Peer-Review comments and authors responses

Reviewer 1
Recommendation: Accept Submission

Congratulations. The issue is highly important and needs to be addressed. The previous studies encourage us to go ahead with testing this novel and hopeful new treatment.

We are deeply grateful for your positive feedback and recommendation to accept our submission. We appreciate your recognition of the importance of the issue and the potential of novelty for the approach. Your encouraging words reinforces us to proceed with our research and motivates our team. We are committed to advancing this work and addressing the critical needs in this field. Thank you once again for your valuable time and consideration.

Reviewer 2
Recommendation: Revisions Required

1. *METHODS*

   The manuscript per se is very good, but I suggest that the authors review their trial design since there are some concerns:

a) *I would suggest recruiting patients with a lower EDSS (currently, the trial suggests 5.5 as the maximum point, but that is bordering a severe disability with MS) since the patients will not have treatment for a long time.*

   Thank you for your feedback regarding the inclusion criteria for our trial. We appreciate your suggestion to recruit patients with a lower EDSS, and we agree that this adjustment will enhance the safety of our study. In response to your recommendation, we have decided to set a new maximum EDSS score of 4.5. This revised criterion corresponds to moderate disability, where patients are able to walk without aid for approximately 350 to 550 yards. We believe this adjustment will balance patient safety with maintaining an adequate recruitment pool. **The corrected inclusion criteria can be found on page 8, in the last paragraph of the section 'Participants. Inclusion Criteria.'**

b) *I also suggest reducing the timing between treatment exposures to avoid ethical concerns about the patients' lack of medication.*

We share your concern and have thoroughly discussed this issue within our team. Based on preclinical and previous pilot trials using MSCs in MS, it has been observed that the biological effect of the therapy on surrogate markers (such as MRI lesions and inflammatory markers) peaks after 2 to 3 months and may last up to 6 to 12 months, depending on the dosage and route of administration (Li et al., 2014; Llufriu et al., 2014). Therefore, reducing the time between treatments would necessitate a longer washout period to cross the arms of the study, ultimately resulting in a duration of at least 6 months to avoid significant carryover effects. This rationale aligns with the MESEMS study (Uccelli et al., 2019), where a 6-month period was approved as a prudent crossover point.

To address the potential ethical issues of having a group of research subjects on placebo for 24 weeks, we have included in the safety monitoring protocol a designated healthcare provider to follow up during the trial, monitoring adverse effects or MS relapses in both arms, thereby avoiding performance bias. **The detailed explanation of the safety monitoring protocol can be found on page 16, in the second paragraph of the section 'Ethical Issues and Reporting Policies.'**

In the event of a multiple sclerosis relapse during the trial, we will immediately intervene with IV steroids, resume the baseline treatment, withdraw the patient from the protocol, and manage the missing data with a statistical imputation method. Additionally, we have established stopping rules in the protocol. **These approaches can be found in the last 2 paragraphs of the same section on page 16.** We believe these measures will mitigate ethical concerns while maintaining the integrity of the study. We appreciate your feedback and the opportunity to clarify our study design.

c) *I suggest including other centers and no single center for higher statistical significance.*

We completely agree that a multicenter trial is the ideal approach for robust statistical power. However, in terms of feasibility, we explored if we could recruit enough patients for the trial, considering specific conditions in the Costa Rican multiple sclerosis population. As the prevalence of the disease in the country is reported to be 9 per 100,000, the target population would roughly be 500 patients. More than 90% of these patients are managed within the social security network, which does not have a well-developed infrastructure for clinical research.

To reach the accessible population, we designed a convenience sampling method, utilizing multiple sclerosis specialist referrals from the main centers of the social security network, as well as from private centers and the national association of multiple sclerosis patients. Practically speaking, potential subjects for the trial will

come from different health centers in the country but will be concentrated in a single research center for trial management. Given that this is the first trial of its kind in the country, we preferred a conservative approach regarding the recruitment rate.

Additionally, we hope that this protocol will generate interest in the international scientific community. If the safety of the intervention is confirmed, it could pave the way for an international, multicenter Phase III study based on this protocol.

Reviewer 3
Recommendation: Revisions Required

1. *ABSTRACT:*
   It is clear and concise, summarizing the key aspects of the study. However, it could be improved if the authors included a statement on the expected implications of the possible findings of the proposal to emphasize the study's significance. In addition, the abstract states that the study "aims to address the effect of UCMSC," while previously, it stated that the objective is to "evaluate the safety and feasibility of intravenous USCMSC," leaving "clinical and paraclinical" outcomes as a secondary endpoint. These differences need to be reconciled.

   We have reconciled the differences in the stated objectives and provided a statement on the expected implications of our potential findings. **The revised Abstract can be found on page 2.**

2. *INTRODUCTION:*
   It is neatly written, outlining the prevalence and impact of multiple sclerosis (MS), particularly the relapsing-remitting form (RRMS), while highlighting some of the limitations of current treatments and introducing the potential benefits of umbilical cord-derived mesenchymal stem cells (UCMSCs). It also mentions and compares UCMSCs with other stem cell therapies currently available. However, this section could be improved if the authors provide a more detailed rationale for why UCMSCs should be chosen over other types of stem cells, which are also mentioned in this section. Some aspects require some clarifications, e.g., the penultimate paragraph of this section states that data from exploratory trials evaluating UCMSCs in MS are promising. In contrast, the last paragraph states that the main objective of the proposal is to evaluate the feasibility of USCMCs. These apparent discrepancies in the section need careful analysis and clarification. In addition, a more detailed explanation of how the authors selected the dose would be helpful to fully understand this choice.

We have reviewed the Introduction section to enhance clarity and provide a more comprehensive rationale for choosing UCMSCs over other types of stem cells. **In paragraph 5 of this section, on page 5, we highlight the practical advantages of using UCMSCs** over other types of stem cells.

We have also reconciled the statements regarding the objectives of our study. The revised text now states that the trial aims to assess the safety and feasibility of intravenous UCMSC administration in RRMS patients, with exploratory outcomes assessing efficacy trends to justify a larger study.

Lastly, we have included an explanation of how we selected the dose for the treatment arm. A single IV infusion of 150 million cells was chosen based on previous research that applied various dose protocols, with most ranging between 1 to 4 million cells per kg. **This information can be found in the last paragraph of the Introduction, on page 6.**

3. *METHODS:*

   a) It is detailed and well-structured, describing the study design, setting, randomization, blinding, participant criteria, recruitment strategy, and interventions. It effectively addresses the primary and secondary outcomes and outlines the data management and monitoring procedures. However, some aspects need further clarification: there is no mention of potential carryover effects that could affect the design choice, possibly confounding the results. In addition, no mention is made about a possible required wash-out period, particularly with a treatment that may have long-lasting effects, as is the case of USCMSs, making the analysis of any possible treatment effect challenging. Finally, logistical concerns may arise given the complexity of implementing an intervention on a group of patients that, according to the selection criteria, may have a debilitating and progressive course of their disease. These aspects require careful planning, which is overlooked in the protocol.

This is an extremely relevant comment for the study design and challenging to address due to the limited pre-existing information. An expanded explanation about the issues of carryover effect and washout period has been added in the last three paragraphs of the Study Design section, on pages 6 and 7.

Furthermore, it is clarified that comparisons within each group at weeks 24 and 48 will be analyzed using the Wilcoxon signed-rank test to address the potential carryover effect for the clinical and paraclinical secondary outcomes. This information can be found in the last paragraph of the Statistical Analysis section, on page 14.

b) *The selection criteria may benefit from rewriting, giving it a clearer description instead of using a bulleted list.*

Following your suggestion to improve the clarity of the selection criteria, we have revised this section to provide a clear and detailed description without using a bulleted list. This updated selection criteria can be found in the section on Participants, Inclusion, and Exclusion Criteria, on pages 8 and 9.

c) *As previously mentioned, the outcomes section appears to contradict previous sections on the document. It states that the primary outcome is to determine the safety of IV therapy, while previous sections state that the objective is to evaluate its feasibility without addressing the terms in which said feasibility would be measured. These differences need to be addressed.*

We have addressed the differences and clarified that the primary outcome is to determine both the safety and feasibility of IV therapy with UC-MSCs 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 UC-MSC treatment. These clarifications ensure consistency across the document, and they can be found in the Abstract, as mentioned in the first correction, and in the first paragraph of the Outcomes section, on page 11.

d) *It is recommended that some sections be reviewed, as they may require additional work on the translation. For instance, the authors mention "sterility" when they refer to "sterile conditions."*

We have thoroughly reviewed the entire document to ensure clear and accurate language use. Our team, including native English speakers, has confirmed that the document is both precise and understandable.

e) *The authors may consider adding more details on the statistical analysis methods and justifying the sample size. In addition, this section similarly presents some inconsistencies, as it states that analysis will only consider descriptive aspects while stating that inferential statistics will be drawn using non-parametric techniques, while later in the text stating that data distribution will be assessed to select the type of statistical techniques to be used. Such inconsistencies need to*

be addressed and rectified. Another aspect of the analysis that raises concerns is that it is stated that ANOVA will be used when, as previously mentioned, authors state they will opt for non-parametric methods. Finally, the analysis of the clinical markers of inflammation mentioned in the discussion section is not mentioned.

After your valuable observation, we have carefully reviewed and completely rewritten the sections on Sample Size Calculation and Statistical Analysis on pages 13-14, to provide a clearer and more precise explanation of the sample size selection and the statistical plan for each outcome. These revisions address the inconsistencies noted in the initial submission and ensure that the statistical analysis methods are detailed and justified appropriately.

4. *DISCUSSION:*

This section provides a well-balanced view of the study's potential strengths and limitations, contextualizing the findings within the broader research field and suggesting future directions. It could be strengthened by including a comparison of the expected results with existing studies and elaborating on how the findings could influence clinical practice. It should also include a critical analysis and discussions of the literature review and limitations of the study.

We have reviewed the entire section to ensure clearer redaction and have also added information to strengthen the potential impact of the trial's findings. This one **can be found in the third paragraph of the section Discussion, on page 14**, where we elaborate on the expected results, considering existing studies and how the findings could influence clinical practice. The section also includes a concise mention of the study's strengths and limitations.

Reviewer 4

Recommendation: Revisions Required

This study has a very detailed and well-designed protocol. The selected study design is appropriate for a phase II trial to investigate feasibility and safety and can also evaluate some preliminary clinical indicators of efficacy. Crossover allows each patient to function as their own control, reduces individual variability, and increases the statistical power of the study. The protocol evaluates ethical and regulatory considerations, informed consent, randomization, and allocation concealment, all in accordance with Good Clinical Practice Guidelines. Safety and feasibility results, together with clinical outcomes, may improve the assessment of the therapeutic potential of UCMSC.

1. METHODS:

a) *I suggest attaching as complementary material all the scales that you will use in the secondary outcomes.*

We have added complementary material with links to access the outcome measure tools: Common Terminology Criteria for Adverse Events (CTCAE) for safety, Expanded Disease Status Scale (EDSS) for disability, Modified Fatigue Impact Scale (MFIS) for fatigue, Multiple Sclerosis International Quality of Life (MUSIQoL) questionnaire, Beck's Depression Inventory (BDI), and Beck Anxiety Inventory (BAI). **This one can be found as an attached supplementary document.**

b) *Regarding intravenous administration, which you cite as a limitation, it is also a point of concern of unblinding. Due to the side effects of the treatment, patients may notice which arm they are currently allocated, and this may interfere with the results, especially in qualitative analysis.*

We acknowledge that IV administration may be seen as a limitation due to the scarcity of information on the optimal route. Previous trials have employed combined IV and intrathecal (IT) routes (Jamali et al., 2024), while others have used a single IV or IT route (Cohen et al., 2018; Uccelli et al., 2021), but comparative trials to define the best method are lacking. However, IV administration can also be viewed as an advantage because it is minimally invasive compared to IT or brain intraarterial administration, which could facilitate broader clinical application if the IV route for UCMSC is proven safe and effective.

c) *Regarding the risk of unblinding due to treatment side effects, previous crossover trials with IV BMMSC in MS (Uccelli et al., 2021; Llufriu et al., 2014) reported no serious adverse effects in the treatment arms, and minor adverse effects were not significantly different from the placebo arms. In parallel group trials with IV infusions of UCMSC versus placebo (Li et al., 2014), no significant adverse effects were reported in any arm after one year of follow-up. While fatigue, headache, and slight fever are potential side effects of MSC application, they are more common with the IT route (Jamali et al., 2024).*

To address these concerns, we have developed a standard protocol for both arms. This protocol includes administering 10 mg of loratadine and 40 mg of famotidine orally 30 minutes before the IV infusion. If symptoms such as fever, pain, or discomfort occur within six hours after the infusion, 1 gram of acetaminophen may be given orally. Steroids will not be administered prior to the infusion as they

can interfere with the biological function of MSC. This approach is highlighted in the Intervention group section, on page 10. Also, independent clinical evaluators will be employed, as well as interpreters for neuroimaging studies, laboratory results, and safety data monitoring. Furthermore, to better manage the possibility of unblinding, the quality of blinding during the trial will be assessed using the Bang index. This approach is described in the Section Blinding, Assessment, and Unblinding section, on page 8.

d) *Regarding the dosage regimen, considering a dose-ranging phase II trial may be more effective at this specific point, as it can help determine the optimal dosage for the best therapeutic response and minimize side effects.*

We do agree that a dose-ranging phase II trial can be effective in determining the optimal dosage for the best therapeutic response while minimizing side effects. However, our approach is based on the current evidence and specific considerations for this study. Although a dose-finding trial for UCMSC was recently published (Jamali et al., 2024), it included IV and IT infusions in a repeated manner, which does not allow for conclusions on the effectiveness of each route or the real effect of a single infusion. Consequently, we based our protocol's dosage on doses that have already been proven safe for MSCs (whether bone marrow or umbilical cord-derived) in previous trials (Cohen et al., 2018; Uccelli et al., 2021; Li et al., 2014; Llufriu et al., 2014). These trials demonstrated that doses ranging from 1 to 4 million cells per kg are safe, with only minor adverse events reported.

Considering that our route of administration is IV through peripheral access, we aimed to avoid pain and inflammation of the veins. Previous studies have shown that doses up to 200 million cells are safe for a single IV administration. Thus, we decided on a dose of 150 million cells to avoid access complications and the risk of unblinding, while still achieving a range of 1 to 3 million cells per kg in individuals weighing between 50 to 150 kg. We believe this approach balances safety and efficacy, while addressing the practical considerations of the trial.

e) *I've also made some corrections over grammar and punctuation and attached the file below. Two sentences are marked due to plagiarism, and it would be nice if you try to re-write them with your own words. Overall, this protocol is very good and will contribute significantly to the knowledge of UCMSC in patients with RRMS.*

Thank you for your meticulous review and the corrections regarding grammar and punctuation. We have addressed the issues you highlighted and have rewritten the

two sentences marked for plagiarism to ensure originality. The entire document has been reviewed to maintain correct use of language, readability, and logical flow while avoiding any risk of plagiarism.

To all the reviewers, your insights have greatly contributed to the improvement of our manuscript, and we deeply appreciate the time and effort you have dedicated to reviewing our work. We are confident that the revisions have strengthened our study, and we look forward to contributing to the ongoing research in this field.