PRINCIPLES AND PRACTICE OF CLINICAL RESEARCH
A Global Journal of Clinical Research

Protocol for a Pilot Phase IIa Randomized, Placebo-Controlled, Double-Blind, Crossover Study of Umbilical Cord-Derived Mesenchymal Stem Cells for the Treatment of Relapsing-Remitting Multiple Sclerosis: The MSCell Study

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Abstract

Introduction: *Multiple sclerosis (MS) is a chronic autoimmune, demyelinating, and neurodegenerative disease of the central nervous system. The relapsing-remitting (RRMS) form affects around 85% of patients initially. While current disease-modifying therapies (DMTs) reduce relapses and slow disability progression, they have significant side effects and do not promote neural repair. Umbilical cord-derived mesenchymal stem cells (UCMSC) offer the potential for immunomodulation, neuroprotection, and repair in MS without the intensive immunoablation required for autologous hematopoietic stem cell transplantation (AHSCT).*

Methods: *This single-center, randomized, double-blind, crossover phase IIa trial will evaluate the safety and feasibility of intravenous UCMSC administration in active RRMS patients. Participants will receive either UCMSC or placebo in a 1:1 ratio, with a crossover to the alternate treatment after 24 weeks. Primary outcomes include safety and feasibility, measured by adverse events assessed through recruitment, retention, and dropout rates. Secondary outcomes involve clinical and paraclinical indicators, including disability, quality of life, fatigue, mood disorders, and inflammation markers.*

Discussion: *This trial aims to address the safety and feasibility of UCMSCs in RRMS. Suppose both aspects are corroborated, and a trend of efficacy is noted in the exploratory outcomes. In that case, a more extensive study is warranted to further explore this therapeutic option with fewer side effects and the potential for enhanced neuroprotection and repair. Successful results may also lead to broader applications in other autoimmune and neurodegenerative diseases, fostering interdisciplinary collaboration and advancing MS research and treatment.*

Conclusion: *This exploratory phase IIa study will provide valuable insights into the safety and feasibility of UCMSC in treating RRMS, highlighting the need for further research to optimize dosing and administration routes and confirm efficacy in larger, more diverse populations.*

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by autoimmune, inflammatory, demyelinating, and neurodegenerative processes. MS affects about 2.8 million people globally, mostly young adults aged 20-40 (Filippi et al., 2018; Walton et al., 2020). Inflammation and immunologic damage target mainly the myelin in the brain, optic nerve, and spinal cord (Dobson & Giovannoni, 2019). The most common form, Relapsing-Remitting MS (RRMS), occurs in 85% of cases and features neurological relapses and remissions (Filippi et al., 2018). Treatments include symptomatic therapies and disease-modifying therapies (DMTs) to reduce relapses and slow disability. DMTs range from less effective, safer drugs to high-efficacy therapies like monoclonal antibodies. Still, they can have significant side effects, require careful monitoring, and do not stop disability progression nor promote neural

*Corresponding author: jvalverde@rmihealth.com
Received: June 26, 2024 **Accepted:** September 15, 2024
Published: November 28, 2024
Editor: Felipe Fregni
Reviewers: Andre Mateus, Roselyn Martin, Juan Godinez, Thaise Pedreira
Keywords: mesenchymal stem cells, multiple sclerosis, regenerative medicine, demyelinating disease, randomized controlled trial
DOI: https://doi.org/10.21801/ppcrj.2024.103.4

repair (Cross & Riley, 2022).

Stem cells (SCs) are undifferentiated cells capable of proliferation, self-renewal, and differentiation into various mature cell types. They are classified into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and neural stem cells (NSCs), each with unique derivation methods and differentiation potentials (Sivandzade & Cucullo, 2021). MSCs are multipotent non-hematopoietic cells that can differentiate into mesodermal, ectodermal, and endodermal lineages. Sourced from bone marrow, adipose tissue, umbilical cord, and dental pulp, MSCs are particularly promising for treating autoimmune diseases like MS due to their immunomodulatory properties and ability to promote tissue repair and neuroprotection. They can migrate to injury sites, suppress T cell activation, and enhance angiogenesis, making them suitable for regenerative medicine (Margiana et al., 2022; Musiał-Wysocka et al., 2019).

Autologous hematopoietic stem cell transplantation (AHSCT) is an approved therapy for severe or refractory MS, ideal for young patients with refractory relapsing MS who remain active despite DMTs and have no significant comorbidities. AHSCT shows high disease-free survival rates, reduced MRI activity, and improved or stabilized disability. The process includes harvesting stem cells from the patient's bone marrow, a myeloablative regimen, and reinfusing the stem cells to reset the immune system. While offering long-term remission and reduced relapses, it involves severe side effects like nausea, hair loss, infection risks, and long-term issues such as new autoimmune diseases and organ toxicity. Specialized centers and high costs limit accessibility, though treatment-related mortality has decreased (Miller et al., 2021; Sharrack et al., 2020; Muraro, 2017).

Over the past two decades, significant evidence supports using autologous bone marrow-derived mesenchymal stem cells (BMMSCs) in treating MS. BMMSCs are an excellent alternative to AHSCT as they do not require immunosuppression and have fewer severe side effects. Freedman et al. (2010) and the International MSCT Study Group convened an international expert panel to establish a consensus on using MSCs for treating MS and to develop protocols for cell culture and patient treatment. Llufriu et al. (2014) ran a phase II double-blind placebo-controlled trial in RRMS patients refractory to conventional therapies, showing safety and immunomodulatory benefits. Cohen et al. (2018) performed an open-label phase I study on expanded and cryopreserved MSCs in RRMS or secondary progressive MS, finding them safe and tolerable with preliminary benefits. The MESEMS trial (Uccelli et al., 2019, 2021) confirmed BMMSCs' safety in active MS across 15 centers in nine European countries despite not showing significant gadolinium-enhanced lesions (GEL) reduction compared to placebo.

Due to several practical advantages, previous exploratory clinical trials have recently focused on allogeneic umbilical cord-derived mesenchymal stem cells (UCMSCs) instead of BMMSCs. The procurement of UCMSCs is more accessible as they can be obtained from discarded healthy umbilical cords, avoiding ethical concerns associated with embryonic sources. The collection process is non-invasive, eliminating the need for bone punctures or liposuctions. Additionally, UCMSCs have a higher expansion capacity than other MSCs and can be cryopreserved for immediate use, thereby reducing the time required for harvesting autologous MSCs (4-6 weeks). Another advantage is that UCMSCs are unaffected by donor age or genetic predisposition to autoimmune diseases, which can impact the efficacy of autologous MSCs. Finally, UCMSCs exhibit robust immunomodulatory properties, making them a more attractive option for developing new treatment approaches for MS (Gugliandolo, 2020).

Although UCMSCs are allogeneic, they have reduced immunogenicity, which lowers the risk of immune rejection. However, some theoretical challenges include the potential for immune rejection, especially with repetitive applications. Additionally, there is a need for informed donor consent, the risk of transmitting infectious diseases, and potential tumorigenic or mutagenic risks. These necessitate stringent screening and safety protocols to mitigate these risks (Shokati et al., 2021).

Regarding the results of previous trials with UCMSC in MS, they include signals of improved disability, reduced relapse frequency, and positive MRI observations with minimal adverse events (Lu et al., 2013; Li et al., 2014; Meng et al., 2018; Riordan et al., 2018). However, data on the profile of the ideal patient to be selected for this therapy, the best route of administration, recommended dosage or application scheme, outcome measures, and follow-up are scarce or lacking. There are no comparative studies between the effect of BM and UCMSC in MS. Additionally, it is unknown whether UCMSCs are more efficacious when administered to a RRMS population with patients in a more inflammatory than degenerative stage of the disease.

This exploratory phase IIa pilot study aims to assess the safety and feasibility of intravenous UCMSC administration in RRMS patients compared to placebo. Participants will act as their controls, and placebo exposure will be limited to 24 weeks with rescue strategies available. The protocol is based on

the design of the MESEMS trial but uses UCMSCs instead of BMMSCs. We have selected a population with only the relapsing form of the disease, characterized by a more inflammatory stage, shorter disease duration, and less clinical disability compared to previous trials, considering the described mechanisms of action of MSCs. For the treatment arm, a single IV infusion of 150 million cells was selected, as the last research applied several dose protocols, with most ranging between 1 to 4 million cells per kg.

Materials and Methods

Study Design

This study is a single-center, randomized, double-blind, crossover, phase IIa trial designed to evaluate the safety and feasibility of UCMSCs in RRMS patients. Participants in the intervention arm will receive an intravenous (IV) infusion of UCMSC, while the control group will receive an IV 0.9% saline solution in a 1:1 allocation ratio. Based on recommendations from the MSCT Study Group (Freedman et al., 2010) and MESEMS (Uccelli et al., 2019), the study adopts a crossover design to address variability in MS progression. Each participant is their own control, enhancing statistical power without increasing sample size and ensuring all participants receive the potential treatment. The trial duration is 68 weeks, including a 12-week recruitment period, followed by 24 weeks for the first intervention period and a subsequent 24-week crossover to the alternate intervention (Figure 1 shows the timeline and stages of the trial).

This design ensures ethical considerations by minimizing the time without therapeutic effects. An additional 8-week period is allocated for statistical analysis and to compare outcomes between the two groups, culminating in the final report. Since the duration of the effect of a single intravenous dose of UCMSC in patients with RRMS is unclear, there is a potential risk of a carryover effect. In the protocols that serve as the basis for this study (Freedman et al., 2010; Uccelli et al., 2019), as well as in other preliminary studies with a similar design (Lublin et al., 2014; Petrou et al., 2020), a washout period, at the crossover point, is also not established. A recent meta-analysis on the use of stem cells in the treatment of MS included these four trials up to the crossover point to avoid the possible carryover effect (Nawar et al., 2024).

Only one study (Llufriu et al., 2014) showed a significant carryover effect after six months for GEL but not for the measured immunoregulatory cells. The authors used statistical methods with repeated measures in both intervention periods for each arm (24 weeks with MSC vs. 24 weeks with placebo) to try to quantify the carryover effect, as well as mixed-effects models, with carryover effect and participants as random variables, as a method to correct this effect on the results. No specific washout period was recommended for future studies.

Considering that this protocol is aimed at assessing the safety and feasibility of the intervention, and GEL is an exploratory outcome, it will follow the design recommendations of the international expert consensus (Freedman et al., 2010) and the European MESEMS study (Uccelli et al., 2019), without a washout period between the two interventions. The statistical section describes the methods that will be used to measure the potential carryover effect of the intervention on the secondary outcomes.

Search Setting

This trial will be conducted at the Regenerative Medicine Institute in San José, Costa Rica, a center for research and innovation in regenerative medicine. A convenience sample will be used. Recruitment will involve MS clinics within the Costa Rican public and private medical network, utilizing referrals from neurologists and MS patient associations. The protocol will be submitted to the CEC FUNIN (INCIENSA Foundation Institutional Review Board) for ethical and research approval. INCIENSA stands for the Costa Rican Institute for Research and Education on Nutrition and Health. The Regenerative Medicine Institute has permission from the Costa Rican Health Ministry to use MSCs for MS research, adhering to the national General Health Law, Biomedical Research Regulatory Law (No. 9234), and Good Clinical Practice guidelines.

Randomization and Allocation Concealment

Randomization into the UCMSC or placebo arm will be done using block randomization with block sizes of 3 and 5 blocks per arm to ensure adequate sample size balance. To ensure allocation concealment, the randomization process will be centralized and conducted by a Site Management Organization (SMO) using a computer-based random number generator (SealedEnvelope). The SMO, ICIMED (Institute for Research in Medical Sciences), is in San José, Costa Rica. The randomization list will remain unrevealed until the last included individual completes their follow-up evaluations.

Blinding, Assessment, and Unblinding

Participants and investigators will be blinded to the treatment assignments. All participants in the study

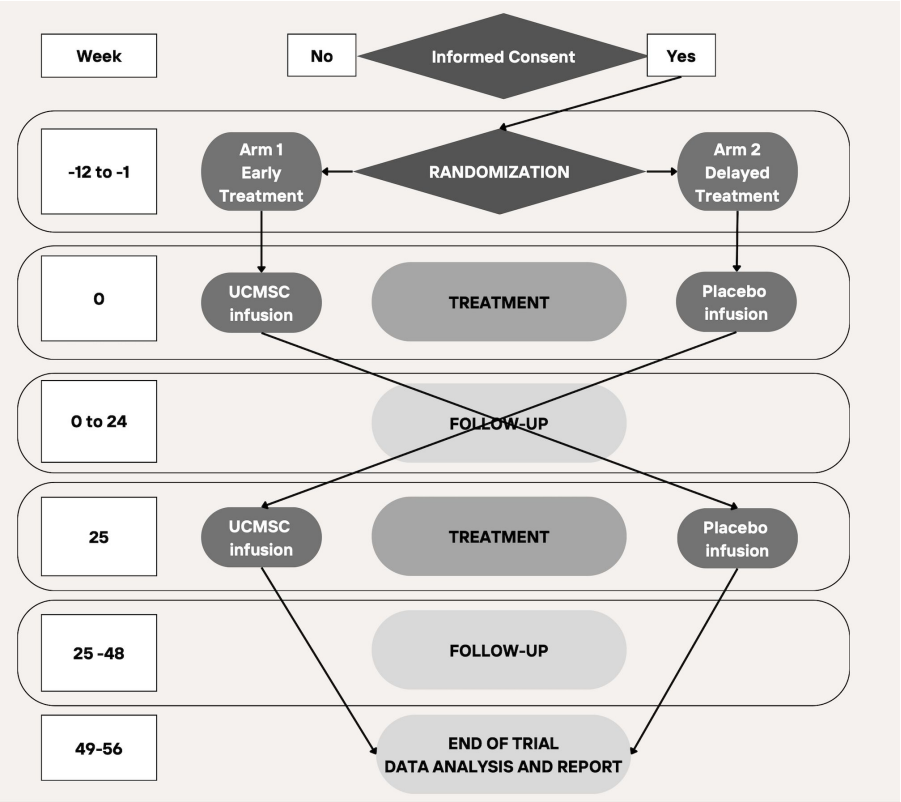

Figure 1: *Timeline and stages of the trial.*

will remain unaware of their treatment allocation until the end of the research and the completion of the locked data analysis. The Regenerative Medicine Institute will provide the UCMSC or placebo infusions in identical materials to avoid unblinding during application. The evaluators responsible for administering the clinical scales will also be blinded. The results of the neuroimaging and laboratory studies will be managed by centralized providers, who will be unaware of the participants' intervention assignments. Upon completing the research or exiting for any condition, sub-investigators will survey to determine if participants can predict their allocation group (Bang index), and the result will be used to assess the quality of the blinding.

In case of an emergency involving any study participant, it is recommended that the research team hold an extraordinary session to discuss the need for unblinding and proceed according to the best medical judgment to address the situation. The principal investigator is responsible for deciding on the need for unblinding. In their absence, a qualified physician attending the emergency may request this procedure. The principal investigator will report the event to an independent Safety Monitoring Committee (SMC). Details of the unblinding will be recorded in the case report form (CRF), including the reason for unblinding, the date of envelope opening, and the signature of the responsible personnel.

Participants

Inclusion Criteria

Participants must have a diagnosis of RRMS according to the 2017 McDonald criteria and be between 18 and 50 years old. The disease duration must be between 1 and 10 years from the first recorded clinical relapse. Eligible participants should have mild to moderate disability defined by an Expanded Disability Status Scale (EDSS) score of 1 to 4.5 points and exhibit disease activity within the last year. Disease activity is characterized by one or more clinical relapses (with the most recent relapse occurring more than 60 days before randomization), an increase in disability sustained for six months (EDSS increase of 1 point), or new or enlarging lesions on T2 sequence or GEL on MRI compared to a previous MRI within the same year. Participants must also be cognitively competent to provide informed consent, as judged by the investigator, and agree to participate in follow-up evaluations throughout the study.

Exclusion Criteria

Participants are excluded if they have had a clinical relapse within the last 60 days, have progressive

forms of MS (secondary progressive [SPMS] or primary progressive [PPMS]), or have an EDSS disability scale score of 5 or more points. Pregnant or breastfeeding women, as well as those without contraception, are excluded. Severe renal, cardiopulmonary, or hepatic disease, neurological deficits caused by diseases other than MS, and uncompensated metabolic or toxic disorders (including blood glucose, electrolytes, and medications) are grounds for exclusion. Participants with chronic or active infections (such as HIV, hepatitis B or C virus, or tuberculosis) or active neoplastic or hematological disease are not eligible.

Recent use of certain medications will result in exclusion: interferons, glatiramer, or corticosteroids within one month; immunosuppressants (including azathioprine, cyclophosphamide, mitoxantrone, cyclosporine, and mycophenolate), natalizumab, or fingolimod within three months; and anti-CD20 therapies (ocrelizumab, ofatumumab, rituximab), alemtuzumab, and cladribine. Cognitive impairment or any condition that limits the ability to provide informed consent, being part of a vulnerable population, or having any affiliation with the sponsor, institution, or researchers related to the study (including family members) are also exclusion criteria. Participants must reside in the country where the study is conducted and be able to attend follow-up visits. Finally, participation in other research that could affect the results or the ability to meet the follow-up requirements of this study is grounds for exclusion.

Recruitment Strategy

Participants will be enrolled using a convenience sampling technique with a directly accessible population of individuals with MS. A mixed recruitment strategy, both targeted and broad-based, will be employed. Among the former, email invitations to participate in the trial will be sent to the Costa Rican Association of Patients with Multiple Sclerosis (ASOCOEM), and referral letters will be sent to neurologists in public and private tertiary centers who manage MS in the country. Among the latter, study announcements will be made in traditional media (newspapers, TV) and digital platforms (The Regenerative Medicine Institute's website, social media) as per the media plan organized by the Regenerative Medicine Institute marketing department. Informative brochures will also be distributed in clinics and hospitals serving people with MS.

Screening and Consent

After recruitment, candidates meeting the 2017 McDonald criteria for RRMS and other inclusion criteria will receive detailed study information from a trained team member, either virtually or in person. A neurologist specializing in MS will review their diagnostic documentation. Candidates who agree to participate will undergo the informed consent (IC) process in a comfortable, private setting. The document will be read thoroughly, with all study points explained and questions addressed. Candidates may have support from a family member or trusted person via phone or video. Written IC is required before any study interventions. Participation is voluntary, and declining will not affect current services or their relationship with their physician. Participants can withdraw anytime without impacting their rights or quality of care.

Intervention

Control Group

The control arm will receive a placebo via an IV infusion of 0.9% saline solution. The saline will be delivered in bags identical in appearance and volume to those used for the intervention group. After 24 weeks, the control group will receive the actual intervention infusion.

Intervention Group

Participants in the intervention arm will receive an infusion of UCMSCs at a single dose of 150 million cells. After 24 weeks, this group will receive the placebo infusion (Kabat et al., 2019).

Before administering the intervention or control infusions, a pre-medication protocol will be followed to optimize patient comfort and minimize mild adverse reactions without affecting the treatment or disease. Thirty minutes before the IV infusion, participants will receive 10 mg of loratadine and 40 mg of famotidine orally. Steroids are excluded from the pre-medication regimen to avoid interfering with the anti-inflammatory and immunomodulatory properties of UCMSCs. If symptoms like fever, pain, or discomfort occur within six hours after the infusion, 1 gram of acetaminophen may be given orally. DMTs will be discontinued before the intervention, during the recruitment period, once the subject grants IC.

The UCMSCs used in this study are produced locally at the Regenerative Medicine Institute in San José, Costa Rica. Umbilical cords are obtained from voluntary, non-remunerated donations from mothers who give birth at local clinics and hospitals. These tissues are collected immediately after birth from

healthy mothers who have been given IC. To be eligible for donation, the newborns must be full-term and healthy. Additionally, the mother's family history is thoroughly reviewed to rule out significant hereditary diseases, including certain types of cancer and genetic disorders. Once collected, the umbilical cords undergo rigorous screening and analysis to ensure no signs of infection or contamination. Only tissues that pass these tests are processed for cultivation and expansion for therapeutic use.

UCMSCs are obtained using the explant technique. First, the cells are washed with phosphate-buffered saline (DPBS), and blood vessels are removed. The remaining tissue is cut into pieces about 2 mm in size and transferred to a tissue culture flask containing alpha-MEM medium supplemented with 10% fetal bovine serum. The tissue is incubated at 37°C with 5% CO2 in a humidified chamber. The tissue remains in culture for eight days and is monitored daily for cell colonies. Each enzymatic separation step is considered a passage, during which cell morphology and division rate are observed.

In passage five, cells undergo characterization, differentiation, review of sterile conditions, and karyotype analysis. Each batch is tested for sterile conditions, ensuring the absence of endotoxins, fungi, mycoplasma, and aerobic and anaerobic bacteria and confirming cell viability over 75% after thawing. On harvest day, cells are detached using TrypLE Express. UCMSCs are characterized by surface markers CD105, CD73, CD44, and CD90 (>90%) and the absence of CD45, CD34, HLA-DR, CD19, and CD11b (<5%) through flow cytometry. They are also tested for their differentiation potential into adipocytes, chondroblasts, and osteoblasts. The cells are cryopreserved in vials at a concentration of 4×10^6/ml in PlasmaLyte A with 10% dimethyl sulfoxide and Hestar starch and stored in the gas phase of liquid nitrogen below -150°C.

Outcomes

The primary outcome is to determine the safety and feasibility of IV therapy with UCMSCs in subjects with RRMS. Safety will be assessed by the number and severity of adverse events in each study arm, classified according to the Common Terminology Criteria for Adverse Events (CTCAE). The feasibility of the intervention will be measured as a co-primary outcome, including the recruitment, retention, and dropout rates of participants, as well as the costs associated with the necessary supplies per patient, to assess the logistic and economic viability of UCMSC treatment.

The secondary outcomes are divided into clinical and paraclinical indicators of efficacy. For clinical variables, the number of demyelinating relapses in each research group will be recorded for each study period to compare relapses before and after the treatment. Disability will be assessed by changes in the EDSS from baseline to the end of each study period. Quality of life will be evaluated using the Multiple Sclerosis International Quality of Life (MUSIQoL) scale, with scores from baseline to the end of the study. Fatigue will be assessed by changes in the Modified Fatigue Impact Scale (MFIS) from baseline to the end of each study period. Depression and anxiety will be measured using the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI), respectively, with scores compared from baseline to the end of each study period. All evaluations will be conducted by researchers who have been trained to standardize measurements and ensure result reliability.

For paraclinical variables, active demyelinating lesions will be evaluated by counting GEL in brain MRI. The number of lesions will be compared from baseline to the end of each study period. Proinflammatory and anti-inflammatory cytokines will be evaluated by changes in levels of proinflammatory cytokines (TNFα, INFγ, IL17) and anti-inflammatory cytokines (IL4, IL10) in serum, measured before and after treatment in each study period. Neuroaxonal damage markers will be assessed by changes in serum neurofilament light chain (NfL), a marker of neuroaxonal damage, measured at baseline and each study period.

Data Management and Monitoring

The personal data of participants will not be exposed in any research documents. Each subject will be assigned a unique code exclusively for this study to protect clinical, laboratory, and imaging information. Data will be collected using an approved standardized electronic Case Report Form (CRF) at each contact and review point. The study will use a secure, validated electronic medical record (EMR) system compliant with RMI's and IRB's data protection policies. An external data monitor provided by the SMO will review discrepancies in the CRF, ensuring data verification, correction, and validation.

The database maintained by the research team will be validated and secured according to RMI's standard operating procedures. Upon completion, the database will be locked and transferred for analysis. After the research concludes, participants' personal information and essential documents will be coded, archived, and monitored by RMI's Research Unit (UDI) for 30 years, per national

regulations. Published study data must comply with the publication policy and national law. All archived documents must remain available for inspection by appropriate authorities upon request.

Sample Size Calculation

This trial plans to randomize 30 subjects, with 15 individuals per study arm, following Billingham et al.'s general recommendation for sample size in a pilot trial (2013). Previous pilot trials involving MSCs for MS in single centers have recruited between 3 and 24 patients (Meng et al., 2018; Cohen et al., 2018; Llufriu et al., 2014; Li et al., 2014). No power calculation was performed.

Statistical Analysis

Data will be analyzed as per protocol. It will be assumed that the rate of missing data is low (<10%) and follows a mechanism of missing completely at random (MCAR), allowing for complete case analysis to be performed to avoid substantial bias. Statistical analyses will be conducted using STATA/IC software version 17. The distribution of continuous data will be assessed using the Shapiro-Wilk normality test, skewness, and kurtosis. Given the small sample size, it is expected that continuous variables will not have a normal distribution shape; therefore, non-parametric tests will be applied.

The primary outcome of safety, specifically the incidence and severity of adverse events between treatment groups, will be compared using the chi-square test at weeks 24 (period one or crossover point) and 48 (period 2, end of the study). Feasibility will be measured by calculating the trial's recruitment, retention, and dropout rates, as well as the average cost per patient.

The number of demyelinating relapses in each research group will be compared at weeks 24 and 48 using negative binomial regression analysis. The remaining clinical secondary outcomes will be measured at three-time points: baseline, 24 weeks, and 48 weeks. All variables expressed as changes in clinical scales are measured relative to the baseline score, including the scales used for measuring disability (EDSS), quality of life (MUSIQoL), fatigue (MFIS), depression (BDI), and anxiety (BAI). Comparisons of the results of these measures between the two arms at weeks 24 and 48 will be conducted using the Mann-Whitney U test. To evaluate the potential carryover effect of the intervention with UCMSC, comparisons within the same group at weeks 24 and 48, relative to their baseline, will be conducted using the Wilcoxon signed-rank test.

Regarding the paraclinical secondary outcomes, the total number of GEL in the MRI at weeks 24 and 48 will be compared between treatment groups using negative binomial regression analysis, adjusting for the number of GEL at baseline. A potential carryover effect will also be analyzed by comparing the number of GEL within each arm at weeks 24 and 48 using the Wilcoxon signed-rank test. The changes in the levels of proinflammatory cytokines (TNFα, INFγ, IL17), anti-inflammatory cytokines (IL4, IL10), and markers of neuroaxonal damage (NfL) in serum relative to their baseline levels will be measured between groups at weeks 24 and 48 using the Mann-Whitney U test. Comparisons within each arm of the trial will be conducted at weeks 24 and 48 using the Wilcoxon signed-rank test to check for any potential carryover effect.

Discussion

The application of UCMSC in treating MS offers a promising and innovative approach to addressing this debilitating disease. Previous trials with mesenchymal stem cells have demonstrated the safety and feasibility of using IV infusions of autologous BMMSC without immunosuppression in treating RRMS (Llufriu et al., 2014; Cohen et al., 2018; Uccelli et al., 2021). Additionally, some exploratory trials have addressed the safety of IV UCMSC infusions in individuals with MS. Still, they have used different dose schemes, routes of administration, and mixed stages of the disease. None of these trials has a crossover design or has tested the feasibility of this intervention (Li et al., 2014; Meng et al., 2018; Riordan et al., 2018; Jamali et al., 2024).

Moreover, there is a significant knowledge gap regarding the efficacy of UCMSC in humans, particularly in comparison to autologous hematopoietic stem cell transplantation with immunosuppression, an already approved therapy for refractory MS. This study aims to confirm the safety and feasibility of using UCMSC in subjects with RRMS, a more inflammatory and immune-active phase of the disease, considering the main biological mechanisms of action of the cell therapy and the necessary logistics and resources to develop this new treatment. Secondary outcomes are set to explore trends in crucial clinical variables (disability, fatigue, quality of life, mood) and surrogate markers (active lesions on MRI, inflammatory and neuroaxonal damage markers).

The impact on clinical practice and patient outcomes could be significant if the trial yields positive results regarding safety, feasibility, and trends in improving clinical and paraclinical variables. Demonstrating UCMSC therapy as a safe and feasible treatment option for RRMS could offer a new, viable al-

ternative for patients, potentially reducing the side effects of immunosuppression. Establishing the feasibility and optimal logistics, including dose and administration routes, would help develop standardized treatment protocols, facilitating wider adoption in clinical practice. Positive trends in clinical scales of disability, fatigue, mood, and quality of life would justify larger, multicenter trials to validate efficacy and safety further, potentially leading to regulatory approval and widespread clinical use. Moreover, the neuroprotective and reparative effects suggested by improvements in surrogate markers, such as active lesions on MRI and the regulation of inflammatory and neuroaxonal damage markers, could indicate a disease-modifying capability. This could expand the treatment landscape for RRMS, offering a new, effective, safer therapeutic option that enhances patient outcomes and quality of life.

One of the strengths of this study is its refined patient selection, which improves upon previous trials by including only patients in the inflammatory phase of the disease, defined by the relapsing-remitting stage within the first decade of evolution. A broader range of UCMSC doses (1-3 million per kg) is also applied to detect efficacy signals without compromising the safety profile. The crossover design reduces individual variability with a smaller sample size, allowing for more affordable recruitment. Additionally, the trial aims to provide efficacy signals in both clinical and laboratory settings, exploring the impact of UCMSC on validated clinical scales and surrogate markers.

However, the study has potential limitations. As a single-center pilot trial with a small sample size, it may not fully represent the broader population, and the results may not be sufficient to draw general conclusions. The intravenous route of administration, while less invasive and more accessible, lacks definitive evidence of being the most effective method. Additionally, based on previous trials and reports, the selected dose may not represent the optimal dose, necessitating larger studies to establish the best dosing regimen. There is also a risk of recruitment failure and the possibility of severe relapses or adverse effects related to the intervention. It should also be noted that progressive forms of MS are not within the scope of this study.

In conclusion, this trial is expected to yield new information on the safety and potential efficacy of UCMSC in treating RRMS. It highlights the need for further research to establish the optimal dosing and administration routes and to confirm the efficacy of UCMSC in larger, more diverse populations. If the findings of this study demonstrate clinical or paraclinical benefits, they could lead to more extensive and definitive clinical trials, potentially resulting in improved therapeutic options for MS patients.

Ethical Issues and Reporting Policies

As stated before, the study protocol will be submitted to the Institutional Review Board of INCIENSA (CEC FUNIN). IC will be obtained from all participants. The results of this study will be disseminated through peer-reviewed journals and scientific conferences.

For ethical reasons, since the placebo arm will spend 24 weeks without a baseline DMT, the safety monitoring plan includes a designated trained health provider (nurse or physician) for follow-up surveillance, including adverse events or multiple sclerosis relapses. This monitoring will occur immediately after the infusion and for the next 3 hours on days 3 and 7 after the injection and on weeks 4, 8, 12, 16, and 24. The same approach will be applied to the treatment arm to avoid performance bias.

Non-serious adverse events will be treated symptomatically in any participant. In the event of a multiple sclerosis relapse during the trial, immediate intervention is warranted by the research team. This includes using IV steroids when needed, resuming the baseline treatment, withdrawing the patient from the protocol, and managing the missing data with a statistical imputation method. Should the participant discontinue study treatment, they will be followed for safety according to the protocol safety monitoring plan. The study participant will receive follow-up communication to ascertain the outcome until it is resolved.

This study will be stopped if any of the following events occur: the subject's death, any serious adverse events determined to be related to the study (including a CTCAE version 5 grade 4 or 5 adverse effects, irrespective of attribution), any CTCAE grade 2 adverse event that persists for more than two weeks, or any grade 3 adverse event that occurs within 72 hours after product administration.

Acknowledgement

José Rojas, MD., Johanna Barrantes, Natalia Jiménez. All part of the Research Unit of the Regenerative Medicine Institute, who made kind recommendations for this protocol. To the staff of Regenesis Labs SA and all participants involved in the study.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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