STATISTICAL ANALYSIS PLAN

(Version 2.0)

Protocol Title:	A single arm, non-randomised device trial to assess the effect of the Theranova Dialyser on albumin and uraemic solutes in patients with Stage V chronic kidney disease requiring haemodialysis (Version 1.3)
Short Title:	REMOVAL-HD (A tRial Evaluating Mid cut-Off Value membrane clearance of Albumin and Light chains in HaemoDialysis patients)
Protocol Date:	11 November 2016
Trial Registration:	Australian New Zealand Clinical Trials Registry number ANZCTRN 12616000804482p

Australasian Kidney Trials Network

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DOCUMENT HISTORY

Version	Reason(s) for change	Date
1.0	Initial document	14/05/2018
2.0	Minor change in Section 7. Table and Figure **numbers** changed to match the final statistical report and the statistical analysis programs. Note, the actual tables and Figures remain unchanged.	06/09/2018

PREFACE

This Statistical Analysis Plan (SAP) describes the planned analyses and reporting for the Australasian Kidney Trials Network (AKTN) trial number 15.01, "A single arm, non-randomised device trial to assess the effect of the Theranova Dialyser of albumin and uraemic solutes in patients with Stage V chronic kidney disease requiring haemodialysis".

The planned analyses identified in this SAP will be included in future manuscripts. Exploratory analyses not necessarily identified in this SAP may be performed to support planned analyses. Any post-hoc or unplanned analyses not specified in this SAP will be clearly identified as such in the Final Statistical Report (FSR) and manuscripts for publication.

The following documents were reviewed when preparing this SAP:

- Clinical Trial Protocol for AKTN Trial Number 15.01 [(1)].
- Case report forms (CRFs) for AKTN Trial Number 15.01.
- Data Management Plan for AKTN Trial Number 15.01 [(2)].
- ICH Guidance on Statistical Principles for Clinical Trials [(3)].
- ICH Guidance on Structure and Content of Clinical Study Reports [(5)].

ABBREVIATIONS

ABBREVIATION	DEFINITION
ADE	Adverse Device Effect
AKI	Acute Kidney Injury
AKTN	Australasian Kidney Trials Network
CKD	Chronic Kidney Disease
CVC	Central Venous Catheter
DSMB	Data and Safety Monitoring Board
ESAS-R	Edmonton Symptom Assessment System Revised
ESKD	End Stage Kidney Disease
FSR	Final Statistical Report
HD	Haemodialysis
ITT	Intention-To-Treat
λ-FLC	Lambda free light chains
k-FLC	kappa-Free Light Chains
MIS	Malnutrition Inflammation Score
MCO	Mid Cut-Off
TSC	Trial Steering Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SADE	Serious Adverse Device Effect
USADE	Unanticipated Serious Adverse Device Effect

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1. INTRODUCTION

1.1 Background

Haemodialysis is a principal renal replacement modality for patients with end stage kidney disease (ESKD). The morbidity and mortality of patients receiving haemodialysis remains high when compared with the general population. The inadequate removal of some uraemic solutes could be a reason for high morbidity and mortality. The survival associated with ESKD is linked to middle molecules which are a well described class of uraemic solutes. To date larger middle molecules have been inadequately removed by haemodialysis strategies. The mid cut-off dialyser Theranova represents a new class of dialysis membrane with the ability to remove nearly all middle molecules.

1.2 Study synopsis

REMOVAL-HD is a pivotal, open label, non-randomised, single-arm, multi-centre device study. The primary objective of the study is to determine if regular haemodialysis using the Baxter Theranova mid cut-off (MCO) dialyser in a chronic haemodialysis population is safe and will not result in a significant loss of serum albumin. The study will also assess the efficacy of large middle molecule removal using this MCO dialyser.

The Baxter Theranova MCO dialyser has been designed to provide increased clearance of these larger middle-molecules in chronic haemodialysis (HD) patients, compared with high flux HD. Short term clinical studies (2 weeks) have demonstrated effective removal of molecules up to the molecular weight of 50kDa. However, these studies also identified a significant amount of albumin (molecular weight around 66kDA) loss during each dialysis session (approximately 5g per 4 hours HD). It is currently unknown if this degree of albumin loss will be tolerated in chronic dialysis patients. But at 5g per session (3x per week) this amount of albumin loss is comparable to that tolerated in peritoneal dialysis patients (4g per 24 hours – 7 days per week)[13]. Sustained albumin loss is a concern for chronic dialysis treatment as hypoalbuminemia is strongly associated with poor morbidity and mortality outcomes in patients receiving HD. No clinical outcomes have yet been studied with this dialyser.

Figure 1 shows the study schema. Patients will have a 4-weeks wash-in period of high flux HD, then 24 weeks of treatment with Theranova MCO dialyser and then 4 weeks wash-out with high flux HD.

2. STUDY DESIGN

2.1 Overview

The Theranova MCO dialyser is a novel therapy in patients with chronic HD. There is insufficient evidence regarding the safety and efficacy of this treatment in HD. A single arm study design is used to monitor the immediate and long-term effects following exposure to the dialyser.

2.2 Study population

Patients will be selected from in-centre HD units in Australia and New Zealand based on their interest and ability to recruit. Nine units are involved in this study and their feasibility to participate in the trial was assessed using a feasibility survey.

2.2.1 Inclusion criteria

Patients were eligible for inclusion in the study if all the following criteria were met:

1. Established chronic in-centre HD patient (>12 weeks on HD)
2. Aged over 18 years
3. Has a functioning Arterio Venous Fistula or Graft
4. Either oliguric (<500mls/24hrs based on 24hr urine collection within 12 weeks of screening) or anuric
5. Able to give informed consent

2.2.2 Exclusion criteria

Patients were excluded from the study if any one of the following conditions was met:

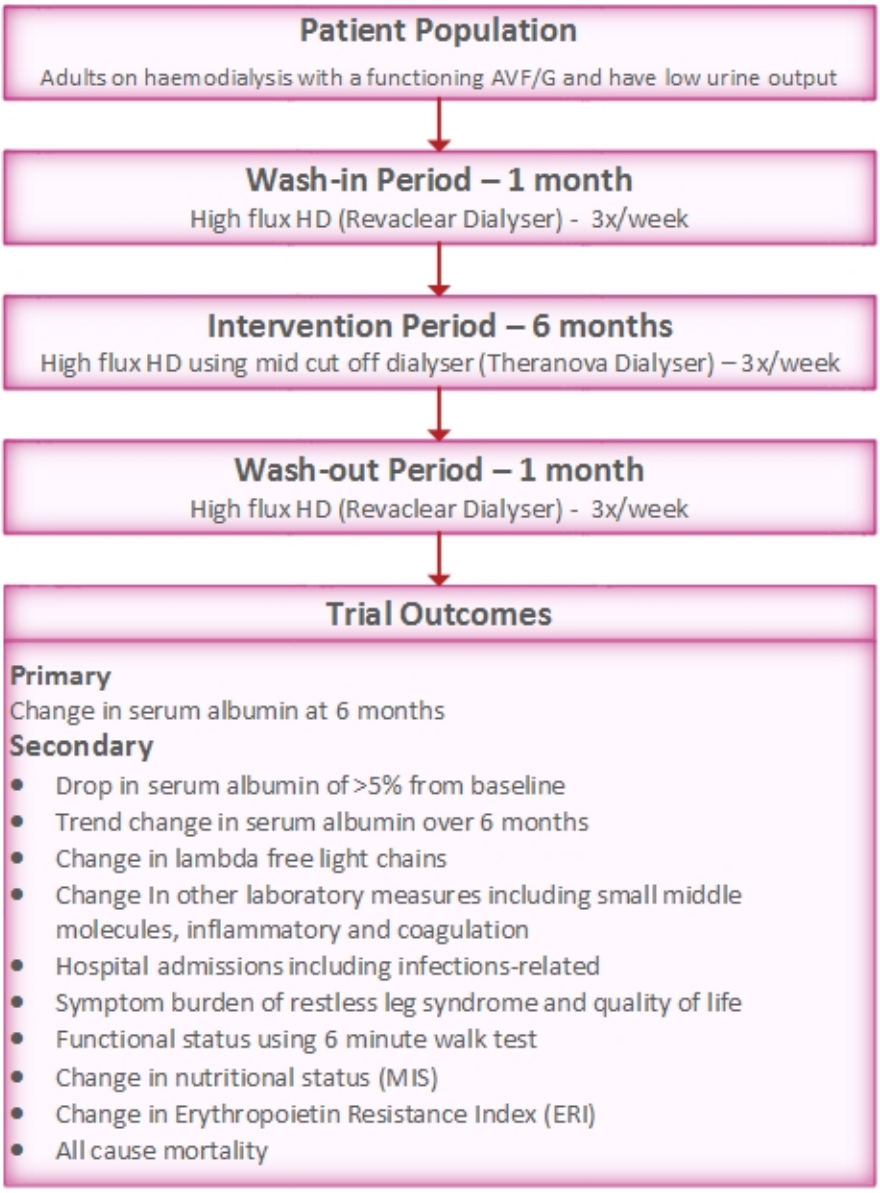
1. Planned renal transplant within study intervention period
2. Planned conversion to peritoneal dialysis or transfer to another dialysis unit within study intervention period
3. Active chronic infection or significant active inflammatory conditions including autoimmune disease, inflammatory arthritis and active malignancy
4. Life expectancy <12 months
5. Pregnancy or breast feeding
6. Indication for haemodiafiltration (HDF) according to treating physician
7. Dialysis catheter in situ
8. Receiving immunosuppressant medication
9. Current use of nutritional or dietary supplements to increase or reduce protein intake including protein powder or weight loss supplements and is unable to cease the supplement
10. Serum albumin <30g/L (within 4 weeks of screening)
11. Inability to complete study assessments

2.3 Study design and wash-in and out periods

Patients will receive six months' treatment with the Theranova dialyser three times per week. The Theranova dialyser use is not blinded. Prior to trial intervention period, patients will have 4-week wash-in period using a Revaclear high flux dialyser. After the intervention period, there will be a 4-week wash-out period using the Revaclear high flux dialyser.

A four week wash-in period is set for all study patients using a standardised dialysis membrane. The wash-in periods ensure equilibrium is reached before the intervention begins. Middle molecule concentrations are monitored during the wash-out period as the patients return to "standard care" (high flux dialysis).

Figure 1: Study schema



2.4 Sample size

The study was powered to detect a change of at least 5% change in central serum albumin concentrations between the baseline and six month visits. The observed serum albumin data collected during the FAVOURED trial (AKTN trial number 06.01) was used in the power calculation. The median baseline serum albumin observed in the FAVOURED trial was 35 g/L. Expected 5% change is 1.75 g/L. The observed standard deviation of albumin change in the FAVOURED trial over 12 weeks was 4.67 g/L. Power analysis was carried out using a one sample t-test for a mean difference of 1.75 with varying standard deviations of 3.67, 4.17, 4.67, 5.17 and 5.67. Required samples at 5% level of significance with varying power of 0.8, 0.85 and 0.90 were calculated. To detect a 5% difference in central serum albumin with 80% power, 5.17 standard deviation and a 5% significance level a sample size of 72 is required. To allow for a 15% loss to follow-up, the study needed to recruit 85 patients. Eighty percent power is associated with a recruitable sample size within the recruitment period for this pivotal study.

2.5 Schedule of assessments

Study Phase	Screening	Wash in Period		Intervention							Wash out
Visits	Visit 1	Visit 2	Visit 3 Baseline	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
	Week -1	Wk 0º	Wk 4	Wk 6	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24	Wk 28	Wk 32
Screening											
Inclusion/exclusion criteria	X	X									
Informed Consent	X										
24hr Urine collection	X										
Demographics/Medical History/Physical exam		X									
Primary Outcome											
Centrally tested serum albumin*		X	X	X	X	X	X	X	X	X	X
Trial Intervention Use											
Revaclear dialyser‡		X	X							X	X
Theranova dialyser‡			X	X	X	X	X	X	X	X	
Clinical assessments											
Erythropoietin Resistance Index			X				X			X	
Weight (pre & post HD)			X				X			X	X
Duration of HD			X				X			X	X
Restless Leg Symptom Rating Scale			X				X			X	
Malnutrition Inflammation Score (MIS)			X				X			X	
Edmonton Symptom Assessment System Revised			X				X			X	
6 minute walk test*			X				X			X	
Adverse events (as required)		X	X	X	X	X	X	X	X	X	X
Local lab assessments											
Albumin*		X	X	X	X	X	X	X	X	X	X
Urea (pre & post HD)			X				X			X	
Hb, transferrin, INR, APTT*			X				X			X	
Central lab samples											
Lambda free light chains (λ-FLC)*		X	X	X	X	X	X	X	X	X	X
K-FLC, β2M, hsCRP*			X				X			X	
Substudy – MGP, fetuin A, CPP FGF-23*			X				X			X	

*Collected pre-dialysis prior to the mid-week HD session ºAll visits calculated from Week 0 ‡ Dialyser use will start at the HD session after the study visit indicated

3. STUDY OUTCOME VARIABLES

3.1 Primary outcome

The primary outcome is change in pre-dialysis concentrations of central serum albumin between baseline and 6 month visits. Centrally tested serum albumin is collected pre-dialysis prior to the mid-week HD session from visit 2 (wash-in) to visit 11 (wash-out).

3.2 Secondary outcomes

Data are collected on a range of secondary outcome variables. The full list of secondary outcome is as follows:

1. Proportion of population with a drop in serum albumin of >5% from baseline value
2. Trend changes in serum albumin levels over the 6-month treatment period
3. Change in lambda free light chain molecules
4. Changes in other laboratory measures including:
    a) Smaller middle molecules - B2-microglobulin and kappa free light chains
    b) Inflammatory marker – hsCRP
    c) Coagulation factors – INR/APTT
5. Number and duration of all-cause hospitalisations
6. Number of infection-related hospitalisations
7. Symptom burden:
    a) Restless leg syndrome (Restless Legs Syndrome Rating Scale [RLSRS])
    b) Quality of life (Edmonton Symptom Assessment System Revised [ESAS-R])
8. Functional status with 6-minute walk test – the distance a patient can achieve by walking on a flat surface in 6 minutes is measured using trundle wheel
9. Malnutrition Inflammation Score (MIS)
10. Erythropoietin resistance index in patients taking erythropoietin ([EPO] - ERI ≥1.0 IU/kg/week/gHb) or darbepoetin ([DPO] - ERI ≥0.005 µg/kg/week/gHb)
11. All-cause mortality

3.3 Exploratory outcomes

Exploratory outcomes are two protein-bound uraemic toxins, indoxyl sulphate and p-cresyl sulphate, and calcification inhibitors (e.g., fetuin-A) measured pre-dialysis.

3.4 Safety outcomes

The following safety outcome variables are recorded from the start of the wash-in period (Visit 2) until the end of the wash-out period (visit 11):

1. Any Serious Adverse Event (SAE)
    a. led to death
    b. a life-threatening illness or injury
    c. a permanent impairment of a body structure or a body function
    d. in-patient or prolonged hospitalization
    e. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
    f. led to foetal distress, foetal death or a congenital abnormality or birth defect
2. Any Serious Adverse Device Effect (SADE): adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event
3. Any Unanticipated Serious Adverse Device Effect (USADE): Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified

An investigator's opinion of the relationship of the SAE to the Theranova device was recorded as none, unlikely, possible or probable.

4. SEQUENCE OF PLANNED ANALYSES

4.1 Interim analyses

Serum albumin analysed at local laboratories was monitored on a regular basis. Any drop in serum albumin from one visit to the next that exceeded 25% was reported to the Trial Steering Committee (TSC). Descriptions of SAEs and their relatedness to the Theranova device were regularly reported to the (TSC).

4.2 Final analyses and reporting

All final analyses described in this SAP will be performed after the database has been cleaned and locked. The database will be cleaned and locked within three months of the last patient completing the wash out visit. Final analyses will commence once this SAP is approved by the lead trial statistician, chair of the AKTN Executives Operations Secretariat and the lead principal investigator. Any post-hoc exploratory analyses not identified in this SAP will be clearly identified as such and reported as supplementary results in the Final Statistical Report (FSR). In manuscripts for publication, any post-hoc analyses will be clearly identified as unplanned analyses.

4.3 Changes to statistical information in the protocol

There are no changes to statistical information given in the final version of the trial protocol [(1)].

5. ANALYSIS PRINCIPLES

The purpose of the REMOVAL-HD study is to determine the *safety* and *efficacy* of the Theranova MCO dialyser in a chronic HD population. The analysis data set will include only the subset of all the enrolled patients who started the baseline visit and used the Theranova MCO dialyser for at least 80% of intended treatments.

The analysis data set would maximise the opportunity for the Theranova MCO dialyser to show safety and efficacy while closely reflecting the scientific model underlying the protocol. All patients excluded from the analysis data set will be listed.

5.1 Subgroup analyses

Pre-specified subgroups are formed by the following baseline characteristics: BMI in Kg/m^2 (<16, 16-20, ≥ 20), gender (female, male), albumin in g/L (< 30, 30-40, ≥40), diabetes type (type 1, type 2).

5.2 Centre effect and heterogeneity

Nine centres recruited between six and nineteen patients. Centre will be considered as a factor in the analysis of albumin and lambda free light chains.

5.3 Multiple comparisons and multiplicity

Type of each variable and their form in each statistical model is discussed in this SAP as per clinical importance. A limited number of subgroups are pre-specified. Unplanned variable conversions, categorisation and other modification in order to obtain statistical significance will not be performed. No adjustments for multiplicity will be made.

5.4 Missing data handling

Missing central lab values will be replaced by the last observation carried forward method.

5.5 Covariate adjustment of main analyses

All the analyses are un-adjusted.

5.6 Data manipulation and computing

The main trial data are in electronic case report forms in a REDCap database. Results from analysis of central blood data will be entered into an Excel spreadsheet. All data manipulation, tables, figures and analyses will be conducted using SAS software version 9.4. List of Tables and Figures are shown in Section 7.

6. STATISTICAL ANALYSES

6.1 Patient characteristics

All enrolled patients will be accounted for in the FSR. Patient progress through the trial will be presented in a CONSORT flow diagram. A monthly recruitment bar chart will be presented with target and cumulative

recruitment. Patient enrolment and completion of trial by site will be presented. Reason for study exit will be shown.

Demographic and baseline characteristics will be summarised for patients who entered the baseline visit. Categorical variables will be summarised by frequencies and percentages. Continuous variables will be summarised by mean and standard deviation as well as quartiles.

6.2 Analysis of primary outcome

The distributions of central lab serum albumin at baseline (week 4) and end of intervention (week 28) will be visualised using parallel box plots. The change in central lab serum albumin between baseline (week 4) and end of intervention (week 28) will be tested using dependent t-test. This test assumes the albumin change scores (six month minus baseline) are normally distributed. This assumption will be tested and, if violated, data transformation will be performed in the first instance. If the data transformation is unsuccessful, Wilcoxon signed–rank test will be performed. The primary results will be a mean or median change score and 95% confidence interval.

6.3 Analysis of secondary outcomes

6.3.1 Proportion of study population with a drop in serum albumin of >5% from baseline value

Centrally tested serum albumin is longitudinally collected at 10 time points, starting at wash-in (week zero) and ending at wash-out (week 32). The number of patients who had albumin collected, had an albumin drop of more than 5% and the averages and standard deviations of albumin drops at each visit will be described.

6.3.2 Trend changes in serum albumin levels over 6-month treatment period

The distribution of albumin at each visit will be shown using multiple box plots. This figure would compare spread, skewness and outliers of albumin across visit by depicting the data through quantiles. Change in average albumin over weeks will be presented with vertical 95% confidence interval bars.

A linear mixed effect model that describes change in albumin over time (week 4 to week 28) will be developed. The model will include time as a fixed effect and patient as a random effect. Results for the fixed effect of time will be reported as a coefficient estimate, 95% confidence limits for the estimate.

6.3.3 Absolute and trend changes in lambda free light chain over 6-month treatment period

Lambda free light chains (λ-FLC) collected at 10 visits, starting at wash-in (week zero) and ending at wash-out (week 32). Distribution of λ-FLC at each visit will be shown using multiple box plots. This figure would compare spread, skewness and outliers of λ-FLC across visit by depicting the data through quantiles. Change in average λ-FLC over weeks will be presented with vertical 95% confidence interval bars.

A linear mixed effect model that describes the change in λ-FLC over time (week 4 to week 28) will be developed. The model will include time as a fixed effect and patient as a random effect. Results for the fixed effect of time will be reported as a coefficients estimate, 95% confidence limits for the estimate.

6.3.4 Change in other laboratory markers

Other laboratory measures (β2M, k-FLC, hsCRP) are centrally collected at weeks 4, 16 and 28. INR or APTT is locally collected at weeks 4, 16 and 28. Distribution of those central lab measures will be shown using panels with box plot. Averages, standard deviation, and change from baseline at weeks 16 and 28 will be calculated. The change from baseline to end of intervention will be tested using dependent t-tests. Normal distribution of the change scores will be assessed. In the event of non-normality, data transformation will be performed in the first instance. If the data transformation is unsuccessful Wilcoxon signed–rank test will be performed.

6.3.5 Number and duration of hospital admissions and Incidence of all infection-related hospitalisations

Number of events and patients of all-cause and infection related hospitalisation will be shown. Average duration of all-cause hospitalisation per patient will be calculated.

6.3.6 Improvement in symptom burden

Restless leg syndrome is collected at weeks 4, 16 and 28. Severity scores of restless legs syndrome will be described in a table and shown as a multiple stack bar plot.

Edmonton symptom assessment system revised (ESAS-R) is used at weeks 4, 16 and 28. Percentage of respondents, median and mode ESAS scores will be described.

6.3.7 Improvement in functional status using 6-minute walk test

A six minute walk test is conducted at weeks 4, 16 and 28. The distribution of distance walked will be shown using multiple box-plots. Number of participants, average distance walked and standard deviation at each visit will be shown. In addition, change in average distance walked at week 16 and 28 from week 4 will be calculated. The change from baseline to end of intervention will be tested using dependent t-tests. Normal distribution of the change scores will be assessed. In the event of non-normality, data transformation will be performed in the first instance. If the data transformation is unsuccessful Wilcoxon signed–rank test will be performed.

6.3.8 Change in nutritional status using Malnutrition Inflammatory Score

Malnutrition-inflammation score (MIS) is collected at weeks 4, 16 and 28. Each MIS component has possible score of zero to three. Percentage of respondents for each MIS score will be displayed.

6.3.9 Change in erythropoietin resistance index in participants taking erythropoietin (EPO - ERI ≥1.0 IU/kg/week/gHb) or darbepoetin (DPO - ERI ≥0.005 µg/kg/week/gHb)

Erythropoietin resistance index (ERI) is calculated at weeks 4, 16 and 28 for patients who were taking Erythropoietin (EPO) or Darbepoetin (DPO). The formula for ERI is shown below,

$$ERI = \begin{cases} \frac{EPO}{Hb}; If\ EPO\ is\ taken \\ \frac{DPO \times 200}{Hb}; If\ DPO\ is\ taken \end{cases}$$

where, EPO is erythropoietin dose, DPO is darbepoetin dose and Hb is haemoglobin. Doses are in IU/kg/week/gHb. Average ERI, number of patients taking EPO or DPO and the change in averages from week 4 will be listed.

6.3.10 All-cause mortality rates

Number of all-cause mortality from week 4 to week 28 will be shown in a bar graph.

6.4 Analysis of safety outcomes

The relationship (none, unlikely, possible, and probable) of each category of SAE to Theranova dialyser will be summarised by frequencies and percentages at each visit . The SAE body system (10 categories) will be summarised by visit. The detail of each SAE will be listed with the description of event as entered by the site coordinator.

Safety measures were implemented on local lab serum albumin. The number of patients who had a drop of more than 25% of local lab albumin in consecutive visits, or had a sustained (over two consecutive visits) albumin drop of more than 25%, and the averages and standard deviations of albumin drops at each visit will be listed. Reason for significant (drop over 25% from baseline) local lab albumin drop will be listed.

7. LIST OF TABLES AND FIGURES

8. REFERENCES

(1) The REMOVAL-HD Trial Protocol, Version 1.3, 11 November 2016.
(2) The REMOVAL-HD Trial Data Management Plan, Version 1.0, 09 February 2018.
(3) ICH harmonised tripartite guideline: statistical principles for clinical trials E9. 1998.
(4) ASA. Ethical guidelines for statistical practice. Prepared by the Committee on Professional Ethics. 1999. Available from: http://www.amstat.org/about/ethicalguidelines.cfm.
(5) ICH Harmonised tripartite guideline: structure and content of clinical study reports E3. 1995.

9. APPROVAL

SAP Version Number being approved: Version 1.0

Lead Trial Statistician

Name Ms Elaine Pascoe

Signed  Date 14/05/18

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