Genome Editing for Scalable Production of Alloantigen-free Lentiviral Vectors for in Vivo Gene Therapy

Michela Milani, Andrea Annoni, Sara Bartolaccini, Mauro Biffi, Fabio Russo, Tiziano Di Tomaso, Andrea Raimondi, Johannes Lengler, Michael C. Holmes, Friedrich Scheiflinger, Angelo Lombardo, Alessio Cantore, Luigi Naldini

Corresponding author: Luigi Naldini, San Raffaele Telethon Institute for Gene Therapy

Review timeline:
Submission date: 30 June 2017
Editorial Decision: 14 July 2017
Revision received: 21 July 2017
Accepted: 26 July 2017

Transaction Report:
(Note: This manuscript was transferred from another journal where it was originally reviewed. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published.)

Editor: Céline Carret

1st Editorial Decision 14 July 2017

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. As discussed, and based on the initial set of reviews you provided us with, we have evaluated your revised article and asked an expert external advisor to look at your paper and responses to the referees (comments pasted below). We have now received our advisor's comments and following editorial discussions, including with our chief editor, we have decided to accept your manuscript pending the following final editorial amendments:

1) Animal work and ethical statements:
- please provide the gender of mice used
- human samples: please indicate where you got them from when not purchased (hospital?) and confirm in the main text compliance to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.

2) Source Data:
We now encourage the publication of source data, particularly for electrophoretic gels, blots, but also microscopy images with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide a PDF file per figure that contains the original, uncropped and unprocessed scans of all or key gels used in the figure? The PDF files should be labeled with the appropriate figure/panel number (1 file/figure), and should have molecular weight markers; further annotation may be useful but is not essential. The PDF files will be published online with the article as supplementary "Source Data" files. If you have any questions regarding this just contact me.

© EMBO
***** Advisor's comments *****

I read carefully the paper by Milani et al. It is a very good paper, carefully written and reporting solid and beautifully controlled data. There is very little I would suggest to improve the paper. Frankly, I don't see the point of asking for in vivo data, they would be anyway irrelevant in proving the immunogenicity and complement sensitivity of the viral particles produced by these stable cell lines in a human situation. The only real problem with these lines is their endpoint titer, which is one log lower than that achieved by transient transfection in a typical GMP manufacturing process. For in vivo applications, this generates serious manufacturing problems, since there would be the need to produce ten-fold higher volumes and concentrate the supernatant ten times more compared to a transient transfection system, a formidable challenge in terms of process scalability and cost.

1st Revision - authors' response 21 July 2017

We have now submitted a revised version of the manuscript, according to all your requests below.

We have also updated figure EV4 with improved flow cytometry analysis of LV packaging cell line and adjusted the corresponding sentence in the text.
In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself.

**A. Figures**

**1. Data**

The data shown in figures should satisfy the following conditions:

- The data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- Figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- Graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- If n<5, the individual data points from each experiment should be plotted and any statistical test employed should be justified.

Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

**2. Captions**

Each figure caption should contain the following information, for each panel where they are relevant:

- A specification of the experimental system investigated (e.g. cell line, sample name).
- The assay(s) and method(s) used to carry out the reported observations and measurements.
- An explicit mention of the biological and chemical entity(ies) that are being measured.
- An explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- The exact sample size [n] for each experimental group/condition, given as a number, not a range.
- A description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- A statement of how many times the experiment shown was independently replicated in the laboratory.
- Definitions of statistical methods and measures:
  - Common tests, such as t-test (please specify whether paired vs. unpaired), simple t-tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section.
  - Are tests one-sided or two-sided?
  - Are there adjustments for multiple comparisons?
  - Exact statistical test results, e.g., P values = x but not P values < x;
  - Definition of "center values" as median or average;
  - Definition of error bars as s.s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (see applicable).

We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

**B. Statistics and general methods**

1. a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? Methods, “Experimental design” subsection

1. b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. Methods, “Experimental design” subsection

2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? Methods, “Experimental design” subsection

3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe. Methods, “Experimental design” subsection

For animal studies, include a statement about randomization even if no randomization was used. Methods, “Experimental design” subsection

4. a. Were any steps taken to minimize the effects of subjective bias during group allocation in/and when assessing results (e.g. blinding of the investigator)? If yes please describe. Methods, “Experimental design” subsection

4. b. For animal studies, include a statement about blinding even if no blinding was done Methods, “Experimental design” subsection

5. For each figure, are statistical tests justified as appropriate? Methods, “Statistical analysis” subsection; Figure Legends

6. Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Methods, “Statistical analysis” subsection; Figure Legends

5. a. How do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Methods, “Statistical analysis” subsection; Figure Legends

7. Is there an estimate of variation within each group of data? Methods, “Statistical analysis” subsection; Figure Legends

8. Is the variance similar between the groups that are being statistically compared? Methods, “Statistical analysis” subsection; Figure Legends

**C. Reagents**
<table>
<thead>
<tr>
<th>D- Animal Models</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.</td>
<td>Methods, &quot;Non experiments&quot; subsection</td>
</tr>
<tr>
<td>7. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.</td>
<td>Methods, &quot;Non experiments&quot; subsection</td>
</tr>
<tr>
<td>8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.</td>
<td>Methods, &quot;Non experiments&quot; subsection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E- Human Subjects</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal’s data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under ‘Expanded View’ or in unstructured repositories such as Dryad [see link list at top right] or FigShare [see link list at top right]).</td>
<td></td>
</tr>
<tr>
<td>22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F- Data Accessibility</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>18. Provide a “Data Availability” section at the end of the Materials &amp; Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39420, Proteomics data: PRIDE P20003220 etc.) Please refer to our author guidelines for ‘Data Deposition’.</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G- Dual use research concern</th>
<th></th>
</tr>
</thead>
</table>