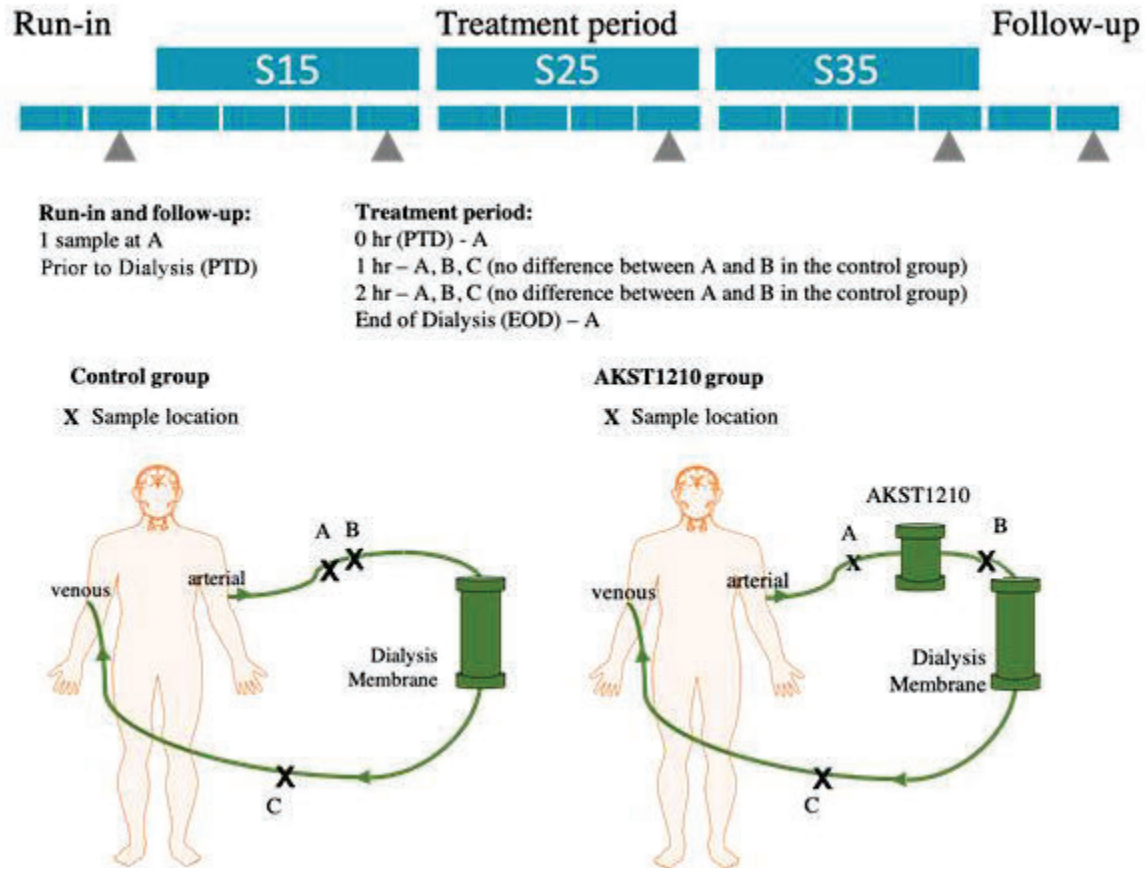


Figure 2. The b2M sample locations in the control and the AKST1210 group.

Table 1. Collection schedule of b2M samples

Study period	**Run-in**	**Treatment period**												**End of study**
Study visit	V1.5	V2.11				V3.11				V4.11				V6
Study week(day)		Week 4 (D4)				Week 8 (D52)				Week 12 (D80)				Week 14 (D96)
Column size		150 mL column (S-15)				250 mL column (S-25)				350 mL column (S-35)				
Timepoint	PTD	PTD	1 hour	2 hours	EOD	PTD	1 hour	2 hours	EOD	PTD	1 hour	2 hours	EOD	PTD
Sample location	A	A	A,B, C	A,B, C	A	A	A,B, C	A,B, C	A	A	A,B,C	A,B, C	A	A

PTD: prior to dialysis; EOD: end of dialysis. Sample location A is before the AKST1210 column. Sample location B is after the AKST1210 column and before the hemodialyzer. Sample location C is after both the AKST1210 column and the hemodialyzer. In the control group, because there is no AKST1210 column, sample location A is the same as sample location B (Figure 2).

Three hypotheses will be tested to evaluate the effects of AKST1210 columns on b2M removal, including overall removal after AKST1210 treatment and instantaneous removal during AKST1210 treatment.

The first hypothesis is that active treatment with AKST1210 columns will change b2M levels significantly compared to the b2M changes in the placebo group. The changed b2M levels will be calculated as b2M levels from samples collected at sample location A prior to dialysis at V2.11, V3.11, V4.11, and V6 (EOS), minus the b2M levels at baseline (V1.5) at sample location A, respectively.

The second hypothesis is that the treatment duration (and/or column size) will significantly affect the b2M removal from baseline. The same changed b2M levels as in the first hypothesis will be used.

The third hypothesis is that the sizes of the columns will significantly affect the instantaneous clearance of b2M. The instantaneous clearance of b2M will be evaluated by 3 ratios in logarithmic scale: 1) B/A ratio at the active group, which is the instantaneous b2M removal ratio of AKST1210 (b2M levels at location B/ b2M levels at location A after 1 hour and after 2 hours, respectively); 2) C/A ratio at the active group, which is the instantaneous b2M removal ratio of AKST1210 and the hemodialyzer (b2M levels at location C/ b2M levels at location A after 1 hour and after 2 hours, respectively); and 3) C/B ratio at both treatment groups (C/A ratio in the control group), which is the instantaneous b2M removal ratio of the hemodialyzer (b2M levels at location C/ b2M levels at location B after 1hour and after 2 hours, respectively). The B/A ratio is solely for the effect of AKST1210 column sizes. The C/A ratio is for the combined effect of AKST1210 columns and hemodialyzer. And the C/B ratio (or the C/A ratio in the control group) is for the effect of hemodialyzer. The B/A ratio and C/A ratio will be analyzed to test the effects of column size on the instantaneous clearance of b2M. The analysis of C/B ratio (or the C/A ratio in the control group) will be only descriptive summary.

For the first hypothesis, the null and alternative are:

$$H0: \mu 1C = \mu 1T \text{ vs. } HA: \mu 1C \neq \mu 1T.$$

The null hypothesis states that there is no difference in mean change (μ1) from baseline b2M levels as measured by the b2M levels at sample location A prior to dialysis between the control (C) and treated (T) groups after each of 4-week treatment and after the whole treatment period. The alternative hypothesis states that there is a difference in mean change from baseline b2M levels between the control and treated groups.

A mixed-effect model for repeated measures (MMRM) will be employed to analyze the change from baseline b2M levels at V2.11, V3.11, V4.11 and V6. The model will include the fixed effects of treatment (reference=control group),

baseline b2M levels, visit (as a categorical variable), treatment by visit interaction, and the random effect of intercept and dialyzer types (or sites, if ≥ 5 subjects per site). Depending on the data, compound symmetry, heterogeneous compound symmetry, an unstructured covariance structure will be used to model the within subject error. Least-square (LS) means, standard error (SE), and 95% confidence interval (CI) of the LS means will be provided for each treatment arm at each visit. P-values testing whether the LS means at each visit is significantly different from 0 will be provided, which indicates the significance of AKST1210 treatment on b2M removal from baseline within group over time. The difference in LS means between treatment arms and 95% CI of the difference will also be calculated. P-value for testing whether the difference is equal to zero at all these 4 visits will be provided and compared against the alpha significance level of 0.1, which indicate significance of AKST1210 treatment on b2M removal compared to the control group.

For the second hypothesis, the null and alternative are:

$$H0: \mu 2C = \mu 2T \text{ vs. } HA: \mu 2C \neq \mu 2T.$$

The null hypothesis states that there is no difference in mean change (μ_2) from baseline b2M levels as measured by the changed b2M levels at sample location A prior to dialysis at V2.11, V3.11 and V4.11 in the active group. The alternative hypothesis states that there is a difference in mean change from baseline b2M levels at least in one between-visits comparison.

The same MMRM will be used. But additional LS means of three between-visit comparisons in the active group, standard error (SE), and 95% CI of the LS means will be calculated. P-value for testing whether the difference is equal to zero at all three between-visit comparisons at the active group will be provided and compared against the alpha significance level of 0.1, which indicate the significance of different AKST1210 treatment durations (and/or column sizes) on b2M removal.

For the third hypothesis, the null and alternative are:

$$H0: \mu iC = \mu iT \text{ vs. } HA: \mu iC \neq \mu iT.$$

The null hypothesis states that there is no difference in mean instantaneous ratios (μi) as measured by B/A ratio and C/A ratio in the logarithmic scale among three AKST1210 columns, alone or combined with hemodialyzer, respectively. The alternative hypothesis states that there is a difference in instantaneous ratio among the three AKST1210 columns.

A mixed-effect models for repeated measures (MMRM) will be employed in the active group to analyze the instantaneous ratios of B/A ratio and C/A ratio. The

model will include the fixed effects of b2M levels at sample location A, column size (as categorical variable), hours (reference=1 hour), ratio types (B/A ratio or C/A ratio), column size by hours interaction, column size by ratio types interaction, hours by ratio types interaction, and three-way interaction of column size, hours and ratio types, as well as the random effects of intercept and dialyzer types (or sites). Depending on the data, compound symmetry, heterogeneous compound symmetry, an unstructured covariance structure will be used to model the within subject error. Least-square (LS) means, standard error (SE), and 95% CI of the LS mean will be provided for each AKST1210 column at each hour. The pairwise difference in LS means among three AKST1210 column size at 1 hour and 2 hours for both ratio types, and 95% CI of the difference will also be calculated. P-value for testing whether the difference is equal to zero will be provided and compared against the alpha significance level of 0.1, which indicates the significance of different AKST1210 column sizes on instantaneous b2M removal.

For the C/B ratio (or the C/A ratio in the control group), summary statistics will be provided for the active group and the control group at 3 visits.

Model diagnostics including residual and noniterative influence analysis will be conducted to ensure the MMRM assumptions (e.g. residual normality, and homogeneity of variance) and to identify potential outliers and influential observations. If residuals are not normal, logarithmic transformation will be applied to raw b2M levels before MMRM. No multiple comparison corrections will be performed as the aim of this study is to find trends in secondary endpoints instead of confirmation.

Sensitivity Analyses

The main analyses with the above MMRMs will use all available data. To check the robustness of the results, the following sensitivity analyses will be performed:

- The same MMRM models will be utilized for data from the PP subjects.
- For the first and the second hypotheses, the same MMRM models will be utilized, but focused on data at V2.11, V3.11 and V4.11 (removing V6).
- For the second hypothesis, a new MMRM model using the data from only the active group will also be performed with the fixed factors of baseline b2M levels and visits (V2.11, V3.11 and V4.11), and random effects of intercept and dialyzer types (or sites).

5.7.8 Duration of Treatment Based on Column Size

The duration of treatment will be summarized by column size (AKST1210 S-15, S-25, and S-35), overall AKST1210 and control at baseline, and at each scheduled post-baseline time point. The duration of each HD session in hours, the number of days of HD session, and the total duration of HD session (hours) will be summarized in each treatment period.

5.7.9 Change from Baseline in MoCA

The MoCA is a 1-page, 30-point test designed as a rapid scoring instrument for mild cognitive dysfunction. The questionnaire assesses several different cognitive domains, including attention and concentration, executive functions, memory, language, visuospatial skills, conceptual thinking, calculations and orientation. Higher scores indicate better cognitive function; the total possible score is 30 and a score of 26 or more is considered normal. Derivation for the MoCA total score=sum of individual item scores, with a range from 0 to 30.

Observed MoCA sub-scores and the MoCA summary score will be summarized by treatment group and Total at baseline, and at Visit 6 EOS. Change from baseline at Visit 6 EOS will also be summarized for sub-scores and summary score. The MoCA summary score are calculated within the EDC; this score will be obtained directly from the EDC data.

Within-group comparison of MoCA summary scores between baseline and Visit 6 EOS will be performed by Wilcoxon signed-rank test and associated P-values will be reported. Additionally, if the change in the MoCA summary score is normal based on Q-Q plot, the mean change from baseline in MoCA total score will be compared between treatment groups using an ANCOVA model with treatment as the main effect and baseline MoCA summary score as the covariate.

5.7.10 Change in Cognitive Domains by Cogstate Battery

Cogstate battery (i.e., verbal learning, psychomotor function, visual attention, working memory, executive function, and verbal delayed recall) will be assessed at Visit 1, Visit 2 Treatment Period 1 prior to HD treatment, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS.

Observed scores will be summarized by treatment group and Total at baseline, and at each scheduled post-baseline time point (Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS for the following:

- Global Composite Score
- Episodic Memory Domain Composite Score
- Attention Domain Composite Score

- Executive Function Domain Composite Score
- Psychomotor Function
- Working Memory
- Verbal Learning

Within-group comparison of the global composite scores between baseline and each scheduled post-baseline time point will be performed by Wilcoxon signed-rank test and associated P-values will be reported. See Appendix B for instructions on composite score calculation.

Change from baseline at each scheduled post-baseline time point will be summarized. A mixed-effect model for repeated measures (MMRM) will be employed to analyze the changed scores. The model will include the fixed effects of treatment (reference=control group), baseline scores, visit (as a categorical variable), treatment by visit interaction. An unstructured covariance structure will be used to model the within subject error. Least-square (LS) means, standard error (SE), and 95% CI of the LS means will be provided for each treatment arm at each post-baseline visit. P-values testing whether the LS means at each post-baseline visit is significantly different from 0 will be provided.

5.7.11 Change in Quality of life using Short-Form Health Survey (SF-36)

The SF-36 yields 8 scales and 2 summary scores for mental health (MCS) and physical health (PCS). SF-36 will be assessed at Visit 2 Treatment Period 1 prior to HD treatment, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS.

Scores from the eight scales (i.e., physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH)), as well as the summated MCS and PCS will be summarized by treatment group and Total at baseline and at each scheduled post-baseline time point. Change from baseline at each scheduled post-baseline time point will also be summarized.

Within-group comparison for both the MCS and PCS scores between baseline and each scheduled post-baseline time point will be performed by Wilcoxon signed-rank test and associated P-values will be reported.

The eight scales will be calculated using the RAND method available from the RAND website https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/scoring.html.

The MCS and PCS will be scored using the norm-based scoring methods based on the SF-36® summary measures manual (Jenkinson et al., 1998) in three steps.

[redacted]

A listing of SF-36 assessments, including the summary scores for mental and physical health, will be provided.

5.7.12 Change in Patient Health Questionnaire-9 (PHQ-9)

Scores will be summated for each subject across the 9 items and the calculated total score will be summarized by treatment group and Total at baseline, and at Visit 6 EOS. Within-group comparison of calculated total scores between baseline and Visit 6 EOS will be performed by Wilcoxon signed-rank test and associated P-values will be reported. Change from baseline at Visit 6 EOS will also be summarized. A listing of PHQ-9, including the total score, will be provided.

Additionally, if the change PHQ-9 total score is normal based on Q-Q plot, the mean change from baseline in PHQ-9 total score will be compared between treatment groups using an ANCOVA model treatment with treatment as the main effect and baseline PHQ-9 total score as the covariate.

5.7.13 Change in Sleep Quality, Measured by Pittsburgh Sleep Quality Index (PSQI)

The PSQI 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. PSQI will be assessed at Visit 2 Treatment Period 1 prior to HD treatment, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS.

Scores will be summated for each subject across all items. The calculated total score, the number and percentage of subjects who are considered “poor” sleepers, and the scores for each domain will be summarized by treatment group and Total at baseline and at each scheduled post-baseline time point. See Appendix C for instructions on the calculation of total score. Change from baseline in the total score and for each domain at each scheduled post-baseline time point will also be summarized.

Within-group comparison of the total score between baseline and each scheduled post-baseline time point will be performed by Wilcoxon signed-rank test and

associated P-values will be reported. A fisher's exact test will be used to compare the proportion of subjects considered "poor" sleepers.

A listing of PSQI, including the total score and a variable identifying if a subject is a poor sleeper, will be provided.

5.7.14 Change in Fatigue, Measured by Fatigue Questionnaire – Functional Assessments of Chronic Illness Therapy (FACIT)

The level of fatigue is measured on a 4-point Likert scale (4 = not at all fatigued to 0 = very much fatigued). The score ranges from 0 to 52. A score of less than 30 indicates severe fatigue. The higher the score, the better the quality of life. FACIT-Fatigue subscale will be assessed at Visit 2 Treatment Period 1 prior to HD treatment, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS.

The fatigue subscale score, and the number and percentage of subjects with severe fatigue will be summarized by treatment group and Total at baseline and at each scheduled post-baseline time point. Change from baseline in the fatigue subscale score at each scheduled post-baseline time point will also be summarized.

Within-group comparison of the fatigue subscale score between baseline and each scheduled post-baseline time point will be performed by Wilcoxon signed-rank test and associated P-values will be reported. A fisher's exact test will be used to compare the proportion of subjects with severe fatigue.

A listing of FACIT, including the fatigue subscale score and a variable identifying if a subject has severe fatigue, will be provided.

5.8 Safety Analyses

Safety analyses include hemodialysis parameters, adverse events, laboratory parameters, vital signs, weight, 12-lead electrocardiogram (ECG), physical examination, pregnancy tests, cognitive assessments during IDH and prior and concomitant medications. Except where indicated, safety parameters will be summarized by treatment group (AKST1210 and Control) using the safety population. A subset of the safety tables defined below will also be provided to the

DSMB for their periodic assessment of safety. The DSMB tables are identified within the analysis description.

5.8.1 Hemodialysis Parameters

Hemodialysis parameters consist of normal saline prime, normal saline bolus, normal saline rinse back, initial fluid removal goal, total fluid removal goal, total fluid output, Kt/V, blood flow rate, dialysate flow rate, arterial pressure, venous pressure, ultrafiltration rate, TMP, dialysate Temp, oxygen Sat pre-dialysis, oxygen Sat post-dialysis, sodium pre-dialysis, sodium post-dialysis, bicarbonate, hematocrit pre-dialysis, hematocrit post-dialysis, estimated dry weight, type of dialyzer, consciousness, eyes open, best verbal response, best motor response, respirations, lungs, lung ultrasound monitoring of Kerley B-line The hemodialysis parameters will be summarized by visit and treatment period.

Additionally, URR (%) will be summarized. URR (%) is calculated as:
URR (%) = (1 – postdialysis BUN / predialysis BUN) X 100

A listing of hemodialysis will be provided (included in DSMB).

5.8.2 Fluid Assessments

Fluid Assessments will not be summarized but will be provided as a listing.

5.8.3 Heparin Infusion

The amount of heparin used in each HD session in each treatment period will be summarized by column size (AKST1210 S-15, S-25, and S-35) and overall.

5.8.4 Study Device Accountability

Study Device Accountability will not be summarized but will be provided as a listing.

5.8.5 Adverse Events

All AEs that occur after the time of treatment with the study device will be considered TEAEs and will be reported. Events meeting this definition will be those events that are a change from subject's baseline condition, including an increase in frequency or severity (these will be entered as new AEs). For AEs occurring on the date of first treatment, if the time of onset is missing, the AE will be assumed to be treatment emergent.

All AEs, TEAEs, and SAEs will be coded using the MedDRA Version 23.0 or higher coding dictionary. The AE analyses will focus on those that are treatment emergent; however, any AEs that are reported after consent has been signed and before initial treatment, will be considered intercurrent events and flagged in the listings as such.

The causal relationship of the AE to the study device is determined by the investigator as Unrelated, Possibly Related, or Definitely Related. These can be

mapped to Unrelated (*Unrelated*) or Related (*Possibly Related or Definitely Related*). Adverse event summaries will be repeated for treatment-related TEAEs.

Adverse events severity grade will be reported as mild, moderate, or severe.

Summaries of incidence rates (frequencies and percentages) of individual TEAEs will be presented by SOC, PT, and treatment group. Such summaries will be displaced for all TEAEs, TEAEs maximum severity, and TEAEs by relationship.

Each subject will be counted only once within each summation level (SOC and PT). If a subject experience more than 1 TEAE within each summation level only, the TEAE with the strongest relationship or the maximum severity, as appropriate, will be included in the summaries of relationship and severity. If a particular event is missing the severity and/or relationship, then the strongest possible severity or relationship will be assumed for analysis (severity=severe, relationship=definitely related).

Per ISO 14155:2011, an adverse device effect (ADE) is an AE that is assessed as possibly or definitely 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.

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

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 a 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 a SADE is considered unanticipated by the investigator, it must be reported to the Sponsor or designee.

A summary of all TEAEs, as reported in the EDC, by the number and percentages of subjects who experienced any of the following will be provided:

- Any TEAE, including severity (Mild, Moderate, and Severe), relationship to study treatment (Unrelated, Possibly Related, Definitively Related), AEs leading to discontinuation of study participation.
- Any serious TEAE, including severity (Mild, Moderate, and Severe), relationship to study treatment (Unrelated, Possibly Related, Definitively Related), AEs leading to discontinuation of study participation
- Any fatal TEAEs, including relationship to study treatment (Unrelated, Possibly Related, Definitively Related)

5.8.5.1 Summary for All TEAEs

The number and percentage of subjects who experience TEAEs will be tabulated by SOC and PT using MedDRA dictionary version 23.0. Adverse events will be counted only once for a subject within each PT and SOC. Since a subject may have more than one PT within a SOC, percentages of PT may not sum to the percentages in the SOC (included in DSMB report).

Incidences will be presented by descending frequency of SOC and PT within SOC, and then alphabetically within PT where the incidence is same; this is based on overall subjects then alphabetically in case of a tie.

Missing and partially missing AE start and/or stop dates will be imputed, for the purpose of statistical analysis. All collected data will be presented in listings. Data not subject to analysis according to this plan will not appear in any tables or graphs but will be included only in the data listings.

Adverse events will be coded using the MedDRA version 23.0 or higher.

A treatment related AE is any AE with a relationship to the study device of possibly related or definitely related.

For all TEAEs the following will be summarized:

- Overall summary of any TEAEs presented by SOC/PT
- Overall summary of any TEAEs by worst severity presented by SOC/PT
- Overall summary of any TEAEs by most causal relationship presented by SOC/PT
- Overall summary of any ADE/SADE/UADEs

A Listing of all AEs will be provided. This listing will include a flag identifying if an AE is a TEAE.

5.8.5.2 Summary for serious TEAEs

For all serious TEAEs the following will be summarized (included in DSMB report):

- Overall summary of any serious TEAEs by worst severity presented by SOC/PT
- Overall summary of any serious TEAEs by closest relationship presented by SOC/PT

A listing of all serious AEs will be provided. This listing will include a flag identifying if a serious AE is a TEAE.

A test of hypothesis comparing the proportions of subjects reporting a TEAE throughout the study will be conducted using a Chi-square or Fisher's exact test, as appropriate. The null hypothesis is that there is no difference in proportion between treatment groups, with a two-sided alternative that considers the possibility of a difference in either direction. The number and percentage of subjects reporting a TEAE and the 95% Wilson confidence interval (CI) for the proportion will be presented for each treatment group. In addition, the difference in proportions between the device and control groups and the 95% Wilson CI will be presented. The same statistics and hypothesis testing will be performed for subjects reporting a SAE throughout the study.

All *P* values will be displayed in four decimals and rounded using standard scientific notation (e.g., 0.XXX). If a *P* value less than 0.0001 occurs it will be shown in tables as <0.0001.

5.8.5.3 Adverse Events Leading to Withdrawal

A summary of incidence rates (frequencies and percentages) of TEAEs leading to withdrawal of study device, by treatment group, SOC, and PT will be prepared for Safety Dataset. No inferential statistical tests will be performed.

A data listing of AEs leading to withdrawal of study device provided, displaying details of the event(s) captured on the CRF.

5.8.5.3 Deaths

Deaths, if any, will be provided in a listing only (included in DSMB report).

5.8.6 Laboratory Data

Analyses on hematology, chemistry, coagulation, and other clinical lab tests will consist of the following (included in DSMB).:

- Observed values at baseline and each scheduled post-baseline time point, along with change from baseline at each scheduled post-baseline time point

- Number and percentage of subjects with abnormal laboratory tests indicating serious condition at each scheduled time point
- Shift tables, by analyte and by out-of-range flag at each scheduled post-baseline time point compared to baseline per subject, may be produced

Hematology: complete blood count (CBC), neutrophils, total lymphocytes, monocytes, eosinophils, basophils, iron, hemoglobin (Hgb) will be collected at Visit 1, Visit 2 Treatment Period 1, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, Visit 5 End of Treatment, and Visit 6 EOS.

Chemistry: sodium, calcium (Total), potassium, chloride, bicarbonate, protein (total), albumin, bilirubin (total), aspartate transaminase (AST), alanine aminotransferase (ALT), glucose, and phosphate will be collected at Visit 1, Visit 2 Treatment Period 1, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, and Visit 6 EOS. Blood urea nitrogen (BUN), creatinine will be collected at Visit 1, Visit 2 Treatment Period 1 Day 1/3/10/17/26, Visit 3 Treatment Period 2 Day 29/31/38/45/54, Visit 4 Treatment Period 3 Day 57/59/66/73/78, Visit 5 End of Treatment, and Visit 6 EOS. Gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), amylase, lipase, lactate dehydrogenase (LDH), creatinine kinase (CK), hemoglobin A1c (HbA1c), magnesium, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and will be collected at Visit 1 and Visit 6 EOS.

Coagulation: Prothrombin time/ international normalized ratio (PT/INR) will be collected at Visit 1, Visit 2 Treatment Period 1, Visit 3 Treatment Period 2, Visit 4 Treatment Period 3, and Visit 6 EOS. Activated partial thromboplastin time (aPTT) will be collected at Visit 1, Visit 2 Treatment Period 1 Day 1/3/10/17/26, Visit 3 Treatment Period 2 Day 29/31/38/45/54, Visit 4 Treatment Period 3 Day 57/59/66/73/78, and Visit 6 EOS.

Other: Thyroid-stimulating hormone (TSH ultrasensitive) will be collected at Visit 1 and Visit 6 EOS.

The tests will be presented in the standardized units. Any data contains '>' or "<" sign will be converted to numeric for the summary tables. Subject listings will present all data collected (the '>' and '<' signs will be kept) along with toxicity grading. If the standardized value contains the "<" sign, the data used for the summary tables will be derived by extracting the numeric value from the standardized value and subtracting 0.1(N+1) to that numeric value, where N is the number of decimals in the numeric value. For example, if the standardized value = "< 0.3", it will be converted to 0.29 for summary statistics in the table. If the original value contains the ">" sign, the data used for the summary tables will be derived by extracting the numeric value from the standardized value and adding 0.1(N+1)

to that numeric value, where N is the number of decimals in the numeric value. For example, if the standardized value =">1000", it will be converted to "1000.1".

Results for hematology, chemistry, coagulation, and other clinical lab tests will be provided in separate listings. A listing of abnormal laboratory tests indicating serious condition will also be provided. If any laboratory tests are collected at an unscheduled visit post-baseline, the results will be included in the listings.

Labs that can indicate serious condition if outside reference range:

Albumin (g/L) < 1
AST > 3xULN
ALT > 3xULN
Alkaline Phosphatase (U/L) > 400
Amylase (U/L) > 120
Bicarbonate (mmol/L) < 18 or > 34
Total Bilirubin (mg/dL) > 1.2
BUN (mg/dL) < 7 or > 20
Calcium (mg/dL) < 8.5 or > 10.5
Chloride (mmol/L) < 90 or > 110
Creatine kinase (U/L) > 300
GGT (U/L) > 125
Glucose (mg/dL) < 50
Hematocrit (%) < 30 or > 58
Hemoglobin (g/dL) < 9
Lactate Dehydrogenase (U/L) > 250
Lipase (U/L) > 120
Magnesium (mg/dL) < 1 or > 2.8
Neutrophils (%) < 20 or > 90
Phosphate (mg/dL) < 1 or > 7
Platelets (x10E3/uL) < 100 or > 800
Potassium (mmol/L) < 3.2 or > 5.6
PT INR > 1.3
PT (sec) > 20
Sodium (mmol/L) < 130 or > 150
WBC count (x10E3/uL) < 2.5

5.8.7 Vital Signs

Vital signs, including blood pressure (mmHg), heart rate (beats/min), respiratory rate (breaths/min), and body temperature (C), will be summarized. 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 as described in the Schedule of Events, Protocol Section 15.1. Observed values will be tabulated at baseline, and at each scheduled post-baseline time point. Change from baseline at

each scheduled post-baseline time point will also be summarized (included in DSMB report). Change from baseline in blood pressure may also be presented in figures.

Additionally, a 'Repeated SBP < 90 mmHg or action taken to HD' listing will be populated if there are two SBP < 90 within 30 minutes of the start of the hemodialysis, or if in the hemodialysis data we have additional saline use entered as 'Hypotension', or there is a log record in the hemodialysis data within 30 minutes of a < 90 SBP with a change in blood flow rate, dialysate flow rate, or ultrafiltration rate (this would include if the hemodialysis was stopped). Abnormal vital sign values will be flagged in the listings. Abnormal vital sign values are as follows:

- Seated systolic blood pressure value < 90 mmHg
- Seated systolic blood pressure value > 180 mmHg
- Seated diastolic blood pressure value < 50 mmHg
- Seated diastolic blood pressure value > 110 mmHg
- Incidence of orthostatic hypotension (a decrease in systolic blood pressure of >20 mmHg and/or a decrease in diastolic blood pressure of > 10 mmHg between supine and standing)
- Heart rate > 100 beats per minute
- Temperature > 37.5°C
- Temperature < 36.5°C
- Respiration rate > 20 breaths per minute
- Respiration rate < 12 breaths per minute

5.8.8 Weight

Weight will be collected as described in the Schedule of Events, Protocol Section 15.1. Observed values in lb. will be tabulated at pre-HD and post-HD per HD session. Change from pre-HD at post-HD per HD session will also be summarized (included in DSMB report). A listing of weight (lb.) will be provided.

The initial fluid removal goal, actual fluid removal, amount differs from the goal, the proportion of subjects achieved the fluid removal goal will also be summarized by visit and treatment period. Actual fluid removal = total fluid output - total fluid intake. The amount differs from the goal = actual fluid removal – fluid removal goal.

5.8.9 12-lead ECG

A 12-lead ECG will be performed to obtain Heart rate (beats/min), QT interval (msec), and QT interval corrected by the Fridericia formula (QTcF (msec)). ECGs will be collected at Visit 1 and Visit 6 EOS.

Observed value at baseline and Visit 5, along with the change from baseline value at Visit 5 will be summarized (included in DSMB report).

A Shift table, by ECG test and by overall interpretation from Visit 1 to Visit 6 EOS will be provided.

A listing of all 12-lead ECG will be provided. A listing of abnormal 12-lead ECG will also be provided.

5.8.10 Physical Examination

Physical Examinations and targeted physical examinations will be conducted at Visit 1, and, for the abnormal tests, across all post-baseline visits, as described in the Schedule of Events, Protocol Section 15.1.

The number and percentage of subjects with abnormal physical examination results will be summarized by the body systems.

5.8.11 Pregnancy tests

Pregnancy test results will be provided for women of childbearing potential (WOBCP) in a listing only.

5.8.12 Cognitive Assessments

A listing will be provided presenting if Subjects are able to answer the following questions during IDH:

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

5.8.13 Prior and Concomitant Medications

The number and percentage of subjects taking any prior and concomitant medications will be summarized by ATC level 3 terms and preferred medication names (included in DSMB).

5.9 Exploratory Analyses

The following exploratory analysis may be performed on the Cogstate Battery data, to augment the endpoint analysis described in Section 5.7.10:

Summary of the number and percentage of subjects with test completion failures and test performance check failures, by study visit, for each treatment group.

In addition, test scores may be presented graphically (e.g., box and whisker plots) to inspect the normality of test data and occurrence of outliers. Scores from card-based tests (i.e., Detection, Identification, One Back tests) have already been transformed. The non-card-based tests (i.e., International Shopping List – Immediate Recall, International Shopping List – Delayed Recall, and modified Groton Maze Learning tests) have not. Based on the outlier occurrence for the non-card-based tests, a mathematical transformation (e.g., log10) may be performed to reduce the influence of extreme scores and to approach normal distribution, and summary tables produced.

Document Version Control

Revision History:

REVISION	RELEASE DATE	AUTHOR	SUMMARY OF CHANGES
A	24Mar2021	[redacted]	Initial Release
B	25Aug2021		Scoring methodologies, specifications for statistical testing of endpoints added; other general clarifications.

Appendix A - Programming Specifications for Tables and Listings

The following specifications will be used in the production of tables and listings.

1. Page Setup
   Unless otherwise noted, tables and listings will use landscape orientation. Margins will be at least 3/4 of an inch on the left side of page, at least 3/4 of an inch at the top, and 3/8 of an inch on the other sides.
   The following header information should be included:
   - Upper left: Sponsor name and protocol number
   - Center: CONFIDENTIAL; Database Download Date: ddmmyy
   - Upper right: Page number shown as Page n of N. Page numbers should be sequential within a table or listing.

   The footer should include:
   - Left: the name of the SAS program used to generate the output
   - Center: run date/time and the words [redacted]
   - Right: output file name.

2. Footnotes
   Unless otherwise specified, footnotes should appear on all pages within the table.

3. Font
   Font will be 9-point Arial, or smaller if needed for space constraints. If possible, small tables should appear on one page. If tables continue on to multiple pages, there should be a page break after an assessment so that all the statistics for an assessment appear on the same page.

4. Tables
   Table titles should reflect the content of the table. Under the main title, in parentheses, the name of the analysis population being summarized should appear.

   4.1 Summary Statistics - Continuous Data

   Unless otherwise noted, the mean and median and confidence interval (CI) of a set of values should be printed out to one decimal place more than the original value. The standard deviation should be printed out to 2 decimal places more than the original value. The number of subjects on whom the parameter is assessed should appear. Minimum and maximum should be consistent with the original value.

   4.2 Summary Statistics - Categorical Data
   Numbers of subjects are reported as whole numbers. Null counts are represented as 0. Table percentages should be reported to one decimal unless otherwise noted. Null percentages should be reported as 0.0. For all categories, the total number of subjects with data will be presented as N.

5. Subjects Included in Listings
   In general, subject data listings should include all subjects who are randomized. If a listing includes a subset of subjects who meet a certain condition (eg, subjects with SAEs) then this should be clear from the title of the listing. If there is no record for a listing, then a statement, such as There is no serious adverse events in any of the treatment groups, will be presented.

[redacted]

[redacted]

[redacted]

4. Global Composite Score

4.1 Tests Included in the Global Composite

- Detection Test (DET)
- Identification Test (IDN)
- One Back Test (ONB)
- Modified Groton Maze Learning Test (GMLTM)
- International Shopping List Test – Immediate Recall (ISLT)
- International Shopping List Test – Delayed Recall (ISRL)

4.2 [redacted]

[redacted]

6. Attention Domain Composite Score

6.1 Tests Included in the Attention Domain Composite

- Detection Test (DET)
- Identification Test (IDN)

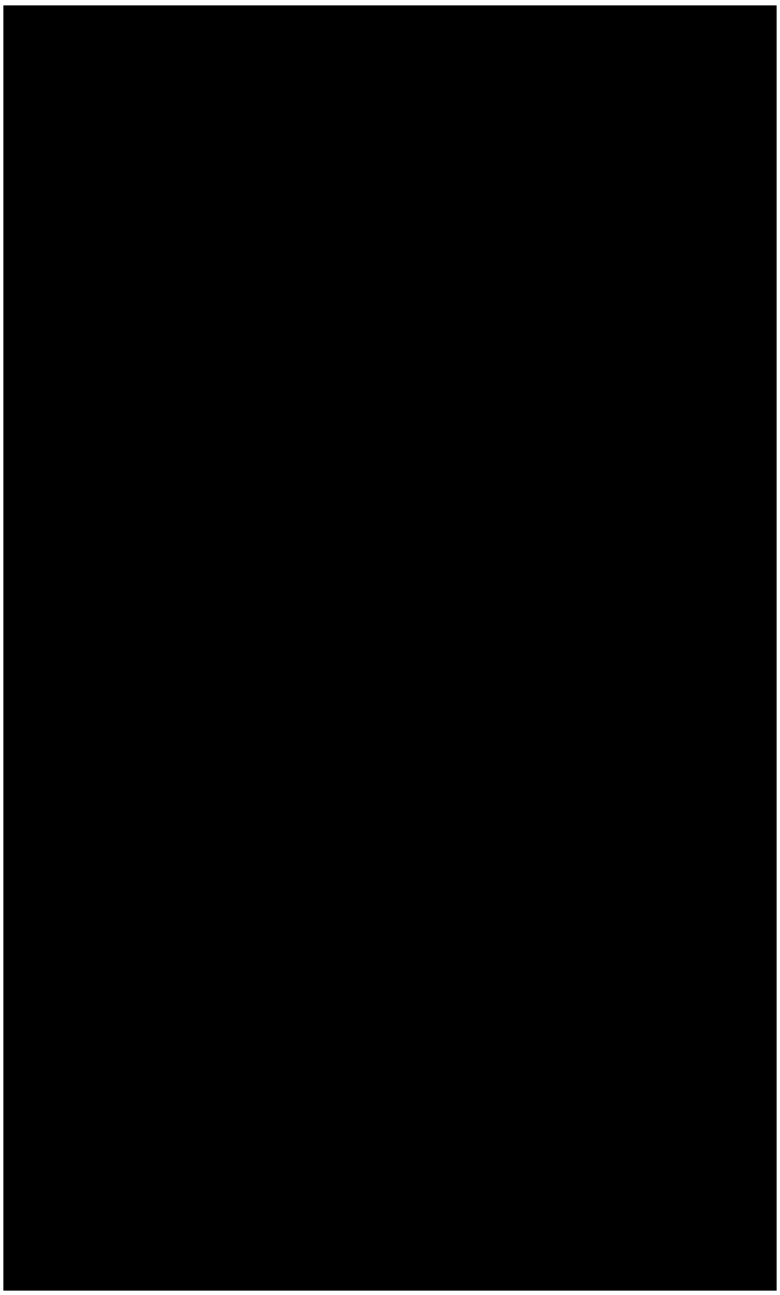
[redacted]

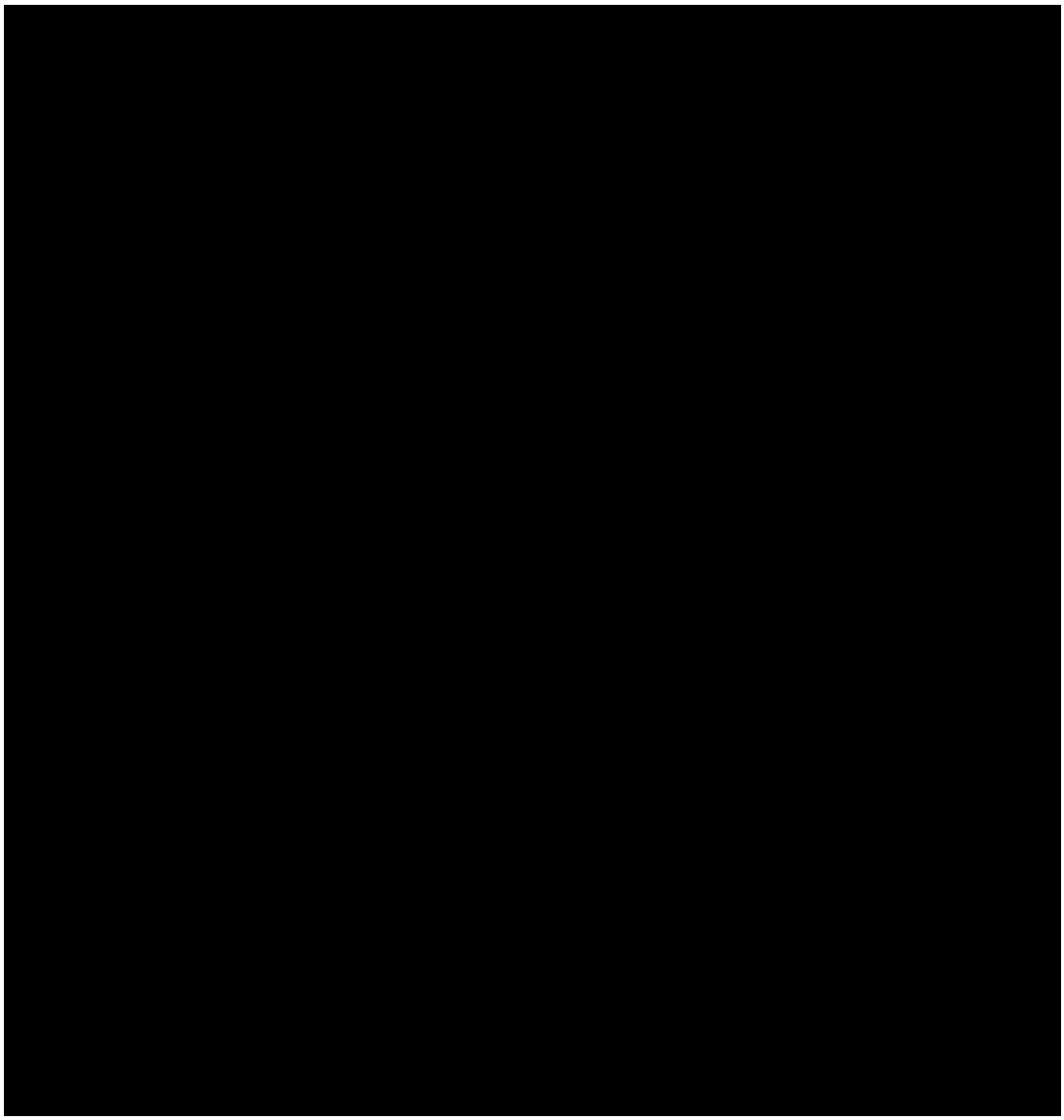
7. Executive Function Domain Composite Score

7.1 Tests Included in the Executive Function Domain Composite

- One Back Test (ONB)
- Modified Groton Maze Learning test (GMLTM)

[redacted]





Pittsburgh Sleep Quality Index (PSQI)

Form Administration Instructions, References, and Scoring

Form Administration Instructions

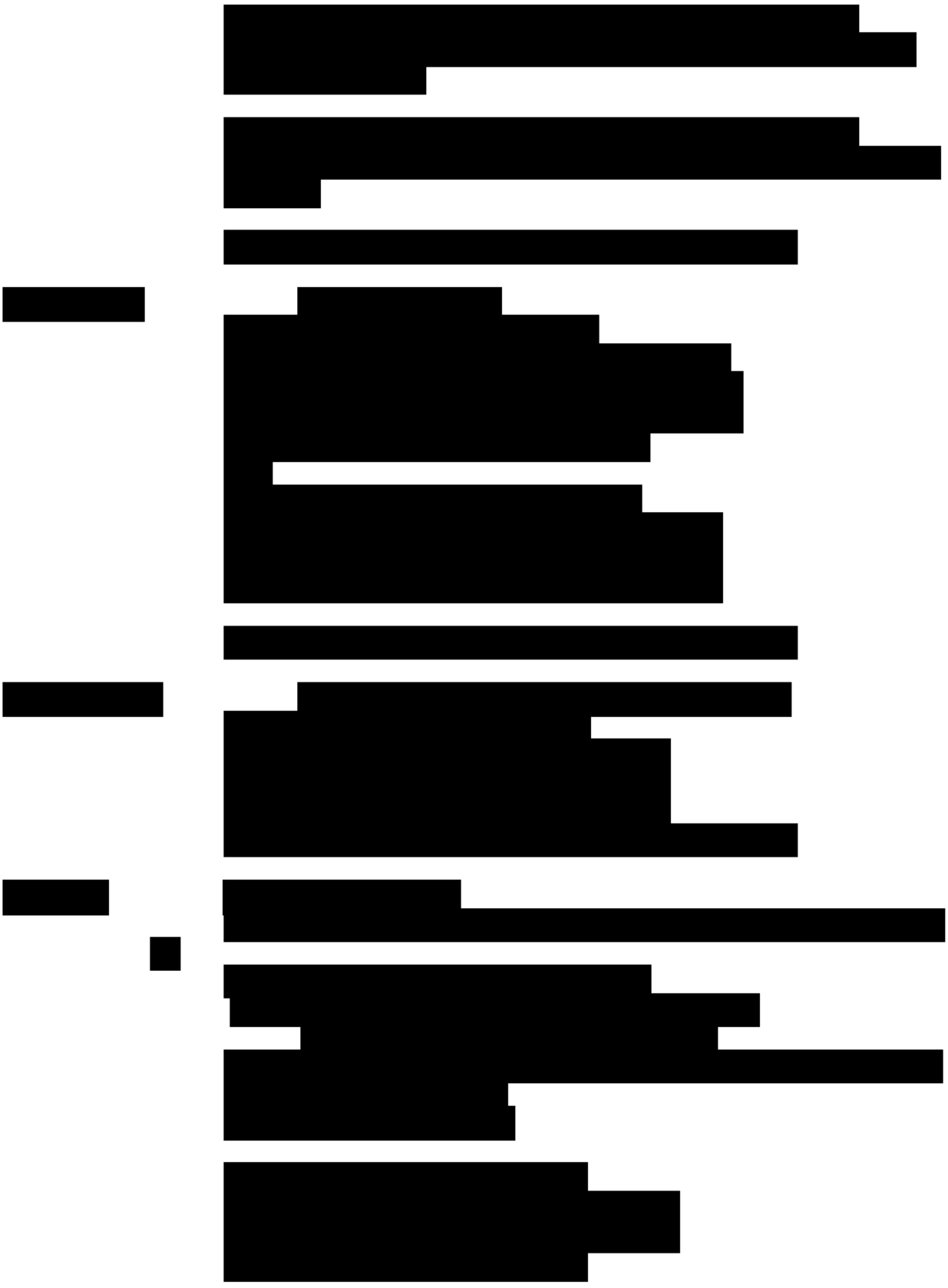
The range of values for questions 5 through 10 are all 0 to 3.

Questions 1 through 9 are not allowed to be missing except as noted below. If these questions are missing then any scores calculated using missing questions are also missing. Thus it is important to make sure that all questions 1 through 9 have been answered.

In the event that a range is given for an answer (for example, '30 to 60' is written as the answer to Q2, minutes to fall asleep), split the difference and enter 45.

Reference

Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research* 28:193-213, 1989.



References

1. US Federal Register. (1998) International Conference on Harmonization; Guidance on Statistical Principles for Clinical Trials. Department of Health and Human Services: Food and Drug Administration [Docket No. 97D-0174]. Federal Register Volume 63, Number 179, pages 49583-49598. September 16, 1998.
2. ASA. (2016) Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, April 2016. http://www.amstat.org/about/ethicalguidelines.cfm
3. RSS. (2014) The Royal Statistical Society: Code of Conduct, 2014. http://www.rss.org.uk/Images/PDF/join-us/RSS-Code-of-Conduct-2014.pdf
4. 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.
5. Jenkinson C. The SF-36 Physical and Mental Health Summary Measures: An Example of How to Interpret Scores. Journal of Health Services Research & Policy. 1998;3(2):92-96. doi:10.1177/135581969800300206
6. Ware, John & Snoww, Kk & MA, Kosinski & BG, Gandek. (1993). SF36 Health Survey: Manual and Interpretation Guide. Lincoln, RI: Quality Metric, Inc, 1993. 30.
7. 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.