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 aspect, 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 aspect, 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 another 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 p53 dominant negative peptide comprising a p53 homo-oligomerisation domain, 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 as defined herein;

(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 treatable by gene therapy, 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 is to be administered to a subject

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.

CLAIMS

1. 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.

2. 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.

3. ~~U~~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 p53 dominant negative peptide comprising a p53 homo-oligomerisation domain, or a nucleotide sequence encoding therefor.

4. The use according to any one of claims 1 to 3, wherein the agent is ~~an inhibitor of p53 activation, preferably wherein the inhibitor is an inhibitor of p53 phosphorylation, more preferably an inhibitor of p53 Serine 15 phosphorylation, more preferably wherein the inhibitor is a p53 dominant negative peptide, an ataxia telangiectasia mutated (ATM) kinase inhibitor or an ataxia telangiectasia and Rad3-related protein (ATR) inhibitor~~ GSE56 or a variant thereof, or a nucleotide sequence encoding therefor.

5. The use of ~~claim 4~~any one of claims 1 to 3, wherein the ~~inhibitor is pifithrin-α or a derivative thereof; KU-55933 or a derivative thereof; GSE56 or a variant thereof; KU-60019, BEZ235, wortmannin, CP-466722, Torin 2, CGK 733, KU-559403, AZD6738 or derivatives thereof; or an siRNA, shRNA, miRNA or antisense DNA/RNA~~ agent is 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.

6. The use of any one of claims 1 to 5, wherein the agent is in the form of an mRNA.

~~6~~7. The use of ~~claim 4 or 5~~any one of claims 1 to 6, wherein the inhibition of p53 in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is transient.

~~7~~8. The use of any one of claims ~~4~~1 to ~~6~~7, wherein the transient inhibition of p53 occurs during gene editing of the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

~~8~~9. The use of any one of claims ~~4~~1 to 7, wherein the inhibitor is added to the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cells at a concentration of ~~about~~ 1-50 µM.

10. The use of any one of claims 1 to 9, wherein the agent further comprises: (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.

~~9~~11. The use of ~~any one of~~ claim~~s 1-8~~ 10, wherein ~~the agent comprises a nucleic acid sequence encoding at least one adenoviral protein, preferably wherein~~ the ~~at least one~~ 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~~, preferably the at least one adenoviral protein is from an Adenovirus of serotype 5~~.

~~10~~12. The use of claim ~~9~~10 or claim 11, wherein the~~ at least one adenoviral protein is: (a)~~ E4ORF1~~, preferably wherein the~~comprises an amino acid sequence ~~of E4ORF1 is~~as set forth in SEQ ID No. 1, 57-76, or a sequence with at least 80% identity thereto; or ~~(b)~~ the adenoviral protein E4ORF6/7~~, preferably wherein the~~comprises an amino acid sequence ~~of E4ORF6/7 is~~as set forth in SEQ ID No. 2, 77-107, or a sequence with at least 80% identity thereto.

~~11. The use of any one of claims 1-10, wherein the agent comprises: adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76; and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107.~~

~~12~~13. The use of any one of claims 1~~-11~~ to 12, wherein the agent comprises~~:~~

~~(a) the inhibitor of p53 activation and an adenoviral protein;~~

~~(b) the inhibitor of p53 activation and adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76;~~

~~(c) the inhibitor of p53 activation and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107; or~~

~~(d)~~ p53 dominant negative peptide~~the inhibitor of p53 activation~~, adenoviral protein E4ORF1 or a nucleic acid sequence encoding therefor, ~~preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76,~~ and adenoviral protein E4ORF6/7 or a nucleic acid sequence encoding therefor~~,~~ ~~preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107,~~

~~preferably wherein the inhibitor of p53 activation is GSE56 or a variant thereof~~.

~~13~~14. The use of any one of claims ~~9~~10 to ~~12~~13, wherein the nucleic acid encoding the adenoviral protein is an mRNA.

~~14~~15. The use of any one of claims ~~9~~10 to ~~13~~14, wherein the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell or haematopoietic progenitor cell~~, preferably wherein the transient expression occurs during gene editing of the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cell~~.

~~15~~16. The use of any one of claims 1 to ~~14~~15, 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.

~~16~~17. 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 as defined in any one of claims 1 to 16;

(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.

~~17. The method of claim 16, wherein the agent which promotes homology directed DNA repair is an inhibitor of p53 activation, preferably wherein the inhibitor of p53 activation is a~~

p53 dominant negative peptide, an ataxia telangiectasia mutated (ATM) kinase inhibitor or an ataxia telangiectasia and Rad3-related protein (ATR) inhibitor.

18. The method of claim 17, wherein the inhibitor of p53 activation is pifithrin-α or a derivative thereof; KU-55933 or a derivative thereof; GSE56 or a variant thereof; KU-60019, BEZ235, wortmannin, CP-466722, Torin 2, CGK 733, KU-559403, AZD6738 or derivatives thereof; or an siRNA, shRNA, miRNA or antisense DNA/RNA.

19. The method of claim 18, wherein the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells at a concentration of about 1-50 μM.

20. The method according to any one of claims -16 to 19, wherein the agent comprises a nucleic acid sequence encoding at least one adenoviral protein, preferably wherein the at least one adenoviral proteins is from an Adenovirus of serotype 5.

21. The method according to claim 20, wherein the at least one adenoviral protein is: (a) E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76; or (b) E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107.

22. The method according to any one of claims 16 to 21, wherein the agent comprises: adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76; and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107.

23. The method according to any one of claims 16 to 22, wherein the agent comprises:

(a) the inhibitor of p53 activation and adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76;

(b) the inhibitor of p53 activation and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107; or

(c) the inhibitor of p53 activation, adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, 57-76, and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, 77-107,preferably wherein the inhibitor of p53 activation is GSE56 or a variant thereof.

~~24. The method according to any one of claims 20 to 23, wherein the nucleic acid encoding the adenoviral protein is an mRNA.~~

~~25. The method according to any one of claims 20 to 24, wherein the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell, and/or haematopoietic progenitor cell.~~

~~26~~18. The method of ~~any one of claims 16 to 25~~ claim 17, 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.

~~27~~19. The method of ~~any one of claims 16 to 26~~ claim 17 or claim 18, wherein the population of haematopoietic stem and/or progenitor cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

~~28~~20. The method of any one of claims ~~16 to 27~~17 to 19, which includes a further step of enriching the population for haematopoietic stem and/or progenitor cells.

~~29~~21. The method of any one of claims ~~16 to 28~~17 to 20, 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.

~~30~~22. Use of a composition in the manufacture of a medicament for treating a disease treatable by gene therapy, 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 ~~19-35~~17 to 21; and

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

~~31~~23. The use of claim ~~30~~22, 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.

~~32~~24. A population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method of any one of claims ~~16 to 29~~17 to 21.

~~33~~25. A pharmaceutical composition comprising the population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells of claim ~~32~~24.

~~34~~26. A composition comprising a population of gene edited haematopoietic cells, haematopoietic stem cells or haematopoietic progenitor cells prepared according to the method of any one of claims ~~16 to 29~~ 17 to 21 for use in therapy.

~~35~~27. 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 of any one of claims ~~16 to 29~~17 to 21.