## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Allogeneic adipose tissue-derived mesenchymal stem cells in ischaemic stroke (AMASCIS-02): A phase IIb, multicentre, double-blind, placebo-controlled clinical trial protocol |
| **AUTHORS** | de Celis-Ruiz, Elena; Fuentes, Blanca; Moniche, Francisco; Montaner, J.; Borobia, Alberto; Gutiérrez-Fernández, M.; Díez-Tejedor, Exuperio |

## VERSION 1 – REVIEW

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| **REVIEWER** | Stefanom Ricci<br>UO Neurologia, USL Umbria 1, Città di Castello, Italy |
| **REVIEW RETURNED** | 20-Apr-2021 |

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| **GENERAL COMMENTS** | This is an interesting protocol of a phase 2 study on AD-MSCs in acute ischaemic stroke. I have just two questions to Authors:<br>1) Can they clarify why they decided to accept to start treatment within 4 days (and not 36h, as suggested by previous work)?<br>2) Can Authors tell us who will score the Rankin scale during follow ups and how (i.e. telephone interview? face to face?, interview with the carer?)? |

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| **REVIEWER** | Raffaele Ornello<br>University of L'Aquila Department of Clinical Sciences and Applied Biotechnology |
| **REVIEW RETURNED** | 22-Apr-2021 |

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| **GENERAL COMMENTS** | Authors proposed an interesting protocol of treatment with adult allogeneic adipose tissue-derived stem cells in patients with ischemic stroke randomized within the first 4 days from onset.<br>The study has important translational implications. I only have some minor concerns regarding the inclusion and exclusion criteria.<br>1 - Please explain the reasons for the NIHSS score range (8-20) to include patients in the trial.<br>2 - Authors state that, according to previous literature, patients benefit the most from the administration of stem cells within 36 hours from stroke onset. Therefore, it is unclear why Authors decided to extend their treatment window to 4 days.<br>3 - Please report whether the study will include patients treated with revascularization procedures, as it is not clear. |

## VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Stefano Ricci, UO Neurologia, USL Umbria 1, Città di Castello, Italy
Comments to the Author:

This is an interesting protocol of a phase 2 study on AD-MSCs in acute ischaemic stroke. I have just two questions to Authors:

Answer: Thank you very much for your interest in our work. Below are the answers to both questions.

1) Can they clarify why they decided to accept to start treatment within 4 days (and not 36h, as suggested by previous work)?

Answer: Indeed, the MASTERS trial included patients treated within a 24–48-h window, and the authors suggested according to a post hoc analysis that the early administration window (<36 h) could provide better functional outcomes. However, there is not enough evidence to restrict the time window to a 36- or 48-h period. In fact, the MASTERS-2 trial is currently exploring this <36 h time window. From a practical point of view, to ensure feasibility of AD-MSC administration, a 4-day time window seems appropriate, given the cells need to be defrosted and undergo cell maintenance and security checks before they can be packed and sent to the recruiting centres.

2) Can Authors tell us who will score the Rankin scale during follow ups and how (i.e. telephone interview? face to face?, interview with the carer?)?

Answer: The modified Rankin Scale will be evaluated by the investigators (stroke neurologist certified and with broad experience in rating the following stroke scales: NIHSS, ERm). The evaluation will be performed face-to-face whenever possible. In suboptimal situations in which the patient is unable to attend clinics, evaluation will be performed by telephone or by using a tablet that permits videoconference. Evaluations can be performed interviewing the patient's caretaker in those situations in which they themselves cannot appropriately transmit the required information.

Reviewer: 2
Dr. Raffaele Ornello, University of L'Aquila Department of Clinical Sciences and Applied Biotechnology
Comments to the Author:
Authors proposed an interesting protocol of treatment with adult allogeneic adipose tissue-derived stem cells in patients with ischemic stroke randomized within the first 4 days from onset.
The study has important translational implications. I only have some minor concerns regarding the inclusion and exclusion criteria.

Answer: We appreciate your comments and hope that our answers can clarify any matter related to the inclusion and exclusion criteria.

1 - Please explain the reasons for the NIHSS score range (8-20) to include patients in the trial.

Answer: It is common practice to choose this range of NIHSS score, given it reflects enough stroke severity to be able to detect improvement after treatment with AD-MSCs. Lower NIHSS scores would likely correspond to smaller infarct sizes; thus, a possible improvement from study treatment might not be noted. Also, higher NIHSS scores reflect very large infarct sizes with lower survival rates that could also underestimate treatment effect.

2 - Authors state that, according to previous literature, patients benefit the most from the administration of stem cells within 36 hours from stroke onset. Therefore, it is unclear why Authors decided to extend their treatment window to 4 days.

Answer: Indeed, the MASTERS trial included patients treated within a 24–48-h window, and the authors suggested according to a post hoc analysis that an early administration window (<36 h) could provide better functional outcomes. However, there is not enough evidence to restrict the time window to a 36- or 48-h period. In fact, the MASTERS-2 trial is currently exploring this <36 h time window. From a practical point of view, to ensure feasibility of AD-MSC administration, a 4-day time window seems appropriate, given the cells need to be defrosted and undergo cell maintenance and security checks before they can be packed and sent to the recruiting centres.

3 - Please report whether the study will include patients treated with revascularization procedures, as it is not clear.

Annswer: Acute stroke management of all patients included in this trial will follow standard of care. Patients undergoing reperfusion therapies such as intravenous thrombolysis or mechanical thrombectomy will be accepted as long as all inclusion and no exclusion criteria are met prior to screening and randomisation. Modifications have been made in the manuscript to the main text on page 8 (lines 8-12).