STATISTICAL ANALYSIS PLAN

Based on:
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PROTOCOL TITLE:	An Open Label Phase 1 Study to Compare the Safety and Tolerability of the [redacted] Column at Different Blood-Flow Rates in Patients with End-Stage Renal Disease Undergoing Hemodialysis
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[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]	10/15/2021
[redacted]	[redacted]	[redacted]	10/15/2021

Abbreviations

Abbreviation	Definition
ACTH	Adrenocorticotropic hormone
ADE	Adverse device effect
AE	Adverse event
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
ATC	Anatomical therapeutic chemical
b2M	Beta 2-microglobulin, β2-microglobulin
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
CBC	Complete blood count
CH50	Total hemolytic complement
CK	Creatinine kinase
Cl	Chloride
CRO	Contract Research Organization
CSR	Clinical study report
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
EOS	End of study
ESRD	End-stage renal disease
FDA	Food and Drug Administration
HbA1c	Hemoglobin A1c (glycated hemoglobin)
HBsAg	Hepatitis B surface antigen
HCO_3	Bicarbonate
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HD	Hemodialysis
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
HIV Ab	Human immunodeficiency virus antibody
Hp	Haptoglobin
IDH	Intradialytic hypotension/hypotensive event
IGFBP1	Insulin-like growth-factor binding protein 1
K	Potassium
Kt/V	Fractional urea clearance
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Magnesium
Na	Sodium
PP	Per-Protocol
PT	Preferred term
PT/INR	Prothrombin time/international normalized ratio
PTH	Parathyroid hormone

QTc	QT interval corrected for heart rate
SAE	Serious adverse event
SAP	Statistical analytical plan
SBP	Systolic blood pressure
SC5b-9	Soluble membrane attack complement
SD	Standard deviation
SOC	System organ class
TEAE	Treatment-emergent adverse event
TFL	Tables, figures, and listings
TSH	Thyroid-stimulating hormone
URR	Urea reduction ratio
WOCBP	Women of childbearing potential

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1 Introduction

This Statistical Analysis Plan (SAP) describes the planned statistical analyses to be performed for data from Protocol AKST1210-101, "An Open Label Phase 1 Study to Compare the Safety and Tolerability of the [redacted] Column at Different Blood-Flow Rates in Patients with End-Stage Renal Disease Undergoing Hemodialysis". This SAP was created using Clinical Protocol AKST1210-101 Version 2.1 dated 13May2021.

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

2 Objectives

2.1 Primary Objectives

To evaluate the safety and tolerability of the [redacted] column at blood-flow rates greater than 250 mL/min in subjects with end-stage renal disease (ESRD) undergoing hemodialysis (HD). The proposed study will assess the safety, tolerability, and impact on HD parameters when the [redacted] column is used at blood-flow rates of up to 450 mL/min.

2.2 Secondary Objectives

Exploration of the performance of the column by measuring beta-2 microglobulin (b2M) concentrations before and after HD as well as the potential impact on other plasma proteins, including complement factors.

2.3 Primary Endpoint

- Safety and tolerability of each column size and each blood-flow rate.
  - Evaluation of safety and tolerability will include assessment of the rate and severity of all treatment emergent adverse events (TEAEs), with analyses of adverse device effects (ADEs), number of intradialytic hypotensive (IDH) events, and changes in total and free hemoglobin (Hgb).
  - Tolerability will be assessed by the proportion of subjects who can escalate to and tolerate each column size and blood-flow-rate combination.

2.4 Secondary Endpoints

- Weekly fractional urea clearance (Kt/V) and urea reduction ratio (URR).
- Total fluid balance for each column-size flow-rate combination.
- Proportion of subjects who are able to achieve the dry weight goal during the allotted HD duration for each column size and flow rate combination.
- Change in plasma b2M concentrations (before and after HD) and contribution of the [redacted] column to b2M removal at each column size and blood-flow rate.
- Number of occurrences of visible thrombosis (clotting) in the [redacted] column, dialyzer, and/or tubing.
- Changes in plasma levels of complement factors, including total hemolytic complement (CH50), soluble membrane attack complex (SC5b-9), and C5a.

- Changes in plasma levels of other proteins, including insulin, adrenocorticotropic hormone (ACTH), insulin-like growth-factor binding protein 1 (IGFBP1), Gastrin, Osteocalcin, Parathyroid hormone (PTH), and Thyroid stimulating hormone (TSH).

3 Study Overview

3.1 Study Design

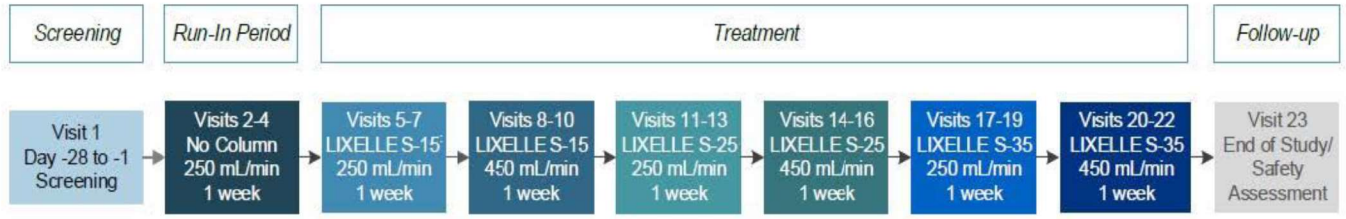
This is an open-label study in subjects between 40 and 75 years of age who have ESRD requiring HD. Assuming a drop-out rate (unrelated to safety and tolerability) of 20%, approximately 15 subjects will be enrolled to obtain 12 evaluable subjects. The [redacted] column will be connected in series before (upstream of) the dialysis cartridge for the duration of each HD session. Only single-use dialyzers will be used during the study.

After screening (Visit 1), subjects will undergo HD at a lower blood-flow rate (250 mL/min) for a 1-week run-in period to determine the HD duration required to achieve adequate Kt/V (>1.2). Daugirdas' formula will be used to calculate Kt/V (See Protocol References Daugirdas 1993, Daugirdas 1995, Daugirdas and Schneditz 1995).

The [redacted] column size will be increased from S-15 (150 mL) to S-25 (250 mL) to S-35 (350 mL), as tolerated, and blood-flow rates will be evaluated at 250 mL/min and up to 450 mL/min as tolerated (see Protocol Appendix 17.1: Escalation, De-escalation, and Discontinuation Rules for Treatment with the [redacted] Column). Each column-size and blood-flow rate combination will be evaluated during 3 consecutive HD sessions (1 week) before proceeding with the next combination, as follows (and as shown in the Protocol Schematic of Study Design):

- Week -1 (Visits 2-4) HD run-in period (no column) at 250 mL/min to determine Kt/V
- Week 1 (Visits 5-7) [redacted] column S-15 at 250 mL/min
- Week 2 (Visits 8-10) [redacted] column S-15 at up to 450 mL/min
- Week 3(Visits 11-13) [redacted] column S-25 at 250 mL/min
- Week 4 (Visits 14-16) [redacted] column S-25 at up to 450 mL/min
- Week 5 (Visits 17-19) [redacted] column S-35 at 250 mL/min
- Week 6 (Visits 20-22) [redacted] column S-35 at up to 450 mL/min
- Week 7 (Visit 23) End of study (EOS), no column, HD at patient's standard rate

SCHEMATIC OF STUDY DESIGN



The first treatment with the [redacted] column S-15 (150 mL) will occur mid-week (e.g., on Wednesday for subjects on a Monday/Wednesday/Friday schedule and on Thursday for subjects on a Tuesday/Thursday/Saturday schedule).

The escalation to the subsequent column-size and flow-rate combination will occur mid-week (e.g., on Wednesday for subjects on a Monday/Wednesday/Friday schedule and on Thursday for subjects on a Tuesday/Thursday/Saturday schedule).

The duration of HD time will be adjusted for flow rate (approximately 4-6 hours for 250 mL/min, 3-4 hours for up to 450 mL/min). During each HD session, measurements of HD effectiveness will include Kt/V>1.2 (calculated from measurements of blood urea) and URR (weekly URR >65%).

The mean and max blood flow rate will be calculated/pulled from the entries on the HDE CRF rather than the HD CRF.

The incidence of IDH events, as defined in Protocol Appendix 17.1: Escalation, De-escalation, and Discontinuation Rules for Treatment with the [redacted] Column, will be one factor used to determine column size/blood-flow rate escalation/de-escalation as well as discontinuation from the study.

The planned duration of subject participation is approximately 12 weeks (up to 4 weeks for screening and 8 weeks on-study, which is inclusive of the 1-week run-in period).

3.2 Statistical Hypothesis

The primary objective of the study is to evaluate the safety and tolerability of the [redacted] column when used at blood-flow rates up to 450 mL/min. The safety and tolerability will be assessed at each column size and blood-flow rate to enable comparisons across those variables in each subject (intrasubject) and across all subjects. Because the primary objective of the study is safety and tolerability, the study is not designed to detect statistically significant differences and will be primarily descriptive.

3.3 Sample Size Justification

The sample size for this study was based on FDA Guidance for the Content for Premarket Notifications for Conventional and High Permeability Hemodialyzers (FDA 1998) which recommends that data be collected for a minimum of 12 subjects for a minimum of 36 treatments. In this study, approximately 15 subjects between 40 and 75 years of age with ESRD will be enrolled. Assuming a drop-out rate of 20% (unrelated to safety), enrollment will yield approximately 12 evaluable subjects. With regard to the most important risk of

[redacted] IDH, a sample of 12 subjects will provide approximately 70% probability of detecting at least 1 IDH event in each week (i.e., for each column size and blood-flow rate combination). This estimate is based on the assumption that the rate of IDH in the general ESRD population, using the Nadir 90 definition, is 10%, based on a study in over 10,000 US patients undergoing HD (Flythe 2015).

3.4 Randomization/Unblinding

This is a non-randomized, open-label study.

3.5 Interim Analyses

Not applicable.

3.6 Study Assessment Time Points

The study consists of 23 protocol-specified visits, which will be assessed as nominal visits from an analysis perspective:

1) Visit 1 Screening: screening will be conducted at Days -35 to -7.
2) Visits 2 to 4, Run-In Period (Week -1): HD run-in period (no column) at 250 mL/min to determine Kt/V. Run-in will be conducted at Days -6 to -1.
3) Visits 5 to 22, Treatment Period (Week 1 to 6):
    a. Visits 5 to 7 (Week 1): [redacted] column S-15 at 250 mL/min. Visit 5 Day 1 is the day of the first use of the column. The escalation to the subsequent column-size and flow-rate combination will occur mid-week (e.g., on Wednesday for subjects on a Monday/Wednesday/Friday schedule and on Thursday for subjects on a Tuesday/Thursday/Saturday schedule).
    b. Visits 8 to 10 (Week 2): [redacted] column S-15 at up to 450 mL/min
    c. Visits 11 to 13 (Week 3): [redacted] column S-25 at 250 mL/min
    d. Visits 14 to 16 (Week 4): [redacted] column S-25 at up to 450 mL/min
    e. Visits 17 to 19 (Week 5): [redacted] column S-35 at 250 mL/min
    f. Visits 20 to 22 (Week 6): [redacted] column S-35 at up to 450 mL/min
4) Visit 23 EOS (Week 7): no column, HD at subject's standard rate. In the event of early withdrawal or discontinuation of a subject who has received at least 1 HD treatment with the [redacted] column, the EOS procedures will be performed unless the subject has withdrawn consent.

3.7 Schedule of Events

Refer to Protocol Section 15.

3.8 Data Safety Monitoring Board (DSMB)

Safety oversight will be provided by the Sponsor's Program Physician or designee and the CRO's Medical Monitor(s) in concert with the site investigators. There will be no formal DSMB established. No DSMB analyses are planned in the SAP.

4 Statistical Methodology

4.1 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 from this feasibility study will be analyzed mostly in a descriptive manner. Limited statistical comparison of outcomes across the various column-size and blood-flow-rate combinations will be performed. Details for any statistical tests are provided in the endpoint analysis sections (4.7, 4.8).

All safety endpoints will be summarized descriptively and tabulated as necessary, unless otherwise stated. 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) and change from baseline values (or percent change from baseline) will be presented as descriptive summary.

- For endpoints that are categorical in nature:
  - Frequency counts and percentages will be presented as descriptive summaries.

Descriptive statistics will be utilized for trial conduct: screen failures, enrollment, withdrawal of consent, and study completion. Primary and secondary endpoints will be assessed at the beginning and end of each week of treatment for a particular column size and blood-flow rate combination. The effect of blood-flow rate will be assessed for a given column size as well as intrasubject and across-subject variability. The sample size was chosen based on clinical consideration of the study designed to test the safety and functionality of the [redacted] column operated at higher blood-flow rates (up to 450 mL/min).

In general, all summary tables will be supported by a relevant subject data listing. 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 and blood-flow rate combination as applicable.

Study day 1 is the first day of HD treatment with the [redacted] column. Study day is the day relative to the study day 1.

Baseline is the first value after the start of HD with the [redacted] column. For the assessments that do not take place during the treatment weeks (e.g., ECG, physical examination, etc.), the value from Screening will be the baseline. Baseline for lab values will be the last non-missing value prior to the start of HD with the [redacted] column.

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

Subjects who de-escalate (i.e., do not proceed to the next column size and blood-flow rate combination) will only be included in analysis up to the point of de-escalation.

4.2 Study Populations for Analysis

4.2.1 Safety Population

The Safety population will consist of all subjects who received at least 1 (or partial) HD treatment with the [redacted] column.

4.2.2 Evaluable Population

The Evaluable population will consist of all subjects who completed blood-flow rates up to 450 mL/min for at least 1 column size (e.g., S-15 at 250 and up to 450 mL/min).

4.2.3 b2M Population

The b2M Population will consist of a subset of the evaluable population that excludes any subjects with Major Protocol Deviations pertaining to blood flow rate as well as any subjects who have documented blood flow rate deviations exceeding +/- 20 mL/min of the protocol-specified blood flow rate at Visits 4, 7, 10, 13, 16, 19 and/or 22.

4.2.4 Per-Protocol Population

The Per-Protocol (PP) population will consist of all subjects who completed the study without any Protocol Deviations that impact analyses.

4.2.5 Subgroup Analyses

Not applicable.

4.3 Subject Disposition

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 Safety, Evaluable, b2M, and PP populations
- Subjects who complete the study, and who discontinued at each phase along with the reason for discontinuation

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

4.4 Demographics and other Baseline Characteristics

4.4.1 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 column size and blood-flow rate combinations, and Total, for the Safety 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.

4.4.2 Baseline Disease Characteristics

Baseline disease characteristics will be summarized by column size and blood-flow rate combinations and total for the Safety population for the following:

- Baseline Historical IDH Rate (0,1,2,3,4)
- Dialysis Vintage
- Baseline b2M Level

4.4.3 Medical History

Medical history will be coded by system organ class (SOC) and preferred terms (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) version 23.0. A listing of medical history data will be provided.

4.4.4 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.

4.5 Protocol Deviations

Protocol deviations will be summarized by column size and blood-flow rate combinations with each deviation category broken down into major and minor for the Safety population.

4.6 Methods for Handling Missing Data

No imputation of missing data will be performed.

4.7 Primary Analyses

Primary analyses will evaluate safety and tolerability of the [redacted] column at blood-flow rates greater than 250 mL/min in subjects with ESRD undergoing HD.
Except where indicated, primary endpoints will be summarized by each column size and blood-flow rate combination (S-15 250 mL/min, S-15 up to 450 mL/min, S-25 250 mL/min, S-25 up to 450 mL/min, S-35 250 mL/min, S-35 up to 450 mL/min) and total using both Safety and Evaluable populations.

4.7.1 Adverse Events

All treatment emergent adverse events (TEAEs) will be reported. A TEAE is defined as an AE that occurs on or after the date of the first column use.

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 for the Safety population:

- Any TEAE, including severity (Mild, Moderate, and Severe), relationship to study device and procedures (Unrelated, Possibly Related, Definitively Related), TEAEs leading to discontinuation of study participation.
- Any serious adverse events (SAEs), including severity (Mild, Moderate, and Severe), relationship to study device and procedures (Unrelated, Possibly Related, Definitively Related), SAEs leading to discontinuation of study participation
- Any ADEs, including severity (Mild, Moderate, and Severe), relationship to study procedures (Unrelated, Possibly Related, Definitively Related), ADEs leading to discontinuation of study participation
- Any fatal TEAEs, including relationship to study device and procedures (Unrelated, Possibly Related, Definitively Related)

4.7.1.1 Summary for All TEAEs

The number and percentage of subjects who experience TEAEs will be tabulated by SOC and PT using the MedDRA version 23.0 or higher.
Adverse events will be counted only once for a subject within each PT and SOC at the highest severity and strongest attribution to the study device; thus, since a subject may have more than one PT within a SOC, percentages of PT may not sum to the percentages in the SOC.

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 to device and procedures presented by SOC/PT

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

4.7.1.2 Summary for Serious TEAEs

For all serious TEAEs the following will be summarized:

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

- Overall summary of any serious TEAEs by closest relationship to device and procedures 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.

4.7.1.3 Deaths

Deaths, if any, will be provided in a listing only.

4.7.1.4 Summary for Adverse Device Effects

The number and percentage of subjects who experience any ADEs will be tabulated by SOC and PT using the MedDRA version 23.0 or higher.

An ADE will be counted only once for a subject within each PT and SOC at the highest severity and strongest attribution to the study device; thus, since a subject may have more than one PT within an SOC, percentages of PT may not sum to the percentages in the SOC.

For all ADEs the following will be summarized:

- Overall summary of any ADEs presented by SOC/PT
- Overall summary of any ADEs by worst severity presented by SOC/PT
- Overall summary of any ADEs by most causal relationship to procedures presented by SOC/PT

A Listing of all ADEs will be provided.

4.7.2 IDH Events

The number and percentage of subjects will be summarized for the Safety, Evaluable and PP populations, by column size and blood-flow rates combination, for the following:

- Historical IDH Rate (0,1,2,3,4)
- Number of IDH Events (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.

Protocol Defined IDH
Definitions:

Intradialytic hypotension/hypotensive event (IDH)	A systolic blood pressure (SBP) < 90 mmHg that occurs during hemodialysis and is confirmed by repeat measurement.
Expected IDH Rate	The expected number of occurrences of IDH per weekly treatment period derived from a subject's Historical IDH rate (see section below and Protocol Appendix 17.1). The expected IDH Rate is compared with the actual IDH rate and used to assess 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 8-week period prior to the run-in period. Subjects with 7 or more IDH events during this 8-week interval will be excluded.

4.7.3 Change in Total and Free Hemoglobin

Plasma levels of total Hgb and free Hgb at baseline (i.e., the last non-missing value prior to the start of HD with the [redacted] column) and each scheduled post-baseline timepoint, along with change from baseline at each scheduled post-baseline timepoint will be summarized for the Safety, Evaluable and PP populations, by each column size and blood-flow rate combination and total.

A listing of total and free Hgb level, as well as the changes from baseline at each scheduled post-baseline timepoint will be provided.

4.7.4 Tolerability of each Column Size and Blood-flow-rate Combination

The number and percentage of subjects will be summarized for the Safety, Evaluable and PP populations, by each column size and blood-flow rate combination and total for the following:

- Subjects entered each column size/blood-flowrate combination
- Subjects who de-escalated to a lower column size categorized by reason: IDH, anemia, or other. Anemia (as it pertains to de-escalation) is defined as having an Hgb lab value of < 9 g/dL despite receiving optimized treatment with erythropoietin stimulating agents (ESA).
- Subjects who were discontinued due to safety, tolerability, and/or investigator discretion
- Subjects who completed all the visits

4.8 Secondary Analyses

Secondary analyses include HD parameters, thrombosis events, weight, b2M, complement factors, and other plasma protein levels. Except where indicated, secondary endpoints will be summarized by each column size and blood-flow rate combination (S-15 250mL/min, S-15 up to 450 mL/min, S-25 250mL/min, S-25 up to 450 mL/min, S-35 250mL/min, S-35 up to 450 mL/min) using both Safety and Evaluable populations.

4.8.1 Hemodialysis Parameters

Kt/V and URR (%) will be summarized for the Safety, Evaluable and PP populations, by visit and each column size/flow-rate combination.

Kt/V will be calculated with Daugirdas' formula from the pre-dialysis to post-dialysis urea nitrogen ratio (R), the weight loss (UF), session length in hours (t), and anthropometric or modeled volume (V) using the equation: Kt/V = In (R - 0.008 x t) + (4 - 3.5 x R) x 0.55 UF/V (Daugirdas 1995).

URR (%) is calculated as:
URR (%) = (1 – post-dialysis BUN / pre-dialysis BUN) * 100

For evaluation of total fluid balance, the initial fluid removal goal (mL), actual fluid removal (mL), and amount differs from the goal will be summarized by column size and blood-flow rate combination.

Actual fluid removal is calculated as follows: Weight (kg) – Weight Post-Dialysis (kg) and subsequently converting weight to volume (1 kg = 1000 mL).

In addition, the number and percent of subjects who achieved the dry weight goal (i.e., Weight Post-Dialysis (kg) is less than or equal to Estimated Dry Weight) will be summarized by column size and blood-flow rate combination.

A listing of HD parameters will be provided. Other HD parameters will not be summarized but will be provided in the listing only.

4.8.2 Thrombosis Events

Number of thrombosis events will be summarized for the Safety, Evaluable and PP populations, at each column size and blood-flow rate combination. A listing will also be provided.

4.8.3 Weight

Weight will be collected as described in the Schedule of Events, Protocol Section 15.1. Observed values in kg, 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. Summaries will be provided for the Evaluable and PP populations.

Interdialytic weight gain will also be calculated as:

Interdialytic weight gain (kg) = Pre-HD weight (kg) – post-HD weight (kg) from previous HD session.

Interdialytic weight gain will be provided in a listing and will be summarized by visit and each column size/blood-flow rate combination.

A listing of weight (kg) will be provided.

4.8.4 b2M

Plasma b2M levels will be measured at V4 (baseline), V7, V10, V13, V16, V19, V22, and V23. At V4 and V23 (EOS), one blood sample will be collected at the arterial port pre-HD. Summaries will be provided for the b2M population.

At V7, V10, V13, V16, V19, and V22, blood samples will be collected at two locations: arterial port and venous port. At the arterial port, samples will be collected pre-HD, 15 min into HD, mid-HD, and end-HD. At the venous port, samples will be collected 15 min into HD, mid-HD, and end-HD.

Research question 1: Is the b2M overall removal different between the different flow rates for each column?
Research question 2: Is the b2M overall removal different between the column sizes at each flow rate?

Analysis methods: a mixed-effect model for repeated measures (MMRM) will be performed at the whole study group for the changed b2M levels (comparing to levels at V4) at arterial port at V7, V10, V13, V16, V19, and V22, with fixed effect of flow rates (binary), column size (categorical) and flow rates-column size interaction, baseline b2M levels, and random effect of intercept and sites (or dialyzer types). Least-square means of changed b2M levels and associated 95% CI at each flow rate and at each column size will be calculated based on the analysis. The comparisons of estimated changed b2M levels between flow rates in each column size will be conducted, which answer question 1. The pair-wise comparisons of estimated changed b2M levels between column sizes at each flow rate will be conducted, which answer question 2. We will use Pre-HD arterial b2M levels to calculate overall removal from baseline for each time point.

Research question 3: Is b2M instantaneous clearance different between the different flow rates for each column?
Research question 4: Is b2M instantaneous clearance different between the column sizes at each flow rate?

Analysis methods: a mixed-effect model for repeated measures (MMRM) will be performed at the whole study group for the b2M ratios (levels at venous port/levels at arterial port) in the logarithmic scale after 15 min, at mid-HD and end-HD at V7, V10, V13, V16, V19, and V22, with fixed effect of flow rates (binary), column size

(categorical), flow rates-column size interaction, time (categorical, after 15 min, at mid-HD and end-HD), time-flow rates interaction, time-column size interaction, time-flow rates-column size three-way interaction and baseline b2M levels, and random effect of intercept and sites (or dialyzer types). Least-square means of the 3 b2M ratios and associated 95% CI at each flow rate and at each column size will be calculated based on the analysis. The comparisons for each of 3 estimated b2M ratios between flow rates in each column size will be conducted, which answer question 3. The pair-wise comparisons for each of 3 estimated b2M ratios between column sizes at each flow rate will be conducted, which answer question 4. The comparisons would be repeated for each measuring time-point (after 15 min, at mid-HD and end-HD).

> Research Question 5: Is there a significant difference in the arterial port end-HD/pre-HD b2M removal ratios for V7, 10, 13, 16, 19, 22?

Analysis methods: a mixed-effect model for repeated measures (MMRM) will be performed at the whole study group for the end-HD/pre-HD arterial ratio in the logarithmic scale, with fixed effect of visits (V7, 10, 13, 16, 19, 22), baseline protein levels, and random effect of intercept and sites (or dialyzer types). Least-square means of end-HD/pre-HD arterial ratios and associated 95% CI at two visits will be calculated based on the model. P value testing whether the difference is equal to 0 will be provided, which indicates whether there is a significant difference in instantaneous removal ratios for V7, 10, 13, 16, 19, and 22.

4.8.5 Complement Factors

Actual values and changes from baseline in plasma levels of complement factors, including total CH50, SC5b-9, and C5a, will be summarized by visit for the Safety and Evaluable populations.

4.8.6 Other Plasma Proteins

Actual values and changes from baseline in plasma levels of insulin, adrenocorticotropic hormone (ACTH), insulin-like growth-factor protein 1 (IGFBP1), Gastrin, Osteocalcin, Parathyroid hormone (PTH), and Thyroid stimulating hormone (TSH) will be summarized for the Safety and Evaluable populations, by visit, at each column size and blood-flow rate combination.

Other proteins will be measured at V4 (baseline), V7, and V22. At V7 and V22, these proteins will be measured only at the arterial port before and after HD.

> Research question 1: Does treatment significantly remove other proteins? If yes, are there significant differences in removal between V7 and V22?

Analysis methods: mixed-effect models for repeated measures (MMRM) will be performed at the whole study group for changed protein levels after HD at V7 and V22, with fixed effect of visits (V7 vs. V22), baseline protein levels, and random

effect of intercept and sites (or dialyzer types). Least-square means of changed protein levels associated 95% CI at two visits will be calculated based on the model. P value testing whether the LS means at each visit is significantly different from 0 will be provided, which indicates whether the treatment significantly removed these proteins. The difference in LS means between the two visits and 95% CI of the difference will also be calculated. P value testing whether the difference is equal to 0 will be provided, which indicates whether there is a significant difference in protein removal at V7 and V22.

Research question 2: Are the instantaneous removal ratios of other proteins different at V7 and V22?

Analysis methods: mixed-effect model for repeated measures (MMRM) will be performed at the whole study group for the End-HD/Pre-HD ratio in the logarithmic scale, with fixed effect of visits (V7 vs. V22), baseline protein levels, and random effect of intercept and sites (or dialyzer types). Least-square means of End-HD/Pre-HD ratios and associated 95% CI at two visits will be calculated based on the model. The difference in LS means between the two visits and 95% CI of the difference will also be calculated. P value testing whether the difference is equal to 0 will be provided, which indicates whether there is a significant difference in instantaneous removal ratios between V7 and V22.

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 this study only aims to find trends in secondary endpoints instead of confirmation.

4.9 Other Safety Analyses

Other safety analyses include physical examination, ECG, vital signs, pregnancy tests, laboratory data, fluid assessment, prior and concomitant medications, heparin infusion, and study device accountability. Except where indicated, safety parameters will be summarized for the Safety population, by column size and blood-flow rate combination.

4.9.1 Physical Examination

Physical Examinations and targeted physical examinations will be conducted at Visit 1, and 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.

4.9.2 12-lead ECG

A 12-lead ECG will be performed to obtain heart rate (beats/min), QT (msec), and QT interval corrected by the Fridericia formula (QTcF (msec)). ECGs will be collected at Visit 1 and Visit 23 End of Study.

Observed value at baseline and Visit 23, along with the change from baseline value will be summarized.

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

4.9.3 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 at the beginning and end of each visit. If screening and/or EOS occur on a day in which a subject is not receiving HD, vital signs will be measured after the subject has been seated/reclined for ~5 minutes. 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. Change from baseline in blood pressure will 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 HD, or if in the HD data we have additional saline use entered as 'Hypotension', or there is a log record in the HD 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 HD was stopped).

4.9.4 Pregnancy tests

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

4.9.5 Laboratory Data

Analyses on hematology, chemistry, coagulation, and other clinical lab tests will consist of the followings:

- 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

Hematology: complete blood count (CBC), haptoglobin (Hp), Hgb, plasma-free Hgb.

Chemistry: Alanine aminotransferase (ALT), albumin, aspartate transaminase (AST), bicarbonate (HCO_3), bilirubin (total), blood urea nitrogen (BUN), calcium (Total), chloride (Cl), creatine kinase (CK), creatinine, glucose, hemoglobin A1c (HbA1c), lactate dehydrogenase (LDH), magnesium (Mg), potassium (K), phosphate, protein (total), sodium (Na).

Coagulation: Activated partial thromboplastin time (aPTT), prothrombin time/ international normalized ratio (PT/INR).

Other: Complement C3 and C4, gastrin, Heparin Anti-Xa (only for patients on low molecular weight heparin), osteocalcin, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH).

Please refer to Protocol Section 15.3 Schedule of Laboratory Tests for scheduled time points of each test.

The tests will be presented in the standardized units. Any data contains '>' or "<" sign will be converted to numeric for the summary tables. 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". Subject listings will present all data collected (the '>' and '<' signs will be kept) along with toxicity grading.

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.

Labs that can indicate serious condition if outside reference range:

Analyte	Sex	MAX YEARS	Units	Critical Low	Critical High
Chemistry					
Albumin	M/F	60	g/dL	2.5	
	M/F	90	g/dL	2.5	
	M/F	150	g/dL	2.5	
ALT	Male	150	IU/L		190
	Female	150	IU/L		160
AST	M/F	150	IU/L		120
Bilirubin Total	M/F	60	mg/dL		5.0
	M/F	90	mg/dL		5.0
	M/F	150	mg/dL		5.0

Bicarbonate (CO2 or HCO3)	M/F	150	mmol/L	9	
Calcium	M/F	60	mg/dL	6.9	12.1
	M/F	90	mg/dL	6.9	12.1
	M/F	150	mg/dL	6.9	12.1
Glucose Fasting	M/F	150	mg/dL	39	401
Glucose Random	M/F	150	mg/dL	39	401
Magnesium	M/F	59	mg/dL	0.9	7.1
	M/F	90	mg/dL	0.9	7.1
	M/F	150	mg/dL	0.9	7.1
Phosphorus (phosphate)	M/F	150	mg/dL	1.4	
Potassium	M/F	150	mmol/L	2.7	6.1
Sodium	M/F	90	mmol/L	119	156
	M/F	150	mmol/L	119	156
Total Protein	M/F	150	g/dL		10.1
Hematology					
WBC	M/F	150	K/uL	1.4	25.1
Hemoglobin	Male	150	g/dL	8.0	20.1
	Female	150	g/dL	8.0	20.1
Hematocrit	Male	150	%	25.0	60.1
	Female	150	%	25.0	60.1
Platelet	M/F	150	K/uL	50	1001
Abs Neutrophil	M/F	150	K/uL	1.0	

4.9.6 Fluid Assessments

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

4.9.7 Prior and Concomitant Medications

Prior and concomitant medications taken during the study participation will be categorized by World Health Organization classification for therapeutic class and medication name. The number and percentage of subjects taking any prior and concomitant medications will be summarized by anatomical therapeutic chemical (ATC) level 3 terms and preferred medication names. A listing will also be provided.

4.9.8 Heparin Infusion

The amount of heparin used in each HD session will be summarized by column size and blood-flow rate combination.

4.9.9 Study Device Accountability

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

4.10 Adherence and Retention Analyses

Subject adherence with the study visits schedule, visit procedures, HD procedures, and subject retention will be assessed and provided in tables and listings. Subject adherence will be assessed for each column size and blood-flow rate. Reasons for study

discontinuation will be compared across column-sizes and blood-flow rates and across subgroups of interest, as appropriate.

Document Version Control

Revision History:

REVISION	RELEASE DATE	AUTHOR	SUMMARY OF CHANGES

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: ddmmyyyy
   - 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 "by CTDS".
   - 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 (e.g., 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 column size/blood-flow rate combinations, will be presented.

References

1. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume KtV: an analysis of error. J Am Soc Nephrol. 1993;4:1205-1213.
2. Daugirdas JT. Simplified equations for monitoring Kt/V, PCRn, eKt/V, and ePCRn. Adv Renal Replace Ther. 1995;2:295-304.
3. Daugirdas JT, Schneditz D. Overestimation of hemodialysis dose depends on dialysis efficiency by regional blood flow but not by conventional two pool urea kinetic analysis. Am Soc Art Intern Org. 1995;41:M719-M724.
4. Flythe JE, Xue H, Lynch KE, Curhan GC, Brunelli SM. Association of mortality risk with various definitions of intradialytic hypotension. J Am Soc Neprhol. 2015;26:724-734.