PROTOCOL FULL TITLE

RANDOMISED DOUBLE-BLIND PLACEBO-CONTROLLED PHASE 3 TRIAL OF TRIUMEQ IN AMYOTROPHIC LATERAL SCLEROSIS

Protocol Short Title/ Acronym

LIGHTHOUSE II

Trial Identifiers

ISRCTN:	ISRCTN88446415		
EudraCT:	2020-005069-15		
Protocol Version Number:	4.0	**Date:**	19.10.2023

Co-Sponsors

Name:	King's College London (UK)
Address:	Great Maze Pond SE1 9RT
Telephone:	+44 20 7818 8330
Email:	amy.holton@kcl.ac.uk

Name:	Macquarie University (Australia)
Address:	Level 3, 75 Talavera Road , NSW 2109, Australia
Telephone:	+61298122956
Email:	clinicaltrials@mq.edu.au

Name:	Stichting TRICALS Foundation (European Union)
Address:	Goeman Borgesiuslaan 77, 3515 ET, Utrecht, The Netherlands
Telephone:	+31 88 75 554 94
Email:	operations@tricals.org

Chief Investigators

Lead Investigator (UK)

Name:	Ammar Al-Chalabi	
Address:	Maurice Wohl Clinical Neuroscience Institute, King's College London, London SE5 9RX, UK	
Telephone:	+44 20 7848 5174	
Email:	ammar.al-chalabi@kcl.ac.uk	
Signature		Date: 7 November 2023

Lead Investigator (Australia)

Name:	Julian Gold	
Address:	150 Albion Street, Surry Hills, Sydney NSW 2010	
Telephone:	+61 411 110451	
Email:	julian.gold@health.nsw.gov.au	
Signature		Date:

Lead Investigator (European Union)

Name:	Leonard van den Berg	
Address:	UMC Utrecht, Heidelberglaan 100, 3584CX, Utrecht, The Netherlands	
Telephone:	+31 88 75 554 94	
Email:	l.h.vandenberg@umcutrecht.nl	
Signature		Date:

Methodologist

Name:	Ben Carter	
Address:	IoPPN, King's College London, London SE5 8AF, UK	
Telephone:	+44 20 7836 5454	
Email:	ben.carter@kcl.ac.uk	
Signature		Date:

Clinical Trials Unit

Name:	King's Clinical Trials Unit
Address:	IoPPN, King's College London, London SE5 8AF, UK
Telephone:	+44 20 7848 0532
Email:	ctu@kcl.ac.uk

Emergency Code Break Service

Name:	Emergency Scientific and Medical Services Global (eSMS)
Address:	Apex Yard, 29 Long Lane, London SE1 4PL
Telephone:	+44 20 7113 7878 (Administration)
Email:	info@esmsglobal.com

Study Synopsis

TITLE OF CLINICAL TRIAL:	Randomised Double-Blind Placebo-Controlled Phase 3 Trial Of Triumeq In Amyotrophic Lateral Sclerosis
Protocol Short Title/ Acronym:	The Lighthouse II Trial
Study Phase:	3
Sponsor Name(s):	King's College London (UK), Macquarie University (Australia), Stichting TRICALS Foundation (European Union)
Chief Investigator(s):	Ammar Al-Chalabi, Leonard H. van den Berg, Julian Gold
Medical Condition or Disease Under Investigation:	Amyotrophic Lateral Sclerosis (ALS)
Purpose of Clinical Trial:	To determine if Triumeq improves survival in ALS compared with placebo
Primary Objective:	The primary objective of this study is to assess the efficacy of Triumeq versus placebo on overall survival, defined as death from any cause, in participants with ALS at 24 months or after a minimum of 212 events.
Secondary Objective(s):	To assess the effect of Triumeq versus placebo on a combined assessment of survival and measures of daily functioning (CAFS) To assess the effect of Triumeq versus placebo on measures of daily functioning To assess the effect of Triumeq versus placebo on respiratory function To assess the effect of Triumeq versus placebo on plasma creatinine To assess the effect of Triumeq versus placebo on the time to reach advanced disease stages To evaluate the safety of Triumeq administered orally to participants with ALS To evaluate the tolerability of Triumeq administered orally to participants with ALS To assess the effect of Triumeq versus placebo on change in cognitive functioning To assess the effect of Triumeq versus placebo on change in quality of life To collect research blood and urine samples for post-trial explanatory analyses; markers will be included e.g. urinary P75ECD, plasma neurofilament light and heavy chain, HERV-K and genotyping

Trial Design:	Double blind (participant, investigators), 2:1 randomised, placebo controlled, international, multi-centre.
Sample Size:	390 participants
Summary of Eligibility Criteria:	Participants with diagnosed ALS who have a specific survival prediction and who meet the standard criteria of ALS trials
Intervention (Description, frequency, details of delivery)	The antiretroviral triple therapy, Triumeq, as a combined capsule taken orally; 4 capsules once daily.
Comparator Intervention:	Matched placebo taken orally; 4 capsules once daily
Maximum Duration of Treatment of a Participant:	24 months
Version and Date of Final Protocol:	Version 4.0 19 October 2023

Revision History

Protocol version	Description of changes from previous revision	Effective Date
Protocol Version 1.0	New protocol	June 2020
Protocol Version 2.0	Protocol refined, and ambiguities clarified	February 2021
Protocol Version 2.1	Research sample section refined. Macquarie University address and logo amended	April 2021
Protocol Version 2.2	Protocol refined	May 2021
Protocol Version 2.3	Gold Coast criteria referenced and appended contraception guidance refined	Aug 2021
Protocol Version 2.4	Participant identifiers refined	Sept 2021
Protocol Version 2.5	ALS-FTD-Q Removed Wording amended in Section 3.3	Sept 2021
Protocol Version 2.6	VAS removed from section 4.1 Participant timeline and removed text '4.4.5 VISUAL ANALOGUE SCALE (QUALITY OF LIFE)'	Oct 2021

Protocol Version 3.0	• Added ISRCTN in 'TRIAL IDENTIFIERS'; • Added regions for each Sponsor on pages 1 and 4 • Updated King;s College London Logo in the header • Added TUDCA to Eligibility criteria. 3.2.1 • Added New Zealand to the 3.3.1. List of Countries; 9.2 Adverse Event processing responsibilities, 12. Ethics & Regulatroy Approval, 14.4 Monitoring • Added reference to HMA CTFG Guidelines on highly effective contraceptive methods and a definition of WOCBP in '3.2 Eligibility Criteria' • Added remote consenting in '3.3 INFORMED CONSENT' • Removed 'Status Form' and added 'Adverse Events' and 'Concomittant Medications' for weeks 2 and 6 in '4.1 PARTICIPANT TIMELINE', Schedule of Events table 1 Clarification +/- 7 day window added 4.1.1 VISIT WINDOWS • Added clarification on Hepatitis B & C tests if rescreening is required • Added clarification the collection of blood samples if consent conducted remotely in section '4.1.2 SCREENING VISIT'; • Indicated the equipment is CE marked, under warranty and used within the intended purpose • Added further details in section 4.5.3 TWELVE-LEAD ECG • Corrected the timepoint (screening) for the Neurological screening in 4.5.2 Neurological exam • Added reference to Pathology Manual for blood samples the sample sizes in 4.6 • Added clarification that Research bloods and urine samples are for future analysis and updated the centrifuge process in 4.6.5. Research and Urine Samples • Added clarification on taking the study drug immediately after opening the capsules and if administered in water: 5.2.3 Dose, Packaging and Labelling • Added that magnesium supplements should be avoided around the time of taking the study drug in section 5.6 Concomittant medicaiton • Added initials to the patient data collected in sections 6.3.3 ASSIGNMENT OF PARTICIPANTS TO INTERVENTION'; '7.2. DATA SECURITY'; '15.4. CONFIDENTIALITY'; • Amended wording to instances when the SAE reporting is required is clear in '9. Adverse Events Management and Reporting' • 7.2 DATA SECURITY Master participant has been replaced with subject ID log • Amended names of the KCTU trial manager, junior statistician, patient representative and Macquarie coordinator, and added KCTU Senior Trial Manager in '14.1 TRIAL MANAGEMENT GROUP (TMG)'	May 2023

	• Added clarification on the regional responsibilites for amendment submisisons in '12. Ethics and Regulatory Approval' • Added data storage length • Added specifics about the GDPR and DPA 2018 • Removed Appendix I Detailed Guidance on Randomisation procedure and amended relevant sections in the Protocol • Clarified that the KCL is responsible for no fault liability insurance in the event of harm arising from the study design in the UK in 15.8 Insurance and Indemnity	

Protocol version 4.0	• 3.1.1. List of study countries: Addition of a new study regions Slovenia and Spain; Belgium has been removed as no longer participating • In 3.2.1 Inclusion Criteria removed serum pregnancy testing at baseline • In 3.2.2 Exclusion Criteria removed lipase as amylase test is sufficient to determine eligibility, clarified that Bilirubin ≥ 1.5 times ULN excludes from participation only with clinical indicators of liver disease, added that patients who are on Tofersen should stop taking 3 months prior enrolling to Lighthouse II • Removed 'Study Medication Dosing Log' for Week 2 and Week 6 in table for Schedule of Events in 4.1 Participant Timeline • In 4.1 Participant Timeline in the footnote under Schedule of Visits table clarified that if serum pregnancy testing was already performed at screening, a serum pregnancy test does not have to be repeated at baseline. Clarified that safety bloods and urinalysis at baseline are optional if already performed at screening visit • In 4.1.3 Baseline Visit also clarified that serum pregnancy test is not required at baseline if already performed at screening • In 4.1.2 Screening visit added the term primary care clinic to accommodate terminology in other countries • In 4.5.2 Neurological Exam added that this can be performed at baseline visit if not performed at screening visit • In 4.5.3 Twelve-Lead ECG clarified that this can be performed by a medically trained professional • In 4.6.1 clarified that HLA-B*5701 is a one-off genetic test that does not need to be repeated in the event of a re-screen • In 4.6.2 Safety Bloods (Haematology and Biochemistry) removed Lipase and Cystatin C and clarified that sites can perform lipase tests to monitor pancreas function if they have capacity. • In 4.6.3. Pregnancy test: also clarified that serum pregnancy test is not required at baseline if already performed at screening • In 4.6.5 Research Blood and Urine Samples added that samples may be used for future unspecified analysis and is optional • In 4.7.2 Study Medication Dosing Log added that dose adjustments are permitted for patients unable to tolerate full dose • In 5.6 Concomittant Medications clarified that antacids containing magnesium/aluminium should not be take 6 hours before or 2 hours after taking Triumeq • In 14.1. Trial Management Group (TMG) updated membership	18 July 2023

	• In 15.4 Confidentiality: Clarified that patient identifiable data will be kept for as long as is required for the rules and regulations for each country.	

Glossary of terms

AE/AR	Adverse Event/Adverse Reaction		**ITT**	Intention to Treat
ALS	Amyotrophic Lateral Sclerosis		**KCTU**	King's Clinical Trials Unit
ALSFRS-R	ALS Functional Rating Scale Revised		**KHP-CTO**	King's Health Partners Clinical Trials Office
ALS-MITOS	ALS Milano-Torino staging (ALS-MITOS)		**LDH**	Lactate Dehydrogenase
APAP	Acetaminophen test		**LLN**	Lower Limit of Normal
ALT	Alanine Aminotransferase		**MHRA**	Medicines and Healthcare products Regulatory Agency
AST	Aspartate aminotransferase		**MND**	Motor Neuron Disease
CA	Competent Authority		**MTA**	Material Transfer Agreement
DCR	Data Clarification Request		**NIHR**	National Institute for Health Research
CAFS	Combined assessment of survival and measures of daily functioning		**NIMP**	Non-Investigational Medicinal Product
CI	Chief Investigator		**NIV**	Non-Invasive Ventilation
CONSORT	Consolidated Standards of Reporting Trials		**PEG**	Percutaneous Endoscopic Gastrostomy
CPK	Creatine phosphokinase		**PI**	Principal Investigator (at site)
CRF	Case Report Form		**PIN**	Participant Identification Number
C-SSRS	Columbia Suicide Severity Rating Scale		**PIS**	Participant Information Sheet
CSV	Comma-Separated Values		**PP**	Per Protocol
CTIMP	Clinical Trial of Investigational Medicinal Product		**PSRAE**	Possible Suicidality-Related Adverse Event
CTU	Clinical Trials Unit		**R&D**	Research and Development
DMC	Data Monitoring Committee		**RA**	Regulatory Agency
DSUR	Development Safety Update Report		**REC**	Research Ethics Committee
ECG	Electrocardiogram		**RIG**	Radiologically Inserted Percutaneous Gastrostomy
eCRF	Electronic Case Report Form		**RN**	Research Nurse
ECAS	Edinburgh Cognitive and Behavioural ALS Screen		**RNA**	Ribonucleic acid
EDC	Electronic Data Capture		**SAE**	Serious Adverse Event/ Serious Adverse Reaction
EQ-5D-5L	EuroQol – 5 dimensions – 5 levels		**SAP**	Statistical Analysis Plan
eSMS	Emergency Scientific and Medical Services		**SDV**	Source Data Verification
EU	European Union		**SmPC**	Summary of Product Characteristics
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database		**SS**	Senior Statistician
GGT	Gamma-glutamyl transferase		**SDW**	Source Data Worksheets
GP	General Practitioner		**SUSAR**	Suspected Unexpected Serious Adverse Reaction
GCP	Good Clinical Practice		**SVC**	Slow Vital Capacity
GLI	Global Lung Function Initiative		**TGA**	Therapeutic Goods Administration
FDA	Food and Drug Administration		**TM**	Trial Manager
hCG	Human chorionic gonadotropin		**TMG**	Trial Management Group
HDPE	High Density Polyethylene		**TRICALS**	Treatment Research Iniative to Cure ALS
HERV-K	Human Endogenous Retrovirus Type K		**TS**	Trial Statistician
HIV	Human Immunodeficiency Virus		**TSC**	Trial Steering Committee
HLA	Human Leukocyte Antigen		**UK**	United Kingdom
ICF	Informed Consent Form		**ULN**	Upper Limit of Normal
ID	Identification		**US**	United States
IgM	Immunoglobulin M		**USA**	United States of America
IMP	Investigational Medicinal Product		**VAS**	Visual Analogue Scale
IMPD	Investigational Medicinal Product Dossier		**WOCBP**	Women of child bearing potential
INR	International Normalized ratio			

PROTOCOL CONTENT

1. Introduction

1.1 BACKGROUND AND RATIONALE

Amyotrophic lateral sclerosis (ALS, MND) is a progressive neurodegenerative disease of corticospinal tract, bulbar and anterior horn motor neurons, leading to severe weakness and death from respiratory failure within 3-5 years[1]. It is estimated that there are 50,000 people living with ALS in Europe and upwards of 10,000 deaths per year[2]. Mean age of onset in clinic populations is 56 years, and it is the most common neurodegenerative disease of mid-life. ALS is more frequent in men than women[3]. The lifetime risk of ALS is 1 in 300, but the prevalence remains low because the prognosis is so bleak[4]. By 2040 the incidence of ALS is predicted to increase by 20% across the UK and Europe[5]. The average survival time after diagnosis of ALS in most European countries is 17.7 months (95% CI 17.0 – 18.4), with death resulting from neuromuscular respiratory failure when the diaphragm muscles are involved[6]. ALS is a greatly feared diagnosis, with severe concomitant anxiety and distress, and it is the most frequent cause of assisted suicide[7]. The extensive care and support needs generated by ALS can place an enormous burden on family members, friends and loved ones providing unpaid care. In addition to these individual costs, the monthly cost to society of ALS has been estimated as €7000 in the Netherlands, which provides analogous levels of care to England[8]. In Australia, the annual per patient cost is estimated at A$205,000 (£100,000). Despite more than 280 clinical trials, the only drug licensed in Europe is Riluzole, which prolongs life by around 38% at 18 months[9]. In the US another treatment, Edaravone, was recently approved by the FDA[10]. Edaravone is a free-radical scavenger administered by infusion on 10-14 days a month for six months. It has not been shown to improve survival, but does marginally improve function when compared with placebo, although it may be that the impact on function of the infusion apparatus is simply countered by the drug, leaving an overall lack of benefit[11]. Nevertheless, because there are no other therapies available, many patients are attracted to this option despite the annual cost of around US$124,000. Edaravone is only approved in South Korea and Japan outside the USA, with a compassionate use programme in Italy.

In this study we will trial a new therapy for ALS using an innovative approach, targeting a proven motor neuron toxic pathway with a treatment that is already shown to have a good safety profile, and as a repurposed drug, would be quickly available to patients.

Although the heritability of ALS is about 60% and identifiable gene mutations account for about 80% of those with familial ALS, they have only been so far found in 14% of those without a family history[1], [12]. In all patients, there is a multistep pathogenesis[13], suggesting that even in those with a large effect mutation, environmental and epigenetic factors affecting gene expression are important. It has been suggested for more than 40 years that a retrovirus may have a role, and a naturally occurring mouse model of ALS likewise is a multistep process, and involves the activation of an endogenous retrovirus on the background of two additional steps involving genetic risk and infection[14], [15], [16].

About 10% of the human genome codes for endogenous retroviruses (HERVs)[17], relics of ancient retrovirus infections that have incorporated into the genome. We, and others, have previously shown reverse transcriptase activity in the serum of people with ALS, and studies of their spouses and relatives suggest the reverse transcriptase is endogenous[18], [19], [20], [21]. Thus, in ALS there may be activation of an endogenous retrovirus[22].

HERV-K is the most recent HERV to be integrated into the human genome[23]. Its reverse transcriptase is significantly elevated in motor neurons from people with ALS[24]. In a study of post-mortem human ALS brain tissue, transcripts of three proteins encoded by HERV-K (gag, pol and env) were detected, and

immunostaining showed expression of HERV-K in motor neurons but not in non-neuronal cells[25]. These findings were not seen in a similar number of Alzheimer disease brains or in patients with other neurological conditions[18]. Technical differences might explain why a recent further study was not able to replicate this HERV-K expression difference between ALS brains and others[26], but in any case, the central, replicated finding of an endogenous retroviral signature remains, and there is considerable additional data implicating HERV-K. In in vitro studies, transfection of HERV-K genome or the env gene into cultured human neurons triggers neuronal death, as does activation of the endogenous HERV-K genome[27]. In one important study, active loci of HERV-K were found in ALS, and HERV-K expression was strongly correlated with TDP-43 expression[22]. Furthermore, HERV-K env protein is neurotoxic in the brains of mice in utero, and transgenic mice expressing HERV-K env develop a reduction in the number of cortical and spinal motor neurons with impaired motor function[18].

See section 11 for information on previous clinical studies.

2. TRIAL DESIGN

Lighthouse II is an international, phase 3, multi-centre, parallel group, placebo-controlled, blinded (participant, investigators, analyst) randomised controlled trial of Triumeq (Abacavir 600mg, Lamivudine 300mg and Dolutegravir 50mg) or matched placebo, once daily in addition to standard care, in a 2:1 treatment/placebo allocation ratio in participants with ALS, in order to determine superiority of treatment versus placebo. If the trial is not terminated at a planned interim analysis, the trial will continue until 24 months after the last enrolled participant or a minimum of 212 events have occurred, whichever is first.

2.1 OBJECTIVES

2.1.1 PRIMARY OBJECTIVE

The primary objective of this study is to assess the efficacy of Triumeq versus placebo on overall survival, defined as death from any cause, in participants with ALS at 24 months or after a minimum of 212 events.

2.1.2 SECONDARY OBJECTIVES

The secondary objectives of this study are:

1. To assess the effect of Triumeq versus placebo on a combined assessment of survival and measures of daily functioning (CAFS)
2. To assess the effect of Triumeq versus placebo on measures of daily functioning
3. To assess the effect of Triumeq versus placebo on respiratory function
4. To assess the effect of Triumeq versus placebo on plasma creatinine
5. To assess the effect of Triumeq versus placebo on the time to reach advanced disease stages
6. To evaluate the safety of Triumeq administered orally to participants with ALS
7. To evaluate the tolerability of Triumeq administered orally to participants with ALS
8. To assess the effect of Triumeq versus placebo on change in cognitive functioning
9. To assess the effect of Triumeq versus placebo on change in quality of life
10. To collect research blood and urine samples for post-trial explanatory analyses; markers will be included e.g. urinary $P75^{ECD}$, plasma neurofilament light and heavy chain, HERV-K, and genotyping

3. PARTICIPANTS

3.1 STUDY SETTING & RECRUITMENT

Participants will be recruited from secondary care settings recognised in their countries as centres of excellence for the diagnosis and management of amyotrophic lateral sclerosis. Participants will be approached regarding the trial, where possible, in routine follow-up clinics or recruiting sites will write to potentially eligible participants to invite them for screening.

Participating sites must have access to a -80°C freezer to store research samples. Where a site does not have capacity to store research samples, the site may be opened only with the explicit permission of the TMG and in those cases, research blood samples will not be collected.

3.1.1 LIST OF STUDY COUNTRIES

United Kingdom
Netherlands
Australia
Ireland
Slovenia
Sweden
Spain
New Zealand

A full list of participating sites within country can be obtained from the Chief Investigators.

3.2 ELIGIBILITY CRITERIA

3.2.1 INCLUSION CRITERIA

1. Age ≥ 18 years at the time of screening
2. Diagnosis of ALS according to the Gold Coast Criteria (Please see Table 1 of Shefner et al Clinical Neurophysiology 2020, also available in Appendix 1)
3. Capable of providing informed consent and complying with trial procedures
4. TRICALS risk profile > -6.0 and < -2.0 (See Section 4.3.4)
5. Those taking Riluzole must be on a stable dose for at least 30 days prior to the baseline visit or must have stopped taking Riluzole at least 30 days prior to the baseline visit
6. Women must not become pregnant (e.g., post-menopausal, surgically sterile, using highly effective birth control methods or not having potentially reproductive sex) for the duration of the study plus five days. *Highly effective methods of birth control are those with a failure rate of < 1% per year when employed consistently and correctly, e.g. Combined (oestrogen and progestogen containing) hormonal contraception or progestogen-only hormonal contraception*. For more information, please refer to the HMA CTFG Guidelines: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf?fbclid=IwAR3AY5Ha0ESDyqIBeUaYI9VTFWmx9bbt8NZ-80N-5ME6pkBb1UHvFsTwqlQ
7. Women of childbearing potential must have a negative serum pregnancy test at screening and be non-lactating. Patients will be advised regarding appropriate contraception. A menstruation history will be taken at each visit. *Women of childbearing potential are defined as females who are fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy (https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf?fbclid=IwAR3AY5Ha0ESDyqIBeUaYI9VTFWmx9bbt8NZ-80N-5ME6pkBb1UHvFsTwqlQ)*

8. For participants taking antacids (regularly or as required), participant is willing and able to avoid taking antacids for at least 6 hours before and 2 hours after Triumeq
9. Participant taking taurursodiol supplements (TUDCA) can participate in this trial if the supplement does not contain sodium phenylbutyrate.
10. Participants taking taurursodiol supplements (TUDCA) that also contain sodium phenylbutyrate must be willing to stop supplementaiton 30 days prior randomisation.

3.2.2 EXCLUSION CRITERIA

1. People who are HLA-B*5701 positive
2. Known hypersensitivity to Dolutegravir, Abacavir or Lamivudine, or to any of the excipients
3. Safety Laboratory Criteria at screening:

   ALT ≥ 5 times upper limit of normal (ULN)

   AST ≥ 3 times ULN

   Bilirubin ≥ 1.5 times ULN with clinical indicators of liver disease

   Creatinine clearance < 30 mL / min

   Platelet concentration of < 100 x109 per L

   Absolute neutrophil count of < 1x109 per L

   Haemoglobin < 100 g/L

   Amylase ≥ 2 times ULN

   Lactate ≥ 2 times ULN
4. Moderate to severe hepatic impairment, as defined by local clinical guidelines
5. Presence of HIV antibodies at screening
6. Presence of Hepatitis C antibodies at screening unless participants have had effective treatment for Hepatitis C
7. Presence of Hepatitis B core or surface antigen at screening
8. Participation in any other investigational drug trial or using investigational drug within 30 days prior to screening
9. Use of NIV ≥22 h per day or having a tracheostomy
10. Edaravone dose within 30 days prior to screening. *Edaravone is approved by the FDA and in Japan, but remains an investigational product in Europe and Australia*
11. Clinically significant history of unstable or severe cardiac, oncological, psychiatric, hepatic, or renal disease or other medically significant illness
12. Taking medication contraindicated with Triumeq: Dofetilide or Fampridine (dalfampridine)
13. Taking Tofersen within 3 months prior to screening.

3.3 **INFORMED CONSENT**

Informed consent will be obtained by the Principal Investigator or delegated physician at each site, following personal explanation of the trial procedures. Informed consent can be obtained by the Principal Investigator or delegated physician remotely via the telephone or videoconference after a hard copy of the approved Patient Information Sheet and Informed Consent Form have been supplied to the participant. If the consent has been obtained remotely, the participants will indicate in the form the means by which the remote consent has been obtained and the name of the investigator. The electronic record of the signature page will be

captured at the time (e.g. a scan, photograph, or a screenshot) either by the investigator or by the participant who will email the copy to the investigator and will supply the signed hard copy of the consent during the next study visit. The investigator will record in the source document that the consent has been obtained remotely and retain the electronic copy of the signature page in the Investigator Site File until the signed hard copy of the document is supplied. Once the signed hard copy has been provided, the investigator will add their signature and today's date. Potential participants may be contacted by the investigator only after consent has been provided by their treating physician.

If a participant is unable to sign the consent form due to hand weakness, verbal consent in the presence of a witness can be documented on the consent form and the participant signature should be marked with an X. The following text should be written on the form: 'Participant physically unable to sign consent but has given verbal consent in the presence of [name, relationship to clinic or participant, contact information]'. The witness should sign the statement and the physician should sign the consent form as normal.

Participants who lack capacity to consent are not eligible to participate.

Blood and urine collection for future unspecified analysis in ALS research or other related neuromuscular diseases will be optional for participants and consent will be within the main trial consent.

4. DATA COLLECTION & DATA ENTRY

4.1 PARTICIPANT TIMELINE

Timepoint	Screening (Day -28 to Day 0)	Baseline (Day 0)	Week 2	Week 6	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Primary outcome Month 24	Ongoing*
1. Registration form & consent	x												
2. Eligibility		x											
3. Medical history	x												
4. ALS history and TRICALS risk score	x												
5. Demographic data	x												
6. Randomisation		x											
7. Status form					x	x	x	x	x	x	x	x	
8. Slow vital capacity	x	x			x	x	x	x	x	x	x	x	
9. ECAS		x						x				x	
10. ALSFRS-R	x	x			x	x	x	x	x	x	x	x	
11. King's staging system		x			x	x	x	x	x	x	x	x	
12. EQ-5D-5L		x			x	x	x	x	x	x	x	x	
13. Columbia suicide severity rating		x						x				x	
14. All-cause mortality													x
15. Physical exam	x	x			x	x	x	x	x	x	x	x	
16. Neurological exam	x				x	x	x	x	x	x	x	x	
17. Twelve-lead ECG		x						x				x	
18. Vital signs		x			x	x	x	x	x	x	x	x	
19. Weight		x			x	x	x	x	x	x	x	x	
20. Adverse events log			x	x									x

Timepoint	Screening (Day -28 to Day 0)	Baseline (Day 0)	Week 2	Week 6	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Primary outcome Month 24	Ongoing*
21. Concomitant medications log			x	x									x
22. Withdrawal form													x
23. Serology bloods***	x												
24. Safety bloods****	x	x			x	x	x	x	x	x	x	x	
25. Pregnancy test (wocbp)**	x	x			x	x	x	x	x	x	x	x	
26. Urinalysis****	x	x			x	x	x	x	x	x	x	x	
27. Research bloods & urine		x			x	x	x	x	x	x	x	x	
28. Study medication dispensing		x			x	x	x	x	x	x	x		
29. Study medication dosing log													x

TABLE 1 SCHEDULE OF EVENTS

* *All ongoing forms to be reviewed and updated at each visit*

** *Women of child bearing potential only. Serum test required at screening ; urine test may be taken at baseline if serum test was alrady performed at screening to permit randomisation and medication preparation prior to serum results being available*

*** *HIV serology results within a year of screening will be accepted for all participants with a low risk for exposure. When re-screening is required, Hepatitits B and C can be omitted for low risk participants. HLA-B*5701 is a one-off genetic test and can be omittied when re-screening.*

**** *Safety bloods and urinalysis are optional at Baseline if performed during screening visit.*

4.1.1 VISIT WINDOWS

The screening visit can be scheduled up to 28 days prior to randomisation or may be combined with the baseline visit. If the baseline visit occurs after more than 28 days after the screening visit, screening assessments must be repeated. Screening and baseline assessments may be done on the same day as randomisation if a participant has received the participant information sheet in advance of the visit; where assessments are scheduled for both the screening and baseline visit but a single visit is undertaken, the data should be entered in the baseline visit and missing data recorded in the screening visit. Visits are scheduled every 3 months (13 weeks) post-randomisation, with a visit window of +/- 7 days. In the event that a visit is later than the target visit window of 7 days, the visit should be scheduled as soon as possible and the data should be entered into the intended visit. Subsequent visits should be scheduled as per the original visit schedule. A timepoint is considered missed if the visit is after the start of the subsequent visit window.

4.1.2 SCREENING VISIT

Participants will be screened for the study only after signing an approved Informed Consent Form. Screening data will be collected as per the Schedule of Events in Table 1 above for the screening visit and ongoing section. Relevant blood results can be requested from the GP surgery/primary care clinic in patients who consent remotely, to minimise the requirement for patients with mobility issues to travel to the recruiting sites repeatedly.Any screening procedures missed can be performed at baseline visit. The screening and baseline visits might be held on the same day. Patients can be re-screened at the principal investigator's discretion.

4.1.3 BASELINE VISIT

Eligibility criteria will be confirmed at the Baseline visit (Week 0). Laboratory results must be reviewed by a physician prior to randomisation. A urine pregnancy test may be undertaken pre-randomisation instead of a serum pregnancy test, if a serum pregnancy test was performed at screening. This will allow the site pharmacy adequate time post-randomisation to prepare the study medication for dispensing on the same day. Study medication must be dispensed on the day of randomisation and participants must be instructed to return all unused capsules and empty medication bottles. Participants must be given a card

to carry during trial, with their study PIN, details of the emergency code break procedure and the risk of hypersensitivity reactions. Baseline data will be collected as per the Schedule of Events in Table 1 above for the baseline visit and ongoing section.

4.1.4 WEEK 2 AND WEEK 6 VISITS

All participants will be followed for safety at week 2 and week 6. Site personnel will conduct follow-up phone calls at week 2 and week 6 to collect adverse events, concomitant medications, and to check patient dosing compliance.

4.1.5 MONTH 3, 6, 9, 12, 15, 18, 21 VISITS

Follow up data will be collected as per the Schedule of Events in Table 1 above for the relevant quarterly visit and ongoing section. At each follow up timepoint, a status form is completed; in the event of a missed visit, the status form must be completed.

4.1.6 MONTH 24 OR END-OF-STUDY VISIT

Month 24 data will be collected as per the Schedule of Events in Table 1 above for the M24 visit and ongoing section. In the event a participant wishes to stop study medication and withdraw from further data collection, a withdrawal form must be completed. Where possible a withdrawal visit should be scheduled to collect unused medication and undertake a final set of outcome assessments. In cases where the participant completes the study to month 24, a withdrawal form should be completed at the final visit to indicate they never withdrew.

4.2 DATA ENTRY

Randomisation of participants will be undertaken as per the instructions in the Randomisation and Intervention Allocation Site User Guide (provided separately).

Authorised staff at sites will transcribe baseline and follow up participant data from source data (SDs) to the study eCRF by going to www.ctu.co.uk and clicking the link to access MACRO Version 4. A full audit trail of data entry and any subsequent changes to entered data will be automatically date and time stamped, alongside information about the user making the entry/changes within the system.

Study site staff will be delegated by the site PI to access the eCRF and randomisation systems via a Study Site Delegation Log. The request for user access must go to the UK Trial Manager, who will submit user requests for all sites to the KCTU team upon receipt of completed Study Site Delegation Logs. Requests for user access will be processed within a maximum of 5 working days.

Training videos for data entry staff, study site monitors and trial managers / trial co-ordinators are available at www.ctu.co.uk under the 'Training' section. Users can self-register and should select the MACRO related training videos.

4.3 PRE-RANDOMISATION DATA COLLECTION

4.3.1 REGISTRATION

When the participant has signed consent, the study site staff should register the participant in the MACRO eCRF system. Upon registration, the system will assign a unique study PIN, to be used for the participant throughout the study.

4.3.2 ELIGIBILITY

All eligibility checks must be completed and a physician must confirm eligibility prior to randomisation.

4.3.3 MEDICAL HISTORY

Relevant medical history must be recorded. If the participant is taking any medications at baseline, the relevant condition should be recorded in the medical history unless it is prophylactic treatment.

4.3.4 ALS HISTORY AND TRICALS RISK SCORE

The TRICALS risk score must be calculated at screening and is used both to determine eligibility and in the randomisation process. A secure online risk calculator is accessible through the TRICALS design platform (https://tricals.shinyapps.io/risk-profile/). The tool provides a simple "eligible" or "not eligible" output based on the participant parameters entered. The following clinical variables are required for risk estimation: month and year of birth, date of ALS diagnosis, date of ALS symptom onset, date of Lighthouse II screening, ALSFRS-R[30] total score at screening, SVC % predicted at screening, site of ALS symptom onset, El Escorial diagnostic criteria category and presence of ALS-FTD. The site PI will record the raw data in the source data worksheets, transcribe to the TRICALS risk profile system and a timestamped summary document will be generated, including the participant Lighthouse II PIN, which must be stored with the source data worksheets. The risk profile is based on the ENCALS survival model and can be conceptualized as a relative summary of prognostic information. The risk profile indicates how participants compare to each other (i.e., who is faster or slower progressing than average) without estimating the absolute survival time or probability.

4.3.5 DEMOGRAPHICS

Relevant demographic information will be collected prior to randomisation.

4.3.6 RANDOMISATION

Sites must confirm in the eCRF system whether participants were randomised into the study or not. Age at randomisation will be entered in the eCRF.

4.4 EFFICACY DATA

Participant self-report measures should ideally be completed in the absence of the caregiver; in all cases it should be documented in the eCRF whether the caregiver was present.

4.4.1 SLOW VITAL CAPACITY (SVC)

Evaluated by using a non-invasive spirometer to determine the Slow Vital Capacity (SVC). The participant is asked to inhale deeply and exhale maximally until no more air can be exhaled while maintaining an upright posture. This procedure is repeated at least 3 times or until a steady recording is obtained; the highest score is recorded. Locally available equipment that is CE marked under warranty and used within intended purpose, and normal clinical practice for assessment will be used. The obtained estimates of the slow vital capacity will be recorded in litres and as a % of predicted.

4.4.2 EDINBURGH COGNITIVE AND BEHAVIOURAL ALS SCREEN (ECAS)[29]

The ECAS[29] is a clinician administered evaluation of five cognitive domains: language functions, executive functions, and letter fluency, memory and visuospatial functioning. Site staff will only be delegated to complete this assessment if they have completed the relevant training and are certified to complete the assessments.

4.4.3 ALS FUNCTIONAL RATING SCALE – REVISED (ALSFRS-R)[30]

The ALSFRS-R[30] is a 12-item participant self-report measure that monitors ALS disease progression.

4.4.4 KING'S STAGING SYSTEM[31]

A clinical staging system, which defines four stages of ALS (1-4), assessed by a clinician via a semi-structured interview with the participant.

4.4.5 EUROQOL (ED-5D-5L)[32]

The EQ-5D-5L[32] is a 5-item participant self-report measure that assesses the health status in terms of five dimensions: mobility, self-care, usual activities, pain and depression and includes a visual analogue scale (EQ VAS). A validated telephone version is available for remote assessments. It will be recorded in the eCRF whether administration was telephone or face-to-face.

4.4.6 COLUMBIA SUICIDE SEVERITY RATING SCALE (C-SSRS)[33]

The C-SSRS[33] is a 6-item participant self-assessment tool that evaluates suicidal ideation and behaviour. Training will be provided to research staff conducting this questionnaire via a youtube video produced by Columbia University Centre for Suicide Risk Assessment. See https://www.youtube.com/watch?v=Ted_gl-UXi8&list=PLf-Iad1cmFb_Osx4U9HpLjdK8VNNOjUNS&index=14.

An answer of yes of any of the six questions must be followed up with both the participant and a health care professional. It is recommended that the investigator consider mental health consultation or referral for participants who experience signs of suicidal ideation or behaviour.

4.4.7 ALL-CAUSE MORTALITY

In the event of death, an SAE form and a withdrawal form must be completed. Available information on the date and circumstances must be recorded in the participants medical notes. Death certificates or other formal confirmation of the death are not required but it must be recorded in the eCRF whether the death was ALS-related, or unconnected to ALS (such as a road traffic accident).

4.5 SAFETY DATA

4.5.1 PHYSICAL EXAM

The physical examination will be the standard exam conducted by a qualified physician at baseline. The extent of subsequent examinations will be at the discretion of the physician, as clinically indicated. Any examinations not required should be recorded as ‘not done’.

4.5.2 NEUROLOGICAL EXAM

The neurological examination will be the standard exam conducted by a qualified neurologist at screening, or at baseline if not performed at screening. If the test was performed at baseline, the results will be entered on EDC for screening vistit. The extent of subsequent examinations will be at the discretion of the physician. Any examinations not required should be recorded as ‘not done’.

4.5.3 TWELVE-LEAD ECG

The twelve-lead ECG will be the standard procedure conducted by an investigator or an appropriately trained professional. No special study-specific requirements. ECG printouts will be retained with the Source Data Worksheets.

4.5.4 VITAL SIGNS

Systolic and diastolic blood pressure, respiratory rate, heart rate and temperature and body weight will be obtained. Vital signs will be measured in upright position after the participant has been rested for five minutes.

4.5.5 WEIGHT

Body weight is measured at each visit and recorded in kilograms. As ALS progresses, participants may become unable to stand on a scale. In those cases, a chair scale will be used as alternative. If the

participant becomes unable to be transferred for weighing, this information must be documented in the source data.

4.5.6 ADVERSE EVENTS

During each visit, participants will be asked about adverse events. Additionally, ALS specific interventions such as gastrostomy placement and non-invasive ventilation (NIV) use will be recorded. All adverse events will be recorded in an ongoing adverse event log. Symptoms of disease progression need only be recorded if treatment is required or if the physician is concerned that the rate of progression is unexpected.

4.5.7 CONCOMITANT MEDICATIONS

During each visit, participants will be asked about their current medication. All concomitant medication will be recorded in an ongoing concomitant medication log.

4.5.8 WITHDRAWAL

A withdrawal form must be completed in the event of participant death or at M24 or where the participant has stopped taking study medication and is no longer prepared to provide any follow up data or have their caregiver or family doctor provide any follow up data, including survival status. Where the participant has stopped study medication but is still being followed for survival status and/or any other secondary outcome data, a withdrawal form should not be completed, but a status update must be recorded in the eCRF every 3 months.

4.6 LABORATORY DATA

All tests listed below will be performed as per the time points indicated in the participant timeline in section 4.1. In addition, laboratory safety tests may be performed at unscheduled times, if deemed necessary by the PI and abnormal results recorded as an adverse event if clinically significant. A local laboratory will be used to perform the tests at each trial site. If samples are requested elsewhere (eg via a community based doctor) the laboratory results report should be requested. Results must be printed and retained with the source data worksheets, after being reviewed and signed by a physician. Where the results indicate an adverse event or medical condition, this should be recorded in the medical history (at baseline) or on the adverse event log if occurring after randomisation.

Clinically significant abnormalities will be recorded in the medical history form at screening and baseline or the adverse event form post-randomisation.

Individual result values will not be transcribed to the eCRF unless outside the normal range.

Approximately 10ml of blood and 20ml of urine per patient will be collected to perform all tests listed below.

4.6.1 SEROLOGY

HLA-b*57:01
HIV 1 and 2
Hepatitis B and C

HIV serology results within a year of screening will be accepted for all participants with a low risk for exposure. If the patient has previously been tested for HLA-B*57:01 this result will be accepted.

4.6.2 SAFETY BLOODS (HAEMATOLOGY & BIOCHEMISTRY)

<u>HAEMATOLOGY</u>

Haemoglobin
Haematocrit
Total and differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
Red blood cell count
Platelet count

BIOCHEMISTRY

Prothrombin time	Amylase	Glucose
International normalized ratio (INR)	Lactate	Urea
Alkaline phosphatase	Albumin	Creatinine
Alanine aminotransferase (ALT)	Sodium	Creatine phosphokinase
Aspartate transaminase (AST)	Potassium	Chloride
Gamma-GT (GGT)	Calcium	Phosphate
Bilirubin	Bicarbonate	

Amylase will be used to monitor pancreas function. If possible, lipase test can also be performed however this is not essential..

4.6.3 PREGNANCY TESTS

SERUM PREGNANCY TEST

Serum beta-hCG will be determined for female study participants of child-bearing potential prior to randomisation. Serum testing is mandated at screening. Thereafter urine pregnancy testing is permitted but if not available, serum testing is acceptable.

URINE PREGNANCY TEST

On the day of randomisation, if serum pregnancy test was performed at screening, a urine beta-hCG pregnancy test may be used the serum pregnancy test to allow the site pharmacy adequate time post-randomisation to prepare the study medication for dispensing on the same day. After randomisation, urine pregnancy testing is adequate.

4.6.4 URINALYSIS

pH	Ketones	Urobilinogen
Specific gravity	Bilirubin	Leukocyte esterases
Protein	Blood	
Glucose	Nitrite	

If urinalysis is positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination (for red blood cells, white blood cells, bacteria, casts, and epithelial cells) will be performed.

4.6.5 RESEARCH BLOOD AND URINE SAMPLES (INCLUDING FUTURE UNSPECIFIED ANALYSIS)

Blood (20ml) will be collected for DNA, biomarkers and stored locally as whole blood, serum/plasma in -80c freezers for the duration of the trial or processed and stored as processed samples. Blood and urine samples for biomarkers should be collected in a clear tube and centrifuged at 2200-2500RPM (blood) or at 2000G (urine) for 15 minutes and then transferred to aliquots. Each aliquot or processed sample should be labelled with the participants study PIN and the study visit (M0, M3, M6 etc) or with a pre-prepared label supplied by the coordinating team in the relevant region.

Urine will be collected to determine urinary $P75^{ECD}$ (20ml), labelled with the study PIN and study visit, and frozen until the end of the study in a standard freezer (0^{o} to -20^{o}).

Any remaining blood and urine samples will be kept for future unspecified analysis. This will be optional and with the participants' consent.

In regions where barcodes are used for samples, relevant information must be recorded in the eCRF to ensure samples can be linked to individual participants. KCL, Macquarie and TRICALS will provide appropriate instructions and/or materials for sites to collect and store the research data and blood/urine samples.

The Investigators will convene an internal advisory group to determine the distribution of biological samples and develop relevant SOPs for sample transport and analysis. If appropriate, transport of stored specimens will be approved after material transfer agreements (MTA) are in place between participating institutions.

4.7 IMP DOSING DATA

4.7.1 STUDY MEDICATION DISPENSING LOG

The unique study medication bottle numbers dispensed at each visit will be recorded, along with details of which bottles have been returned at subsequent visit and the number of capsules returned in each bottle. This information will be used to measure compliance with treatment.

4.7.2 STUDY MEDICATION DOSING LOG

Study medication dosing, including any active temporary or permanent discontinuation of study medication, will be recorded on the Study Medication Dosing Log. Participants must take 4 capsules per day. Dose adjustements are permitted for patients unable to tolerate the full dose. Missed doses at the end stage of palliative care, when medication cannot be administered, is not considered to be 'treatment discontinuation'.

4.8 MEASURES TO PROMOTE PARTICIPANT RETENTION

Where possible data collection will be via routine follow up clinics, minimising the need for additional research visits. Much of the data are collected in routine care in recruiting sites and are not time consuming to complete. In the event a participant wishes to stop taking study medication, follow up visits should still be completed. If the participant no longer wishes to attend site, these visits can be completed via phone. If the participant is no longer able to attend follow up visits but remains on study medication, then study medication may be posted or couriered to the participant and follow up data collected remotely. In some cases, the General Practitioner / Community Doctor may need to be asked for blood samples for safety follow up, if the participant cannot physically attend secondary care. In the event the participant wishes to cease contact with the site, study medication must be discontinued but permission will be sought to contact the participants General Practitioner for follow up data on survival.

4.8.1 END-OF-LIFE ACCOMMODATIONS FOR CLINICAL VISITS

As ALS progresses, participants may experience increasing difficulty to adhere to the visit schedule. To reduce discontinuations due to non-compliance, we provide an alternative option for debilitated participants. If, in the judgment of the site Investigator, the participant cannot reasonably be expected to travel to the clinic (e.g., housebound or under hospice care), the minimum data required for continued participation in the study (i.e. remain on the study medication) are: (1) laboratory tests, (2) adverse event reporting and (3) ALSFRS-R[30], all of which can be obtained remotely by a trial nurse (either home visit or by phone). Blood samples for safety assessments may be obtained by the participants general practitioner. These data are to be obtained according to the visit schedule until the participant withdraws, reaches the primary endpoint (death) or the study completes, whichever occurs first. If the participant becomes unfit to travel to clinic, this must be documented in the source data. If no

accommodations can be made for adequate safety monitoring, participants are discontinued from study medication. The monitoring of ALSFRS-R[30], SAEs and ALS related interventions may continue by telephone according to the visit schedule.

5. INTERVENTIONS

5.1 EXPLANATION FOR THE CHOICE OF COMPARATORS

The comparator for this trial will be a matched capsulated placebo. Participants in both arms will be permitted to take riluzole, the only approved treatment for ALS, during the trial and this will be recorded in the concomitant medications log.

5.2 INTERVENTION AND COMPARATOR DESCRIPTION, DOSING AND LABELLING

5.2.1 ACTIVE TRIUMEQ DESCRIPTION

The intervention for the active (treatment group) will be four size 0 Swedish Orange capsules containing between them the equivalent of a single Triumeq daily dose tablet (Abacavir 150mg, Lamivudine 75mg and Dolutegravir 12.5mg) taken anytime once a day, with or without food. The process of crushing and encapsulating Triumeq is done by Tiofarma NL, under contract. A manufacturing technical agreement can be found in the UK Trial Master File. The Triumeq tablets are supplied to Tiofarma by ViiV Healthcare. Stability testing of the crushed capsules has been performed by Tiofarma and confirms stability for at least 18 months.

5.2.2 PLACEBO DESCRIPTION

The placebo is supplied as matched size 0 Swedish Orange capsules in matched plastic bottles. The matched placebo capsules will contain inactive excipients as used in the active medication plus colour and bitrix to match the taste of Triumeq. The Investigational Medicinal Product Dossier (IMPD) supplied by Tiofarma details composition of the placebo.

5.2.3 DOSE, PACKAGING AND LABELLING

The intervention will be supplied to sites and to participants in white HDPE plastic child-resistant closure bottles with a desiccant, containing 200 capsules per bottle. Two bottles will be dispensed every 3 months (13 weeks), providing 91 day's supply of 4 capsules a day, plus 'overage' to allow sufficient medication for a 7-day visit window. The capsules can be taken whole or split open and taken immediately with water or other liquid or sprinkled on food. For participants with a gastric feeding tube, the powder can be administered in water and should be administered immediately. Each bottle will have a unique ID number supplied to the manufacturer by the KCTU pharmacist prior to labelling. The information presented on the IMP labels will be annex 13 compliant. Multi-language labels will be applied to the medication bottles, allowing manufactured IMP and placebo to be shipped to any recruiting site globally. The Sponsor organisation/s will be responsible for approving the label content in each country.

5.3 IMP ACCOUNTABILITY

Study medication will be shipped to the hospital pharmacy of each recruiting site.

The pharmacy clinical trials team must maintain accurate accountability records of the study medication, including, but not limited to, the number of bottles received, the number of bottles dispensed to each participant (including the unique bottle numbers), batch number, expiry date, and the date of the transaction in addition to the quantity of study medication returned by each participant.

Participants will be asked to return any unused study medication and/or empty packaging at each study visit and at the end of the active study period. The study drug returns will be returned to pharmacy by the research team for accountability. The returns will be verified by the pharmacy clinical trials team and the sponsor representative prior to disposal at site.

A site pharmacy file, including a pharmacy manual, will be supplied to each site by the relevant CRA at the site initiation visit.

5.4 IMP STORAGE

The study medication does not require any special temperature storage conditions. Store at ambient room temperature in a secure location. Temperature monitoring and reporting is not required. Store in the original container to protect from moisture. Study medication will be shipped from Tiofarma to a central facility managed by Eramol (UK) Limited. It will be distributed to sites by Eramol (UK) Limited on the request of the KCTU pharmacist.

5.5 DISCONTINUING ALLOCATED INTERVENTIONS

Participants may temporarily or permanently stop taking study drug at the discretion of the site PI. In all cases follow up data should be collected per protocol to the end of the trial, unless the participant is formally withdrawing from further data collection. Where the site PI is considering stopping study medication, they are encouraged to contact the UK CI to discuss the circumstances in advance. The clinical study report will include reasons for participants discontinuing study medication. The Study Medication Dosing Log must be updated if a participant temporarily or permanently discontinues study medication.

5.6 CONCOMITANT MEDICATIONS PERMITTED OR PROHIBITED DURING THE TRIAL

Antacids containing magnesium/aluminium should not be taken for at least 6 hours before and/or 2 hours after taking Triumeq. Similarly, magnesium supplements should be avoided 6 hours before and/or 2 hours after taking Triumeq.

Prohibited medication includes:

- dofetilide (or pilsicainide [available in Japan]); dolutegravir may inhibit its renal tubular secretion resulting in increased dofetilide concentrations and potential for toxicity.
- fampridine (dalfampridine); dolutegravir may decrease the renal tubular secretion of this drug and result in increased levels which have the potential of causing seizures.

Medications listed in the Summary of Product Characteristics (SmPC) for Triumeq as contraindicated will not be permitted in this study.

All other usual care therapy and medications typically used in the management of ALS, including Riluzole, are allowed during the trial.

5.7 PROVISIONS FOR POST-TRIAL CARE

Standard-of-care will continue to be provided for each participant during all phases of the trial, including post-trial. Any participant who suffers harm from trial participation has access to all available services and relevant compensation offered by their participating institution. And by the country-specific sponsor of the trial.

6. ASSIGNMENT OF INTERVENTIONS

6.1 RANDOMISATION METHOD

The method of allocation sequence is stratified block randomisation with randomly varying block sizes, stratified by recruiting study site and by the TRICALS risk profile score, defined as low (less than -4.5) or high (greater than or equal to -4.5). See section 4.3.4.

6.2 CONCEALMENT MECHANISM

Randomisation and treatment allocation will be via the web based 'KCTU randomisation and IMP management system', maintained by the King's Clinical Trials Unit for the duration of the project. Study site staff and participants will be blind to treatment allocation throughout the study. Randomly varying block sizes will be implemented to assure allocation concealment of subsequent participants in a block, to safeguard against the impact of obvious IMP side effects.

The KCTU pharmacist will liaise with Tiofarma to provide a blinding list for each batch of IMP and placebo manufactured. That list will be uploaded by KCTU to the randomisation system for each batch released. At the point of randomisation or dispensing follow up medication, sites will access the system and input the relevant participant parameters and the system will generate an email to the site and to the trial manager, indicating the study drug bottle numbers to be dispensed by pharmacy. A second email will be generated and sent to the emergency code break service, eSMS, specifying the trial arm to which the participant has been randomised in addition to the information provided to the recruiting sites.

6.3 RANDOMISATION IMPLEMENTATION

6.3.1 ALLOCATION SEQUENCE GENERATION

The randomisation sequence will be generated dynamically by the KCTU team via the KCTU web based randomisation system, in accordance with the specification agreed with the CI and Senior Statistician. The Chief Investigator, Senior Statistician and TMG will be blinded to the sequence generation.

6.3.2 ENROLMENT OF PARTICIPANTS

Participants will be enrolled in specialist secondary care centres. Participants will be enrolled in the study for the purpose of CONSORT reporting at the point of signing a consent form to being screened for eligibility and will be part of the target N=390 at the point of randomisation.

6.3.3 ASSIGNMENT OF PARTICIPANTS TO INTERVENTIONS

Recruiting sites will assign participants to interventions by logging into the 'KCTU randomisation and IMP management system' at www.ctu.co.uk (click 'randomisation' and select 'Lighthouse II') and entering the participant's initials (in countries where this is permitted), year of birth and age, and stratifiers. The system will randomise the participants to active Triumeq or placebo in a ratio of 2:1. Once allocated to a trial arm, the system will then access details of which study medication bottles are currently available in the recruiting site pharmacy and will email the bottle numbers of the two bottles to be dispensed to relevant individuals at site and at the emergency code break service (eSMS).

6.3.4 RANDOMISATION & STUDY DRUG ALLOCATION PROCEDURE

See Randomisation and Intervention Allocation Site User Guide (provided separately).BLINDING STATUS OF RESEARCHERS

Individual blinding status	Blinded	Unblinded
Chief Investigators	**x**	
Principal Investigators and all other staff at site	**x**	
Co-applicants (except KCTU Pharmacist and KCTU Operations Director)	**x**	

KCTU Pharmacist and KCTU Operations Director		**x**
Trial Manager/Trial Co-ordinators	**x**	
Senior Statistician	**x**	
TRICALS Statistician	**x**	
Junior Statistician		**x**
Pharmacists at site	**x**	
Trial Participants	**x**	
Outcome Assessors/Research Nurses	**x**	
Treating clinicians	**x**	
Sponsor (CRA/Monitors)	**x**	
Trial Steering Committee (TSC)	**x**	
Data Monitoring Committee (DMC)		**x**

TABLE 2 BINDING STATUS

The blinding status of the research team is detailed in Table 2 above.

**For roles not listed please refer to study delegation logs.*

6.4 EMERGENCY UNBLINDING

All participants will remain blind to treatment allocation until the primary analyses are complete and the primary paper has been accepted. Sites will be informed of the participants treatment allocation at this point.

Treating physicians should only request emergency code break when information about the participant's trial treatment is necessary for the appropriate medical management of the participant and where stopping the blinded medication is not sufficient. The treating physician or investigator will have the primary right to break the blind in any moment in case of emergency and they will be able to unblind immediately and without delay.

The Lighthouse II Trial has commissioned a 24-hour telephone-based emergency code break service, Emergency Scientific Medical Services (eSMS) for all countries involved in the study. Participants will be asked to carry emergency cards during the study, which will have details of the code break telephone number. The code break number will also be printed on the study medication bottles. The caller can request code break from eSMS using either the IMP treatment bottle number or the participants study PIN, which is allocated by the MACRO eCRF system after consent. It must be explained to participants that due to the risk of hypersensitivity reactions to Triumeq, it is particularly important they carry the card at all times in this particular study.

7. DATA MANAGEMENT

7.1 DATA MANAGEMENT

There are two datasets in the trial: the KCTU randomisation dataset and the KCTU Elsevier Macro 4 eCRF system dataset. The CI will act as custodian for the trial data.

Source data worksheets will be supplied to all recruiting sites by the co-ordinating centre for the region. These will be prepared after the database specification is finalised and database testing is complete. The UK Trial Manager will send the master version to the Macquarie and TRICALS co-ordinating teams, who will be

responsible for adding validated versions of country-specific participant-reported outcome measures in local languages.

Laboratory results may be reviewed directly in hospital laboratory systems where appropriate and need not be transcribed in full to the source data work. The source data worksheet must confirm that the samples were processed, and any abnormal results must be recorded and transcribed. Normal results need not be transcribed.

Data will be transcribed from the source to the MACRO eCRF system, ideally within 7 days of the study visit.

Participating Sites will complete source data location lists defining the source data at their site.

7.2 DATA SECURITY

Clinical trial will involve the sharing of deidentified data and samples of subjects for research purposes, both during and after the trial for the purposes of monitoring and analysis. All applicable statutory requirements and mandatory codes of practice in respect of confidentiality (including, where applicable, medical confidentiality) in relation to such trial subjects or their legal guardians. Data flow will be governed by country-specific requirements.

Data Management Plans will be provided to the UK Trial Manager for the Global Trial Master File, detailing relevant security information about both data systems. Systems access will be strictly restricted through user-specific passwords to the authorised research team members. It is a legal requirement that passwords are not shared, and that only those authorised to access the system are allowed to do so. If new staff members join the study, a user-specific username and password must be requested and a request for access to be revoked must be requested when staff members leave the project.

Participant initials (in countries where this is permitted), year of birth and age will be entered into the systems. No more identifiable data will be entered into the eCRF system. Trial sites will maintain a subject ID log linking participant identifiers to study numbers. No data will be entered unless a participant has signed a consent form to participate in the trial

7.3 DATA QUALITY PROCESSES

At the database design stage, validations will be programmed into the systems to minimise data entry errors by querying the data entered in real time with sites.

The CI team will undertake appropriate reviews of the entered data, in consultation with the project analyst, where appropriate for the purpose of data cleaning and will request amendments to the MACRO eCRF system data as required. No data will be amended independently of the study site responsible for entering the data.

No data can be amended in the randomisation system, however CI or delegate (e.g., Trial Manager) may request King's Clinical Trials Unit to add notes against individual participant entries to clarify data entry errors. Any errors should be reported by site staff to the Trial Manager as soon as possible once they are detected. The trial manager will onward report errors to KCTU and retain records in the TMF.

The KCTU will provide the Trial Manager with Data Management Plans for both the Elsevier Macro eCRF system and the randomisation system once the systems are made live. Those documents will be filed in the Trial Master File.

A regular Data Management Report will be produced by KCTU and passed to the Trial Manager, who will raise Data Clarification Requests (DCRs) with sites in the eCRF system. The UK Trial Manager will raise DCR's. Study sites will periodically review raised DCR's and respond to the queries raised.

During site monitoring visits, the CRA will raise any queries with sites via the Source Data Verification (SDV) function.

7.4 DATABASE LOCK

At the end of the trial, the site PI's will review all the data for each participant in the MACRO eCRF system and provide electronic sign-off to verify that all the data are complete and correct.

The trial manager will confirm all checks are complete and all monitors queries have been resolved prior to database lock. At this point, with the agreement of the senior statistician, all data can be formally locked for analysis.

When the final data extract is requested, KCTU will remove all data entry user access prior to data extract and will retain only 'monitor' access for site PI's and other relevant individuals.

Upon request, KCTU will provide a copy of the final exported dataset to the CI in .csv format and the CI will onward distribute to sites as appropriate. Once sites have received copies of their individual datasets and confirmation of receipt has been received, the Trial Manager will request that all user access is removed from the MACRO eCRF system. A copy of the dataset will be stored in the TMF at the end of the study.

8. SUMMARY OF KNOWN AND POTENTIAL RISK OF TRIUMEQ

Administration of Triumeq for this study is not anticipated to induce any potential risk other than the known potential side effects listed below. However, in patients with concurrent ALS, there may be some unknown, infrequent or unforeseeable risks associated with the use of Triumeq in this population, which have not to date been characterized for the use of Triumeq in its licensed indication to treat HIV infected patients. Our phase IIa study did not indicate a markedly different AE profile as compared to earlier reports of HIV positive patients treated with Triumeq. The following adverse reactions have been noted with Triumeq in HIV positive patients:

8.1 COMMON SIDE EFFECTS OF TRIUMEQ

Insomnia, headache, fatigue, diarrhoea, nausea, vomiting, fever, loss of appetite, low energy, nightmares or abnormal dreams, abnormal body fat distribution, numbness and tingling, hypersensitivity reactions (fever, rash, shortness of breath, cough, or sore throat), joint pain or swelling, muscle pain, extremity swelling, depression, dizziness, and spinning sensation (vertigo).

8.2 LESS COMMON OR RARE SIDE EFFECTS OF TRIUMEQ

Neuropathy, anxiety, difficulty moving, disorders in digestive system, dizziness, weight loss, muscle and/or bone pain, itching, hair loss, high blood sugar and high amounts of triglyceride in the blood.

8.2.1 HYPERSENSITIVITY REACTIONS TO DOLUTEGRAVIR AND ABACAVIR

Both abacavir and dolutegravir are associated with a risk for hypersensitivity reactions and share some common features such as fever and/or rash with other symptoms indicating multi-organ involvement. Clinically it is not possible to determine whether a hypersensitivity reaction with Triumeq would be caused by abacavir or dolutegravir. Hypersensitivity reactions have been observed more commonly with abacavir, some of which have been life-threatening, and in rare cases fatal, when not managed appropriately. The risk for abacavir hypersensitivity reactions to occur is high for patients who test positive for the HLA-B*5701 allele. However, abacavir hypersensitivity reactions have been reported at a low frequency in patients who do not carry this allele.

8.2.2 ABACAVIR HYPERSENSITIVITY

The signs and symptoms of this hypersensitivity reaction are listed below. These have been identified either from clinical studies or post marketing surveillance. Almost all patients developing hypersensitivity reactions will have fever and/or rash (usually maculopapular or urticarial) as part of the syndrome, however reactions have occurred without rash or fever. Other key symptoms include gastrointestinal, respiratory or constitutional symptoms such as lethargy and malaise.

Skin Rash:	Usually maculopapular or urticarial
Gastrointestinal tract:	Nausea, vomiting, diarrhoea, abdominal pain, mouth ulceration
Respiratory tract:	Dyspnoea, cough, sore throat, adult respiratory distress syndrome, respiratory failure
Miscellaneous:	Fever, lethargy, malaise, oedema, lymphadenopathy, hypotension, conjunctivitis, anaphylaxis
Neurological/Psychiatry:	Headache, paraesthesia
Haematological:	Lymphopenia Liver/pancreas, Elevated liver function tests, hepatitis, hepatic failure
Musculoskeletal:	Myalgia, rarely myolysis, arthralgia, elevated creatine phosphokinase
Urology:	Elevated creatinine, renal failure. Symptoms related to this hypersensitivity reactions worsen with continued therapy and can be life-threatening and in rare instance, have been fatal.

Restarting abacavir following an abacavir hypersensitivity reaction results in a prompt return of symptoms within hours. This recurrence of the hypersensitivity reaction is usually more severe than on initial presentation and may include life threatening hypotension and death. Similar reactions have also occurred infrequently after restarting abacavir in patients who had only one of the key symptoms of hypersensitivity (see above) prior to stopping abacavir; and on very rare occasions have also been seen in patients who have restarted therapy with no preceding symptoms of a hypersensitivity reaction (i.e., patients previously considered to be abacavir tolerant).

8.2.3 DOLUTEGRAVIR HYPERSENSITIVITY

Symptoms have included rash, constitutional findings, and sometimes, organ dysfunction, including severe liver reactions.

8.3 **RARE SIDE EFFECTS OF TRIUMEQ**

Abnormal liver function tests, abnormally low blood pressure, acute inflammation of the pancreas, anaemia, adult respiratory distress syndrome, enlarged fatty liver, Graves' disease, hepatitis, neuropathy, Stevens-Johnson's syndrome. There have been rare post-marketing reports of liver failure.

8.3.1 SUICIDE IDEATION AND BEHAVIOUR

Psychiatric disorders including suicide ideation and behaviours are common in HIV-infected patients. In clinical trials, the psychiatric profile for dolutegravir (including suicidality, depression, bipolar and hypomania, anxiety and abnormal dreams) was generally similar or favourable compared with other antiretroviral therapy, although a higher incidence of depression and suicidal ideation/behaviours with dolutegravir compared with darunavir/ritonavir was noted in the FLAMINGO clinical study. An evaluation of aggregate data, including post-marketing data concluded that a causal association between dolutegravir and depression and suicidal behaviours could not be ruled out. These events occur primarily in patients with a prior history of psychiatric illness. Therefore, there is conflicting evidence

whether antiretroviral therapy increases the prevalence of suicide ideation. To screen for suicide ideation and behaviour we have added a yearly screening tool.

8.3.2 TERATOGENESIS

Human experience from a birth outcome surveillance study in Botswana shows a small increase of neural tube defects; 7 cases in 3,591 deliveries (0.19%; 95% CI 0.09%, 0.40%) to mothers taking dolutegravir-containing regimens at the time of conception compared to 21 cases in 19,361 deliveries (0.11%: 95% CI 0.07%, 0.17%) to women exposed to non-dolutegravir regimens at the time of conception. The incidence of neural tube defects in the general population ranges from 0.5-1 case per 1,000 live births (0.05-0.1%). Most neural tube defects occur within the first 4 weeks of embryonic development after conception (approximately 6 weeks after the last menstrual period). If a pregnancy is confirmed in the first trimester while on Triumeq, the benefits and risks of continuing Triumeq versus switching to another antiretroviral regimen should be discussed with the patient taking the gestational age and the critical time period of neural tube defect development into account. 15 Data analysed from the Antiretroviral Pregnancy Registry do not indicate an increased risk of major birth defects in over 600 women exposed to dolutegravir during pregnancy but are currently insufficient to address the risk of neural tube defects. In animal reproductive toxicology studies with dolutegravir, no adverse development outcomes, including neural tube defects, were identified. Dolutegravir was shown to cross the placenta in animals. More than 1000 outcomes from exposure to dolutegravir during second and third trimester pregnancy indicate no evidence of increased risk of foeto/neonatal toxicity. Triumeq may be used during the second and third trimester of pregnancy when the expected benefit justifies the potential risk to the foetus. Concerning lamivudine, a large amount of data (more than 5200 outcomes from first trimester) indicates no malformative toxicity. A moderate amount of data (more than 1200 outcomes from first trimester) indicates no malformative toxicity for abacavir.

8.4 DRUG INTERACTIONS

Dolutegravir is primarily metabolized by UGT1A1, and is also a substrate for UGT1A3, -1A9, CYP3A4, 'breast cancer resistance protein' (BCRP) and P-glycoprotein (Pgp). Simultaneous administration of drugs that induce or inhibit these enzymes and transports could affect plasma concentrations of dolutegravir. Abacavir is metabolized by UGT2B7 and alcohol dehydrogenase; simultaneous administration of inducing drugs (rifampicin, carbamazepine or phenytoin) or inhibiting drugs (valproic acid) of UGT-enzymes or drugs that interact with alcohol dehydrogenase could affect plasma concentrations of abacavir. Chronic use of polyalcohol (e.g. sorbitol, xylitol, mannitol or lactitol) could lower the exposure to lamivudine. For more information on drug-drug interactions see http://hivinsite.ucsf.edu/insite?page=ar-00-02&post=1 (University of California, San Francisco)

9. ADVERSE EVENT MANAGEMENT AND REPORTING

All adverse events will be recorded in the participants medical notes, the study source data worksheets and the eCRF. SAE's will be additionally reported, within 24 hours of site becoming aware of the event, to the KHP-CTO.

All SAEs, SARs and SUSARs (except those specified in this protocol as not requiring reporting) will be reported immediately (and certainly no later than 24hrs) by the Investigator to the KHP-CTO for review in accordance with the current Pharmacovigilance Policy and as per the instructions on the SAE report form.

The sponsor will report SAEs and/or suspected unexpected serious adverse reactions as required to regulatory authorities, investigators/institutions, and IRBs/IECs in compliance with all reporting requirements according to local regulations and good clinical practice.

Admission to hospice or equivalent hospital facility for respite care,symptoms management, or end of life care, planned hospital admission for Percutaneous Endoscopic Gastrostomy (PEG) or Radiologically Inserted Gastrostomy (RIG) tube insertion or initiation of Non-Invasive Ventilation (NIV) that may require hospitalisation, other planned admissions for expected ALS adverse events, unless the event results in death, or in the opinion of the PI the event was serious, are not considered to be SAEs. They should be reported on the ALS interventions log.

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

- Adverse Event (AE): Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
- Adverse Reaction (AR): Any untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.
- Unexpected Adverse Reaction (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the summary of product characteristics (SmPC) for that product
- Serious adverse Event (SAE) (must be reported to KHP-CTO within 24 hours), Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR): Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:
    - results in death, including death due to the disease progression
    - is life-threatening
    - required unplanned hospitalisation or unplanned prolongation of existing hospitalisation
    - results in persistent or significant disability or inca pacity
    - consists of a congenital anomaly or birth defect
- Important Medical Events (IME) & Pregnancy: Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious. Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.
- Investigators are encouraged to report any pregnancy that occurs during study participation to the Antiretroviral Pregnancy Registry. More information including is available at www.apregistry.com

9.1 EVALUATING AES AND SAES

9.1.1 ASSESSMENT OF INTENSITY

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgement. The intensity of each AE and SAE recorded in the eCRF should be assigned to one of the following categories:

- Mild; An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities
- Moderate; An event that is sufficiently discomforting to interfere with normal everyday activities
- Severe; An event, which is incapacitating and prevents normal everyday activities

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilised for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

9.1.2 ASSESSMENT OF CAUSALITY

The Investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. The Investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated.

The causal relationship to the study product assessed by the Investigator (or medically qualified delegate) should be assessed using the following classifications:

- Not Related: In the Investigator's opinion, there is not a causal relationship between the study product and the AE.
- Unlikely: The temporal association between the AE and study product is such that the study product is not likely to have any reasonable association with the AE.
- Possible: The AE could have been caused by the study participant's clinical state or the study product.
- Likely: The AE follows a reasonable temporal sequence from the time of study product administration, abates upon discontinuation of the study product and cannot be reasonably explained by the known characteristics of the study participant's clinical state.
- Definitely: The AE follows a reasonable temporal sequence from the time of study product administration or reappears when study product is reintroduced.

There may be situations when an SAE has occurred, and the Investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the Investigator always assesses causality for every event prior to transmission of the SAE form to the Sponsor. The Investigator may change his/her opinion of causality considering follow-up information, amending the SAE form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

9.1.3 ASSESSMENT OF EXPECTEDNESS

A reasonable possibility of a relationship conveys that there are facts, evidence and/or arguments to suggest a causal relationship, rather than a relationship that cannot be ruled out.

- Expected: An adverse reaction, the nature or severity of which is consistent with the applicable Reference Safety Information in the Summary of Product Characteristics for an approved medicinal product
- Unexpected: An adverse reaction, the nature or severity of which is not consistent with information in the relevant source document

9.1.4 FOLLOW-UP OF AES AND SAES

After the initial AE/SAE report, the Investigator is required to proactively follow each participant and provide further information to the Sponsor on the participant's condition. All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts. All AEs and SAEs will be followed until resolution, until the condition stabilises, until the event is otherwise explained, or until the participant is lost to follow-up. Once resolved, the adverse event log will be updated. The Investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. New or updated information will be recorded on the originally completed SAE form, with all changes signed and dated by the Investigator. The updated SAE form should be resent to the Sponsor.

9.1.5 POST-STUDY AES AND SAES

A post-study AE/SAE is defined as any event that occurs outside the AE/SAE detection period. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the Investigator learns of any SAE, including death, at any time after a participants has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the Investigator will promptly notify the Sponsor.

9.2 ADVERSE EVENT PROCESSING RESPONSIBILITIES

TRICALS, Macquarie University and KCL has delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP-CTO).

The Chief Investigators will report to the relevant ethics committee in Australia, New Zealand, Europe and the UK. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days;
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

The KHP-CTO will report SUSARs to the MHRA, TRICALS will report SUSARs to relavent EEA regulatory authorities (competent authorities of other EEA (European Economic Area) states in which the trial is taking place).

In Australia, Macquarie Univeristy will be responsible for reporting all SUSARs occurring in Australian participants to the Therapeutic Goods Administration. Fatal or life threatening Australian SUSARs will be reported immediately, and no later than 7 calendar days after being made aware of the case, with any follow-up information within a further 8 calendar days. For all other Australian SUSARs, reporting will be no later than 15 calendar days after the KHP-CTO being made aware of the case.

In New Zealand (sponsored by Macquarie University), Macquarie University, or Macquarie University's designee, will be responsible for reporting all fatal or life-threatening SUSARS occurring in New Zealand participants to Medsafe within 7 days of being made aware of the case, in accordance with Part 11 (Clinical Trials Regulatory Approval and good clinical practice requirements) of the Guideline on the Regulation of Therapeutic Products in New Zealand

The Chief Investigator and KHP-CTO (on behalf of the co-sponsors) will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually. Macquarie university will be responsible for disseminating the annual DSUR to local trial sites within Australia. Each local trial site in Australia will be responsible for submitting the DSUR to their approving REC. In the EU, TRICALS will be responsible for disseminating the relevant reports to participating country authorities and to the EMA.

The Trial Statistician will report relevant adverse events to the Data Monitoring Committee.

10. TOXICITY MANAGEMENT

10.1 LIVER CHEMISTRY ABNORMALITIES

Liver chemistry threshold stopping criteria have been designed to assure participant safety and to evaluate liver event aetiology during administration of Investigational Product and the follow-up period. Study

medication will be stopped and an SAE will be reported if any of the following liver chemistry criteria are met:

- ALT ≥3xULN and bilirubin ≥2xULN (>35% direct bilirubin; bilirubin fractionation required)
  NOTE: serum bilirubin fractionation should be performed if testing is available. If testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury. If testing is unavailable and a participant meets the criterion of total bilirubin ≥ 2xULN, then the event meets liver stopping criteria.
- ALT ≥8xULN
- ALT ≥3xULN (if baseline ALT is < ULN) with symptoms or worsening of acute hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia, OR
- ALT ≥3x baseline ALT with symptoms or worsening of acute hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia
- ALT ≥5xULN and <8xULN that persists > 2 weeks (with bilirubin <2xULN and no signs or symptoms of acute hepatitis or hypersensitivity)
- ALT ≥5xULN but <8xULN and cannot be monitored weekly for >2 weeks
- participants who develop ALT ≥5xULN should be followed up with repeat blood samples at least weekly until resolution or stabilization (ALT <5xULN on 2 consecutive evaluations)

When a liver chemistry stopping criterion is met, do the following:

- Immediately discontinue study medication. Participants should not restart study medication due to the risk of a recurrent reaction. In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed
- Monitor the participant until liver chemistries resolve, stabilize, or return to baseline values as described below
- Make every reasonable attempt to have participants return to clinic within 24 hours for repeat liver chemistries and monitoring
- Monitor participants twice weekly until liver chemistries (ALT, AST, alkaline phosphatase, bilirubin) resolve, stabilize or return to within baseline values
- Consider additional investigations

The following additional tests may be considered for follow up at the investigators discretion to further evaluate the liver event:

- Viral hepatitis serology including:
  - Hepatitis A IgM antibody
  - HBsAg and Hepatitis B Core Antibody (IgM)
  - Hepatitis C RNA
  - Hepatitis E IgM antibody
- Cytomegalovirus IgM antibody
- Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing)
- Syphilis screening
- Drugs of abuse screen including alcohol
- Serum acetaminophen test (APAP adduct test)
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)
- Fractionate bilirubin, if total bilirubin is greater than 1.5xULN
- Obtain complete blood count with differential to assess eosinophilia

- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies
- Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease

10.2 HYPERSENSITIVITY REACTION

Both dolutegravir and abacavir are associated with a risk for hypersensitivity reactions and share some common features such as fever and/or rash with other symptoms indicating multi-organ involvement. Clinically it is not possible to determine whether a hypersensitivity reaction with Triumeq is caused by dolutegravir or abacavir. Hypersensitivity reactions have been observed more commonly with abacavir, some of which have been life-threatening, and in rare cases fatal, when not managed appropriately. More detailed clinical descriptions of these reactions are included in the Summary of Product Characteristics.

The risk for abacavir hypersensitivity reactions to occur is significantly increased for patients who test positive for the HLA-B*5701 allele. However, abacavir hypersensitivity reactions have been reported at a lower frequency in patients who do not carry this allele.

Investigators must ensure that participants are fully informed regarding the risk of abacavir hypersensitivity reactions prior to commencing abacavir therapy.

The following should be adhered to in the management of participants presenting with signs and symptoms suggesting a possible hypersensitivity reaction:

- In any participant treated with Triumeq, the clinical diagnosis of suspected hypersensitivity reaction must remain the basis of clinical decision making.
- Triumeq must be stopped without delay and an SAE will be reported, even in the absence of the HLA-B*5701 allele, if a hypersensitivity reaction is suspected. Delay in stopping treatment with Triumeq after the onset of hypersensitivity may result in a life-threatening reaction.
- Participants who have experienced a hypersensitivity reaction should be instructed to return their remaining Triumeq/placebo capsules, in order to avoid restarting abacavir.
- After stopping treatment with Triumeq for reasons of a suspected hypersensitivity reaction, Triumeq or any other medicinal product containing abacavir or dolutegravir must never be re-initiated.

Restarting abacavir-containing products following a suspected abacavir hypersensitivity reaction can result in a prompt return of symptoms within hours and may include life-threatening hypotension and death.

If a hypersensitivity reaction is ruled out, participants may restart Triumeq. Rarely, patients who have stopped abacavir for reasons other than symptoms of hypersensitivity reaction have also experienced life-threatening reactions within hours of re- initiating abacavir therapy. Participants must be made aware that hypersensitivity reactions can occur with reintroduction of Triumeq or any other medicinal product containing abacavir and that reintroduction of Triumeq or any other medicinal product containing abacavir should be undertaken only if medical care can be readily accessed.

In the event of discontinuation of Triumeq due to a hypersensitivity reaction, it should not be restarted. In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed.

10.2.1 SKIN REACTIONS WITHOUT OTHER SYMPTOMS TYPICAL OF ABACAVIR HYPERSENSITIVITY REACTION

Participants should be instructed to contact the Investigator as soon as possible if they develop a rash while on study.

Participants who develop rash of any grade should be evaluated for the possibility of an abacavir hypersensitivity reaction or a serious skin reaction such as Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis (TEN) or Erythema Multiforme. These have been reported very rarely in patients taking abacavir-containing products. These patients generally do not have the cluster of additional symptoms (e.g., gastrointestinal and respiratory) that characterize the abacavir hypersensitivity reactions, but they do have features typical of these serious skin reactions.

If a serious skin reaction develops, abacavir (and / or all other concurrent medication(s) suspected in the Investigators causality assessment) should be discontinued, and the participant should not be re-challenged with any abacavir-containing medicinal product (i.e., Triumeq, Ziagen, Trizivir, Epzicom or Kivexa). In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed.

As many products other than abacavir also cause rash and/or serious skin reactions, all other medicinal products that the participant is receiving should also be reviewed and discontinued as appropriate.

Mild to moderate rash is an expected adverse reaction for dolutegravir - containing ART. Episodes generally occur within the first ten weeks of treatment, rarely require interruptions or discontinuations of therapy and tend to resolve within two to three weeks. The index case of hypersensitivity with dolutegravir involved a profuse, purpuric and coalescing leukocytoclastic vasculitis as well as clinically significant liver chemistry elevations. Other than this case, no other instances of serious skin reaction, including SJS, TEN and erythema multiforme, have been reported for dolutegravir in clinical trials.

The following guidance is provided for clinical management of participants who experience rash alone in the absence of accompanying diagnosis of abacavir hypersensitivity reaction, systemic or allergic symptoms or signs of mucosal or target lesions.

- Participants with an isolated Grade 1 rash may continue Triumeq at the Investigator's discretion. The participant should be advised to contact the Investigator immediately if there is any worsening of the rash, if any systemic signs or symptoms worsen, or if mucosal involvement develops.
- Participants may continue Triumeq for an isolated Grade 2 rash. However, Triumeq (and all other concurrent medication(s) suspected in the Investigators causality assessment) should be permanently discontinued for any Grade ≥2 rash that is associated with an increase in ALT. In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed.
- The participant should be advised to contact the physician immediately if rash fails to resolve (after more than two weeks), if there is any worsening of the rash, if any systemic signs or allergic symptoms develop, or if mucosal involvement develops.
- Participants should permanently discontinue Triumeq (and all other concurrent medication(s) suspected in the Investigators causality assessment) for an isolated Grade 3 or 4 rash. Participants should be treated as clinically appropriate and followed until resolution of the AE. In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed.

The rash and any associated symptoms should be reported as adverse events. If the aetiology of the rash can be definitely diagnosed as being unrelated to IMP and due to a specific medical event or a concomitant non-study medication, routine management should be performed, and documentation of the diagnosis provided.

10.3 DECLINE IN RENAL FUNCTION

Treatment with Triumeq must be discontinued in any participant developing moderate to severe renal impairment during the study. Cases with creatinine clearance of <30mL/min (Cockroft-Gault method) will be reported as an SAE. In the event the site PI believes a re-challenge is warranted, with the Chief Investigator in the region must be consulted in advance and the KHP-CTO CRA must be informed

10.4 SUICIDALITY

If any participant experiences a possible suicidality-related adverse event (PSRAE) while participating in this study that is considered by the Investigator be serious, the Investigator will report the event on a SAE report form. A PSRAE may include, but is not limited to, an event that involves suicidal ideation, a preparatory act toward imminent suicidal behaviour, a suicide attempt, or a completed suicide. The investigator will exercise his or her medical and scientific judgment in deciding whether an event is possibly suicide related.

10.4.1 SAFETY PLAN FOR PSRAE

- In the case of immediate suicidal threat, call an ambulance or escort the participant to the nearest Accident and Emergency Department
- In the case of obvious suicidal intent, a study physician must be called for an urgent consultation and based on that assessment, consider immediate psychiatric referral or urgent General Practitioner appointment
- Consider informing the General Practitioner of suicidal ideation in all cases
- Refer, if appropriate, for any additional psychological support available within the ALS clinical service

11. PREVIOUS CLINICAL STUDIES OF TRIUMEQ IN ALS

The Lighthouse Study, a Phase 2a non-randomised open label safety and tolerability trial of Triumeq in 40 HIV negative patients with ALS, was conducted in Australia to investigate combination antiretroviral therapy (https://clinicaltrials.gov/ct2/show/NCT02868580; paper uploaded to NIHR portal). Triumeq is one of the most widely used and well-tolerated antiretrovirals and is a combination of two nucleoside reverse transcriptase inhibitors (Abacavir and Lamivudine) and an integrase inhibitor (Dolutegravir). Patients were observed for 10 weeks pre-treatment and then treated with Triumeq for six months. The primary outcome was met, with Triumeq demonstrated to be safe and well tolerated in people with ALS.

In the Lighthouse Study, secondary analysis of efficacy indicated that for all accepted parameters of outcome in ALS clinical trials, Triumeq showed biological activity during the treatment phase, compared with the pre-treatment observation phase. Moreover, Triumeq demonstrated a possible survival advantage for patients, when compared with a matched cohort of ALS patients in a large European ALS survival prediction study. However, it is important to state this is an open label study with no matched placebo group. An example of the key outcome results follows: the most widely accepted outcome indicator in ALS trials is the ALS Functional Rating Scale-revised (ALSFRS-R)[30]. This is a 12-question scale with a number of sub-scales, generally administered by a clinician. The scale starts at 48 and declines from 0.8 to around 1.0 points a month. Faster decline indicates more rapid disease progression.

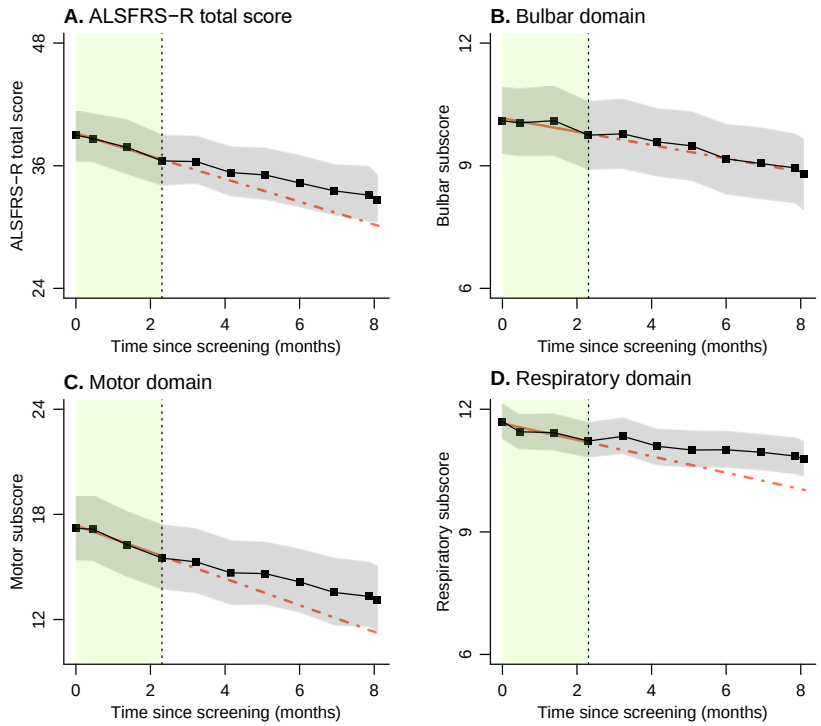

FIGURE 1. ALSFRS-R DECLINE LIGHTHOUSE I

Figure 1 indicates the ALSFRS-R[30] decline during the 10-week observation period compared with the 6-month treatment phase. The monthly rate of change in ALSFRS-R[30], as marker for disease progression, was 1.12 (95% CI 0.63 – 1.60) points per month during the lead-in period (i.e., visit 1 to 4) and 0.76 (95% CI 0.49 – 1.04,) points per month during the Triumeq period (i.e., visit 5 to 11), suggesting a 31.7% (95% CI 6.6 – 56.4) slope reduction after treatment initiation. This effect remained after adjusting for the observed pattern during the lead-in period: adjusted slope reduction of 21.8% (95% CI -4.8 – 48.6).

There is mounting evidence for the use of Triumeq in ALS. We selected Triumeq for this trial because the dose is already licensed and available worldwide, and in a Phase 2a trial, was shown to be safe, well tolerated, and with a low side-effect profile. Thus, repurposing of this widely used therapy would provide ready access for patients with ALS if this Phase 3 trial proves positive. There are about 600,000 patients with HIV currently taking Triumeq on a daily basis. Preliminary data from the National Institutes of Health indicate that the dose of Triumeq we propose to use significantly suppresses HERV-K (Figure 2).

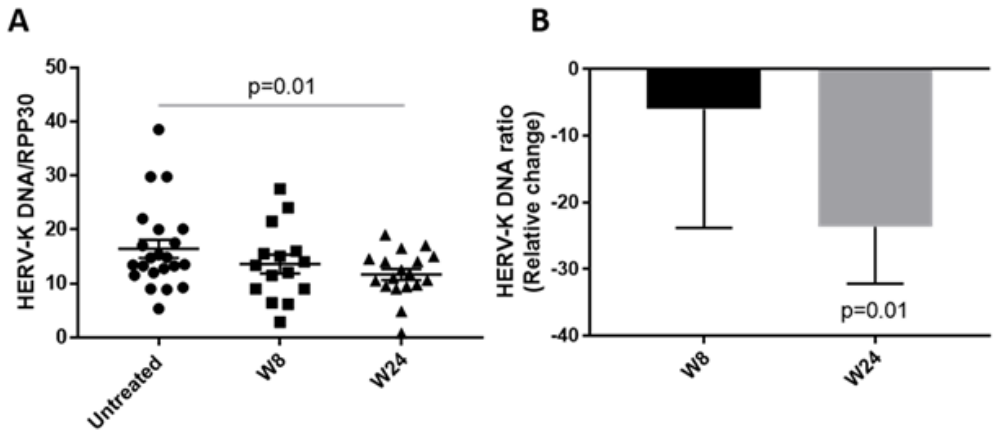

FIGURE 2. EFFECT OF THE ANTIRETROVIRAL TREATMENT ON HERV-K DNA/RPP30 RATIO

HERV-K DNA copy numbers were measured in all samples by digital PCR, and the ratio HERV-K/RPP30 was calculated as a measure of non-genomic HERV-K. A) Absolute value of HERV-K DNA /RPP30 in untreated samples and at weeks 8 and 24 of treatment. B) The relative change as a percentage, with respect to the ratio in the untreated visits (average of screening and baseline) for each follow-up visit (W8 and W24). Untreated samples, n=22; W8 samples, n=15; W24 samples, n=18.

In addition to its safety profile and efficacy against the target, Triumeq is also an optimal antiretroviral agent for this study for other reasons. First, the components of Triumeq have very efficient CNS penetration. Abacavir and Lamivudine, which are nucleoside reverse transcriptase inhibitors, show excellent CNS levels when taken orally [(40)], as does the integrase strand inhibitor, dolutegravir [(41)]. Second, although there are other available antiretroviral combination therapies, these require boosting to increase their absorption e.g. Genvoya which is boosted with Cobicistat. In HIV practice, Cobicistat, which is a CYP3A inhibitor, has a considerable number of drug-drug interactions and can cause ongoing gastrointestinal disturbance.

11.1 SUMMARY OF FINDINGS FROM NON-CLINICAL STUDIES

No carcinogenicity studies have been conducted with the combination of dolutegravir, abacavir and lamivudine.

11.2 SUMMARY OF FINDINGS FROM CLINICAL STUDIES

Safety and tolerability has not been definitively evaluated in an adequate number of patients, but previous research in HIV patients has indicated Triumeq may be well tolerated among patients with ALS. During the lead-in period of 10 weeks, 16 subjects reported 22 AEs. This resulted in 1.38 AEs per week per 10 patients. During the treatment phase with Triumeq, 37 subjects reported 124 AEs. This resulted in 1.33 AEs per week per 10 patients and did not indicate an increased incidence of AEs between the observation and the treated phase. All AEs are summarized in Table 3 below. There were no deaths during the study medication period.

Body Code Category	Number of AEs (*n*)
Infections and Infestations	46
Gastrointestinal Disorders	23
Musculoskeletal and Connective Tissue Disorders	16
Injury, Poisoning and Procedural Complications	14
Nervous System Disorders	13
Investigations	9
Skin and Subcutaneous Tissue Disorders	8
Respiratory, thoracic and mediastinal disorders	8
Psychiatric Disorders	6
Vascular Disorders	1
Reproductive system and breast disorders	1

TABLE 3 SUMMARY OF ADVERSE EVENTS BY BODY CODE CATEGORY IN LIGHTHOUSE PHASE IIA STUDY

The majority of AEs reported in the study were mild in intensity (67 AEs reported by 36 subjects); 28 AEs (reported by 37 subjects) were moderate in intensity, and 10 SAEs (reported by 10 subjects) were severe in intensity. No patient was withdrawn due to a medication associated AE. There were no deaths of trial participants during the trial period. One death occurred in a participant who had been withdrawn and occurred 3 months after withdrawal and unrelated to the study medication.

SAEs were:

- Elevated liver function & withdrawal from study (1 subject, *possibly related*)
- Hospitalization for increased anxiety (1 subject, *possibly related*)

- Headache (1 subject, *not related*)
- Cellulitis (1 subject, *not related*)
- Aspiration pneumonia and choking episode (2 subjects, *not related*)
- Gastrostomy placement (2 subjects, *not related*)
- Overdose atropine eye drops (1 subject, *not related*)
- Discectomy (1 subject, *not related*)

In total, five patients prematurely withdrew from the study. The Investigator withdrew one patient due to elevated liver enzymes. The other four patients stopped due to loss-to-follow-up (two moved away) and Adverse Events unrelated to study medication (one elective cholecystectomy and one bowel obstruction).

12. ETHICS AND REGULATORY APPROVAL

Ethical and regulatory approval will be sought in each participating region. The co-ordinating teams in the participating regions will be repsonsible for submitting the trial documentation for regulatory approval to relevant regulatory authorities. The KHP-CTO will be responsible for authorising the submission packs for regulatory approval in all EU countries.

Individual participants will consent to participate. The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996)[34] , the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments[35].

This protocol and related documents will be submitted for review to Health Research Authority (HRA), Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation in the UK. Relevant regulatory authorities in Australia, New Zealand and EU countries will be included in submissions.

The Chief Investigators will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the co-Sponsors) and the REC within the timelines defined in the Regulations. TRICALS will upload the final report to EudraCT on behalf of the co-Sponsors.

12.1 PROTOCOL AMENDMENTS AND VERSION CONTROL OF STUDY DOCUMENTS

The UK Trial Manager and the KHP-CTO CRA will be responsible for preparing and submitting protocol amendments to the ethics committee and the MHRA in the UK. Relevant documentation will be passed to the co-ordinating teams in the EUAustralia to submit and disseminate locally. Team in Australia will also submit and disseminate documentation in New Zealand.

Country-specific participant-facing documents (e.g., participant information sheet, consent form, emergency code break cards) will be prepared by the co-ordinating centre in each region (KCL, TRICALS, Macquarie) and the co-ordinating centres are responsible for maintaining version control and track-changes copies and ensuring the documents contain all relevant information to meet local legal requirements.

Once approved by ethics and regulatory in the relevant country, the documents will be sent to site by the regional co-ordinating team for filing in the Investigator Site File and an acknowledgement will be requested and retained from each site. All correspondence, including submission packs with attachments and approvals,

will be forwarded to the UK Trial Manager for filing in the global Trial Master File. Site staff CVs, GCP certifications and delegation logs will also be retained in the global Trial Master File at KCL.

Recruiting study sites are responsible for communicating relevant information to participants.

The UK Trial Manager will be responsible for updating the ISRCTN register subsequent to relevant protocol amendments.

13. STATISTICAL METHODS

13.1 PRIMARY OUTCOME

1. The primary endpoint is overall survival, defined as time to mortality from any cause

13.2 SECONDARY OUTCOMES

1. Combined assessment of survival and measures of daily functioning using the ALSFRS-R[30] total score (CAFS)
2. Daily functioning using the ALSFRS-R[30] total score
3. Respiratory function measured by slow vital capacity (SVC) (% predicted of normal according to the GLI-2012 reference standard[37]).
4. Plasma creatinine levels
5. Clinical disease stage, defined as mean time spent in each stage of the King's Staging Scale and the ALS Milano-Torino staging systems [36] (MITOS, derived from ALSFRS-R[30]).
6. Safety based on the safety assessments including physical examinations, clinical laboratory evaluations, vital signs and frequency of adverse events (AEs) or serious adverse events (SAEs). (S)AEs will be categorized according seriousness, causality (by study medication), severity and expectedness (as defined in the Triumeq Summary of Product Characteristics).
7. Tolerabilty, as defined by study medication discontinuation
8. Cognitive function, defined as the total scores on the ECAS[32]
9. Quality of life, defined as total scores on the Visual Analogue Scale (single-item scale) and EQ-5D-5L[35].
10. Laboratory parameters e.g. Urine $P75^{ECD}$, plasma neurofilament light and heavy chain, HERV-K expression and genotyping (UNC13a / C9orf72)

13.3 SAMPLE SIZE JUSTIFICATION

We estimate that for those randomised to the placebo arm, a 70% two-year mortality, and this would be decreased to 51.5% of those allocated to the Triumeq arm (Hazard Ratio=0.60). Using a log-rank test, with a randomisation ratio of 2:1 (Triumeq:placebo) and Freedman method to detect this effect. With a minimum of 87% power and 5% two-sided significance 203 events (353 participantss) are needed to be analysed. This will be inflated to 212 participants(368 participants) to adjust for a formal interim analysis, and a further 5% loss to follow up, so 390 participants will be randomised. The trial duration is defined as when the last participant has completed 24-months of follow-up or a minimum of 212 events have occurred, whichever is first. Detail of the interim analysis is found within section 13.5.

13.4 STATISTICAL METHODS FOR PRIMARY AND SECONDARY OUTCOMES

A full statistical analysis plan will be drafted in accordance with the KCTU Standard Operating Procedures authored and reviewed by the Senior Statistician TRICALS Statistician, respectively, and approved by the Trial Steering Committee.

The Junior Statistician will be fully blind until the first version of the Statistical Analysis Plan (SAP) is approved by the Trial Steering Committee (TSC). The SAP should be detailed enough so that it presents a

clear and structured plan for the primary outcome, required data manipulation, and analysis. All changes to the SAP after approval by the TSC should be authored by a statistician who is fully blind, this would be expected to be the Senior Statistician.

After the first version of the SAP is approved by the TSC, the Junior statistician is planned to become partially blinded and access participant level data coded as A/B/C. The Junior Statistician will then have access to the adherence data and be able to monitor and inform the DMC of the trial adherence of the participants. They will present the closed DMC report to the DMC members.

The Junior Statistician will not take part in any discussion that influences the early stopping of the trial at any TMG, TSC, or DMC meetings.

13.4.1 STATISTICAL METHODS FOR PRIMARY OUTCOME

We will model the all-cause mortality between participants allocated to Triumeq and placebo using a multivariable Cox's Proportional Hazards regression, with a frailty for site. The analyses will be adjusted for key design and clinical characteristics, and demographics as defined in the SAP. We will use an intention to treat approach in our primary analysis. Log-log plots will be used to visually assess baseline proportionality.

13.4.2 STATISTICAL METHODS FOR SECONDARY OUTCOMES

13.4.2.1 CONTINUOUS OUTCOMES

Continuous outcomes will be analysed with a multi-level mixed-effects multivariable regression analysis and will be carried out on all follow up time-points. A random intercept and slope over time will be fitted for participant and the analysis adjusted by terms consistent with the primary outcome as well as baseline values of the outcome where appropriate.

13.4.2.2 BINARY OUTCOMES

Binary outcomes will be fitted with a multi-level mixed-effects multivariable logistic regression analysis will be carried out on all follow up time-points. A random intercept will be fitted for participant and the analysis adjusted by terms consistent with the primary outcome as well as baseline values of the outcome where appropriate.

13.4.3 ADHERENCE

To be defined in the Statistical Analysis Plan.

13.5 **INTERIM ANALYSES (STATISTICAL)**

By using an asymmetric two-sided non-binding two-stage group sequential design with 87% power and 2.5% type 1 error we will introduce one formal interim analysis to stop for both futility and efficacy. By applying type 1-error spending using Hwang-Shih-DeCani and Gamma = -2 (stage one spent error =0.003), we will inflate the final sample size to 390 participants (212 events) and conduct one formal interim analysis to detect efficacy after a minimum of 150 events.

13.5.1 INTERIM ANALYSIS 1

Interim analyses to conclude futility carried out after 75 events.

Using a asymmetric two sided non-binding two-stage group sequential design with 87% power and 2.5% type 1 error, applying a lower futility bound cut off rule to conclude futility (H_1:Reject). After a minimum of 76 events, we will conclude futility if Z<-0.24, favouring Triumeq

13.5.2 INTERIM ANALYSIS 2

Interim analyses carried out after 150 events.

Interpretation for superiority:

Using an asymmetric two-sided non-binding two-stage group sequential design with 87% power and 2.5% type 1 error, applying a upper bound for efficacy bound cut off rule to conclude superiority (H0:Reject). After a minimum of 150 events, we will conclude Superiority if Z>2.75 (nominally p-value <0.003).

Interpretation for futility:

We will conclude futility if Z<0.94, favouring Triumeq.

The DMC will provide advice to the TSC, who will advise the Sponsor in making the final decision on continuing or stopping the trial.

13.6 METHODS FOR ADDITIONAL ANALYSES (E.G. SUBGROUP ANALYSES)

We will carry out the following planned subgroup analyses:

Country
Age at randomisation
Sex
TRICALS High/Low
UNC13A and C9orf72 genotype status
Neurofilament (high vs. low)
HERV-K and P75 ECD

13.7 METHODS TO HANDLE MISSING DATA

All participants who are randomised and have at least one follow-up time-point will be analysed as per their allocation group (intention-to-treat) for the primary outcome. Missing baseline will be presented and explored for patterns of missingness. Missing baseline data less than 5% will be assumed to be missing completely at random. Methods for dealing with missing data will be implemented according to previously described methods [38].

13.8 POPULATIONS UNDER INVESTIGATION

A modified intention to treat population (ITT) will include all participants in the outcome that are randomised and followed up with one post baseline measurement.
A per-protocol (PP) population will exclude participants who are protocol violators

13.9 METHODS TO HANDLE COMPLIANCE

Compliance will be defined in the SAP.

13.10 SENSITIVITY ANALYSIS

The primary outcome will be repeated for the following sensitivity analyses:

Using the per protocol population

Using ALS-specific mortality, which will exclude deaths due to non-ALS-related causes e.g. trauma.

13.11 PLANS TO GIVE ACCESS TO THE FULL PROTOCOL AND PARTICIPANT LEVEL-DATA

It is anticipated the full protocol and all results will be available as open access according to the rules of the funding bodies.

14. OVERSIGHT AND MONITORING

14.1 TRIAL MANAGEMENT GROUP (TMG)

Title	Name	Role
KCL Chief Investigator (UK)	Ammar Al-Chalabi	Chair
Macquarie University Chief Investigator (Australia)	Julian Gold	Member
TRICALS Chief Investigator (Europe)	Leonard van den Berg	Member
LIGHTHOUSE-II Senior Statistician (UK)	Ben Carter	Member
TRICALS Statistician (Europe)	Ruben van Eijk	Member
KCTU Junior Statistician (UK)	Rose Tinch-Taylor	Member
KCTU Senior Trial Manager (UK)	Sylvia Wilczynska	Member
KCTU Trial Manager (UK)	Alisha McPherson	Member
TRICALS Co-ordinator (Europe)	Roel Vink	Member
Macquarie Co-ordinator (Australia)	Jenny Slavec	Member
KCTU Pharmacist (UK)	Angela Cape	Member
KCTU Operations Director (UK)	Caroline Murphy	Member
KCTU Data Centre Lead (UK)	Joanna Kelly	Member
KHP-CTO Quality Manager (or delegated CRA) (UK)	Amy Holton	Observer
Macquarie Head of Operations (Australia)	Nicola Chapman	Member
TRICALS CRA (Europe)	Annemarie Janse	Member
LIGHTHOUSE II Collaborator (UK)	Christopher J McDermott	Member
Participant Representative (UK)	Dr Peter Morley	Observer

TABLE 4 TRIAL MANAGEMENT GROUP MEMBERSHIP IN LIGHTHOUSE II STUDY

Members of the TMG are listed in Table 4 above. Changes in individuals filling these roles will not require a protocol update but will be documented in the TMG minutes.

14.2 TRIAL STEERING COMMITTEE (TSC)

The TSC will be composed of six independent members. The TSC is an executive committee, reporting to the funder (NIHR) and the sponsor. The TSC is formally appointed by NIHR and members will receive individual letters from NIHR confirming their role. Independent members will be independent of the Sponsor organisations and of any recruiting study sites.

The trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring Committee / Trial Steering Committee regulatory authority or ethics committee concerned.

If the trial is prematurely discontinued, active participants will be informed, and no further participant data will be collected. The Competent Authority and Research Ethics Committee will be informed within 15 days of the early termination of the trial.

14.3 DATA MONITORING COMMITTEE (DMC)

The DMC will be composed of three independent members: a statistician and two clinicians. The DMC is an advisory committee, reporting to the Trial Steering Committee. The DMC is formally appointed by NIHR and members will receive individual letters from NIHR confirming their role. Members will be independent of the Sponsor organisations and of any recruiting study sites. The DMC will work to the DAMOCLES guidance[39].

14.4 MONITORING

Monitoring of this trial to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained by the KHP-CTO Quality Team at King's College London.

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g., participants' case sheets, blood test reports etc.).

In the UK, KHP-CTO will prepare a monitoring plan for UK, EU, Australian and New Zealand sites in accordance with local regulatory and HREC requirements. The KHP-CTO will conduct site monitoring visits in the UK, TRICALS across Europe and Macquarie University will monitor sites in Australia and New Zealand. At the site initiation visit, the relevant CRA will provide the recruiting site with a Lighthouse-specific investigator site file and a site pharmacy file, to be maintained for the duration of the study.

15. MISCELLANEOUS

15.1 PLANS FOR INDEPENDENT AUDIT

There are no current plans to commission an independent audit study conduct. If Triumeq is found to be beneficial in this participant population and the data is to be used for a licensing application, an independent audit may be commissioned at a later date.

15.2 DISSEMINATION PLANS

The primary and secondary outcomes will be published in a peer reviewed open-source medical journal within 12 months of the end of trial. Recruiting sites will be informed of the results and will be asked to disseminate the findings to participants. Patient groups will be informed of the results for dissemination among their members. The sharing dataset will be passed to the UK Chief Investigator by the analyst and all future data sharing will be managed by the TRICALS consortium and Macquarie University.

15.3 END OF TRIAL

The end of the trial will be defined as last participant last visit.

15.4 CONFIDENTIALITY

When consent forms are signed, a copy will be provided to the participant, a copy will be filed in the medical records and the original will be retained in the Investigator Site File. Participant's initials (in countries where this is permitted), year of birth and age will be entered into the study database, but no more identifying information will be collected outside the recruiting study site. Within site, an Investigator Site File will be maintained by the site PI. Participants will be fully identifiable within these files.

The patients' identifiable data will be kept in the UK for 15 years after the study has finished or for as long as is required for the rules and regulations for each country.

When the study is complete, a data sharing dataset will be created from the raw data by the study analyst, which will not include any other identifiable data and study PIN will be altered so that individuals are not recognisable from the dataset.

The study will comply with the General Data Protection Regulations (GDPR) relying on the 'public task' grounds as the lawful bases for processing personal data, and its UK implementation, Data Protection Act (DPA) 2018 [42, 43].

15.5 COVID-19 CONTINGENCIES

The recent COVID pandemic has impacted clinical trial work. While ALS clinics continued to function throughout lockdowns in Europe, the UK and Australia, and clinical trial enrolment continued in many centres, essential measurements including respiratory evaluation were limited because of the generation of aerosols with associated infection risk. Members of the proposed Lighthouse 2 sites have worked to develop protocols to enable the possibility of remote respiratory and questionnaire assessments. These include the use of home spirometry measures, tele-linked evaluation where appropriate, and home or telephone visits by research staff to evaluate AEs, if participant travel is restricted. Note that these measures are not mandated but are optional for sites to use when appropriate. Data will be collected in the eCRF to indicate whether data was collected at clinic or remotely. If required IMP may be posted to participants using appropriate courier service.

15.6 FUNDING

UK funding is from the NIHR EME Programme (NIHR EME 127921). Australian funding and European funding is from Fight MND. In addition, Australia receives funding from the MND Research Institute of Australia and TRICALS from the ALS Stichting Nederland. VIIV Healthcare Ltd is donating active Triumeq medication for the study.

15.7 AVAILABILITY OF DATA AND MATERIALS

Data will be available for sharing upon request for future scientific research, subject to approval by the TRICALS consortium and Macquarie University.

15.8 INSURANCE AND INDEMNITY

King's College London provides no fault liability insurance in the event of harm arising from the study design in the UK. UK NHS recruiting sites provide indemnity in the event of clinical negligence.
In New Zealand each site institution maintains insurance necessary to provide indemnity to it in relation to any liability which it may incur. In Australia in the public sector, each State and Territory provides indemnity or insurance coverage in relation to their clinical trial activities. The arrangements are implemented and managed through a State or Territory agency and may take the form of insurance or an indemnity fund or a self-insurance scheme.
In Australia, Universities are cover by the Unimutual scheme, which covers trials conducted by university researchers. Unimutual is a discretionary mutual, operating on not-for-profit principles and was formed to offer higher education and research institutions a cost-effective alternative to insurance. TRICALS will provide clinical trial insurance in accordance with applicable national law in the country where the site is located. Each site and/or investigator shall provide indemnity in accordance with mandatory law of the country where the site is located.

15.9 ARCHIVING

At the end of the trial, all trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the 2018 Data Protection Act and archived in line with the Medicines for

Human Use (Clinical Trials) Amended Regulations 2006 as defined in the KHP-CTO Archiving Standard Operating Procedure (SOP). Recruiting sites will be responsible for archiving the source data, Investigator Site Files and Pharmacy Site Files. The KHP-CTO has a contract with an external company that will provide a suitable archiving service for this trial. Off-site archiving will only be arranged with the prior authorisation of the Named Archivist or delegate within the KHP-CTO. Off-site archiving will be only arranged with the prior authorisation of the Named Archivist or delegate within the KHP-CTO. Only the Named Archivist or delegate can instigate the retrieval of trial documents. A written a written retrieval request is required.

16. REFERENCES

1. Brown RH, Al-Chalabi A. Amyotrophic Lateral Sclerosis. N Engl J Med. 2017;377(2):162-72.
2. Hardiman O, Al-Chalabi A, Brayne C, Beghi E, van den Berg LH, Chio A, et al. The changing picture of amyotrophic lateral sclerosis: lessons from European registers. J Neurol Neurosurg Psychiatry. 2017;88(7):557-63.
3. Manjaly ZR, Scott KM, Abhinav K, Wijesekera L, Ganesalingam J, Goldstein LH, et al. The sex ratio in amyotrophic lateral sclerosis: A population based study. Amyotroph Lateral Scler. 2010;11(5):439-42.
4. Johnston CA, Stanton BR, Turner MR, Gray R, Blunt AH-M, Butt D, et al. Amyotrophic lateral sclerosis in an urban setting: a population based study of inner city London. Journal of neurology. 2006;253:1642-3.
5. Alonso A, Logroscino G, Jick SS, Hernán MA. Incidence and lifetime risk of motor neuron disease in the United Kingdom: a population-based study. European journal of neurology. 2009;16:745-51.
6. Westeneng HJ, Debray TPA, Visser AE, van Eijk RPA, Rooney JPK, Calvo A, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. The Lancet Neurology. 2018;17:423-33.
7. Veldink JH, Wokke JH, van der Wal G, Vianney de Jong JM, van den Berg LH. Euthanasia and physician-assisted suicide among patients with amyotrophic lateral sclerosis in the Netherlands. N Engl J Med. 2002;346(21):1638-44.
8. van der Steen I, van den Berg JP, Buskens E, Lindeman E, van den Berg LH. The costs of amyotrophic lateral sclerosis, according to type of care. Amyotroph Lateral Scler. 2009;10(1):27-34.
9. Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. Lancet (London, England). 1996;347:1425-31.
10. Writing G, Edaravone ALSSG. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2017;16(7):505-12.
11. Turnbull J. Is edaravone harmful? (A placebo is not a control). Amyotroph Lateral Scler Frontotemporal Degener. 2018;19(7-8):477-82.
12. Al-Chalabi A, Fang F, Hanby MF, Leigh PN, Shaw CE, Ye W, et al. An estimate of amyotrophic lateral sclerosis heritability using twin data. J Neurol Neurosurg Psychiatry. 2010;81(12):1324-6.
13. Al-Chalabi A, Calvo A, Chio A, Colville S, Ellis CM, Hardiman O, et al. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol. 2014;13(11):1108-13.
14. Gardner MB, Rasheed S, Klement V, Rongey RW, Brown JC, Dworsky R, et al. Lower motor neuron disease in wild mice caused by indigenous type C virus and search for a similar etiology in human amyotrophic lateral sclerosis. UCLA Forum Med Sci. 1976(19):217-34.
15. Engel W. RNA metabolism in relation to Amyotrophic lateral sclerosis. In: Rowland L, editor. Amyotrophic lateral sclerosis and other motor neuron diseases. New York: Raven Press; 1991. P.125-53. NIHR127921
16. Younger DS, Rowland LP, Latov N, Hays AP, Lange DJ, Sherman W, et al. Lymphoma, motor neuron diseases, and amyotrophic lateral sclerosis. Annals of Neurology. 1991;29:78-86.
17. Hughes JF, Coffin JM. Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: Implications for human and viral evolution. Proceedings of the National Academy of Sciences. 2004;101:1668-72.
18. Li W, Lee MH, Henderson L, Tyagi R, Bachani M, Steiner J, et al. Human endogenous retrovirus-K contributes to motor neuron disease. Science Translational Medicine. 2015;7.
19. Steele AJ, Al-Chalabi A, Ferrante K, Cudkowicz ME, Brown RH, Garson JA. Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives. Neurology. 2005;64:454-8.
20. McCormick AL, Brown RH, Cudkowicz ME, Al-Chalabi A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. Neurology. 2008;70:278-83.

21. Andrews WD, Tuke PW, Al-Chalabi A, Gaudin P, Ijaz S, Parton MJ, et al. Detection of reverse transcriptase activity in the serum of patients with motor neurone disease. Journal of medical virology. 2000;61:527-32.
22. Alfahad T, Nath A. Retroviruses and amyotrophic lateral sclerosis. Antiviral research. 2013;99:180-7.
23. Barbulescu M, Turner G, Seaman MI, Deinard AS, Kidd KK, Lenz J. Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans. Current biology : CB. 1999;9:861-8.
24. Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Annals of neurology. 2011;69:141-51.
25. Mayer J, Harz C, Sanchez L, Pereira GC, Maldener E, Heras SR, et al. Transcriptional profiling of HERV-K(HML-2) in amyotrophic lateral sclerosis and potential implications for expression of HML2 proteins. Molecular neurodegeneration. 2018;13:39.
26. Garson JA, Usher L, Al-Chalabi A, Huggett J, Day EF, McCormick AL. Quantitative analysis of human endogenous retrovirus-K transcripts in postmortem premotor cortex fails to confirm elevated expression of HERV-K RNA in amyotrophic lateral sclerosis. Acta Neuropathol Commun. 2019;7(1):45.
27. Hansen DT, Petersen T, Christensen T. Retroviral envelope proteins: Involvement in neuropathogenesis. Journal of the Neurological Sciences. 2017;380:151-63.
28. Raaphorst J, Beeldman E, Schmand B, Berkhout J, Linssen WH, van den Berg LH, Pijnenburg YA, Grupstra HF, Weikamp JG, Schelhaas HJ, Papma JM, van Swieten JC, de Visser M, de Haan RJ. The ALS-FTD-Q: a new screening tool for behavioral disturbances in ALS. Neurology. 2012 Sep 25;79(13):1377-83. Doi: 10.1212/WNL.0b013e31826c1aa1. Epub 2012 Sep 12. PMID: 22972650.
29. Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2014 Mar;15(1-2):9-14. Doi: 10.3109/21678421.2013.805784. Epub 2013 Jun 19. PMID: 23781974.
30. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, Nakanishi A. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999 Oct 31;169(1-2):13-21. Doi: 10.1016/s0022-510x(99)00210-5. PMID: 10540002.
31. Roche JC , Rojas-Garcia R , Scott KM , et al . A proposed staging system for amyotrophic lateral sclerosis. Brain 2012;135:847–52.doi:10.1093/brain/awr351 pmid:http://www.ncbi.nlm.nih.gov/pubmed/22271664
32. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011 Dec;20(10):1727-36. Doi: 10.1007/s11136-011-9903-x. Epub 2011 Apr 9. PMID: 21479777; PMCID: PMC3220807.
33. Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, Mann JJ. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011 Dec;168(12):1266-77. Doi: 10.1176/appi.ajp.2011.10111704. PMID: 22193671; PMCID: PMC3893686.
34. World Medical Association. 1996. Declaration of Helsinki, 4th (Somerset West) amendment
35. Medicines for Human Use (Clinical Trials) Regulations 2004 and shall come into force on 1st May 2004
36. Tramacere I, Dalla Bella E, Chiò A on behalf of the EPOS Trial Study Group, et al The MITOS system predicts long-term survival in amyotrophic lateral sclerosis Journal of Neurology, Neurosurgery & Psychiatry 2015;86:1180-1185.
37. Quanjer PH, Brazzale DJ, Boros PW et al. Implications of adopting the Global Lungs Initiative 2012 all-age reference equations for spirometry. Eur Respir J 2013; 42: 1046–1054
38. Jakobsen JC, Gluud C, Wetterslev J, Winkel P. When and how should multiple imputation be used for handling missing data in randomised clinical trials – a practical guide with flowcharts. BMC Med Res Methodol. 2017;17(1):162.
39. DAMOCLES Study Group, NHS Health Technology Assessment Programme. A proposed charter for clinical trial data monitoring committees: helping them to do their job well. Lancet. 2005 Feb 19-25;365(9460):711-22. Doi: 10.1016/S0140-6736(05)17965-3. PMID: 15721478.
40. Ene L, Duiculescu D, Ruta SM. How much do antiretroviral drugs penetrate into the central nervous system? J Med Life. 2011;4(4):432-9.

41. Letendre SL, Mills AM, Tashima KT, Thomas DA, Min SS, Chen S, et al. ING116070: A study of the pharmacokinetics and antiviral activity of dolutegravir in cerebrospinal fluid in HIV-1-infected, antiretroviral therapy-naïve subjects. Clinical Infectious Diseases. 2014;59:1032-7.
42. General Data Protection Regulations (GDPR) are European Union regulations and can be found here: European Parliament and Council of European Union (2016) Regulation (EU) 2016/679. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32016R0679&from=EN (Accessed: 18th January 2021).
43. Data Protection Act (DPA) 2018 https://www.legislation.gov.uk/ukpga/2018/12/contents (Accessed 28th August 2022)
44. Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, Grosskreutz J, Hardiman O, Henderson R, Matamala JM, Mitsumoto H, Paulus W, Simon N, Swash M, Talbot K, Turner MR, Ugawa Y, van den Berg LH, Verdugo R, Vucic S, Kaji R, Burke D, Kiernan MC. A proposal for new diagnostic criteria for ALS. Clin Neurophysiol. 2020 Aug;131(8):1975-1978. doi: 10.1016/j.clinph.2020.04.005. Epub 2020 Apr 19. PMID: 32387049.

APPENDIX 1 CRITERIA FOR DIAGNOSIS, J.M. SHEFNER ET AL. / CLINICAL NEUROPHYSIOLOGY 131 (2020) 1975–1978 (43)

Table 1
Criteria for diagnosis of ALS.

1. Progressive motor impairment documented by history or repeated clinical assessment, preceded by normal motor function, and
2. Presence of upper[1] and lower[2] motor neuron dysfunction in at least 1 body region[3] , (with upper and lower motor neuron dysfunction noted in the same body region if only one body region is involved) or lower motor neuron dysfunction in at least 2 body regions, and
3. Investigations[4] excluding other disease processes

Footnotes:

[1]Upper motor neuron dysfunction implies at least one of the following:
1. Increased deep tendon reflexes, including the presence of a reflex in a clinically weak and wasted muscle, or spread to adjacent muscles
2. Presence of pathological reflexes, including Hoffman sign, Babinski sign, crossed adductor reflex, or snout reflex.
3. Increase in velocity-dependent tone (spasticity)
4. Slowed, poorly coordinated voluntary movement, not attributable to weakness of lower motor neuron origin or Parkinsonian features

[2]Lower motor neuron dysfunction in a given muscle requires either:
Clinical examination evidence of
Muscle weakness, and
Muscle wasting
or
EMG abnormalities that must include:
Both evidence of chronic neurogenic change, defined by large motor unit potentials of increased duration and/or increased amplitude, with polyphasia and motor unit instability regarded as supportive but not obligatory evidence.
And evidence of ongoing denervation including
Fibrillation potentials or positive sharp waves, or
fasciculation potentials

[3]Body regions are defined as bulbar, cervical, thoracic and lumbosacral. To be classified as an involved region with respect to lower motor neuron involvement, there must be abnormalities in two limb muscles innervated by different roots and nerves, or one bulbar muscle, or one thoracic muscle either by clinical examination or by EMG.

[4]The appropriate investigations depend on the clinical presentation, and may include nerve conduction studies and needle EMG, MRI or other imaging, fluid studies of blood or CSF, or other modalities as clinically necessary.