Replies to the reviewer's comments

Reviewer 1 - reviewer's code 02398400

Comments :

1. In the introduction the authors state that MSCs "can escape immune recognition". This is a highly subjective statement that requires further clarification. Indeed, there is a wealth of published data showing that MSCs stimulate allo-graft responses in rodent, swine, and non-human primate models. Therefore, the authors should elaborate on this statement by adding further context.

We have provided further context on immune responses and MSC. P.5 line 13-23.

2. *The reference list given in Table 1 does not appear to be exhaustive as one citation is provided for many different molecules. The authors should state whether the list reflects initial works and/or is meant to be exhaustive. If the latter is true then a more complete list of citations should be provided.*

This table includes several publications, these often describe expression of a wide range of homing molecules. The list reflects initial works and several references were added. As requested by reviewer 3 and 4 the different MSC sources were added.

Reviewer 2 - reviewer's code 00289357

Introduction Sentence This interest arises from the following MSC characteristics: they can escape immune recognition, they have immunomodulatory capacities. Add the following references: Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Nasef Aet al. Transplantation. 2007 Jul 27;84(2):231-7 Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells. Nasef A et al Gene Expr. 2007;13(4-5):217-26. Selected Stro-1-enriched bone marrow stromal cells display a major suppressive effect on lymphocyte proliferation. Nasef A et al. Int J Lab Hematol. 2009 Feb;31(1):9-19

These references were added. P. 5 line 13 (references 9, 10 and 11).

Sentence Clinical applications have been studied most extensively in Orthopaedics where MSC are used for repair of large bone defects and in Haematology for the treatment of graft-versus-host disease and support of engraftment [4,6] Add the following reference: Infusion of allogeneic-related HLA mismatched mesenchymal stem cells for the treatment of incomplete engraftment following autologous haematopoietic stem cell transplantation. Fouillard L, et al. Leukemia. 2007 Mar;21(3):568-70

This reference was added. P. 5 line 26.

Sentence In this setting homing and persistence of MSC in the target tissue is desirable [9-12]. Add the following reference: Long-Term Quantitative Biodistribution and Side Effects of Human Mesenchymal Stem Cells (hMSCs) Engraftment in NOD/SCID Mice following Irradiation. Fran?ois S, et al. Stem Cells Int. 2014;2014:939275.

This reference was added. P. 6 line 1.

Sentence In haematology MSC are currently mainly tested to control graft versus host disease and to support haematopoiesis after haematopoietic stem cell transplantation. Add the following reference: Innovative cell therapy in the treatment of serious adverse events related to both chemo-radiotherapy protocol and acute myeloid leukemia syndrome: the infusion of mesenchymal stem cells post-treatment reduces hematopoietic toxicity and promotes hematopoietic reconstitution. Fouillard L, et al. Curr Pharm Biotechnol. 2013;14(9):842-8.

The reference was added in the text. P. 7 line 4.

Sentence Devine et al. performed MSC transplantations in baboons and found MSC in a variety of tissues with highest signal in the gastro-intestinal tract, the percentage of MSC in the different tissues was estimated between 0.1 and 2.7% [13]. Modified : Devine et al. and Chapel et al. performed MSC transplantations in Non-Human primate and……..(Chapel A et al. 2003)

The text was modified accordingly. P. 8 line 1-4.

Sentence: Several other groups have also shown that MSC homing is improved after irradiation [13, 31-35] Change reference Reference 32 (Br J Radiol 2007; 80 Spec No 1: S49-55) by Local irradiation not only induces homing of human mesenchymal stem cells at

exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Fran?ois S, et al. Stem Cells. 2006 Apr;24(4):1020-9. Reference 34 (J Gene Med 2003; 5:1028-1038) by New emerging concepts in the medical management of local radiation injury. Benderitter M et al. JJ. Health Phys. 2010 Jun;98(6):851-7

References were adapted. P. 8 line 29 (references 49 and 52).

Reviewer 3 - reviewer's code 02902002

1. The review mainly focuses on homing mechanisms and strategies to improve homing efficiency. This should be stated clearly in the title. If the authors consider to focusing only on stem cell homing to bone marrow, the title also needs to be revised to indicate this intention.

In this review paper we describe the general homing features of MSCs, but indeed, when discussing tissue-specific homing, we do focus more on bone marrow. This has been clarified in the abstract. P. 3 line 12-13.

2. The sources of MSCs, the route of administration and the target tissue in the studies quoted in the manuscript should be stated. Please check throughout the manuscript.

When available source of MSC and administration route and target tissue were quoted in the text and in Table 1 these data were included. P. 8 line 6, P. 8 line 10-11, P. 8 line 20-21, P. 8 line 26, P. 12 line 10-11, P. 13 line 25-26, P. 15 line 17-18, P. 16 line 23, P. 17 line 16-17, P. 18 line 6-7, P. 19 line 16.

3. P.3, Line 4, be consistent in using the term "MSC" or "MSCs" to refer to mesenchymal stem cell"s".

MSC is consistently used to refer to mesenchymal stromal cell, MSCs is used to refer to the plural mesenchymal stromal cell**s**.

4. P.3, line 14-18, please add references.

References are added. P. 5 line 30.

5. P.3, line 18, clarify what "in this setting" means.

"This setting" referred to the use of MSC as vehicles for anticancer treatment and the different modalities that can be used in this scenario described on P. 5 line 26-30. The sentence was rewritten to clarify this. P. 5 line 30 and P. 6 line 1.

6. P.3, line 19-20, rewrite this sentence.

Sentence was rewritten. P. 6 line 2-5.

7. P.3, line 20-22, please add references.

Reference was added. P. 6 line 18.

8. P.3, line 29-32, rewrite this sentence.

Sentence was rewritten. P. 7 line 1-4.

9. P.3, line 34, this reviewer thinks that there may be some differences in the strategies used to improve homing efficiency depending on the target tissues. Hence, it would be better to state clearly if the authors are interested in bone marrow homing.

We have added a paragraph explaining that we are interested in bone marrow homing but that we believe this research might also benefit from studies on homing to other target tissues. P. 11 line 7-14.

10. The section heading "Homing and migration of MSC" should be revised to more specifically reflect the content in that section.

We agree that this section refers to several studies about bone marrow homing but studies analyzing homing to other tissues were included as well. Therefore we changed the section title in "Homing and migration of MSC to bone marrow and other tissues". P. 7 line 9-10.

11. Are there any differences in migration or homing efficiency of MSCs isolated from different sources? Please discuss.

A paragraph discussing this issue was added to the paper. P. 18 line 30 and P. 19 line 1-9.

12. The association between outcomes and route of MSC administration should be discussed.

The advantages and limitations of different routes of MSC administration should be discussed.

Differences between different administration modes and their respective advantages and limitations are discussed in the text. P. 6 line 2-23 and P. 8 line 7-23.

13. P.4, line 10, what does "at 35 years" mean?

At 35 years of age, this has been changed in the text. P. 7 line 24.

14. P.5, line 1, it should be G-protein coupled receptors

This was corrected. P. 9 line 8.

15. P.5, line 1-3, add reference.

Reference was added. P. 9 line 10.

16. P.5, line 3, clarify what "both" means.

"Both" refers to the molecules mentioned in the sentence just before; i.e. CXCR4 and SDF-1. The text was changed to "Both molecules" to clarify this. P. 9 line 10.

17. P.6, line 14, should be "Much effort focuses…"

Text was corrected. P. 12 line 2.

18. P.6, line 28, should be "Treatment of …… MMPs in MSCs…"

Sentence was rewritten accordingly. P. 12 line 20.

19. P.7, line 29-31, was it a pre-treatment?

The text on P. 7 line 29-31 in the originally submitted manuscript describes non-viral transfection of CXCR4 in MSCs, this is by default a pre-treatment. This section is now on P. 14 line 14-16 in the revised manuscript.

20. P.7, line 38-37, should state the source of MSC and indicate that it is an effect of hypoxia.

Line 38 & 37 on P. 7 in the originally submitted manuscript discuss transfection efficiencies of different transfection methods, not effects of hypoxia. Effects of

hypoxia are discussed on **P. 6 lines 35-40** and continue on **P. 7 lines 1-8.** In the revised manuscript culture under hypoxic conditions is discussed on P. 13 line 1-15.

21. P.7, line 4-7, this statement is not clear. Please rewrite.

The sentence has been changed. P. 13 line 13-15.

22. P.7, line 9, should be "… MSC cultured and maintained at completed confluence…"

Changes to the sentence proposed by the NPG language editors were accepted. P. 13 line 17.

23. P.7, line 11, should be "… MMPs that decreases the migration capacity when compared to MSC cultured at low confluence"

This sentence was changed according to this remark and the NPG language editors. P. 13 line 19-20.

What was the seeding density in that study?

The seeding density was not mentioned in the publication of this study.

24. P.7, line 15-16, what was the route of MSC administration in that study?

The route of administration was added in the text. P. 13 line 26.

25. P.7, line 22-23, what was the route of MSC administration in that study?

The route of administration was added in the text. P. 14 line 5

26. P.8, line 2-4, this statement is not clear. Please rewrite.

Sentence was rewritten. P. 14 line 27-29.

27. P.8, line 6-7, This statement is not clear. Please rewrite and add a reference.

This sentence just summarizes what is mentioned in this paragraph and the included references. P. 15 line 2-6.

28. P.8, line 13-14, clarify what "in this study" refers to. Please add a reference.

In this paragraph only one study (reference 54) is discussed. The reference number is mentioned twice. P. 15 line 7-19.

29. Figure 2 should be referred to in the manuscript.

Figure 2 is now referred to in the manuscript. P. 11 line 16.

30. P. 9 line 11-12. More explanations are needed for this statement. Why is the use the US, magnetic or electric field to enhance MSC homing regarded as not very practical?

Possible draw-backs of these techniques are now discussed in the text. P. 17 line 7-12.

31. Suggest adding a section on "future research directions" to increase the impact of the review article.

A "Future research directions" section was added. P. 20 line 4-28.

32. The English of the manuscript should be improved.

A language certificate was obtained through Nature Publishing Group Language Editing.

33. Table 1: the source of MSC and the target tissue should be mentioned.

We have added the source of MSCs in Table 1. Several studies are descriptive and do not provide data on in vivo homing or on migration to a target tissue. When available, functional data were provided.

34. Add a short description to both figure 1 and figure 2. Add an abbreviation list in figure 2.

A short description and a complete list of abbreviations was added to both figures. P. 46, P. 48.

Reviewer 4 - reviewer's code 02446158

1- The proposed table is roughly descriptive. Some parts are not discussed in the main text like the growth factors receptors

Table 1 was meant to be descriptive, but a section on growth factor receptors was added to the text (P. 10 line 10-16). The information in Table 1 now also includes the source of MSCs in the different studies.

2 - The potential pro-coagulant activity of MSC that could hamper both processes should be discussed

This issue is now discussed in the text. P. 8 line 15-23.

3- A simple comparison between all studied MSC should be discussed

We have included the MSC source in Table 1 to provide a clear overview of differences in expression among the different sources. In the text we have also addressed this issue in a new paragraph. P. 18 line 30 and P. 19 line 1-9.

4- Homing could be critical for allowing the transplanted MSC to reach the target organ but, according to what is published both in preclinical and clinical studies, it seems not related to engraftment regulation. Modifying parameters that may improve homing could modulate positively/negatively the plasticity and/or engraftment of the cells. The authors should discuss such issue

This topic is now discussed in the paper. P. 17 line 13-28 and P. 18 line 1-22.

5- The nature of the substrate on which the MSC proliferate in vitro/ the passaging number (different depending on the MSC used) may also significantly impact their homing. The authors should also provide a brief update on what could alter the membrane expression profile of the MSC in vitro

Impact of culture conditions and MSCs used is now discussed in the text. P 18 line 18-30 and P. 19 line 11-30 and P. 20 line 1-3.

6- The CXCR4/CXCL12 axis strategy (major discussed point) is not yet conclusive. Hence, the authors should provide at least of the more attractive current other investigated issues.

We agree that CXCR4-CXCL12 interaction is a major point of discussion in this paper. However we also elaborate on strategies to improve adhesion (P. 14 line 6-7, P. 14 line 26-29, P. 15 line 7-30 and P. 16 line 1-25) and extravasation (P. 12 line 20-24, P. 13

line 7-9 and P. 13 line 18-20) and we touched upon the role of growth factors and their receptors in MSC homing (P. 10 line 10-16). In HSC homing the unique role of the CXCR4-SDF1 axis for bone marrow homing has been challenged in recent years, e.g., by sphingosine-1-phosphatase driven bone marrow homing. However, we could not find published data on this molecule and MSC homing.