## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | A prospective double-blind randomized controlled trial protocol comparing bone marrow aspirate concentrate intra-articular injection combined with subchondral injection versus intra-articular injection alone for the treatment of symptomatic knee osteoarthritis. |
| **AUTHORS** | Silva, Simone; Andriolo, Luca; Boffa, Angelo; Di Martino, Alessandro; Reale, Davide; Vara, Giulio; Marco, Miceli; Cavallo, Carola; Grigolo, Brunella; Zaffagnini, Stefano; Filardo, Giuseppe |

## VERSION 1 – REVIEW

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| **REVIEWER** | Lucio Rovati<br>University of Milan |
| **REVIEW RETURNED** | 25-Apr-2022 |

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| **GENERAL COMMENTS** | This is a randomized controlled trial protocol comparing the intra-articular and subchondral injection to the intra-articular injection only, of presumably mesenchymal stromal cells derived from bone marrow aspirate concentrate in knee osteoarthritis patients.<br>The methods are sound, but there are several aspects that the authors should clarify.<br>1) The authors provide minimal justification of their approach and vague references. They shoud expand the description of their rationale and previous non-clinical studies results, both in terms of efficacy and safety, especially with respect to subchondral injections.<br>2) In addition, they shoud discuss if and how their product differs from other similar approaches.<br>3) It is unclear which femoral and tibial lesions should be located and graded by knee arthroscopy and whether these would be the site of subchondral injection.<br>4) The primary endpoint is represented by symptoms scored by the WOMAC total index. The authors should discuss why they do not use the single pain and/or function domain WOMAC scales, that are usually a preferred outcome compared to the whole (total) WOMAC score.<br>5) The calculation of the sample size is incomplete and the authors should clarify the difference (and variability) they expect on the primary variable (WOMAC values) between groups. They only report the expected effect size (ES), which is apparently huge: few treatments in OA achieve a >0.50 ES vs placebo (especially if intra-articular); assuming such a huge ES between two possibly active treatments implies the risk that the patient sample is actually too small and it will be impossible to see any significant difference between groups, with inconclusive results.<br>6) Blindness is apparently well described, but it is unclear whether the subchondral injection may elicit a placebo effect and how this will be controlled: this crucial aspect should be discussed.<br>7) The primary analysis will be conducted in ITT, but it is unclear |

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| | how drop-outs will be treated (last-observation-carried-forward that may be discouraged, other data input methods?)<br>8) The possible influence of the uncontrolled rescue pain medication on the primary outcome is not discussed.<br>9) Is the imaging analysis also blinded and randomized (i.e. baseline and 12-month assessments randomized for the analysis)?<br>10) There is major confusion between severe and serious adverse events, which casts doubts on all safety assessments.<br>11) The legal basis under which this experimental treatment is authorized for the study is not discussed. |

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| **REVIEWER** | Maryam Azlan<br>Universiti Sains Malaysia |
| **REVIEW RETURNED** | 26-Apr-2022 |

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| **GENERAL COMMENTS** | The paper was properly written although some sentences require the use of past tense. The methodology was clearly presented. However, the outcome of the study was not clearly defined. I would like to suggest to the authors to obtain the study output or results prior to submitting the manuscript. |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1
Dr. Lucio Rovati , University of Milan
Comments to the Author:
This is a randomized controlled trial protocol comparing the intra-articular and subchondral injection to the intra-articular injection only, of presumably mesenchymal stromal cells derived from bone marrow aspirate concentrate in knee osteoarthritis patients.
The methods are sound, but there are several aspects that the authors should clarify.
1) The authors provide minimal justification of their approach and vague references. They should expand the description of their rationale and previous non-clinical studies results, both in terms of efficacy and safety, especially with respect to subchondral injections.
- Thank you very much for the careful review and the thoughtful suggestions. We agree, the rationale about subchondral injections have been summarized for word count reasons but may deserve more space aiming for clarity. Thus, we expanded the introduction according to your observations. Lines 114-122.

2) In addition, they should discuss if and how their product differs from other similar approaches.
- We did not aim at developing a new product, rather to assess the potential of a different application of a product already available on the market and thus usable in the clinical practice. Accordingly, the BMAC production method used in this study is a product commonly used in the clinical practice. This may be actually considered an advantage, because it means that the results of this study may be useful to give indications to clinicians working in the clinical practice and considering to implement subchondral bone BMAC injections. We have clarified it. Lines 179-182.

3) It is unclear which femoral and tibial lesions should be located and graded by knee arthroscopy and whether these would be the site of subchondral injection.
- Thanks for the remark. In fact, arthroscopic evaluation allowed us to better localize the area of both medial femoral condyle and medial tibia plateau mainly involved by osteoarthritic lesions. We have clarified it. Lines 187-188.

4) The primary endpoint is represented by symptoms scored by the WOMAC total index. The authors should discuss why they do not use the single pain and/or function domain WOMAC scales, that are usually a preferred outcome compared to the whole (total) WOMAC score.

- We agree that single pain and/or function domain WOMAC scales are usually a preferred outcome. Nonetheless, we believe every choice of outcome presents pros and cons. In this case, we choose the total index since we believe that single scales may not capture the entire effect of a treatment. For example, patients may feel much better in terms of pain, but only because they limit their function. This outcome would be therefore interpreted as a positive outcome, while the real improvement may be actually less satisfactory. On the other hand, some patients may improve their function with a similar pain level. Also in this case, the pain scale would not capture the improvement in function offered by the treatment. In this light, for this study we aimed at considering a more comprehensive assessment of the patient outcome when choosing the primary endpoint. We elaborated on this aspect in the text, as suggested by the reviewer. Lines 214-216.

5) The calculation of the sample size is incomplete and the authors should clarify the difference (and variability) they expect on the primary variable (WOMAC values) between groups. They only report the expected effect size (ES), which is apparently huge: few treatments in OA achieve a >0.50 ES vs placebo (especially if intra-articular); assuming such a huge ES between two possibly active treatments implies the risk that the patient sample is actually too small and it will be impossible to see any significant difference between groups, with inconclusive results.
- Thank you for this relevant remark. We have provided a more complete explanation. With an expected treatment-related difference of 10.0 points of the WOMAC total score and a SD of 18.2 based on a pilot study, we obtained 0.55 effect size (ES). This is a medium ES according to the Cohen convention, and we verified that this value can be actually obtained for injectable treatments according to literature. We agree this is a rather large difference, but we want to have a large difference, to be able to justify adding a surgical treatment to a simple injection. Lines 313-320.

6) Blindness is apparently well described, but it is unclear whether the subchondral injection may elicit a placebo effect and how this will be controlled: this crucial aspect should be discussed.
- Thanks for the proper observation. Actually, the expectation of receiving an additional subchondral injection, which is the study procedure, could likely elicit a placebo effect compared to the intra-articular injection only. However, the trial setting prevents from such instance because of the blinding, thus the possible placebo effect is equally distributed between the two groups. We have added this aspect to the manuscript. Lines 269-271.

7) The primary analysis will be conducted in ITT, but it is unclear how drop-outs will be treated (last-observation-carried-forward that may be discouraged, other data input methods?)
- This has been now specified. Lines 320-324.

8) The possible influence of the uncontrolled rescue pain medication on the primary outcome is not discussed.
- It is a limitation of the study (we ask the patient if they took other medicines, but we do not offer homogeneous rescue medications, and actually discouraged their use) and it has been correctly added to the appropriate section. Lines 56-57

9) Is the imaging analysis also blinded and randomized (i.e. baseline and 12-month assessments randomized for the analysis)?
- Thanks for the observation. Imaging analysis is blinded and randomized. We added this point to the manuscript. Lines 272-273.

10) There is major confusion between severe and serious adverse events, which casts doubts on all safety assessments.
- Thanks for this significant remark. We have rectified safety considerations. Lines 288-290.

11) The legal basis under which this experimental treatment is authorized for the study is not discussed.
- The study is authorized by the local Ethic Committee and the funding provided by the Ministry of Health, as reported in Lines 355-357 and Lines 412-413.

Reviewer: 2
Dr. Maryam Azlan, Universiti Sains Malaysia
Comments to the Author:
The paper was properly written although some sentences require the use of past tense. The methodology was clearly presented. However, the outcome of the study was not clearly defined. I would like to suggest to the authors to obtain the study output or results prior to submitting the manuscript.
- We appreciate the reviewer feedback on our manuscript. Thanks for the grammatical suggestions, we revised the manuscript in order to adjust this aspect. Please let us know if there are further specific correction suggested.
Since this is an ongoing study and according to the journal guidelines, we cannot provide any result, while we focused on the study protocol. A dedicated paper will analyze the final results and will be submitted for publication in the future.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Lucio Rovati<br>University of Milan |
| **REVIEW RETURNED** | 01-Aug-2022 |

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| **GENERAL COMMENTS** | The authors have satisfactorily answered to most previous remarks. There are still a coupe of point in the method section that may be addressed more properly.<br>- The blinding paragraph (lines 276 onward) indeed clarifies that this is assured by the same number of surgical access between the two groups. On the other hand, the intervention section (lines 201-208) apparently states that only the combination groups will undergo surgical access for subchondral injection. Please clarify whether also the intra-articular group is accessed with the two trocars (without puncturing and injecting, of course), which would seem strange to me because at the limit of ethics. Thus please correct one of the two paragraphs accordingly, since at the moment one seems to contradict the other. If there is no identical surgical access, please state this as a linmitation (not to mention that already the lack of injection is a limitation: this is why with intra-articular administrations we inject saline in the control group, i.e. to prevent the effect of volume, etc.)<br>- It is unclear whether the sequence of imaging reading is randomized beside being blinded.<br>- Please always mention that you are using the TOTAL Womac as an endpoint. The justification of its use is pretty weak (FDA requires both the pain and function subscales to be succesful for a new drug), but acceptable. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 1

Dr. Lucio Rovati , University of Milan

Comments to the Author:

The authors have satisfactorily answered to most previous remarks.

There are still a coupe of point in the method section that may be addressed more properly.

- The blinding paragraph (lines 276 onward) indeed clarifies that this is assured by the same number of surgical access between the two groups. On the other hand, the intervention section (lines 201-208) apparently states that only the combination groups will undergo surgical access for subchondral injection. Please clarify whether also the intra-articular group is accessed with the two trocars (without puncturing and injecting, of course), which would seem strange to me because at the limit of ethics. Thus please correct one of the two paragraphs accordingly, since at the moment one seems to contradict the other. If there is no identical surgical access, please state this as a linmitation (not to mention that already the lack of injection is a limitation: this is why with intra-articular administrations we inject saline in the control group, i.e. to prevent the effect of volume, etc.)

We confirm this is not a limitation of our study, it is actually a strength. In fact, arthroscopy was done in both groups using the standard antero-lateral, antero-medial, and supero-medial portals. The same portals were used to access the subchondral bone in the experimental group in order to maintain blinding. For ethical reason, no bone puncturing and injection was performed in the control group, as correctly pointed out by the reviewer. We clarified this aspect in the text [lines 189-190 and 272-274].

- It is unclear whether the sequence of imaging reading is randomized beside being blinded.

Thank you for allowing us to clarify this aspect, the radiologists are blinded both to type of treatment and evaluation time [line 278].

- Please always mention that you are using the TOTAL Womac as an endpoint. The justification of its use is pretty weak (FDA requires both the pain and function subscales to be succesful for a new drug), but acceptable.

We specified that the primary endpoint is the TOTAL Womac, but we actually also evaluate WOMAC subscales among the secondary outcomes. We better specified this aspect in the text [lines 34, 212-213, 218-219, Table 1, 320].

**VERSION 3 – REVIEW**

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| **REVIEWER** | Lucio Rovati<br>University of Milan |
| **REVIEW RETURNED** | 15-Aug-2022 |

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| **GENERAL COMMENTS** | The authors have satisfactorily answered to the remaining comments and modified the manuscript accordingly. |