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Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol

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List and legends of tables and figures:

- Table 1. Methods flowchart
- Figure 1. Schematic flowchart of the clinical trial

List of abbreviations:

- AD-MSCs: allogeneic adipose tissue-derived mesenchymal stem cells
- MSCs: mesenchymal stem cells
- CT: computed tomography
- MRI: magnetic resonance imaging
- NIHSS: National Institutes of Health Stroke Scale
- mRS: modified Rankin Scale
- AEs: Adverse events
- GM-CSF: granulocyte-macrophage colony-stimulating factor
- PDGF-BB: platelet-derived growth factor BB
- BDNF: brain-derived neurotrophic factor
- VEGF: vascular endothelial growth factor
- TGF-1: transforming growth factor 1
- GFAP: endostatin, glial fibrillary acid protein
- MBP: myelin basic protein
- MMP-3: matrix metalloproteinase 3
- ECG: electrocardiogram
- SCReN: Spanish Clinical Research Network
- IQR: interquartile range

ABSTRACT

Introduction: Stroke is a serious public health problem, given it is a major cause of disability worldwide despite the spread of recanalisation therapies. Enhancement of brain plasticity with stem cell administration is a promising innovative therapy to reduce sequelae in these patients.

Methods and analysis: We have developed a phase IIb, multicentre, randomised, double-blind, placebo-controlled clinical trial protocol to evaluate the safety and efficacy of intravenous administration of allogeneic adipose tissue-derived mesenchymal stem cells (AD-MSCs) in patients with acute ischaemic stroke, concurrently with conventional stroke treatment. Thirty patients will be randomised on a 1:1 basis to receive either intravenous placebo or allogeneic AD-MSCs as soon as possible within the first 4 days from stroke symptom onset. Patients will be followed up to 24 months after randomisation. The primary objective is the safety assessment of early intravenous administration of allogeneic AD-MSCs by reporting all adverse events and neurological or systemic complications in both treatment groups. Secondary objectives assess efficacy of early intravenous AD-MSC treatment in acute ischaemic stroke by evaluating changes in the modified Rankin Scale and the National Institutes of Health Stroke Scale throughout the follow-up period. In addition, brain repair biomarkers will be measured at various visits.

Ethics and dissemination: This clinical trial has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products. It has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003).

Strengths and limitations

- First multicentre clinical trial evaluating safety and possible efficacy of early intravenous treatment with adipose derived mesenchymal stem cells in ischemic stroke.
- Allogeneic adipose derived mesenchymal stem cells are abundant, easily accessible and have low immunogenicity.
- Limitations: early-phase study with only two centres in the same country recruiting patients.

KEY WORDS: *Acute ischaemic stroke, Allogeneic adipose tissue-derived mesenchymal stem cells, Clinical trial, Stem cell therapy, safety.*

INTRODUCTION

Stroke is a serious health problem, given it is one of the most common causes of mortality and the leading cause of disability due to neurologic diseases worldwide. In 2016, the global estimated incidence of stroke ranged between 189 and 218 cases per 100,000 inhabitants.[1] A large proportion of patients with ischaemic stroke have persistent neurological deficits that impair their daily life activities, and many also experience non-neurological complications, such as pulmonary and urinary tract infections, deep vein thrombosis and depression. In the last 2 decades, there has been a breakthrough in ischaemic stroke treatments. However, currently available therapies, such as intravenous thrombolysis and mechanical thrombectomy, focus only on reperfusion in the acute phase and are not accessible to all patients due to a narrow time window and eligibility issues. Furthermore, these cerebral reperfusion-based therapies cannot reverse established ischaemic injury or reduce its sequelae. Therefore, to reduce the burden of this disease, there is an urgent need for therapies that can enhance patient recovery after stroke.

After a brain injury, several interrelated mechanisms, such as neurogenesis, gliogenesis, oligodendrogenesis, synaptogenesis and angiogenesis take place to repair the damaged tissue. In clinical practice, these processes are stimulated through rehabilitation therapies; however, cell therapies promote tissue repair exogenously,[2] which could complement current reperfusion treatments for acute ischaemic stroke.

Stem cells are immature cells with the capacity to differentiate into diverse cell lines. At present, challenges concerning the most suitable cell type, route and time of administration are the main issues for progression from preclinical studies to the design of clinical trials.[3-5]

Regarding the route of administration, preclinical studies have shown that it is not mandatory for stem cells to migrate and nest at the injury site to achieve functional recovery after stroke, given their therapeutic effect is also due to secretion of trophic factors and modulation of the immune response.[5,6] Intravenous administration has been proven to be noninferior to other routes in preclinical studies, such as in intracerebral, intrathecal or intra-arterial deliveries, and it is far less

invasive. Regarding cell type, preclinical and initial clinical trial data [5,7,8,9,10,11] suggest that mesenchymal stem cells (MSCs) constitute a safe and possibly effective therapy in ischaemic stroke. Allogeneic MSCs lack human leucocyte antigen-class II molecules, avoiding the risk of rejection and allowing short timings of administration.

Most studies concerning cell therapies in ischaemic stroke have been performed in chronic stages of the disease, although data from preclinical studies as well as the recent MASTERS trial suggest their early administration could be more effective. In the MASTERS trial,[12] intravenous administration of multipotent adult progenitor cells in patients with acute ischaemic stroke did not provoke any safety concerns or administration issues, and a tendency towards better functional recovery in the early treatment arm (<36 h from symptom onset instead of 36–48 h) versus placebo was observed. This observation was also supported by the fact that the trophic factors released during the acute phase after stroke inhibited the first steps of the ischaemic cascade and enhanced brain protection mechanisms.[3,13,14]

Adipose tissue-derived mesenchymal stem cells (AD-MSCs) are abundant, accessible and easy to obtain by lipoaspiration techniques;[15,16] they also can be administered without ethical concerns.[13] Their intravenous administration has been proven safe in rat models of ischaemic stroke and has also been associated with good functional recovery, reductions in cell death and increased cell proliferation at the peri-infarct zone without observed implantation of the AD-MSCs at the infarct site.[17, 18] To our knowledge, the first clinical trial assessing the safety and efficacy of intravenous administration of allogeneic AD-MSCs in patients with ischaemic stroke is the AMASCIS-01 trial (NCT01678534),[19] which has been performed by our study group. Although the 2-year follow-up results have not yet been published, a safety interim analysis at 6 months showed no safety concerns when the AD-MSCs were administered within the first 2 weeks after symptom onset.[20] Other case reports and clinical trials exploring this cell type in non-ischemic neurological diseases [21] and non-neurological diseases, such as in acute respiratory distress and refractory rheumatoid arthritis, have also shown no safety issues compared with the placebo group.[22, 23] .

In preclinical studies, the MSC dose in ischaemic stroke animal models has been variable, being between 10^4-4.3 x 10^7 MSCs/kg.[7-10] Results appear more beneficial for lower rather than higher cell doses, which could be due to the fact that higher doses could potentially provoke a brain embolus or slow cerebral blood flow.[9] Our study group has used doses of 1-2 x 10^6 cells/kg in preclinical studies without issue. In the MASTERS trial, a single intravenous total dose of 4 or 12 x 10^8 multipotent adult progenitor cells did not cause any safety issues. In the AMASCIS-01 trial performed by our study group, a single dose of 10^6 AD-MSCs/kg was administered intravenously, also without safety concerns.

Therefore, in the AMASCIS-02 trial, we aim to show the safety of early allogeneic AD-MSC intravenous administration (10^6 cells/kg of the patient's weight within the first 4 days from stroke onset) and explore the possible efficacy of this therapy in reducing stroke-associated disability.

MATERIALS AND METHODS

Design

The Allogeneic Adipose Tissue derived Mesenchymal Stem Cells in Ischemic Stroke (AMASCIS-02) study is an academic, randomised, double-blind, placebo-controlled multicentre clinical trial. The two recruiting centres are La Paz University Hospital and Virgen del Rocío University Hospital, both of them in Spain. EudraCT: 2019-001724-35. The study is registered at http://www.clinicaltrials.gov and identified by NCT04280003. Recruitment began on January 2021.

Primary objective

To assess the safety of intravenous administration of allogeneic AD-MSCs within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Secondary objective

To assess potential efficacy of allogeneic AD-MSCs when administered within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Eligibility

Patients will be selected from the Neurology Department of both of the study centres. They will include adults who meet the following inclusion criteria and none of the exclusion criteria:

- **Inclusion criteria**

1. Men and women aged older than 18 years with acute ischaemic stroke.
2. Treatment within 4 days (+/- 1 day) from the onset of stroke symptoms or from the last time observed as asymptomatic.
3. A computed tomography (CT) or magnetic resonance imaging (MRI) scan compatible with the clinical diagnosis of acute nonlacunar ischaemic stroke in the region of the middle cerebral artery.
4. A score on the National Institutes of Health Stroke Scale (NIHSS) of 8–20, with at least 2 of these points in sections 5 and 6 (motor deficit) at the time of inclusion. NIHSS evaluation for screening of these patients will take place after finalisation of reperfusion therapies (if they have been performed) providing that the clinical condition of the patient is stable with no prevision of immediate recovery. A measurable focal neurologic disability must persist to the time of treatment administration.
5. A prestroke score on the modified Rankin Scale (mRS) ≤1 (no significant disability).
6. Women with non-childbearing potential must have either (at least 1 criterion)

   -Undergone a hysterectomy and/or bilateral oophorectomy;

-Have medically confirmed ovarian failure; or

-Achieved postmenopausal status: cessation of regular menses for at least 12 consecutive months with no alternative cause.

7. Women with childbearing potential need a negative pregnancy test and must agree to use adequate contraception during the duration of the study.
8. Signed informed consent.

- **Exclusion criteria**

1. Comatose patients (≥2 score on item 1a of the NIHSS, related to degree of awareness).
2. Evidence on neuroimaging of brain tumour, cerebral oedema with midline shift and a clinically significant compression of ventricles, cerebellar or brainstem infarction and intraventricular, intracerebral or subarachnoid haemorrhage. Small petechial haemorrhages are not exclusion criteria.
3. Current drug or alcohol dependence.
4. Active infectious disease, including human immunodeficiency virus and hepatitis B and C. A controlled infection is not an exclusion criterion.
5. Pre-existing dementia.
6. A health status, clinical condition or other characteristics that preclude appropriate diagnosis, treatment or follow-up in the trial.
7. Inability or unwillingness of the individual or their representative to provide written informed consent.
8. Participation in another clinical trial.

Randomisation

Each patient will have a unique number assigned sequentially as they enter the study. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer when the program was executed. Randomisation will be stratified by age (<65 years or ≥65 years). After verifying the inclusion and exclusion criteria, patients will be assigned the study drug or a placebo solution.

Masking

The study is double-blind. After a patient is randomised by a blinded member of the research team, a nonblinded research member will request the corresponding study medication according to the randomisation code. The study medication and the placebo solution will be indistinguishable, and masking will be made by the nonblinded research team members.

Outcomes

Principal variables. The safety of AD-MSCs will be assessed using the following parameters:

- Adverse events (AEs) reported spontaneously by the patient or in response to questions not addressed, and serious AEs (death, life-threatening events, events that require inpatient hospitalisation or prolongation of existing hospitalisation, events that result in persistent or significant disability, congenital anomalies or any other event that does not meet the definitions above but can be considered potentially serious).
- Neurological and systemic complications (including abnormal laboratory values): deteriorating stroke, stroke recurrences, brain oedema, seizures, haemorrhagic transformation, respiratory infections, urinary tract infections, deep venous thrombosis and pulmonary embolism.

Secondary variables. The efficacy of intravenous treatment with AD-MSCs will be assessed using the following parameters:

- Modified Rankin Scale (mRS): The outcome will be considered positive when the patient obtains a score of 0–3, measured at months 3, 6, 12 and 24. An additional exploratory efficacy analysis will consider mRS shift at months 3, 6, 12 and 24.
- National Institutes of Health Stroke Scale (NIHSS): Measured at all scheduled visits. A successful outcome will occur with an improvement of 75% or more from the baseline score. An additional exploratory efficacy analysis will measure differences in the median (interquartile range [IQR]) distribution and in the frequency of an NIHSS ≤1 between groups.
- Biomarker measurement: Granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor BB (PDGF-BB), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), transforming growth factor 1 (TGF-1), endostatin, glial fibrillary acid protein (GFAP), myelin basic protein (MBP), matrix metalloproteinase 3 (MMP-3) and extracellular vesicles will be measured at baseline, day 7 and month 3 after treatment administration.

Study procedures

After initial screening and randomisation (visit 1) takes place, the study treatment will be administered within the first four days since stroke symptom onset (visit 2). Eight more visits will take place during the 24 month follow-up period (2h, 24h, 7 days or hospital discharge, 3, 6, 12, 18 and 24 months since treatment administration) to assess security and efficacy measures (see table 1 for details of study visits and procedures and figure 1 for schematic flowchart of the clinical trial design).

Table 1. Flowchart of study visits and procedures

FLOWCHART	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
	Screening	Baseline 0 h	2h	24 h	Day 7 or Discharge[4]	M3 (±14 d)	M6 (±14 d)	M12 (±14 d)	M18 (±14 d)	M24 (±14 d)
Informed consent signature	X									
Inclusion and exclusion criteria	X	X [2, 3]								
Past medical/surgical history Physical examination	X									
Pregnancy test	X			X	X	X	X	X	X	X
	X									
NIHSS	X	X [3]	X	X	X	X	X	X	X	X
Modified Rankin Scale	X				X	X	X	X	X	X
Blood pressure	X	X [3]	X	X	X					
Heart rate	X	X [3]	X	X	X					
Body temperature	X	X [3]	X	X	X					
Oxygen saturation	X	X [3]	X	X	X					
Capillary blood glucose	X	X [3]	X	X	X					
12-lead ECG	X	X [3]		X						X
Neuroimaging (brain CT)	X [1]									
Neuroimaging (brain MRI)	X [1]									
Randomisation	X									
Study drug administration		X								
Laboratory assessments: Haematology, coagulation test and biochemistry	X			X	X					X
Blood brain repair markers		X [3]			X	X				
Adverse events register		X [3]	X	X	X	X	X	X	X	X
Concomitant drugs	X	X [3]	X	X	X	X	X	X	X	X

NIHSS: National Institutes of Health Stroke Scale; ECG: electrocardiogram; CT: computed tomography; MRI: magnetic resonance imaging.

[1] Review Neuroimaging (one of them, CT or MRI)

[2] Review selection criteria

[3] Tests, measurements, registry of adverse events and study drug administration and also revision of inclusion and exclusion criteria will take place before study drug administration.

[4] Visit 5 is performed at day 7 after study drug administration or at hospital discharge if it takes place before day 7.

Treatment or intervention

All study medications will be industrially manufactured, tested and released according to current Good Manufacturing Practice Guidelines by Histocell. The experimental drug is an intravenous solution of allogeneic AD-MSCs in a concentration of 10 million cells per millilitre, obtained from a donor by *in vitro* cell culture techniques, conditioned in cryovials of Type I sterile borosilicate glass (with ringer lactate, glucosaline, sodium bicarbonate and human albumin 20% as excipients) and cryopreserved at −150° C. The manufacturing process is detailed in the investigator's brochure, which has been approved by the Spanish Agency of Medicines and Health products. The placebo solution has the same appearance as the study drug and is formed by its excipients. Medication vials containing either solution will be dissolved in 50ml of physiologic saline solution and dispensed as a single intravenous drop administration in approximately 30 minutes, at a dose of 1 million cells per kg of the patient's weight (for the AD-MSCs).

Discontinuation of study medication

The study medication must be discontinued if a suspected anaphylactic reaction or serious adverse event occurs during its administration of if patient participation consent is withdrawn.

Standard treatments for the management of acute stroke

All patients will be managed according to current guidelines for acute stroke management, including treatment with recanalization therapies such as intravenous thrombolysis, mechanical thrombectomy or both.

Data collection and outcome measures

An electronic case report form has been designed using MACRO. This system will maintain patient anonymity, and the data will be transferred to a '*.csv' file to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, data management will be performed by the Spanish Clinical Research Network (SCReN). The data management plan has been

approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size estimates

Given this is a pilot phase II clinical trial focused on the assessment of safety, no systematic sample size calculation applies. Based on the previous clinical trial, AMASCIS-01 (NCT01678534 and EudraCT: 2011-003551-18), it seems feasible to recruit 30 patients between the 2 participating centres.

Statistical analyses

Demographic and clinical characteristics will be summarised in terms of means and standard deviations, median and IQR or relative frequency as appropriate for each variable type. A univariate approach will be employed for calculating nonparametric tests for the continuous variables (Wilcoxon rank sum), and Fisher's or a chi-squared test will be employed for the categorical variables. Given the binary nature of the primary outcome, a logistic regression under the minimum Akaike information criterion using all available variables will be fitted to the data, estimating odds ratios and their confidence intervals. Rules of prediction for the binary outcome will be performed using receiver operating characteristic curves and k-fold cross-validation procedures. Only *P* values less than 0.05 will be considered significant. Statistical computations will be calculated using the statistical computing environment R. The statistical analysis will be performed by the personnel from the Clinical Pharmacology Service and Central Unit of Clinical Investigation and Clinical Trials. The safety analysis will include tables with all AEs and neurological and systemic complications. A descriptive analysis summarising efficacy variables in each group will be performed and an exploratory analysis will be conducted by comparing both treatment groups using the Wilcoxon rank sum test or Student's t-test.

Plans for predefined subgroup analyses:

1) The "intention to treat" population will consist of all patients for whom baseline variables and at least 1 value of a principal or secondary variable are available. If a final visit variable is not obtained for a patient, it will be replaced by the last available value (LOCF=Last Observation Carried Forward).

2) The "per protocol" population will consist of all patients for whom infusion of the study medication had been started with no major protocol violation.

3) The "safety analysis population" will consist of all patients for whom infusion of the study medication had been started.

Data monitoring body

Coordination, management, monitoring, data management and the statistical analysis will be performed by the SCReN.

Auditing

During the progress of the study, audit visits may be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the health authorities.

Patient and Public Involvement

The development of the research objectives and outcomes are based on the neurologist´s experience treating this profile of patients and the desire to optimise brain tissue repair therapies. Patients and their advisors were not involved in the design, recruitment or conduct of this study.

SUMMARY AND CONCLUSIONS

AD-MSCs administered intravenously in the first days from stroke symptom onset could be a safe and promising therapy to enhance recovery from brain ischaemia and to help reduce disability. This type of cell therapy has already demonstrated its efficacy and safety in preclinical research,

but further studies are needed to prove similar results in patients with stroke. The advantages of AD-MSCs over other cell types are their accessibility and abundance, as well as their low immunogenicity. An intravenous infusion appears to be the most appropriate manner of administration, given it is the least aggressive, and cell therapies can be performed by modification of trophic factors and immunomodulation.

This clinical trial aims to demonstrate that early intravenous administration of AD-MSCs has no major safety complications and that it could possibly reduce disability due to stroke. Also, analysis of blood biomarkers and trophic factors will help elucidate and deepen our understanding of the mechanisms of action of cell therapy, along with previous results from other clinical trials.[24,25] The results of the AMASCIS-02 trial, together with those from the AMASCIS-01 trial, will increase our knowledge of the safety of intravenous AD-MSC administration in patients with ischaemic stroke. We will then be able to conduct larger clinical trials to evaluate their efficacy and possibly enable their use in clinical practice in the near future.

DECLARATIONS

Conflicts of Interest

The Funders and Sponsor will not interfere in the selection processes of patients, analysis of data and/or publication of the results, or in any other process that might interfere with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities. The authors declare that they have no conflicts of interest.

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Ethics approval

The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality. The study will follow the principles of Good Clinical Practice. This study (protocol version 2, with date 5thMay 2020) has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003).

All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation.

Consent to participate

Informed consent will be obtained by all individual participants included in this study, or by their family member/representative.

Consent for publication

According to the Royal Decree 1090/2015, the results of this clinical trial will be published once the study is completed.

Availability of data and material

Data will be available from the corresponding author on reasonable request, considered compliant General Data Protection Regulation and the data-sharing agreement is approved by the relevant Spanish authorities. Trial results will be published in Open Access journals.

Authors contributions

The first draft of the manuscript was written by Elena de Celis Ruiz, and all authors commented on previous versions of the manuscript. Blanca Fuentes, Exuperio Díez, Francisco Moniche, Joan Montaner, Alberto Borobia and María Gutiérrez participated in the study conception and design. All authors have read and approved the final manuscript.

Dissemination policy

Output from this study will include open-journal publications, conference presentations and community reporting. Output will not identify participants.

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Short-Term Safety of Allogeneic Adipose-Tissue Derived Mesenchymal Stem Cells in Acute Ischemic Stroke (AMASCIS): A Phase II, Randomised, Double-Blind, Placebo-Controlled, Single-Centre, Pilot Clinical Trial (July 13, 2019). Available at SSRN: https://ssrn.com/abstract=3420416

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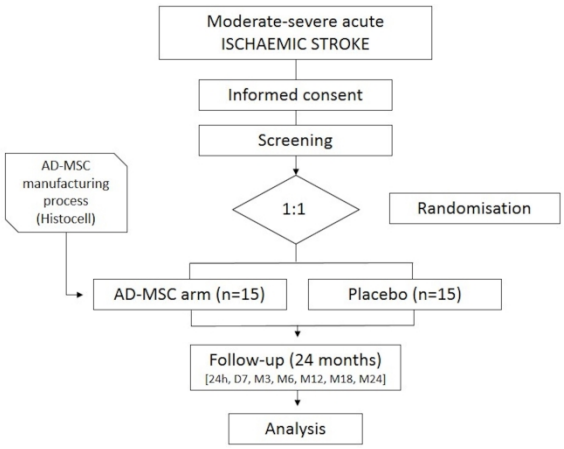

Schematic flowchart of the clinical trial

338x190mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **(Page 1)**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry. **(NCT04280003. EudraCT: 2019-001724-35**)
	2b	All items from the World Health Organization Trial Registration Data Set (not applicable)
Protocol version	3	Date and version identifier **(5thMay 2020, version 2)**
Funding	4	Sources and types of financial, material, and other support **(On page 16)**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **(On page 1 and page 18).**
	5b	Name and contact information for the trial sponsor **(Page 16).**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities **(Page 16).**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **(Page 15).**
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **(Pages 5-7)**
	6b	Explanation for choice of comparators **(NA, placebo-controlled)**

Objectives	7	Specific objectives or hypotheses **(Pages 7-8)**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **(Page 7)**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained). **(Page 7)**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **(Pages 8 and 9)**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **(Page 13)**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **(Page 13)**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **(NA for the study drug, single-doses during hospitalisation; monitoring and audit data on page 15)**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **(Page 13)**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended. **(Pages 7, 8, 10 and 11)**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **(Pages 11-13)**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **(Page 14)**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **(Page 8)**
Methods: Assignment of interventions (for controlled trials)		

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **(Page 10)**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **(Page 10)**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **(Pages 8 and 10)**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **(Page 10)**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial **(specified in original protocol)**
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **(Page 10-11)**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols **(page 15)**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **(Page 15)**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **(Page 14 and 15)**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **(Page 15)**

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **(Page 15)**
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **(Page 15)**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial **(NA)**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **(Page 10)**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **(Page 15)**
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **(Page 17)**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **(specified in original protocol)**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **(Page 17)**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **(Not applicable)**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **(Pages 13 and 15)**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **(Page 16)**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **(Pages 15 and 18)**

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **(insurance provision, specified in original protocol)**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **(Page 18)**
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **(Page 18)**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **(specified in original protocol)**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **(specified in original protocol)**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol

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Primary Subject Heading:	Neurology
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Keywords:	Stroke < NEUROLOGY, Clinical trials < THERAPEUTICS, Adverse events < THERAPEUTICS

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Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol

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Word count: Abstract 241 words, text body without references, abbreviations and author affiliations 3880 words.

List of tables and figures:

- Table 1. Methods flowchart
- Figure 1. Schematic flowchart of the clinical trial

List of abbreviations:

- AD-MSCs: allogeneic adipose tissue-derived mesenchymal stem cells
- MSCs: mesenchymal stem cells
- CT: computed tomography
- MRI: magnetic resonance imaging
- NIHSS: National Institutes of Health Stroke Scale
- mRS: modified Rankin Scale
- AEs: Adverse events
- GM-CSF: Granulocyte-macrophage colony-stimulating factor
- PDGF-BB: platelet-derived growth factor BB
- BDNF: brain-derived neurotrophic factor
- VEGF: vascular endothelial growth factor
- TGF-1: transforming growth factor 1
- GFAP: endostatin, glial fibrillary acid protein
- MBP: myelin basic protein
- MMP-3: matrix metalloproteinase 3
- ECG: electrocardiogram
- SCReN: Spanish Clinical Research Network

1

- IQR: interquartile range

ABSTRACT

Introduction: Stroke is a serious public health problem, given it is a major cause of disability worldwide despite the spread of recanalization therapies. Enhancement of brain plasticity with stem cell administration is a promising innovative therapy to reduce sequelae in these patients.

Methods and analysis: We have developed a phase IIb, multicentre, randomised, double-blind, placebo-controlled clinical trial protocol to evaluate the safety and efficacy of intravenous administration of allogeneic adipose tissue-derived mesenchymal stem cells (AD-MSCs) in patients with acute ischaemic stroke, concurrently with conventional stroke treatment. Thirty patients will be randomised on a 1:1 basis to receive either intravenous placebo or allogeneic AD-MSCs as soon as possible within the first 4 days from stroke symptom onset. Patients will be followed up to 24 months after randomisation. The primary objective is the safety assessment of early intravenous administration of allogeneic AD-MSCs by reporting all adverse events and neurological or systemic complications in both treatment groups. Secondary objectives assess efficacy of early intravenous AD-MSC treatment in acute ischaemic stroke by evaluating changes in the modified Rankin Scale and the National Institutes of Health Stroke Scale throughout the follow-up period. In addition, brain repair biomarkers will be measured at various visits.

Ethics and dissemination: This clinical trial has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003). Study results will be disseminated through peer-reviewed publications in Open Access format and at conference presentations.

Strengths and limitations of this study

- First multicentre clinical trial evaluating safety and possible efficacy of early intravenous treatment with adipose derived mesenchymal stem cells in ischemic stroke.
- The trial is designed as a randomised, placebo-controlled, double-blind study so biases will be minimised, albeit limiting general population applicability.
- Limitations for external validity are the fact that patients are recruited in a single country from only 2 centres and the small sample size.

KEY WORDS: *Acute ischaemic stroke, Allogeneic adipose tissue-derived mesenchymal stem cells, Clinical trial, Stem cell therapy, safety.*

INTRODUCTION

Stroke is a serious health problem, given it is one of the most common causes of mortality and the leading cause of disability due to neurologic diseases worldwide. In 2016, the global estimated incidence of stroke ranged between 189 and 218 cases per 100,000 inhabitants.[1] A large proportion of patients with ischaemic stroke have persistent neurological deficits that impair their daily life activities, and many also experience non-neurological complications, such as pulmonary and urinary tract infections, deep vein thrombosis and depression. In the last 2 decades, there has been a breakthrough in ischaemic stroke treatments. However, currently available therapies, such as intravenous thrombolysis and mechanical thrombectomy, focus only on reperfusion in the acute phase and are not accessible to all patients due to a narrow time window and eligibility issues. Furthermore, these cerebral reperfusion-based therapies cannot reverse established ischaemic injury or reduce its sequelae. Therefore, to reduce the burden of this disease, there is an urgent need for therapies that can enhance patient recovery after stroke.

After a brain injury, several interrelated mechanisms, such as neurogenesis, gliogenesis, oligodendrogenesis, synaptogenesis and angiogenesis take place to repair the damaged tissue. In clinical practice, these processes are stimulated through rehabilitation therapies; however, cell therapies promote tissue repair exogenously,[2] which could complement current reperfusion treatments for acute ischaemic stroke.

Stem cells are immature cells with the capacity to differentiate into diverse cell lines. At present, challenges concerning the most suitable cell type, route and time of administration are the main issues for progression from preclinical studies to the design of clinical trials.[3-5]

Regarding the route of administration, preclinical studies have shown that it is not mandatory for stem cells to migrate and nest at the injury site to achieve functional recovery after stroke, given their therapeutic effect is also due to secretion of trophic factors and modulation of the immune response.[5,6] Intravenous administration has been proven to be noninferior to other routes in preclinical studies, such as in intracerebral, intrathecal or intra-arterial deliveries, and it

is far less invasive. Regarding cell type, preclinical and initial clinical trial data [5,7,8,9,10,11] suggest that mesenchymal stem cells (MSCs) constitute a safe and possibly effective therapy in ischaemic stroke. Allogeneic MSCs lack human leucocyte antigen-class II molecules, avoiding the risk of rejection and allowing short timings of administration.

Most studies concerning cell therapies in ischaemic stroke have been performed in chronic stages of the disease, although data from preclinical studies as well as the recent MASTERS trial suggest their early administration could be more effective. In the MASTERS trial,[12] intravenous administration of multipotent adult progenitor cells in patients with acute ischaemic stroke did not provoke any safety concerns or administration issues, and a tendency towards better functional recovery in the early treatment arm (<36 h from symptom onset instead of 36–48 h) versus placebo was observed. This observation was also supported by the fact that the trophic factors released during the acute phase after stroke inhibited the first steps of the ischaemic cascade and enhanced brain protection mechanisms.[3,13,14]

Adipose tissue-derived mesenchymal stem cells (AD-MSCs) are abundant, accessible and easy to obtain by lipoaspiration techniques;[15,16] they also can be administered without ethical concerns.[13] Their intravenous administration has been proven safe in rat models of ischaemic stroke and has also been associated with good functional recovery, reductions in cell death and increased cell proliferation at the peri-infarct zone without observed implantation of the AD-MSCs at the infarct site.[17, 18] To our knowledge, the first clinical trial assessing the safety and efficacy of intravenous administration of allogeneic AD-MSCs in patients with ischaemic stroke is the AMASCIS-01 trial (NCT01678534),[19] which has been performed by our study group. Although the 2-year follow-up results have not yet been published, a safety interim analysis at 6 months showed no safety concerns when the AD-MSCs were administered within the first 2 weeks after symptom onset.[20] Other case reports and clinical trials exploring this cell type in non-ischemic neurological diseases [21] and non-neurological diseases, such as in acute respiratory distress and refractory rheumatoid arthritis, have also shown no safety issues compared with the placebo group.[22, 23] .

In preclinical studies, the MSC dose in ischaemic stroke animal models has been variable, being between 10^4-4.3 x 10^7 MSCs/kg.[7-10] Results appear more beneficial for lower rather than higher cell doses, which could be due to the fact that higher doses could potentially provoke a brain embolus or slow cerebral blood flow.[9] Our study group has used doses of 1-2 x 10^6 cells/kg in preclinical studies without issue. In the MASTERS trial, a single intravenous total dose of 4 or 12 x 10^8 multipotent adult progenitor cells did not cause any safety issues. In the AMASCIS-01 trial performed by our study group, a single dose of 10^6 AD-MSCs/kg was administered intravenously, also without safety concerns.

Therefore, in the AMASCIS-02 trial, we aim to show the safety of early allogeneic AD-MSC intravenous administration (10^6 cells/kg of the patient's weight within the first 4 days from stroke onset) and explore the possible efficacy of this therapy in reducing stroke-associated disability.

MATERIALS AND METHODS

Design

The Allogeneic Adipose Tissue derived Mesenchymal Stem Cells in Ischemic Stroke (AMASCIS-02) study is an academic, randomised, double-blind, placebo-controlled multicentre clinical trial. The two recruiting centres are La Paz University Hospital and Virgen del Rocío University Hospital, both of them in Spain. EudraCT: 2019-001724-35. The study is registered at http://www.clinicaltrials.gov and identified by NCT04280003.

Recruitment began on January 2021, and the estimated end date is July 2023.

Primary objective

To assess the safety of intravenous administration of allogeneic AD-MSCs within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Secondary objective

To assess potential efficacy of allogeneic AD-MSCs when administered within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Eligibility

Patients will be selected from the Neurology Department of both of the study centres. Acute stroke management of all patients will follow standard of care. Reperfusion therapies such as intravenous fibrinolysis and mechanical thrombectomy are allowed prior to patient screening and randomisation as long as all study inclusion and no exclusion criteria are met after their application. Inclusion and exclusion criteria are as follows:

- **Inclusion criteria**

1. Men and women aged older than 18 years with acute ischaemic stroke.
2. Treatment within 4 days (+/- 1 day) from the onset of stroke symptoms or from the last time observed as asymptomatic.
3. A computed tomography (CT) or magnetic resonance imaging (MRI) scan compatible with the clinical diagnosis of acute nonlacunar ischaemic stroke in the region of the middle cerebral artery.
4. A score on the National Institutes of Health Stroke Scale (NIHSS) of 8–20, with at least 2 of these points in sections 5 and 6 (motor deficit) at the time of inclusion. NIHSS evaluation for screening of these patients will take place after finalization of reperfusion therapies (if they have been performed) providing that the clinical condition of the patient is stable with no prevision of immediate recovery. A measurable focal neurologic disability must persist to the time of treatment administration.

5. A prestroke score on the modified Rankin Scale (mRS) ≤1 (no significant disability).
6. Women with non-childbearing potential must have either (at least 1 criterion)

   -Undergone a hysterectomy and/or bilateral oophorectomy;

   -Have medically confirmed ovarian failure; or

   -Achieved postmenopausal status: cessation of regular menses for at least 12 consecutive months with no alternative cause.
7. Women with childbearing potential need a negative pregnancy test and must agree to use adequate contraception during the duration of the study.
8. Signed informed consent.

- **Exclusion criteria**

1. Comatose patients (≥2 score on item 1a of the NIHSS, related to degree of awareness).
2. Evidence on neuroimaging of brain tumour, cerebral oedema with midline shift and a clinically significant compression of ventricles, cerebellar or brainstem infarction and intraventricular, intracerebral or subarachnoid haemorrhage. Small petechial haemorrhages are not exclusion criteria.
3. Current drug or alcohol dependence.
4. Active infectious disease, including human immunodeficiency virus and hepatitis B and C. A controlled infection is not an exclusion criterion.
5. Pre-existing dementia.
6. A health status, clinical condition or other characteristics that preclude appropriate diagnosis, treatment or follow-up in the trial.

7. Inability or unwillingness of the individual or their representative to provide written informed consent.
8. Participation in another clinical trial.

Randomisation

Each patient will have a unique number assigned sequentially as they enter the study. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer when the program was executed. Randomisation will be stratified by age (<65 years or ≥65 years). After verifying the inclusion and exclusion criteria, patients will be assigned the study drug or a placebo solution.

Masking

The study is double-blind. After a patient is randomised by a blinded member of the research team, a nonblinded research member will request the corresponding study medication according to the randomisation code. The study medication and the placebo solution will be indistinguishable, and masking will be made by the nonblinded research team members.

Outcomes

Principal variables. The safety of AD-MSCs will be assessed using the following parameters:

- Adverse events (AEs) reported spontaneously by the patient or in response to questions not addressed, and serious AEs (death, life-threatening events, events that require inpatient hospitalisation or prolongation of existing hospitalisation, events that result in persistent or significant disability, congenital anomalies or any other event that does not meet the definitions above but can be considered potentially serious).

- Neurological and systemic complications (including abnormal laboratory values): deteriorating stroke, stroke recurrences, brain oedema, seizures, haemorrhagic transformation, respiratory infections, urinary tract infections, deep venous thrombosis and pulmonary embolism.

Secondary variables. The efficacy of intravenous treatment with AD-MSCs will be assessed using the following parameters:

- Modified Rankin Scale (mRS): The outcome will be considered positive when the patient obtains a score of 0–3, measured at months 3, 6, 12 and 24. An additional exploratory efficacy analysis will consider mRS shift at months 3, 6, 12 and 24.
- National Institutes of Health Stroke Scale (NIHSS): Measured at all scheduled visits. A successful outcome will occur with an improvement of 75% or more from the baseline score. An additional exploratory efficacy analysis will measure differences in the median (interquartile range [IQR]) distribution and in the frequency of an NIHSS ≤1 between groups.
- Biomarker measurement: Granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor BB (PDGF-BB), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), transforming growth factor 1 (TGF-1), endostatin, glial fibrillary acid protein (GFAP), myelin basic protein (MBP), matrix metalloproteinase 3 (MMP-3) and extracellular vesicles will be measured at baseline, day 7 and month 3 after treatment administration.

Study procedures

After initial screening and randomisation (visit 1) takes place, the study treatment will be administered within the first four days since stroke symptom onset (visit 2). Eight more visits will take place during the 24 month follow-up period (2h, 24h, 7 days or hospital discharge, 3, 6, 12, 18 and 24 months since treatment administration) to assess security and efficacy measures

(see table 1 for details of study visits and procedures and figure 1 for schematic flowchart of the clinical trial design).

Table 1. Flowchart of study visits and procedures

FLOWCHART	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
	Screening	Baseline 0 h	2h	24 h	Day 7 or Discharge[4]	M3 (±14 d)	M6 (±14 d)	M12 (±14 d)	M18 (±14 d)	M24 (±14 d)
Informed consent signature	X									
Inclusion and exclusion criteria	X	X [2, 3]								
Past medical/surgical history Physical	X									
examination	X									
Pregnancy test	X			X	X	X	X	X	X	X
NIHSS	X	X [3]	X	X	X	X	X	X	X	X
Modified Rankin Scale	X				X	X	X	X	X	X
Blood pressure	X	X [3]	X	X	X					
Heart rate	X	X [3]	X	X	X					
Body temperature	X	X [3]	X	X	X					
Oxygen saturation	X	X [3]	X	X	X					
Capillary blood glucose	X	X [3]	X	X	X					
12-lead ECG	X	X [3]		X						X
Neuroimaging (brain CT)	X [1]									
Neuroimaging (brain MRI)	X [1]									
Randomisation	X									
Study drug administration		X								
Laboratory assessments: Haematology, coagulation test and biochemistry	X			X	X					X
Blood brain repair markers		X [3]			X	X				
Adverse events register		X [3]	X	X	X	X	X	X	X	X
Concomitant drugs	X	X [3]	X	X	X	X	X	X	X	X

NIHSS: National Institutes of Health Stroke Scale; ECG: electrocardiogram; CT: computed tomography; MRI: magnetic resonance imaging.

[1] Review Neuroimaging (one of them, CT or MRI); [2] Review selection criteria; [3] Tests, measurements, register of adverse events and study drug administration and also revision of inclusion and exclusion criteria will take place before study drug administration. **[4]** Visit 5 is performed at day 7 after study drug administration or at hospital discharge if it takes place before day 7.

Treatment or intervention

All study medications will be industrially manufactured, tested and released according to current Good Manufacturing Practice Guidelines by Histocell. The experimental drug is an intravenous solution of allogeneic AD-MSCs in a concentration of 10 million cells per millilitre, obtained from a donor by *in vitro* cell culture techniques, conditioned in cryovials of Type I sterile borosilicate glass (with ringer lactate, glucosaline, sodium bicarbonate and human albumin 20% as excipients) and cryopreserved at −150° C. The manufacturing process is detailed in the investigator's brochure, which has been approved by the Spanish Agency of Medicines and Health products. The placebo solution has the same appearance as the study drug and is formed by its excipients. Medication vials containing either solution will be dissolved in 50ml of physiologic saline solution and dispensed as a single intravenous drop administration in approximately 30 minutes, at a dose of 1 million cells per kg of the patient's weight (for the AD-MSCs).

Discontinuation of study medication

The study medication must be discontinued if a suspected anaphylactic reaction or serious adverse event occurs during its administration of if patient participation consent is withdrawn.

Standard treatments for the management of acute stroke

All patients will be managed according to current guidelines for acute stroke management, including treatment with recanalization therapies such as intravenous thrombolysis, mechanical thrombectomy or both.

Data collection and outcome measures

An electronic case report form has been designed using MACRO. This system will maintain patient anonymity, and the data will be transferred to a '*.csv' file to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, data management will be performed by the Spanish Clinical Research Network (SCReN). The data management plan has been

approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size estimates

Given this is a pilot phase II clinical trial focused on the assessment of safety, no systematic sample size calculation applies. Based on the previous clinical trial, AMASCIS-01 (NCT01678534 and EudraCT: 2011-003551-18), it seems feasible to recruit 30 patients between the 2 participating centres.

Statistical analyses

Demographic and clinical characteristics will be summarised in terms of means and standard deviations, median and IQR or relative frequency as appropriate for each variable type. A univariate approach will be employed for calculating nonparametric tests for the continuous variables (Wilcoxon rank sum), and Fisher's or a chi-squared test will be employed for the categorical variables. Given the binary nature of the primary outcome, a logistic regression under the minimum Akaike information criterion using all available variables will be fitted to the data, estimating odds ratios and their confidence intervals. Rules of prediction for the binary outcome will be performed using receiver operating characteristic curves and k-fold cross-validation procedures. Only *P* values less than 0.05 will be considered significant. Statistical computations will be calculated using the statistical computing environment R. The statistical analysis will be performed by the personnel from the Clinical Pharmacology Service and Central Unit of Clinical Investigation and Clinical Trials. The safety analysis will include tables with all AEs and neurological and systemic complications. A descriptive analysis summarising efficacy variables in each group will be performed and an exploratory analysis will be conducted by comparing both treatment groups using the Wilcoxon rank sum test or Student's t-test.

Plans for predefined subgroup analyses:

1) The "intention to treat" population will consist of all patients for whom baseline variables and at least 1 value of a principal or secondary variable are available. If a final visit variable is not obtained for a patient, it will be replaced by the last available value (LOCF=Last Observation Carried Forward).

2) The "per protocol" population will consist of all patients for whom infusion of the study medication had been started with no major protocol violation.

3) The "safety analysis population" will consist of all patients for whom infusion of the study medication had been started.

Data monitoring body

Coordination, management, monitoring, data management and the statistical analysis will be performed by the SCReN.

Auditing

During the progress of the study, audit visits may be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the health authorities.

Patient and Public Involvement

The development of the research objectives and outcomes are based on the neurologist´s experience treating this profile of patients and the desire to optimise brain tissue repair therapies. Patients and their advisors were not involved in the design, recruitment or conduct of this study.

SUMMARY

AD-MSCs administered intravenously in the first days from stroke symptom onset could be a safe and promising therapy to enhance recovery from brain ischaemia and to help reduce

disability. This type of cell therapy has already demonstrated its efficacy and safety in preclinical research, but further studies are needed to prove similar results in patients with stroke. The advantages of AD-MSCs over other cell types are their accessibility and abundance, as well as their low immunogenicity. An intravenous infusion appears to be the most appropriate manner of administration, given it is the least aggressive, and cell therapies can be performed by modification of trophic factors and immunomodulation.

This clinical trial aims to demonstrate that early intravenous administration of AD-MSCs has no major safety complications and that it could possibly reduce disability due to stroke. Also, analysis of blood biomarkers and trophic factors will help elucidate and deepen our understanding of the mechanisms of action of cell therapy, along with previous results from other clinical trials.[24,25] The results of the AMASCIS-02 trial, together with those from the AMASCIS-01 trial, will increase our knowledge of the safety of intravenous AD-MSC administration in patients with ischaemic stroke. We will then be able to conduct larger clinical trials to evaluate their efficacy and possibly enable their use in clinical practice in the near future.

DECLARATIONS

Competing Interests

There are no competing interests for any author.

Funding

This clinical trial has been promoted by the La Paz University Hospital Institute for Health Research – IdiPAZ (La Paz University Hospital – Autonomous University of Madrid) and sponsored from a competitive grant from the Carlos III Health Institute Health Care Research Fund, and co-funded by the European Regional Development Fund (ERDF) “A way to make Europe”/”Investing in your future” (PIC18/00016). This clinical trial has been supported by Plataforma Española de Investigación Clínica y Ensayos Clínicos, SCReN (Spanish Clinical

Research Network), funded by Carlos III Health Institute-General Subdirection for Evaluation and Promotion of Research, State Plan for Scientific Investigation, Technology and Innovation (2017–2020) and co-funded by European Regional Development Fund/European Social fund "A way to make Europe"/"Investing in your future" (grant ID PT17/0017/0013). The Funders and Sponsor will not interfere in the selection processes of patients, analysis of data and/or publication of the results, or in any other process that might interfere with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities.

Ethics and dissemination

Research ethics approval: The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality. The study will follow the principles of Good Clinical Practice. This study (ID 5528, protocol version 2, dated 5 May 2020) has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003).

Protocol amendments: All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation. All modifications of the study protocol will be communicated by updating the trial registry at ClinicalTrials.gov (NCT04280003).

Consent to participate: Informed consent will be obtained by a recruiting investigator for all individual participants included in this study, or by their family member/representative (if the patient has aphasia or altered consciousness at that moment). Re-consent from the patient will be obtained whenever possible if initial consent was provided by family

members/representatives. The informed consent document is written in Spanish. The informed consent documents are available as supplementary files.

Consent for publication: According to the Royal Decree 1090/2015, the results of this clinical trial will be published once the study is completed in an Open Access format.

Confidentiality: All participant information as well as the electronic database will remain in secure storage at both study centres during the trial and up to 25 years after its finalisation.

Access to data: All investigators and each centres' monitor will have access to the electronic trial data during the data collection period; after completion of the study, the data will also be accessible to statisticians. Only the blinded study nurses and Histocell will have access to randomisation codes until the data collection period is complete.

Availability of data and material: Data will be available from the corresponding author on reasonable request, if it is considered compliant with the General Data Protection Regulation and the data-sharing agreement is approved by the relevant Spanish authorities. Upon study finalisation, anonymised individual patient data will be available on a public repository of the Community of Madrid.

Ancillary and post-trial care: Patients will be treated during and after the clinical trial is completed with the best medical treatments according to the state of the art. Participants will not receive economic benefits beyond compensation from the study insurance company in cases of study treatment-related adverse events (Hannover seguros España).

Dissemination policy: Output from this study will include journal publications, conference presentations and community reporting. Output will not identify participants.

Authors´ contributions

The first draft of the manuscript was written by Elena de Celis-Ruiz, and all authors commented on previous versions of the manuscript. Blanca Fuentes, Exuperio Díez-Tejedor, Francisco

Moniche, Joan Montaner, Alberto Borobia and María Gutiérrez-Fernández participated in the study conception and design. All authors have read and approved the final manuscript.

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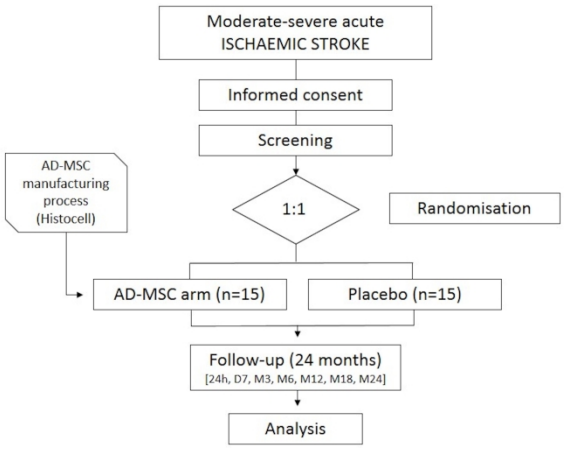

Figure 1. Schematic flowchart of the clinical trial

338x190mm (600 x 600 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

Author comment: Items not mentioned in the manuscript sent for revision are not applicable or otherwise specified in the original study protocol

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **(Page 1)**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry. **(NCT04280003. EudraCT: 2019-001724-35**)
	2b	All items from the World Health Organization Trial Registration Data Set (not applicable)
Protocol version	3	Date and version identifier **(5thMay 2020, version 2)**
Funding	4	Sources and types of financial, material, and other support **(On pages 16-17)**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **(On page 1 and pages 18-19).**
	5b	Name and contact information for the trial sponsor **(Page 16).**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **(Pages 5-7)**

	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses **(Pages 7-8)**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **(Page 7)**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained). **(Page 7)**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **(Pages 8 - 10)**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **(Page 13)**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **(Page 13)**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **(Pages 8 and 13)**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended. **(Pages 10 and 11)**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **(Pages 11-13)**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **(Page 14)**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **(Page 8)**
Methods: Assignment of interventions (for controlled trials)		

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **(Page 10)**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **(Page 10)**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **(Pages 8 and 10)**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **(Page 10)**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **(Page 10-11)**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **(Page 15)**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **(Page 14 and 15)**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **(Page 15)**

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **(Page 15)**
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **(Page 15)**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **(Page 10)**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **(Page 15)**
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **(Page 17)**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **(Page 17)**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **(Page 17)**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **(included in the informed consent form as supplementary material).**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **(Page 18)**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **(Page 16)**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **(Page 18)**

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **(Page 18)**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **(Page 18)**
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **(Page 18)**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **(provided as supplementary data)**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **(included in the informed consent form).**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Stroke < NEUROLOGY, Clinical trials < THERAPEUTICS, Adverse events < THERAPEUTICS

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Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol

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List of tables and figures:

- Table 1. Methods flowchart
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List of abbreviations:

- AD-MSCs: allogeneic adipose tissue-derived mesenchymal stem cells
- MSCs: mesenchymal stem cells
- CT: computed tomography
- MRI: magnetic resonance imaging
- NIHSS: National Institutes of Health Stroke Scale
- mRS: modified Rankin Scale
- AEs: Adverse events
- GM-CSF: Granulocyte-macrophage colony-stimulating factor
- PDGF-BB: platelet-derived growth factor BB
- BDNF: brain-derived neurotrophic factor
- VEGF: vascular endothelial growth factor
- TGF-1: transforming growth factor 1
- GFAP: endostatin, glial fibrillary acid protein
- MBP: myelin basic protein
- MMP-3: matrix metalloproteinase 3
- ECG: electrocardiogram
- SCReN: Spanish Clinical Research Network

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- IQR: interquartile range

ABSTRACT

Introduction: Stroke is a serious public health problem, given it is a major cause of disability worldwide despite the spread of recanalization therapies. Enhancement of brain plasticity with stem cell administration is a promising innovative therapy to reduce sequelae in these patients.

Methods and analysis: We have developed a phase IIb, multicentre, randomised, double-blind, placebo-controlled clinical trial protocol to evaluate the safety and efficacy of intravenous administration of allogeneic adipose tissue-derived mesenchymal stem cells (AD-MSCs) in patients with acute ischaemic stroke, concurrently with conventional stroke treatment. Thirty patients will be randomised on a 1:1 basis to receive either intravenous placebo or allogeneic AD-MSCs as soon as possible within the first 4 days from stroke symptom onset. Patients will be followed up to 24 months after randomisation. The primary objective is the safety assessment of early intravenous administration of allogeneic AD-MSCs by reporting all adverse events and neurological or systemic complications in both treatment groups. Secondary objectives assess efficacy of early intravenous AD-MSC treatment in acute ischaemic stroke by evaluating changes in the modified Rankin Scale and the National Institutes of Health Stroke Scale throughout the follow-up period. In addition, brain repair biomarkers will be measured at various visits.

Ethics and dissemination: This clinical trial has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003). Study results will be disseminated through peer-reviewed publications in Open Access format and at conference presentations.

Strengths and limitations of this study

- First multicentre clinical trial evaluating safety and possible efficacy of early intravenous treatment with adipose derived mesenchymal stem cells in ischemic stroke.
- The trial is designed as a randomised, placebo-controlled, double-blind study so biases will be minimised, albeit limiting general population applicability.
- Limitations for external validity are the fact that patients are recruited in a single country from only 2 centres and the small sample size.

KEY WORDS: *Acute ischaemic stroke, Allogeneic adipose tissue-derived mesenchymal stem cells, Clinical trial, Stem cell therapy, safety.*

INTRODUCTION

Stroke is a serious health problem, given it is one of the most common causes of mortality and the leading cause of disability due to neurologic diseases worldwide. In 2016, the global estimated incidence of stroke ranged between 189 and 218 cases per 100,000 inhabitants.[1] A large proportion of patients with ischaemic stroke have persistent neurological deficits that impair their daily life activities, and many also experience non-neurological complications, such as pulmonary and urinary tract infections, deep vein thrombosis and depression. In the last 2 decades, there has been a breakthrough in ischaemic stroke treatments. However, currently available therapies, such as intravenous thrombolysis and mechanical thrombectomy, focus only on reperfusion in the acute phase and are not accessible to all patients due to a narrow time window and eligibility issues. Furthermore, these cerebral reperfusion-based therapies cannot reverse established ischaemic injury or reduce its sequelae. Therefore, to reduce the burden of this disease, there is an urgent need for therapies that can enhance patient recovery after stroke.

After a brain injury, several interrelated mechanisms, such as neurogenesis, gliogenesis, oligodendrogenesis, synaptogenesis and angiogenesis take place to repair the damaged tissue. In clinical practice, these processes are stimulated through rehabilitation therapies; however, cell therapies promote tissue repair exogenously,[2] which could complement current reperfusion treatments for acute ischaemic stroke.

Stem cells are immature cells with the capacity to differentiate into diverse cell lines. At present, challenges concerning the most suitable cell type, route and time of administration are the main issues for progression from preclinical studies to the design of clinical trials.[3-5]

Regarding the route of administration, preclinical studies have shown that it is not mandatory for stem cells to migrate and nest at the injury site to achieve functional recovery after stroke, given their therapeutic effect is also due to secretion of trophic factors and modulation of the immune response.[5,6] Intravenous administration has been proven to be noninferior to other routes in preclinical studies, such as in intracerebral, intrathecal or intra-arterial deliveries, and it

is far less invasive. Regarding cell type, preclinical and initial clinical trial data [5,7,8,9,10,11] suggest that mesenchymal stem cells (MSCs) constitute a safe and possibly effective therapy in ischaemic stroke. Allogeneic MSCs lack human leucocyte antigen-class II molecules, avoiding the risk of rejection and allowing short timings of administration.

Most studies concerning cell therapies in ischaemic stroke have been performed in chronic stages of the disease, although data from preclinical studies as well as the recent MASTERS trial suggest their early administration could be more effective. In the MASTERS trial,[12] intravenous administration of multipotent adult progenitor cells in patients with acute ischaemic stroke did not provoke any safety concerns or administration issues, and a tendency towards better functional recovery in the early treatment arm (<36 h from symptom onset instead of 36–48 h) versus placebo was observed. This observation was also supported by the fact that the trophic factors released during the acute phase after stroke inhibited the first steps of the ischaemic cascade and enhanced brain protection mechanisms.[3,13,14]

Adipose tissue-derived mesenchymal stem cells (AD-MSCs) are abundant, accessible and easy to obtain by lipoaspiration techniques;[15,16] they also can be administered without ethical concerns.[13] Their intravenous administration has been proven safe in rat models of ischaemic stroke and has also been associated with good functional recovery, reductions in cell death and increased cell proliferation at the peri-infarct zone without observed implantation of the AD-MSCs at the infarct site.[17, 18] To our knowledge, the first clinical trial assessing the safety and efficacy of intravenous administration of allogeneic AD-MSCs in patients with ischaemic stroke is the AMASCIS-01 trial (NCT01678534),[19] which has been performed by our study group. Although the 2-year follow-up results have not yet been published, a safety interim analysis at 6 months showed no safety concerns when the AD-MSCs were administered within the first 2 weeks after symptom onset.[20] Other case reports and clinical trials exploring this cell type in non-ischemic neurological diseases [21] and non-neurological diseases, such as in acute respiratory distress and refractory rheumatoid arthritis, have also shown no safety issues compared with the placebo group.[22, 23] .

In preclinical studies, the MSC dose in ischaemic stroke animal models has been variable, being between 10^4-4.3 x 10^7 MSCs/kg.[7-10] Results appear more beneficial for lower rather than higher cell doses, which could be due to the fact that higher doses could potentially provoke a brain embolus or slow cerebral blood flow.[9] Our study group has used doses of 1-2 x 10^6 cells/kg in preclinical studies without issue. In the MASTERS trial, a single intravenous total dose of 4 or 12 x 10^8 multipotent adult progenitor cells did not cause any safety issues. In the AMASCIS-01 trial performed by our study group, a single dose of 10^6 AD-MSCs/kg was administered intravenously, also without safety concerns.

Therefore, in the AMASCIS-02 trial, we aim to show the safety of early allogeneic AD-MSC intravenous administration (10^6 cells/kg of the patient's weight within the first 4 days from stroke onset) and explore the possible efficacy of this therapy in reducing stroke-associated disability.

METHODS AND ANALYSIS

Design

The Allogeneic Adipose Tissue derived Mesenchymal Stem Cells in Ischemic Stroke (AMASCIS-02) study is an academic, randomised, double-blind, placebo-controlled multicentre clinical trial. The two recruiting centres are La Paz University Hospital and Virgen del Rocío University Hospital, both of them in Spain. EudraCT: 2019-001724-35. The study is registered at http://www.clinicaltrials.gov and identified by NCT04280003.

Recruitment began on January 2021, and the estimated end date is July 2023.

Primary objective

To assess the safety of intravenous administration of allogeneic AD-MSCs within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Secondary objective

To assess potential efficacy of allogeneic AD-MSCs when administered within the first 4 days from stroke onset in patients with acute ischaemic stroke.

Eligibility

Patients will be selected from the Neurology Department of both of the study centres. Acute stroke management of all patients will follow standard of care. Reperfusion therapies such as intravenous fibrinolysis and mechanical thrombectomy are allowed prior to patient screening and randomisation as long as all study inclusion and no exclusion criteria are met after their application. Inclusion and exclusion criteria are as follows:

- **Inclusion criteria**

1. Men and women aged older than 18 years with acute ischaemic stroke.
2. Treatment within 4 days (+/- 1 day) from the onset of stroke symptoms or from the last time observed as asymptomatic.
3. A computed tomography (CT) or magnetic resonance imaging (MRI) scan compatible with the clinical diagnosis of acute nonlacunar ischaemic stroke in the region of the middle cerebral artery.
4. A score on the National Institutes of Health Stroke Scale (NIHSS) of 8–20, with at least 2 of these points in sections 5 and 6 (motor deficit) at the time of inclusion. NIHSS evaluation for screening of these patients will take place after finalization of reperfusion therapies (if they have been performed) providing that the clinical condition of the patient is stable with no prevision of immediate recovery. A measurable focal neurologic disability must persist to the time of treatment administration.

5. A prestroke score on the modified Rankin Scale (mRS) ≤1 (no significant disability).
6. Women with non-childbearing potential must have either (at least 1 criterion)

   -Undergone a hysterectomy and/or bilateral oophorectomy;

   -Have medically confirmed ovarian failure; or

   -Achieved postmenopausal status: cessation of regular menses for at least 12 consecutive months with no alternative cause.
7. Women with childbearing potential need a negative pregnancy test and must agree to use adequate contraception during the duration of the study.
8. Signed informed consent.

- **Exclusion criteria**

1. Comatose patients (≥2 score on item 1a of the NIHSS, related to degree of awareness).
2. Evidence on neuroimaging of brain tumour, cerebral oedema with midline shift and a clinically significant compression of ventricles, cerebellar or brainstem infarction and intraventricular, intracerebral or subarachnoid haemorrhage. Small petechial haemorrhages are not exclusion criteria.
3. Current drug or alcohol dependence.
4. Active infectious disease, including human immunodeficiency virus and hepatitis B and C. A controlled infection is not an exclusion criterion.
5. Pre-existing dementia.
6. A health status, clinical condition or other characteristics that preclude appropriate diagnosis, treatment or follow-up in the trial.

7. Inability or unwillingness of the individual or their representative to provide written informed consent.
8. Participation in another clinical trial.

Randomisation

Each patient will have a unique number assigned sequentially as they enter the study. The randomisation sequence was created using SAS version 9.4 statistical software (procedure 'PROC PLAN') with a 1:1 allocation. No randomisation seed was specified. The randomisation seed was generated taking the hour of the computer when the program was executed. Randomisation will be stratified by age (<65 years or ≥65 years). After verifying the inclusion and exclusion criteria, patients will be assigned the study drug or a placebo solution.

Masking

The study is double-blind. After a patient is randomised by a blinded member of the research team, a nonblinded research member will request the corresponding study medication according to the randomisation code. The study medication and the placebo solution will be indistinguishable, and masking will be made by the nonblinded research team members.

Outcomes

Principal variables. The safety of AD-MSCs will be assessed using the following parameters:

- Adverse events (AEs) reported spontaneously by the patient or in response to questions not addressed, and serious AEs (death, life-threatening events, events that require inpatient hospitalisation or prolongation of existing hospitalisation, events that result in persistent or significant disability, congenital anomalies or any other event that does not meet the definitions above but can be considered potentially serious).

- Neurological and systemic complications (including abnormal laboratory values): deteriorating stroke, stroke recurrences, brain oedema, seizures, haemorrhagic transformation, respiratory infections, urinary tract infections, deep venous thrombosis and pulmonary embolism.

Secondary variables. The efficacy of intravenous treatment with AD-MSCs will be assessed using the following parameters:

- Modified Rankin Scale (mRS): The outcome will be considered positive when the patient obtains a score of 0–3, measured at months 3, 6, 12 and 24. An additional exploratory efficacy analysis will consider mRS shift at months 3, 6, 12 and 24.
- National Institutes of Health Stroke Scale (NIHSS): Measured at all scheduled visits. A successful outcome will occur with an improvement of 75% or more from the baseline score. An additional exploratory efficacy analysis will measure differences in the median (interquartile range [IQR]) distribution and in the frequency of an NIHSS ≤1 between groups.
- Biomarker measurement: Granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor BB (PDGF-BB), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), transforming growth factor 1 (TGF-1), endostatin, glial fibrillary acid protein (GFAP), myelin basic protein (MBP), matrix metalloproteinase 3 (MMP-3) and extracellular vesicles will be measured at baseline, day 7 and month 3 after treatment administration.

Study procedures

After initial screening and randomisation (visit 1) takes place, the study treatment will be administered within the first four days since stroke symptom onset (visit 2). Eight more visits will take place during the 24 month follow-up period (2h, 24h, 7 days or hospital discharge, 3, 6, 12, 18 and 24 months since treatment administration) to assess security and efficacy measures

(see table 1 for details of study visits and procedures and figure 1 for schematic flowchart of the clinical trial design).

Table 1. Flowchart of study visits and procedures

FLOWCHART	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
	Screening	Baseline 0 h	2h	24 h	Day 7 or Discharge[4]	M3 (±14 d)	M6 (±14 d)	M12 (±14 d)	M18 (±14 d)	M24 (±14 d)
Informed consent signature	X									
Inclusion and exclusion criteria	X	X [2, 3]								
Past medical/surgical history Physical	X									
examination	X									
Pregnancy test	X			X	X	X	X	X	X	X
NIHSS	X	X [3]	X	X	X	X	X	X	X	X
Modified Rankin Scale	X				X	X	X	X	X	X
Blood pressure	X	X [3]	X	X	X					
Heart rate	X	X [3]	X	X	X					
Body temperature	X	X [3]	X	X	X					
Oxygen saturation	X	X [3]	X	X	X					
Capillary blood glucose	X	X [3]	X	X	X					
12-lead ECG	X	X [3]		X						X
Neuroimaging (brain CT)	X [1]									
Neuroimaging (brain MRI)	X [1]									
Randomisation	X									
Study drug administration		X								
Laboratory assessments: Haematology, coagulation test and biochemistry	X			X	X					X
Blood brain repair markers		X [3]			X	X				
Adverse events register		X [3]	X	X	X	X	X	X	X	X
Concomitant drugs	X	X [3]	X	X	X	X	X	X	X	X

NIHSS: National Institutes of Health Stroke Scale; ECG: electrocardiogram; CT: computed tomography; MRI: magnetic resonance imaging.

[1] Review Neuroimaging (one of them, CT or MRI); [2] Review selection criteria; [3] Tests, measurements, register of adverse events and study drug administration and also revision of inclusion and exclusion criteria will take place before study drug administration. **[4]** Visit 5 is performed at day 7 after study drug administration or at hospital discharge if it takes place before day 7.

Treatment or intervention

All study medications will be industrially manufactured, tested and released according to current Good Manufacturing Practice Guidelines by Histocell. The experimental drug is an intravenous solution of allogeneic AD-MSCs in a concentration of 10 million cells per millilitre, obtained from a donor by *in vitro* cell culture techniques, conditioned in cryovials of Type I sterile borosilicate glass (with ringer lactate, glucosaline, sodium bicarbonate and human albumin 20% as excipients) and cryopreserved at −150° C. The manufacturing process is detailed in the investigator's brochure, which has been approved by the Spanish Agency of Medicines and Health products. The placebo solution has the same appearance as the study drug and is formed by its excipients. Medication vials containing either solution will be dissolved in 50ml of physiologic saline solution and dispensed as a single intravenous drop administration in approximately 30 minutes, at a dose of 1 million cells per kg of the patient's weight (for the AD-MSCs).

Discontinuation of study medication

The study medication must be discontinued if a suspected anaphylactic reaction or serious adverse event occurs during its administration of if patient participation consent is withdrawn.

Standard treatments for the management of acute stroke

All patients will be managed according to current guidelines for acute stroke management, including treatment with recanalization therapies such as intravenous thrombolysis, mechanical thrombectomy or both.

Data collection and outcome measures

An electronic case report form has been designed using MACRO. This system will maintain patient anonymity, and the data will be transferred to a '*.csv' file to analyse it with R software (3.5.2 version or newer). To ensure the quality of the data, data management will be performed by the Spanish Clinical Research Network (SCReN). The data management plan has been

approved by the principal investigator and the sponsor. Data collection forms will be included in the final report.

Sample size estimates

Given this is a pilot phase II clinical trial focused on the assessment of safety, no systematic sample size calculation applies. Based on the previous clinical trial, AMASCIS-01 (NCT01678534 and EudraCT: 2011-003551-18), it seems feasible to recruit 30 patients between the 2 participating centres.

Statistical analyses

Demographic and clinical characteristics will be summarised in terms of means and standard deviations, median and IQR or relative frequency as appropriate for each variable type. A univariate approach will be employed for calculating nonparametric tests for the continuous variables (Wilcoxon rank sum), and Fisher's or a chi-squared test will be employed for the categorical variables. Given the binary nature of the primary outcome, a logistic regression under the minimum Akaike information criterion using all available variables will be fitted to the data, estimating odds ratios and their confidence intervals. Rules of prediction for the binary outcome will be performed using receiver operating characteristic curves and k-fold cross-validation procedures. Only *P* values less than 0.05 will be considered significant. Statistical computations will be calculated using the statistical computing environment R. The statistical analysis will be performed by the personnel from the Clinical Pharmacology Service and Central Unit of Clinical Investigation and Clinical Trials. The safety analysis will include tables with all AEs and neurological and systemic complications. A descriptive analysis summarising efficacy variables in each group will be performed and an exploratory analysis will be conducted by comparing both treatment groups using the Wilcoxon rank sum test or Student's t-test.

Plans for predefined subgroup analyses:

1) The "intention to treat" population will consist of all patients for whom baseline variables and at least 1 value of a principal or secondary variable are available. If a final visit variable is not obtained for a patient, it will be replaced by the last available value (LOCF=Last Observation Carried Forward).

2) The "per protocol" population will consist of all patients for whom infusion of the study medication had been started with no major protocol violation.

3) The "safety analysis population" will consist of all patients for whom infusion of the study medication had been started.

Data monitoring body

Coordination, management, monitoring, data management and the statistical analysis will be performed by the SCReN.

Auditing

During the progress of the study, audit visits may be conducted at the participating centres. The investigator will allow direct access to the source data/documents for monitoring, auditing, review by the ethical research committee and inspection by the health authorities.

Patient and Public Involvement

The development of the research objectives and outcomes are based on the neurologist´s experience treating this profile of patients and the desire to optimise brain tissue repair therapies. Patients and their advisors were not involved in the design, recruitment or conduct of this study.

SUMMARY

AD-MSCs administered intravenously in the first days from stroke symptom onset could be a safe and promising therapy to enhance recovery from brain ischaemia and to help reduce

disability. This type of cell therapy has already demonstrated its efficacy and safety in preclinical research, but further studies are needed to prove similar results in patients with stroke. The advantages of AD-MSCs over other cell types are their accessibility and abundance, as well as their low immunogenicity. An intravenous infusion appears to be the most appropriate manner of administration, given it is the least aggressive, and cell therapies can be performed by modification of trophic factors and immunomodulation.

This clinical trial aims to demonstrate that early intravenous administration of AD-MSCs has no major safety complications and that it could possibly reduce disability due to stroke. Also, analysis of blood biomarkers and trophic factors will help elucidate and deepen our understanding of the mechanisms of action of cell therapy, along with previous results from other clinical trials.[24,25] The results of the AMASCIS-02 trial, together with those from the AMASCIS-01 trial, will increase our knowledge of the safety of intravenous AD-MSC administration in patients with ischaemic stroke. We will then be able to conduct larger clinical trials to evaluate their efficacy and possibly enable their use in clinical practice in the near future.

DECLARATIONS

Conflicts of Interest

There are no competing interests for any author.

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Research Network), funded by Carlos III Health Institute-General Subdirection for Evaluation and Promotion of Research, research PT17/0017/0013. State Plan for Scientific Investigation, Technology and Innovation (2017–2020). Co-funded by European Regional Development Fund/European Social fund "A way to make Europe"/"Investing in your future" (grant ID PT17/0017/0013). The Funders and Sponsor will not interfere in the selection processes of patients, analysis of data and/or publication of the results, or in any other process that might interfere with the results of the study. Funding will be independent of the results of the study. The Principal Investigator has ultimate authority over any of these activities.

Ethics and dissemination

Research ethics approval: The researchers will adhere strictly to the provisions of this protocol and will complete the case report forms. The study will be performed according to the recommendations for clinical studies and the evaluation of drugs in humans, as contained in the Declaration of Helsinki (revised in successive world assemblies) and in the current Spanish and European legislation on clinical studies and patient data confidentiality. The study will follow the principles of Good Clinical Practice. This study (protocol version 2, dated 5 May 2020) has been approved by the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain) and by the Spanish Agency of Medication and Health Products, and has been registered in Eudra CT (2019-001724-35) and ClinicalTrials.gov (NCT04280003).

Protocol amendments: All protocol amendments will be evaluated by the Ethics Committee and the Spanish Agency of Medication and Health Products, following the principles of Good Clinical Practice and national legislation. All modifications of the study protocol will be communicated by updating the trial registry at ClinicalTrials.gov (NCT04280003).

Consent to participate: Informed consent will be obtained by a recruiting investigator for all individual participants included in this study, or by their family member/representative (if the patient has aphasia or altered consciousness at that moment). Re-consent from the patient will be obtained whenever possible if initial consent was provided by family

members/representatives. The informed consent document is written in Spanish. The informed consent documents are available as supplementary files.

Consent for publication: According to the Royal Decree 1090/2015, the results of this clinical trial will be published once the study is completed in an Open Access format.

Confidentiality: All participant information as well as the electronic database will remain in secure storage at both study centres during the trial and up to 25 years after its finalisation.

Access to data: All investigators and each centres' monitor will have access to the electronic trial data during the data collection period; after completion of the study, the data will also be accessible to statisticians. Only the blinded study nurses and Histocell will have access to randomisation codes until the data collection period is complete.

Availability of data and material: Data will be available from the corresponding author on reasonable request, if it is considered compliant with the General Data Protection Regulation and the data-sharing agreement is approved by the relevant Spanish authorities. Upon study finalisation, anonymised individual patient data will be available on a public repository of the Community of Madrid.

Ancillary and post-trial care: Patients will be treated during and after the clinical trial is completed with the best medical treatments according to the state of the art. Participants will not receive economic benefits beyond compensation from the study insurance company in cases of study treatment-related adverse events (Hannover seguros España).

Dissemination policy: Output from this study will include journal publications, conference presentations and community reporting. Output will not identify participants.

Authors´ contributions

The first draft of the manuscript was written by Elena de Celis-Ruiz, and all authors commented on previous versions of the manuscript. Blanca Fuentes, Exuperio Díez-Tejedor, Francisco

Moniche, Joan Montaner, Alberto Borobia and María Gutiérrez-Fernández participated in the study conception and design. All authors have read and approved the final manuscript.

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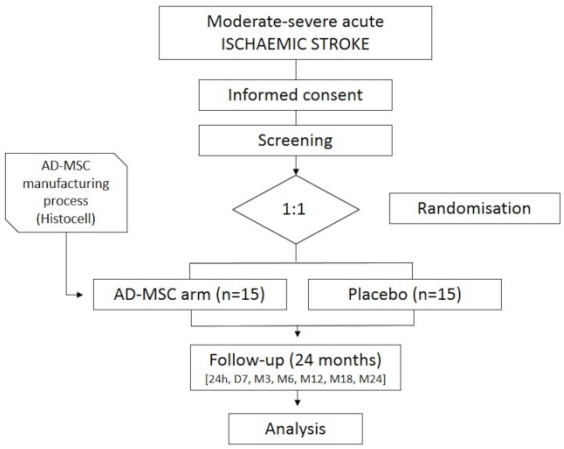

Figure 1. Schematic flowchart of the clinical trial

338x190mm (600 x 600 DPI)

Código de protocolo: AMASCIS 02
Versión 3.0 de 08 de Mayo de 2020

HOJA DE INFORMACIÓN PARA EL PARTICIPANTE

TÍTULO DEL ESTUDIO: ADMINISTRACIÓN INTRAVENOSA DE CÉLULAS TRONCALES MESENQUIMALES ALOGÉNICAS DE TEJIDO ADIPOSO EN EL INFARTO CEREBRAL AGUDO. ENSAYO CLÍNICO FASE IIB MULTICÉNTRICO Y DOBLE CIEGO CONTROLADO CON PLACEBO.
CÓDIGO DEL ESTUDIO: AMASCIS-2
VERSIÓN: 3.0 de 8 de mayo de 2020
PROMOTOR: Fundación para la Investigación Biomédica del Hospital Universitario de La Paz (FIBHULP)
INVESTIGADOR COORDINADOR: Exuperio Díez Tejedor.
CENTRO: _____

INTRODUCCIÓN

Nos gustaría invitarle a participar en este estudio clínico. Antes de decidir si participar en este estudio, tómese todo el tiempo que necesite para revisar toda la información y pregunte todo lo que quiera. El estudio ha sido aprobado por un Comité de Ética con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo con la legislación vigente. Antes de aceptar su participación en este estudio debe comprender por completo porqué se está realizando el estudio y lo que va a implicar. Debe preguntarle al médico si existen otros tratamientos disponibles para su enfermedad. No debe consentir su participación a menos que esté completamente de acuerdo con todos los procedimientos implicados.

ANTECEDENTES Y OBJETIVO DEL ESTUDIO

Le estamos solicitando participar en este ensayo clínico porque ha padecido un ictus agudo. Un ictus está provocado por una obstrucción del flujo sanguíneo a una parte del cerebro. Algunas de las células del área del cerebro que no reciben sangre se mueren inmediatamente. Además, después del ictus en el cerebro se producen una serie de procesos nocivos que provocan que se mueran más células cerebrales. Esto supone que las secuelas provocadas por el ictus podrían aumentar. Pero también se activan procesos que intentan reparar el daño, en la mayoría de las ocasiones son insuficientes para conseguir mejorar el pronóstico.

Las células troncales (también conocidas como células madre) son células inmaduras con capacidad de autoregenerarse y de diferenciarse en múltiples tipos celulares. Hasta ahora se han ensayado tratamientos basados en la administración de células troncales en varias enfermedades neurológicas. La administración intravenosa de células troncales

mesenquimales autólogas ha sido factible y segura, y se ha sugerido una mejoría en la recuperación neurológica en pacientes con infarto cerebral grave. Además, se ha constatado la seguridad de este tratamiento a los 5 años de su administración. En el estudio AMASCIS-01, similar al que se le invita a participar, se incluyeron 19 pacientes y se llegó a administrar las células a 4 de ellos sin que se detectara ningún efecto adverso relacionado con su administración

El objetivo de este estudio de investigación es evaluar la seguridad del tratamiento alogénico (procedentes de un donante) precoz con células troncales procedentes de tejido adiposo en pacientes que han sufrido un ictus agudo. También se recogerán datos sobre su posible eficacia en la reducción de secuelas.

DESARROLLO DEL ESTUDIO

Si usted consiente su participación en el estudio, usted deberá firmar este formulario de consentimiento informado. Su médico realizará algunas pruebas médicas y otros procedimientos para determinar si usted reúne los requisitos para participar en el estudio. Deberá informar si ha padecido problemas médicos en el pasado. Como parte del estudio habitual en pacientes con ictus agudo se le practicará una exploración general y neurológica, incluyendo mediciones de la presión arterial, el pulso, la temperatura, la glucosa en sangre capilar, la saturación de oxígeno y se le realizará un electrocardiograma. Se le tomará una muestra sanguínea para realizar pruebas generales de laboratorio. No podrá participar en el estudio en caso de embarazo o lactancia. En los casos de mujeres en edad fértil sólo podrán participar en el estudio si la prueba de embarazo es negativa en el momento de su inclusión en el estudio y si aceptan usar métodos anticonceptivos adecuados y efectivos (dispositivos intrauterinos no hormonales, oclusión tubárica bilateral, pareja vasectomizada o abstinencia sexual) durante la duración del mismo (24 meses). Previo a su inclusión en este estudio, se le habrá realizado una tomografía computarizada (TC) craneal o una resonancia magnética (RM) cerebral como parte del estudio habitual del ictus, en las cuales se habrá verificado que usted tiene un infarto cerebral.

Se prevé que un total de 30 personas participen en el estudio. Si reúne los requisitos para participar, la duración de su participación en el mismo será de aproximadamente 24 meses. Se le asignará aleatoriamente (como cara o cruz, o sacando un número de un bombo) a recibir el fármaco del estudio (células troncales mesenquimales) o placebo. El placebo es una sustancia que tiene el aspecto del fármaco del estudio pero no contiene sustancia farmacológicamente activa, por lo que no se espera que tenga efecto. Tendrá un 50% de posibilidades de recibir el fármaco del estudio o un 50% de posibilidades de recibir el placebo. Ni usted ni el médico del estudio sabrán si está recibiendo el fármaco del estudio o el placebo. Sin embargo, el médico del estudio podrá identificar su tratamiento en caso de emergencia médica.

El fármaco del estudio o el placebo se administrarán por vía intravenosa (a través de una pequeña vía de perfusión colocada en la vena de su brazo) en una sola dosis alrededor del cuarto día desde el inicio del ictus, mientras permanece hospitalizado. Todos los pacientes recibirán el tratamiento convencional del infarto cerebral de acuerdo a las actuales guías de práctica clínica y vías al uso. Como pruebas adicionales al manejo y tratamiento convencional del ictus, al participar en el estudio se le extraerán en 3 ocasiones muestras de sangre adicionales para el análisis de sustancias relacionadas con la recuperación y regeneración del tejido cerebral.

El estudio tendrá una duración total de **24 meses**, estando previstas **10 visitas**

Primera visita: Tras comprobar que usted cumple los criterios de inclusión y ninguno de los de exclusión para participar en este ensayo, el médico le informará acerca del estudio y le pedirá su consentimiento por escrito. Se le preguntará por su estado funcional previo (actividades que antes del ictus podía realizar de forma independiente o si requería alguna ayuda), y se le realizará un examen clínico que incluirá una medición de las constantes vitales, una exploración clínica general completa y una exploración neurológica detallada. En el caso de las mujeres en edad fértil se realizará además un test de embarazo. Se registrarán los resultados de las pruebas ya realizadas como parte de la práctica clínica habitual en el ictus. La asignación aleatoria de la administración del fármaco del estudio o del placebo se realizará también en esta visita.

Segunda visita: Tendrá lugar en el momento de administración del fármaco. Se repasarán los criterios para la participación en el ensayo. Se le realizará un examen clínico general y una exploración neurológica detallada. Se realizará un nuevo electrocardiograma. Adicionalmente se le tomará una muestra de sangre para analizar sustancias relacionadas con la recuperación y reparación del tejido cerebral tras un infarto cerebral. Cualquier evento adverso se registrará.

Tercera visita: tendrá lugar a las 2 horas de la finalización del tratamiento por vía intravenosa e incluirá la medición de las constantes vitales y una exploración neurológica detallada (escala NIHSS), así como el registro de cualquier acontecimiento adverso.

Cuarta y quinta visitas: se realizarán a las 24h y a los 7 días (o alta hospitalaria en caso de que ocurra antes del día 7) desde la administración del fármaco. Se le realizará una medición de las constantes vitales, así como una exploración clínica general y neurológica detalladas. Se anotarán todos los fármacos que haya recibido además del fármaco del estudio. Se realizará un nuevo electrocardiograma en la cuarta visita y se tomarán muestras de sangre para asegurarnos de su seguridad durante el estudio, además de analizar las sustancias involucradas en regeneración cerebral en la quinta visita. Cualquier evento adverso se registrará

Sexta visita: Se realiza en el mes 3 desde su inclusión en el estudio. Se le realizará una exploración clínica general y neurológica detallada. Se extraerán de nuevo muestras de sangre para analizar las sustancias relacionadas con la regeneración cerebral. Se registrarán los posibles acontecimientos adversos ocurridos y los fármacos que esté usted tomando en ese momento

Séptima a novena visitas: se realizarán a los 6, 12, y 18 meses desde su inclusión en el estudio. De nuevo se realizará una exploración clínica y neurológica completas y se anotarán las posibles reacciones adversas y los fármacos que esté usted tomando.

Última visita: se realizará a los 24 meses. Se repetirá una exploración clínica y neurológica completas. Adicionalmente se realizará una analítica y un electrocardiograma de 12 derivaciones. De nuevo, será preguntado por los fármacos que haya precisado tomar desde la última visita, y se registrará cualquier evento adverso.

RIESGOS POSIBLES

Estudios previos han observado que la infusión intravenosa de células troncales mesenquimales alogénicas es factible y segura, y se ha sugerido una mejoría en la recuperación de las secuelas en pacientes con infarto cerebral grave. Además se ha constatado la seguridad de este tratamiento a los 5 años del tratamiento. El uso de estas células administradas por vía intravenosa en pacientes con otras enfermedades diferentes al ictus también ha sido seguro, siendo los efectos adversos más frecuentes fiebre (<30%), malestar general (10%), síntomas similares a una gripe (10%), infecciones de orina (<35%), infecciones respiratorias (<30%), otitis (<10%), erupción cutánea (<10%), debilidad muscular (<10%), náuseas (<20%), vómitos (<20%), diarrea (<10%), caries dental (<10%), anemia (<10%). Una revisión de 70 estudios con administración de células troncales del tejido adiposo en 1474 pacientes con otras enfermedades distintas al ictus observaron muy pocos efectos adversos relacionados directamente con la terapia celular. No se ha observado incremento del riesgo de cáncer ni de tromboembolismo pulmonar. Sin embargo, puesto que se trata de un fármaco experimental (aún no se encuentra autorizado o comercializado), pueden existir otros efectos secundarios y riesgos que aún no hayan sido descritos. No se espera que ocurran efectos adversos con el placebo, al ser una sustancia no activa farmacológicamente.

La extracción de sangre de su brazo puede provocar dolor, moratones y en pocas ocasiones una infección.

BENEFICIOS POSIBLES

En algún estudio anterior, el tratamiento con células troncales se asoció con una mejoría en la recuperación neurológica en algunos pacientes con infarto cerebral grave. Sin embargo, no es posible predecir ni garantizar si este fármaco en estudio ayudará a mejorar su enfermedad. Sus síntomas pueden mejorar, seguir igual o empeorar. En

cualquier caso, su participación en el estudio puede ayudar a otros pacientes que sufran ictus en el futuro.

TRATAMIENTOS ALTERNATIVOS

Usted recibirá todos los tratamientos actualmente establecidos en el infarto cerebral agudo que requiera según las características de su infarto cerebral (tipo de infarto y duración de los síntomas). Existen otros tratamientos en experimentación diseñados para proteger y reparar el cerebro en los infartos cerebrales, pero ninguno de ellos cuenta todavía con aprobación para su utilización en pacientes fuera de estudios de investigación como el que le proponemos.

DEBERES DEL PARTICIPANTE DEL ESTUDIO

Como participante en el estudio, está obligado a respetar las instrucciones médicas del médico del estudio, informar detalladamente a su médico de la evolución de su enfermedad y de cualquier malestar o dolencia que pueda observar, así como de los tratamientos que esté tomando prescritos por otro médico y de aquellos comprados sin receta, incluyendo los tratamientos homeopáticos y suplementos vitamínicos.

NUEVAS INFORMACIONES

Si aparece cualquier nueva información sobre la seguridad del tratamiento con células troncales mesenquimales alogénicas, le informaremos a usted o a su representante legal autorizado para que pueda decidir si desea continuar con su participación en este estudio de investigación. Si decide seguir participando en el estudio tras haber recibido esta nueva información, se le pedirá que firme un documento actualizado de consentimiento. Si decide retirarse del estudio, esto no tendrá repercusión sobre el tratamiento que reciba en el futuro por su enfermedad.

INTERRUPCIÓN PRECOZ DEL ESTUDIO

Su participación en este estudio de investigación es voluntaria. Puede negarse a participar o abandonar su participación en cualquier momento, sin ningún tipo de penalización y la calidad de su atención sanitaria no se verá alterada y recibirá los cuidados habituales que los pacientes con ictus reciben en su hospital. El médico del estudio puede poner fin a su participación en el estudio en caso de una reacción inesperada, si usted incumple las instrucciones dadas por el personal del estudio o porque todo el estudio sea interrumpido. Si decide no continuar participando en este estudio, el médico realizará los trámites oportunos para que sus cuidados continúen.

OBTENCIÓN Y USO DE MUESTRAS BIOLÓGICAS:

Su participación en este ensayo clínico conlleva la obtención y utilización de muestras biológicas con fines de investigación, para lo que se observará la Ley 14/2007 de

investigación biomédica y el Real Decreto 1716/2011, normativas que garantizan el respecto a los derechos que le asisten. Al firmar este documento, revisado y evaluado por el Comité de Ética de la Investigación con medicamentos que ha aprobado este ensayo clínico, usted acepta que se utilicen sus muestras para las finalidades del presente estudio.

Algunas de las muestras se obtienen durante el seguimiento habitual de su enfermedad o proceso; otras son solicitadas porque son necesarias para cumplir con los objetivos de estudio. Entre las pruebas que se obtienen como parte del seguimiento habitual se encuentran análisis de hemograma, coagulación y datos de bioquímica básica; obtenidas en las visitas 1 4, 5 y 10. Como pruebas adicionales del estudio se encuentran el análisis de factores relacionados con la regeneración y recuperación del tejido cerebral que se obtendrán en las visitas 2,5 y 6.

Las muestras de sangre que forman parte del estudio habitual del ictus permanecerán almacenadas en el hospital donde se realiza la extracción (Hospital Universitario La Paz u Hospital Universitario Virgen del Rocío); y posteriormente parte de las muestras se analizarán en el Hospital Universitario Virgen Macarena y otra parte en el Hospital Universitario La Paz.

La cantidad extraída en cada análisis de sangre de seguimiento habitual serán tres tubos, mientras que en los análisis específicos del estudio serán cinco tubos. Cada tubo contiene aproximadamente 2 ml de sangre. Los posibles riesgos derivados del procedimiento realizado para la obtención de estas muestras estarán cubiertos por el seguro del ensayo clínico.

Las muestras estarán asociadas a un código que solo podrá ser relacionado con su identidad por personal autorizado (médicos, enfermeros y personal de laboratorio). Los datos que se deriven de la utilización de estas muestras se tratarán del mismo modo que el resto de datos que se obtengan durante este ensayo (ver apartado confidencialidad y protección de datos personales). Las muestras y los datos asociados se mantendrán bajo las condiciones de seguridad adecuadas y se garantiza que los sujetos no podrán ser identificados a través de medios considerados razonables por personas distintas a las autorizadas.

Durante el desarrollo del ensayo sus muestras pueden ser analizadas en diversos laboratorios y se mantendrán almacenadas durante 10 años, en previsión de que fuera necesario repetir algún análisis adicional relacionado con los objetivos del estudio. Durante este tiempo, el responsable de las muestras será el promotor del ensayo. En caso de conservación para usos futuros de las muestras, se mantendrán almacenadas en el Instituto de Genética Médica y Molecular (INGEMM), Sección de Farmacogenética Hospital Universitario La Paz, durante 10 años. En el caso del Hospital Universitario Virgen Macarena serán almacenadas en su Biobanco

Una vez finalizado el ensayo, las muestras sobrantes serán destruidas, a no se que usted consienta para que puedan ser almacenadas y utilizadas en futuras investigaciones. La finalidad del almacenamiento de estas muestras es que sean utilizadas en proyectos de investigación en el futuro.

Si cambiara de opinión en relación con la donación de muestras biológicas y la cesión de los datos proporcionados, tiene derecho a solicitar su destrucción o anonimización, a través de su médico/investigador/investigador principal de la colección. No obstante, debe saber que los datos que se hayan obtenido en los análisis realizados hasta ese momento podrán ser utilizados para los fines solicitados y podrán conservarse en cumplimiento de las obligaciones legales correspondientes.

En el caso de que en este ensayo se obtengan datos que pudieran ser clínica o genéticamente relevantes para usted, e interesar a su salud o a la de su familia, podrá solicitar que le sean comunicados por su médico del ensayo si así lo indica en la casilla que aparece al final de este documento. No obstante, si el paciente hubiera indicado su negativa y cuando esta información según criterio del médico responsable, sea necesaria para evitar un grave perjuicio para su salud o la de sus familiares biológicos, se informará a un familiar próximo o a un representante, previa consulta al Comité de Ética Asistencial del centro. La comunicación de esta información se llevará a cabo por profesionales que le podrán explicar adecuadamente su relevancia y las opciones que se pudieran plantear. En caso de información genética clínicamente relevante podrá recibir el preceptivo consejo genético.

Hable con su médico sobre la posibilidad de que pueda establecer restricciones para que su muestra biológica no sea utilizada en determinadas investigaciones.

COSTE

A usted no le costará nada participar en el estudio y no recibirá ningún pago por participar. El coste de la terapia con células troncales la asumirá el hospital promotor del estudio gracias a una ayuda del Instituto de Salud Carlos III.

COMPENSACIÓN

Todas las pruebas médicas y tratamientos (tanto experimentales como de rutina) implican cierto riesgo de lesión. Si nota o experimenta cualquier lesión relacionada con este estudio clínico, y ha seguido las instrucciones de sus médicos y del resto del personal de estudio, el promotor del mismo cubrirá los gastos médicos para tratar su lesión. No se ofrecerá ninguna compensación si su lesión fuera provocada por un incumplimiento de las instrucciones del personal de la investigación. El promotor no ofrecerá ninguna otra compensación.

SEGUROS

El hospital promotor del estudio ha contratado una póliza de seguro de Responsabilidad Civil con la compañía Hannover Seguros España.

CONFIDENCIALIDAD Y PROTECCIÓN DE DATOS PERSONALES:

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los pacientes participantes se ajustará a lo dispuesto en el Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 de Protección de Datos (RGPD).

-De acuerdo con lo que establece la legislación mencionada, usted puede ejercer los derechos de acceso, rectificación, oposición y supresión de sus datos, para lo cual deberá dirigirse a su médico del estudio. También puede limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio. Para ejercitar sus derechos, diríjase al investigador principal del estudio o al Delegado de Protección de datos del centro: José Manuel Laperal González (tfno:91426998/e-mail: protecciondedatos.sanidad@madrid.org).

Le recordamos que los datos no se pueden eliminar aunque deje de participar en el ensayo para garantizar la validez de la investigación y cumplir con los deberes legales y los requisitos de autorización de medicamentos. Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos, pero sí se utilizarán los que ya se hayan recogido. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no quedara satisfecho

Tanto el Centro como el Promotor son responsables respectivamente del tratamiento de sus datos y se comprometen a cumplir con la normativa de protección de datos en vigor. Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no se incluya información que pueda identificarle, y sólo su médico del estudio/colaboradores podrá relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a ninguna otra persona salvo a las autoridades sanitarias, cuando así lo requieran o en casos de urgencia médica. Los Comités de Ética de la Investigación, los representantes de la Autoridad Sanitaria en materia de inspección y el personal autorizado por el Promotor, únicamente podrán acceder para comprobar los datos personales, los procedimientos del estudio clínico y el cumplimiento de las normas de buena práctica clínica (siempre manteniendo la confidencialidad de la información).

El Investigador y el Promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, su información personal solo se conservará por el centro para el cuidado de su salud y por el promotor para otros fines de investigación científica si usted hubiera otorgado su consentimiento para ello, y si así lo permite la ley y requisitos éticos aplicables.

Si realizáramos transferencia de sus datos codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos por las autoridades de protección de datos. Si el participante quiere saber más al respecto, puede contactar al/a la Delegado/a de Protección de Datos del promotor o la institución Delegado/a de Protección de Datos del promotor o la institución. Alaro Avant, S.L.Avda. de Brasil 17, 7G, 28020, Madrid dpo.fiblapaz@alaroavant.com; 91112396**.**

APROBACIÓN DEL ESTUDIO:

Conforme a la legislación en España, este estudio ha sido revisado y aprobado por el Comité Ético de investigación Clínica (CEIC) del Hospital la Paz.

Además, la Agencia española de medicamentos y Productos Sanitarios (AEMPS) ha dado su autorización para la realización de esta investigación.

CONTACTO:

Si desea hacernos alguna pregunta o consulta no dude en contactar con el Dr..... del Servicio de Neurología del Hospital ..... (Teléfono: ……)

CONSENTIMIENTO INFORMADO PARA EL PARTICIPANTE

TÍTULO DEL ESTUDIO: ADMINISTRACIÓN INTRAVENOSA DE CÉLULAS TRONCALES MESENQUIMALES ALOGÉNICAS DE TEJIDO ADIPOSO EN EL INFARTO CEREBRAL AGUDO. ENSAYO CLÍNICO FASE IIB MULTICÉNTRICO Y DOBLE CIEGO CONTROLADO CON PLACEBO.
CÓDIGO DEL ESTUDIO: AMASCIS-2
VERSIÓN: 3.0 de 8 de mayo de 2020
PROMOTOR: Fundación para la Investigación Biomédica del Hospital Universitario de La Paz (FIBHULP)
INVESTIGADOR COORDINADOR: Exuperio Díez Tejedor.
CENTRO: _____
_____

Yo (nombre y apellidos del participante)
…….....,

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.

He hablado con el Dr.....

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

1. Cuando quiera.
2. Sin tener que dar explicaciones.
3. Sin que esto repercuta en mis cuidados médicos.

Deseo que la información derivada de la investigación que pueda ser relevante para mi salud me sea comunicada.

◯ SI ◯ NO

Consiento el almacenamiento y uso de las muestras biológicas y de los datos asociados para futuras investigaciones en las condiciones explicadas en la hoja de información al paciente.

◯ SI ◯ NO

Consiento ser contactado en el caso de necesitar más información o muestras biológicas adicionales

◯ SI ◯ _NO

Recibiré una copia firmada y fechada de este documento de consentimiento informado.

Presto libremente mi conformidad para mi participación en el estudio.

FIRMA DEL PARTICIPANTE **FIRMA DEL INVESTIGADOR**

Fecha Fecha

FIRMA DEL TESTIGO (si procede)

Fecha

Código de protocolo: AMASCIS 02
Versión3.0 de 08 de Mayo de 2020

HOJA DE INFORMACIÓN PARA EL REPRESENTANTE LEGAL / FAMILIAR DEL PARTICIPANTE

TÍTULO DEL ESTUDIO: ADMINISTRACIÓN INTRAVENOSA DE CÉLULAS TRONCALES MESENQUIMALES ALOGÉNICAS DE TEJIDO ADIPOSO EN EL INFARTO CEREBRAL AGUDO. ENSAYO CLÍNICO FASE IIB MULTICÉNTRICO Y DOBLE CIEGO CONTROLADO CON PLACEBO.
CÓDIGO DEL ESTUDIO: AMASCIS-2
VERSIÓN: 3.0 de 8 de mayo de 2020
PROMOTOR: Fundación para la Investigación Biomédica del Hospital Universitario de La Paz (FIBHULP)
INVESTIGADOR COORDINADOR: Exuperio Díez Tejedor.
CENTRO: _____

INTRODUCCIÓN

Nos gustaría invitar a su representado a participar en este estudio clínico, quien en este momento no se considera en plenas facultades para comprender la información y dar su consentimiento. Antes de decidir si desea que participe en este estudio, tómese todo el tiempo que necesite para revisar toda la información y pregunte todo lo que quiera. El estudio ha sido aprobado por un Comité de Ética con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo con la legislación vigente. Antes de aceptar la participación de su representado en este estudio debe comprender por completo porqué se está realizando el estudio y lo que va a implicar. Debe preguntarle al médico si existen otros tratamientos disponibles para la enfermedad de su representado. No debe consentir su participación a menos que esté completamente de acuerdo con todos los procedimientos implicados.

ANTECEDENTES Y OBJETIVO DEL ESTUDIO

Estamos solicitando que su representado participe en este ensayo clínico porque ha padecido un ictus agudo. Un ictus está provocado por una obstrucción del flujo sanguíneo a una parte del cerebro. Algunas de las células del área del cerebro que no reciben sangre se mueren inmediatamente. Además, después del ictus, en el cerebro se producen una serie de procesos nocivos que provocan que se mueran más células cerebrales. Esto supone que las secuelas provocadas por el ictus podrían aumentar. Pero también se activan procesos que intentan reparar el daño, en la mayoría de las ocasiones son insuficientes para conseguir mejorar el pronóstico.

Las células troncales, también conocidas como células madre, son células inmaduras con capacidad de autoregenerarse y de diferenciarse en múltiples tipos celulares. Hasta ahora

se han ensayado tratamientos basados en la administración de células troncales en varias enfermedades neurológicas. La administración intravenosa de células troncales mesenquimales autólogas ha sido factible y segura, y se ha sugerido una mejoría en la recuperación neurológica en pacientes con infarto cerebral grave. Además, se ha constatado la seguridad de este tratamiento a los 5 años de su administración. En el estudio AMASCIS-01, similar al que se le invita a participar, se incluyeron 19 pacientes y se llegó a administrar las células a 4 de ellos sin que se detectara ningún efecto adverso relacionado con su administración

El objetivo de este estudio de investigación es evaluar la seguridad del tratamiento alogénico (procedentes de un donante) precoz con células troncales procedentes de tejido adiposo en pacientes que han sufrido un ictus agudo. También se recogerán datos sobre su posible eficacia en la reducción de secuelas.

DESARROLLO DEL ESTUDIO

Si usted consiente la participación de su representado en el estudio, usted deberá firmar este formulario de consentimiento informado. Su médico realizará algunas pruebas médicas y otros procedimientos para determinar si su representado reúne los requisitos para participar en el estudio. Deberá informar si ha padecido problemas médicos en el pasado. Como parte del estudio habitual en pacientes con ictus agudo se le practicará una exploración general y neurológica, incluyendo mediciones de la presión arterial, el pulso, la temperatura, la glucosa en sangre capilar, la saturación de oxígeno y se le realizará un electrocardiograma. Se le tomará una muestra sanguínea para realizar pruebas generales de laboratorio. Su representado no podrá participar en el estudio en caso de embarazo o lactancia. En caso de que su representado sea una mujer en edad fértil, es necesario que ella se comprometa a usar métodos anticonceptivos adecuados y efectivos (dispositivos intrauterinos no hormonales, oclusión tubárica bilateral, pareja vasectomizada o abstinencia sexual) durante la duración del estudio (24 meses). Previo a la inclusión de su representado en este estudio, se le habrá realizado una tomografía computarizada (TC) craneal o una resonancia magnética (RM) cerebral como parte del estudio habitual del ictus, en las cuales se habrá verificado que tiene un infarto cerebral.

Se prevé que un total de 30 personas participen en el estudio. Si su representado reúne los requisitos para participar, la duración de su participación en el mismo será de aproximadamente 24 meses. Se le asignará aleatoriamente (como cara o cruz, o sacando un número de un bombo) a recibir el fármaco del estudio (células troncales mesenquimales) o placebo. El placebo es una sustancia que tiene el aspecto del fármaco del estudio pero no contiene sustancia farmacológicamente activa, por lo que no se espera que tenga efecto. Su representado tendrá un 50% de posibilidades de recibir el fármaco del estudio y un 50% de posibilidades de recibir el placebo. Ni usted, ni su representado ni el médico del estudio sabrán si está recibiendo el fármaco del estudio o el placebo. Sin embargo, el médico del estudio podrá identificar el tratamiento en caso de emergencia médica.

El fármaco del estudio o el placebo se administrarán por vía intravenosa (a través de una pequeña vía de perfusión colocada en la vena del brazo) en una sola dosis alrededor del cuarto día desde el inicio del ictus, mientras su representado permanece hospitalizado. Todos los pacientes recibirán el tratamiento convencional del infarto cerebral de acuerdo a las actuales guías de práctica clínica y vías al uso. Como pruebas adicionales al manejo y tratamiento convencional del ictus, al participar en el estudio se le extraerán en 3 ocasiones muestras de sangre adicionales para el análisis de sustancias relacionadas con la recuperación y regeneración del tejido cerebral.

El estudio tendrá una duración total de **24 meses**, estando previstas **10 visitas**

Primera visita: Tras comprobar que su representado cumple los criterios de inclusión y ninguno de los de exclusión para participar en este ensayo, el médico le informará a usted acerca del estudio y le pedirá su consentimiento por escrito. Se le preguntará por el estado funcional previo de su representado (actividades que antes del ictus podía realizar de forma independiente o si requería alguna ayuda), y se le realizará un examen clínico que incluirá una medición de las constantes vitales, una exploración clínica completa y una exploración neurológica detallada. En el caso de las mujeres en edad fértil se realizará además un test de embarazo. Se registrarán los resultados de las pruebas ya realizadas como parte de la práctica clínica habitual en el ictus. La asignación aleatoria de la administración del fármaco del estudio o del placebo se realizará también en esta visita.

Segunda visita: Tendrá lugar en el momento de administración del fármaco. Se repasarán los criterios para la participación en el ensayo. Se le realizará a su representado un examen clínico general y una exploración neurológica detallada. Se realizará además un nuevo electrocardiograma. Adicionalmente se le tomará una muestra de sangre para analizar sustancias relacionadas con la recuperación y reparación del tejido cerebral tras un infarto cerebral. Cualquier evento adverso se registrará.

Tercera visita: tendrá lugar a las 2 horas de la finalización del tratamiento por vía intravenosa e incluirá la medición de las constantes vitales de su representado y una exploración neurológica detallada (escala NIHSS), así como el registro de cualquier acontecimiento adverso.

Cuarta y quinta visitas: se realizarán a las 24h y a los 7 días (o alta hospitalaria en caso de que ocurra antes del día 7) desde la administración del fármaco. A su representado se le realizará una medición de las constantes vitales, así como una exploración clínica general y neurológica detalladas. Se anotarán todos los fármacos que haya recibido además del fármaco del estudio. Se realizará un nuevo electrocardiograma en la cuarta visita y se tomarán muestras de sangre para asegurarnos de su seguridad durante el estudio, además de analizar las sustancias involucradas en regeneración cerebral en la quinta visita. Cualquier evento adverso se registrará

Sexta visita: Se realiza en el mes 3 desde la inclusión de su representado en el estudio. Se le realizará una exploración clínica general y neurológica detallada. Se extraerán de nuevo muestras de sangre para analizar las sustancias relacionadas con la regeneración cerebral. Se registrarán los posibles acontecimientos adversos ocurridos y los fármacos que esté tomando en ese momento.

Séptima a novena visitas: se realizarán a los 6, 12, y 18 meses desde la inclusión de su representado en el estudio. De nuevo se realizará una exploración clínica general y neurológica completas y se anotarán las posibles reacciones adversas y los fármacos que esté tomando.

Última visita: se realizará a los 24 meses desde la inclusión en el estudio. Se repetirá una exploración clínica general y neurológica completas. Adicionalmente se realizará una analítica y un electrocardiograma de 12 derivaciones. De nuevo, será preguntado por los fármacos que su representado haya precisado tomar desde la última visita, y se registrará cualquier evento adverso.

RIESGOS POSIBLES

Estudios previos han observado que la infusión intravenosa de células troncales mesenquimales alogénicas es factible y segura, y se ha sugerido una mejoría en la recuperación de las secuelas en pacientes con infarto cerebral grave. Además se ha constatado la seguridad de este tratamiento a los 5 años del tratamiento. El uso de estas células administradas por vía intravenosa en pacientes con otras enfermedades diferentes al ictus también ha sido seguro, siendo los efectos adversos más frecuentes fiebre (<30%), malestar general (10%), síntomas similares a una gripe (10%), infecciones de orina (<35%), infecciones respiratorias (<30%), otitis (<10%), erupción cutánea (<10%), debilidad muscular (<10%), náuseas (<20%), vómitos (<20%), diarrea (<10%), caries dental (<10%), anemia (<10%). Una revisión de 70 estudios con administración de células troncales del tejido adiposo en 1474 pacientes con otras enfermedades distintas al ictus observaron muy pocos efectos adversos relacionados directamente con la terapia celular. No se ha observado incremento del riesgo de cáncer ni de tromboembolismo pulmonar. Sin embargo, puesto que se trata de un fármaco experimental (aún no se encuentra autorizado o comercializado), pueden existir otros efectos secundarios y riesgos que aún no hayan sido descritos. No se espera que ocurran efectos adversos con el placebo, al ser una sustancia no activa farmacológicamente.

La extracción de sangre del brazo de su representado puede provocar dolor, moratones y en pocas ocasiones una infección.

BENEFICIOS POSIBLES

En algún estudio anterior, el tratamiento con células troncales se asoció con una mejoría en la recuperación neurológica en algunos pacientes con infarto cerebral grave. Sin embargo, no es posible predecir ni garantizar si este fármaco en estudio ayudará a mejorar la enfermedad de su representado. Sus síntomas pueden mejorar, seguir igual o empeorar. En cualquier caso, la participación de su representado en el estudio puede ayudar a otros pacientes que sufran ictus en el futuro.

TRATAMIENTOS ALTERNATIVOS

Su representado recibirá todos los tratamientos actualmente establecidos en el infarto cerebral agudo que requiera según las características de su infarto cerebral (tipo de infarto y duración de los síntomas). Existen otros tratamientos en experimentación diseñados para proteger y reparar el cerebro en los infartos cerebrales, pero ninguno de ellos cuenta todavía con aprobación para su utilización en pacientes fuera de estudios de investigación como el que le proponemos.

DEBERES DEL PARTICIPANTE DEL ESTUDIO

Como participante en el estudio, su representado está obligado a respetar las instrucciones médicas del médico del estudio. Se debe informar detalladamente a su médico de la evolución de su enfermedad y de cualquier malestar o dolencia que se pueda observar, así como de los tratamientos que esté tomando prescritos por otro médico y de aquellos comprados sin receta, incluyendo los tratamientos homeopáticos y suplementos vitamínicos.

NUEVAS INFORMACIONES

Si aparece cualquier nueva información sobre la seguridad del tratamiento con células troncales mesenquimales alogénicas, le informaremos a usted para que pueda decidir si desea que su representante continúe participando en este estudio de investigación. Si decide seguir participando en el estudio tras haber recibido esta nueva información, se le pedirá que firme un documento actualizado de consentimiento. Si decide que se retire del estudio, esto no tendrá repercusión sobre el tratamiento que reciba en el futuro por su enfermedad.

INTERRUPCIÓN PRECOZ DEL ESTUDIO

La participación en este estudio de investigación es voluntaria. Puede negarse a que su representado participe o solicitar que abandone su participación en cualquier momento, sin ningún tipo de penalización. La calidad de su atención sanitaria no se verá alterada y recibirá los cuidados habituales que los pacientes con ictus reciben en su hospital. El

médico del estudio puede poner fin a la participación de su representado en el estudio en caso de una reacción inesperada, si éste incumple las instrucciones dadas por el personal del estudio o porque todo el estudio sea interrumpido. Si decide que su representado no continúe participando en este estudio, el médico realizará los trámites oportunos para que sus cuidados continúen.

OBTENCIÓN Y USO DE MUESTRAS BIOLÓGICAS:

La participación en este ensayo clínico conlleva la obtención y utilización de muestras biológicas con fines de investigación, para lo que se observará la Ley 14/2007 de investigación biomédica y el Real Decreto 1716/2011, normativas que garantizan el respeto a los derechos que le asisten. Al firmar este documento, revisado y evaluado por el Comité de Ética de la Investigación con medicamentos que ha aprobado este ensayo clínico, usted acepta que se utilicen las muestras procedentes de su representado para las finalidades del presente estudio.

Algunas de las muestras se obtienen durante el seguimiento habitual de la enfermedad o proceso; otras son solicitadas porque son necesarias para cumplir con los objetivos de estudio. Entre las pruebas que se obtienen como parte del seguimiento habitual se encuentran análisis de hemograma, coagulación y datos de bioquímica básica; obtenidas en las visitas 1 4, 5 y 10. Como pruebas adicionales del estudio se encuentran el análisis de factores relacionados con la regeneración y recuperación del tejido cerebral que se obtendrán en las visitas 2,5 y 6.

Las muestras de sangre que forman parte del estudio habitual del ictus permanecerán almacenadas en el hospital donde se realiza la extracción (Hospital Universitario La Paz u Hospital Universitario Virgen del Rocío); y posteriormente parte de las muestras se analizarán en el Hospital Universitario Virgen Macarena y otra parte en el Hospital Universitario La Paz.

La cantidad extraída en cada análisis de sangre de seguimiento habitual serán tres tubos, mientras que en los análisis específicos del estudio serán cinco tubos. Cada tubo contiene aproximadamente 2 ml de sangre. Los posibles riesgos derivados del procedimiento realizado para la obtención de estas muestras estarán cubiertos por el seguro del ensayo clínico.

Las muestras estarán asociadas a un código que solo podrá ser relacionado con la identidad de su representado por personal autorizado (médicos, enfermeros y personal de laboratorio). Los datos que se deriven de la utilización de estas muestras se tratarán del mismo modo que el resto de datos que se obtengan durante este ensayo (ver apartado confidencialidad y protección de datos personales). Las muestras y los datos asociados se mantendrán bajo las condiciones de seguridad adecuadas y se garantiza que los sujetos no podrán ser identificados a través de medios considerados razonables por personas distintas a las autorizadas.

Durante el desarrollo del ensayo las muestras pueden ser analizadas en diversos laboratorios y se mantendrán almacenadas durante 10 años, en previsión de que fuera necesario repetir algún análisis adicional relacionado con los objetivos del estudio. Durante este tiempo, el responsable de las muestras será el promotor del ensayo. En caso de conservación para usos futuros de las muestras, se mantendrán almacenadas en el Instituto de Genética Médica y Molecular (INGEMM), Sección de Farmacogenética Hospital Universitario La Paz, durante 10 años. En el caso del Hospital Universitario Virgen Macarena serán almacenadas en su Biobanco.

Una vez finalizado el ensayo, las muestras sobrantes serán destruidas, a no ser que usted consienta para que puedan ser almacenadas y utilizadas en futuras investigaciones. La finalidad del almacenamiento de estas muestras es que sean utilizadas en proyectos de investigación en el futuro.

Si cambiara de opinión en relación con la donación de muestras biológicas y la cesión de los datos proporcionados, tiene derecho a solicitar su destrucción o anonimización, a través del médico/investigador/investigador principal de la colección. No obstante, debe saber que los datos que se hayan obtenido en los análisis realizados hasta ese momento podrán ser utilizados para los fines solicitados y podrán conservarse en cumplimiento de las obligaciones legales correspondientes.

En el caso de que en este ensayo se obtengan datos que pudieran ser clínica o genéticamente relevantes para su representado, e interesar a su salud o a la de su familia, podrá solicitar que le sean comunicados por su médico del ensayo si así lo indica en la casilla que aparece al final de este documento. No obstante, si usted hubiera indicado su negativa y cuando esta información según criterio del médico responsable, sea necesaria para evitar un grave perjuicio para la salud de su representado o la de sus familiares biológicos, se informará a un familiar próximo o a un representante, previa consulta al Comité de Ética Asistencial del centro. La comunicación de esta información se llevará a cabo por profesionales que le podrán explicar adecuadamente su relevancia y las opciones que se pudieran plantear. En caso de información genética clínicamente relevante podrá recibir el preceptivo consejo genético.

Hable con su médico sobre la posibilidad de que pueda establecer restricciones para que la muestra biológica de su representado no sea utilizada en determinadas investigaciones.

COSTE

Ni a su representado ni a usted les costará nada participar en el estudio y no recibirán ningún pago por participar. El coste de la terapia con células troncales la asumirá el hospital promotor del estudio gracias a una ayuda del Instituto de Salud Carlos III.

COMPENSACIÓN

Todas las pruebas médicas y tratamientos (tanto experimentales como de rutina) implican cierto riesgo de lesión. Si su representado nota o experimenta cualquier lesión relacionada con este estudio clínico, y se han seguido las instrucciones de sus médicos y del resto del personal de estudio, el promotor del mismo cubrirá los gastos médicos para tratar la lesión. No se ofrecerá ninguna compensación si la lesión fuera provocada por un incumplimiento de las instrucciones del personal de la investigación. El promotor no ofrecerá ninguna otra compensación.

SEGUROS

El hospital promotor del estudio ha contratado una póliza de seguro de Responsabilidad Civil con la compañía Hannover Seguros España.

CONFIDENCIALIDAD Y PROTECCIÓN DE DATOS PERSONALES:

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los pacientes participantes se ajustará a lo dispuesto en el Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 de Protección de Datos (RGPD).

-De acuerdo con lo que establece la legislación mencionada, usted puede ejercer los derechos de acceso, rectificación, oposición y supresión de los datos de su representado, para lo cual deberá dirigirse a su médico del estudio. También puede limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio. Para ejercitar sus derechos, diríjase al investigador principal del estudio o al Delegado de Protección de datos del centro: José Manuel Laperal González [tfno: 91426998/ e-mail: protecciondedatos.sanidad@madrid.org.

Le recordamos que los datos no se pueden eliminar aunque su representado deje de participar en el ensayo para garantizar la validez de la investigación y cumplir con los deberes legales y los requisitos de autorización de medicamentos. Si usted decide retirar el consentimiento para la participación de su representado en este estudio, ningún dato nuevo será añadido a la base de datos, pero sí se utilizarán los que ya se hayan recogido. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no quedara satisfecho

Tanto el Centro como el Promotor son responsables respectivamente del tratamiento de los datos y se comprometen a cumplir con la normativa de protección de datos en vigor. Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no se incluya información que pueda identificar a su representado, y sólo su médico del estudio/colaboradores podrá relacionar dichos datos con él/ella y con su historia clínica. Por lo tanto, su identidad no será revelada a ninguna otra persona salvo a las autoridades sanitarias, cuando así lo requieran o en casos de urgencia médica. Los Comités de Ética de la Investigación, los representantes de la Autoridad Sanitaria en materia de inspección y el personal autorizado por el Promotor, únicamente podrán

acceder para comprobar los datos personales, los procedimientos del estudio clínico y el cumplimiento de las normas de buena práctica clínica (siempre manteniendo la confidencialidad de la información).

El Investigador y el Promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, la información personal solo se conservará por el centro para el cuidado de la salud de su representado y por el promotor para otros fines de investigación científica si usted hubiera otorgado su consentimiento para ello, y si así lo permite la ley y requisitos éticos aplicables.

Si realizáramos transferencia de los datos de su representado codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos por las autoridades de protección de datos. Si el participante o usted quiere saber más al respecto, puede contactar al/a la Delegado/a de Protección de Datos del promotor o la institución Delegado/a de Protección de Datos del promotor o la institución. Alaro Avant, S.L.Avda. de Brasil 17, 7G, 28020, Madrid dpo.fiblapaz@alaroavant.com; 91112396.

APROBACIÓN DEL ESTUDIO:

Conforme a la legislación en España, este estudio ha sido revisado y aprobado por el Comité Ético de investigación Clínica (CEIC) del Hospital la Paz para su puesta en marcha el día

Además, la Agencia española de medicamentos y Productos Sanitarios (AEMPS) ha dado su autorización para la realización de esta investigación.

CONTACTO:

Si desea hacernos alguna pregunta o consulta no dude en contactar con el Dr..... del Servicio de Neurología del Hospital ..... (Teléfono: ……)

Código de protocolo: AMASCIS 02
Versión3.0 de 08 de Mayo de 2020

CONSENTIMIENTO INFORMADO PARA EL REPRESENTANTE LEGAL / FAMILIAR DEL PARTICIPANTE

TÍTULO DEL ESTUDIO: ADMINISTRACIÓN INTRAVENOSA DE CÉLULAS TRONCALES MESENQUIMALES ALOGÉNICAS DE TEJIDO ADIPOSO EN EL INFARTO CEREBRAL AGUDO. ENSAYO CLÍNICO FASE IIB MULTICÉNTRICO Y DOBLE CIEGO CONTROLADO CON PLACEBO.
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INVESTIGADOR COORDINADOR: Exuperio Díez Tejedor.
CENTRO: Servicio de Neurología. Hospital Universitario La Paz.

Yo (nombre y apellidos del representante legal / familiar) ……....., en calidad de (indique parentesco o relación) …….. de (nombre y apellidos del participante) …….. al ser su representante, afirmo que

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.

He hablado con el Dr.....

Comprendo que la participación de mi representado es voluntaria.

Comprendo que puede retirarse del estudio:

1. Cuando quiera.
2. Sin tener que dar explicaciones.
3. Sin que esto repercuta en sus cuidados médicos.

Deseo que la información derivada de la investigación que pueda ser relevante para su salud me sea comunicada.

◯ SI ◯ NO

Consiento el almacenamiento y uso de las muestras biológicas y de los datos asociados para futuras investigaciones en las condiciones explicadas en la hoja de información al paciente.

◯ SI ◯ NO

Consiento ser contactado en el caso de necesitar más información o muestras biológicas adicionales

◯ SI ◯ _NO

Recibiré una copia firmada y fechada de este documento de consentimiento informado.

Presto libremente mi conformidad para la participación de mi representado en el estudio.

FIRMA DEL REPRESENTANTE LEGAL / FAMILIAR	**FIRMA DEL INVESTIGADOR**
Fecha	Fecha

Author comment: Items not mentioned in the manuscript sent for revision are not applicable or otherwise specified in the original study protocol

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym **(Page 1)**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry. **(NCT04280003. EudraCT: 2019-001724-35**)
	2b	All items from the World Health Organization Trial Registration Data Set (not applicable)
Protocol version	3	Date and version identifier **(5thMay 2020, version 2)**
Funding	4	Sources and types of financial, material, and other support **(On pages 16-17)**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **(On page 1 and pages 18-19).**
	5b	Name and contact information for the trial sponsor **(Page 16).**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **(Pages 5-7)**

	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses **(Pages 7-8)**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **(Page 7)**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained). **(Page 7)**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **(Pages 8 - 10)**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **(Page 13)**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **(Page 13)**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **(Pages 8 and 13)**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended. **(Pages 10 and 11)**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **(Pages 11-13)**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **(Page 14)**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **(Page 8)**
Methods: Assignment of interventions (for controlled trials)		

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **(Page 10)**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **(Page 10)**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **(Pages 8 and 10)**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **(Page 10)**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **(Page 10-11)**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **(Page 15)**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **(Page 14 and 15)**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **(Page 15)**

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **(Page 15)**
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **(Page 15)**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **(Page 10)**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **(Page 15)**
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **(Page 17)**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **(Page 17)**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **(Page 17)**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **(included in the informed consent form as supplementary material).**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **(Page 18)**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **(Page 16)**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **(Page 18)**

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **(Page 18)**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **(Page 18)**
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **(Page 18)**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **(provided as supplementary data)**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable **(included in the informed consent form).**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.