Reviewer 00203715

We thank the reviewer for his/her comments. This reviewer had no suggestions for revision, therefore the text was not altered based on these comments.

Martinez et al. “Aneuploidy in stem cells: a deadly CIN” describe chromosomal instability (CIN) in healthy tissues and in pathologies. The authors use several examples to show that CIN is hardly tolerated by stem cells while it can be quite common in differentiated cells like in hepatocytes. In addition, chromosomal instability is raised in aged cells and is dramatically increased in tumors. This will affect the rising field of induced pluripotent stem cells. Although CIN will reduce the proliferation and survival of iPSC, subtle chromosomal arrangements might still be inherited. This risk is even increased with transient ex vivo culture. The review points out that verification of chromosomal fidelity will be a crucial issue for regenerative medicine. The topic chosen is interesting and the manuscript is very well written, concise, clear, and comprehensive.

Reviewer 00180736

We thank the reviewer for his/her comments and suggestions for revision.

The article is very interesting and it puts the emphasis on a problem that may be essential in cellular therapy. However, there are sections that limit the interest reader such as those related to CIN in brain or liver. They should be deleted or summarized. Similar with the paragraph about Down syndrome.

We have decided to compromise between this comment and one made by reviewer 02446158, who suggested further expansion of the sections on specific diseases and tissues, by minimally summarizing the referred sections while at the same time referring to some more relevant literature.

It would be desirable to include in the section "types of stem" a table with the most common chromosomal alterations were found in each cell type.

In addition to naming the reported aneuploidies for some stem cell types (where known/ reported), we also summarize this in a newly added Table (Table 1)

Conclusion is very interesting but What happens to the cell therapies with adult cells (mesenchymal, hematopoietic...)?

This is an important issue: We further discussed the effect of aneuploidy on MSCs and HSCs, but little is known about this particular topic. We now also refer to this gap in knowledge in the main body text.

Reviewer 02446158

We thank the reviewer for the comprehensive overview of suggested revisions.

The current review proposed by Martinez et al, deals with the description of the role that aneuploidy plays in controlling the number and the quality of somatic and stem cells. The topic of the proposed review is quite interesting and actualized for the field of cell therapy and regenerative medicine. The review is easily readable and well written. However, some parts of the manuscript need more comprehensive and updated details. The authors should address the concerns detailed here below before a publication:

- The introduction part should be more developed. A transition paragraph from somatic to stem cells is to add.

We expanded the introduction to smoothen the transition from somatic cells to stem cells.

- The authors should provide additional data regarding the description of aneuploidy in cell types and in the different zones (proximal and/or distal to the corresponding niches) of the discussed organs (brain and liver)?

As suggested by reviewer 00180736 above, expanding on specific organs in greater detail might reduce reader interest. However as a compromise we have included additional information on cytogenetic abnormalities in different stem cell types(where known), and refer to relevant literature regarding the role of aneuploidy in different tissues.

- Is there any correlation between potency level and aneuploidy? or between proliferation rate and aneuploidy? Indeed, these features represent the principal differences between embryonic vs somatic stem cells. Is the quality of differentiation in stem cells impacted by aneuploidy?

This is an excellent point, but unfortunately not yet comprehensively studied in human stem cells. Therefore, we cannot discuss this any further yet. From the animal models (discussed in the text), we know that aneuploidy is detrimental to many cell types, but not all, emphasizing the importance of a tissue-specific/ cell lineage specific approach in human stem cells.

- In the liver, when hepatocytes are no more able to proliferate and stem cells are emerging, is there any documented data involving aneuploidy? A brief liver disease section is to add if documented data related to aneuploidy are available

We discuss the role of tetraploidy in liver homeostasis. Unfortunately, there is –to our knowledge – no data reported on aneuploidy in liver stem cells.

- Because mesenchymal stem cells (MSC) have been currently used in many clinical trials, a comparison with ESC and iPSCs should be added.

We have added a section discussing (aneuploidy in) mesenchymal stem cells.

- A brief description of the technical skills that have been/are used for an accurate analysis of aneuploidy should be added. A table would be more appreciated.

This is quite important, but as this is an extensive topic on itself, it falls out of the scope of this review. We refer a recent review discussing all this (Bakker et al., 2015) that examines the most commonly used methods for karyotyping and lists their advantages and limitations.

- Is there any difference in the aneuploidy molecular level/markers during development? Are the differences observed between somatic and stem cells equivalent in all tissues? This information is very important regarding an accurate comparison with what is described in vitro.

This is another excellent question, but difficult to address with the current state of the field. We have highlighted this issue in the text and stated the need for better tools to measure aneuploidy at the single cell level.

- The authors only propose Nanog transcription factor as aneuploidy driver, other demonstrated and/or proposed factors should be discussed if any

We have included some additional factors that could contribute to selection for the gain of specific chromosome.

- Typographical errors should be corrected

Typographical errors are corrected.

Answer to chief editor:

Dear Fang-Fang,

Thanks for the comments of the chief editor. We have amended the manuscript accordingly. Please infd the manuscript (with track changes visisble) attached. The comments are pasted below, with our proposed changes explained in blue.

The topic of this review is interesting. However, there is a basic mistake in the manuscript to be corrected before publication. The section of 'Stem cell types' is not about stem cell types but cell potency. If the cells mentioned in this section are stem cells, they will be contradictory to the 3 stem cell cardinal features described in the previous section. 1) Embryonic stem cells (ESCs) are not 'omnipotent' (or totipotent) and cannot differentiated into any extraembryonic tissues. That is why ESCs cannot produce an organism on their own. ESCs are pluripotent. 2) Are unipotent cells are stem cells? Do they have the 2nd cardinal feature mentioned above – i.e. the capacity to produce multiple cell lineages? In general, stem cells could be categorised into two types: pluripotent stem cells (incl ESCs and iPSCs, which are cell culture artefacts) and somatic stem cells.

We have amended the text and corrected all of this: please check the tracked changes whether you now agree with the phrasing.

A minor point: it might be better to spell out CIN in the title as it is not very informative to use abbreviation in the title.

The CIN in the title is a word joke that we would really like to keep as it is: CIN sounds like sin and such a title might drive the attention of readers. I hope you can agree to to this. Otherwise I can rephrase the title into: '*Stem cells and aneuploidy*".

Best wishes
Floris