efficiency and limited proficiency of homology directed DNA repair (HDR), likely due to HSC quiescence, to low levels of expression of the HDR machinery and conversely to high activity of the error-prone non homologous end joining (NHEJ) pathway. Thus, it will be crucial to enhance the efficiency of HDR in HSC while fully preserving their long-term repopulating activity.

Similarly, the impact of adeno-associated virus (AAV) as a source of donor template for HDR-mediated gene editing remains poorly investigated, and no clinical application of this vector in HSPC has been reported yet. AAV dose-dependent toxicity has been observed, which is directly related to G-rich regions of ITRs that induce cells accumulation in early S-phase due to p53-mediated induction of apoptosis, as described in a hESCs model.

Substantial difficulties remain with the methods employed for the genetic modification of haematopoietic stem and progenitor cells. In particular, the multiple hits of high vector doses required and prolonged ex vivo transduction times associated with existing methods give rise to problems with survival of the transduced haematopoietic stem and progenitor cells during culture and potentially impact their biological properties. Furthermore, improvements in the engraftment of transduced cells will greatly benefit clinical applications.

SUMMARY

The inventors have surprisingly found that the use of an agent which promotes homology directed DNA repair, (such as an inhibitor of p53 activation, or an adenoviral protein) improves gene editing efficiency of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells and improves the survival and/or engraftment of treated gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

The inventors determined that advantageously, the agent which promotes homology directed DNA repair enhanced gene targeting efficiency in haematopoietic stem and progenitor cells, particularly in the more primitive CD34+CD133+CD90+ fraction. Furthermore, the agent does not significantly affect the differentiation status of the haematopoietic stem and/or progenitor cells, thus preserving their long-term re-population capacity.

In one example, the present disclosure refers to a use of an agent which promotes homology directed DNA repair in the manufacture of a medicament for treating a disease treatable by haematopoietic cell gene therapy, haematopoietic stem cell gene therapy, and/or haematopoietic progenitor cell gene therapy, wherein said gene therapy comprises gene editing, and wherein said agent is a p53 dominant negative peptide comprising a p53 homo-oligomerisation domain, or a nucleotide sequence encoding therefor.

In another example, the present disclosure refers to a use of an agent which promotes homology directed DNA repair in the manufacture of a medicament for treating a disease treatable by increasing the survival and/or engraftment of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, and wherein said agent is a p53 dominant negative peptide comprising a p53 homo-oligomerisation domain, or a nucleotide sequence encoding therefor.

In one aspect, the present disclosure refers to an *in vitro* use of an agent which promotes homology directed DNA repair, for increasing the survival and/or engraftment of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, or for increasing the efficiency of gene editing of haematopoietic cells, haematopoietic stem cells, and/or haematopoietic progenitor cells, and wherein said agent is: (a) a p53 dominant negative peptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 9, or a nucleotide sequence encoding therefor; or (b) GSE56, or a nucleotide sequence encoding therefor.

In yet another aspect, the present disclosure refers to an *ex vivo* or *in vitro* method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells comprising the steps:

(a) contacting the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells with an agent which promotes homology directed DNA repair, wherein the agent is (a) a p53 dominant negative peptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 9, or a nucleotide sequence encoding therefor; or (b) GSE56, or a nucleotide sequence encoding therefor;

(b) introducing gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells with one or more vectors; and

(c) editing the genome of said haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells,

In yet another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for treating a disease, wherein:

(a) the composition comprises a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells that is gene edited according to the method as disclosed herein; and

(b) that the gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells are to be administered to a subject, and wherein the disease is selected from the group consisting of Hyper IgM syndrome, chronic granulomatous disease, SCID, atypical SCID, Omenn syndrome, mucopolysaccharidoses disorders, mucopolysaccharidosis type I (MPS-1), Fanconi anaemia (FA), sickle cell disease, metachromatic leukodystrophy (MLD), globoid cell leukodystrophy (GLD), GM2 gangliosidosis, thalassemia, haemophilia, a lysosomal storage disorder and cancer.

In yet another aspect, the present disclosure refers to a population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells as disclosed herein.

In yet another aspect, the present disclosure refers to a composition comprising a population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method as disclosed herein for use in therapy.

In yet another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for treating a disease treatable by an autologous stem cell transplant procedure or an allogeneic stem cell transplant procedure, wherein the composition comprises a population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method as disclosed herein.

In one example, the present disclosure provides an agent which promotes homology directed DNA repair for use in haematopoietic cell gene therapy, haematopoietic stem cell gene therapy, and/or haematopoietic progenitor cell gene therapy, wherein said gene therapy comprises gene editing.

CLAIMS

1. In vitro use of an agent which promotes homology directed DNA repair, for increasing the survival and/or engraftment of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, or for increasing the efficiency of gene editing of haematopoietic cells, haematopoietic stem cells, and/or haematopoietic progenitor cells, and wherein said agent is: (a) a p53 dominant negative peptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 9, or a nucleotide sequence encoding therefor; or (b) GSE56, or a nucleotide sequence encoding therefor.

2. The use of claim 1, wherein the agent is in the form of an mRNA.

3. The use of claim 1 or 2, wherein the inhibition of p53 in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is transient.

4. The use of any one of claims 1 to 3, wherein transient inhibition of p53 occurs during gene editing of the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

5. The use of any one of claims 1 to 3, wherein the agent is added to the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cells at a concentration of 1-50 µM.

6. The use of any one of claims 1 to 5, wherein the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells are contacted with the agent and: (a) adenoviral protein E4ORF1 or a nucleic acid sequence encoding therefor; and/or (b) adenoviral protein E4ORF6/7 or a nucleic acid sequence encoding therefor.

7. The use of claim 6, wherein the adenoviral protein E4ORF1 and/or E4ORF6/7 is from an Adenovirus of serotype 4, Adenovirus of serotype 5, Adenovirus of serotype 7 and/or Adenovirus of serotype 9.

8. The use of claim 6 or claim 7, wherein the E4ORF1 comprises an amino acid sequence as set forth in SEQ ID No. 1, 57-76, or a sequence with at least 80% identity thereto; or the adenoviral protein E4ORF6/7 comprises an amino acid sequence as set forth in SEQ ID No. 2, 77-107, or a sequence with at least 80% identity thereto.

9. The use of any one of claims 1 to 8, wherein the agent is in combination with adenoviral protein E4ORF1 or a nucleic acid sequence encoding therefor, and adenoviral protein E4ORF6/7 or a nucleic acid sequence encoding therefor.

10. The use of any one of claims 6 to 9, wherein the nucleic acid encoding the adenoviral protein is an mRNA.

11. The use of any one of claims 6 to 10, wherein the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell or haematopoietic progenitor cell.

12. The use of any one of claims 1 to 11, wherein the target of the gene editing is selected from the group consisting of CD40L, RAG-1, IL-2RG, CYBA, CYBB, NCF1, NCF2, and NCF4, preferably CD40L.

13. An *ex vivo* or *in vitro* method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells comprising the steps:

(a) contacting the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells with an agent which promotes homology directed DNA repair, wherein the agent is: (a) a p53 dominant negative peptide comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 9, or a nucleotide sequence encoding therefor; or (b) GSE56, or a nucleotide sequence encoding therefor;

(b) introducing gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells with one or more vectors; and

(c) editing the genome of said haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

14. The method of claim 13, wherein the agent is in the form of an mRNA.

15. The method of claim 13 or 14, wherein the inhibition of p53 in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is transient.

16. The method of any one of claims 13 to 15, wherein transient inhibition of p53 occurs during gene editing of the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

17. The method of any one of claims 13 to 16, wherein the agent is added to the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cells at a concentration of 1-50 µM.

18. The method of any one of claims 13 to 17, wherein the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells are contacted with the agent and: (a) adenoviral protein E4ORF1 or a nucleic acid sequence encoding therefor; and/or (b) adenoviral protein E4ORF6/7 or a nucleic acid sequence encoding therefor.

19. The method of claim 18, wherein the adenoviral protein E4ORF1 and/or E4ORF6/7 is from an Adenovirus of serotype 4, Adenovirus of serotype 5, Adenovirus of serotype 7 and/or Adenovirus of serotype 9.

20. The method of claim 18 or claim 19, wherein the E4ORF1 comprises an amino acid sequence as set forth in SEQ ID No. 1, 57-76, or a sequence with at least 80% identity thereto; or the adenoviral protein E4ORF6/7 comprises an amino acid sequence as set forth in SEQ ID No. 2, 77-107, or a sequence with at least 80% identity thereto.

21. The method of any one of claims 13 to 20, wherein the agent is in combination with adenoviral protein E4ORF1 or a nucleic acid sequence encoding therefor, and adenoviral protein E4ORF6/7 or a nucleic acid sequence encoding therefor.

22. The method of any one of claims 18 to 21, wherein the nucleic acid encoding the adenoviral protein is an mRNA.

23. The method of any one of claims 18 to 22, wherein the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell or haematopoietic progenitor cell.

24. The method of any one of claims 13 to 23, wherein the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is contacted with the agent which promotes homology directed DNA repair concurrently with gene editing.

25. The method of any one of claims 13 to 24, wherein the population of haematopoietic stem and/or progenitor cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

26. The method of any one of claims 13 to 25, which includes a further step of enriching the population for haematopoietic stem and/or progenitor cells.

27. The method of any one of claims 13 to 26, wherein the target of the gene editing is selected from the group consisting of CD40L, RAG-1, IL-2RG, CYBA, CYBB, NCF1, NCF2, and NCF4, preferably CD40L.

28. Use of a composition in the manufacture of a medicament for treating a disease, wherein:

(a) the composition comprises a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells that is gene edited according to the method of any one of claims 13 to 27; and

(b) that the gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells are to be administered to a subject,

and wherein the disease is selected from the group consisting of Hyper IgM syndrome, chronic granulomatous disease, SCID, atypical SCID, Omenn syndrome, mucopolysaccharidoses disorders, mucopolysaccharidosis type I (MPS-1), Fanconi anaemia (FA), sickle cell disease, metachromatic leukodystrophy (MLD), globoid cell leukodystrophy (GLD), GM_2 gangliosidosis, thalassemia, haemophilia, a lysosomal storage disorder and cancer.

29. The use of claim 28, wherein the gene edited cells are to be administered to a subject as part of an autologous stem cell transplant procedure or an allogeneic stem cell transplant procedure.

30. A population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method of any one of claims 13 to 27.

31. A pharmaceutical composition comprising the population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells of claim 30.

32. A composition comprising a population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method of claim 27 for use in therapy.