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.

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.

In another aspect, the present disclosure refers to a 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.

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;

(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.

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.

In a preferred example, the haematopoietic cells, haematopoietic stem cells and haematopoietic progenitor cells are human cells.

In one example, the cells are HSCs. In one example the cells are HSPCs.
In one example, the haematopoietic stem and/or progenitor cells are CD34+ cells.

In one example, the population of haematopoietic stem and/or progenitor cells comprises, is enriched in or substantially consists of CD34+ cells. The population of cells may be further enriched for a particular sub-population of cells, for example CD34+CD38- cells. The population of cells may be further enriched for a particular sub-population of cells, for example CD34+CD133+ and CD90+ cells.

In a preferred example, the gene therapy is haematopoietic stem cell therapy and haematopoietic stem cells are gene edited.

In one example, the present disclosure provides an agent which promotes homology directed DNA repair, for use in increasing the survival and/or engraftment of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

In one example, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 50%, 75% or 90%, preferably at least 70%, more cells survive in culture for a period of time (e.g. about 6 or 12 hours, or 1, 2, 3, 4, 5, 6, 7 or more days, preferably about 2 days) when the cells have been exposed to the agent rather than in its absence. Preferably, the period of time begins with transduction of the cells with a viral vector.

The present disclosure may allow the timing of the pretreatment /conditioning of cells with cytokines to prime the cells (otherwise quiescent) for transduction to be reduced. Standard timing is 3 days. This time may be reduced to less than 3 days, or less than 2 days, or less than 1 day, for example 0, 1 or 2 days.

In one example, the agent substantially prevents or reduces apoptosis in the haematopoietic stem and/or progenitor cells, in particular during in vitro culture.

In one example, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50% or 75%, preferably at least 25%, fewer cells become apoptotic following culture for a period of time (e.g. about 6 or 12 hours, or 1, 2, 3, 4, 5, 6, 7 or more days, preferably about 2 days) when the cells have been exposed to the agent rather than in its absence. Preferably, the period of time begins with the transduction of the cells with a viral vector.

In one example, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50% or 75%, preferably at least 10%, more transplanted haematopoietic stem cells and/or haematopoietic progenitor cells and/or their descendant cells (e.g. graft-derived cells) engraft in a host subject when the cells have been exposed to the agent rather than in its absence.

In another example, the present disclosure provides the use of an agent which promotes homology directed DNA repair, for increasing the survival and/or engraftment of gene edited haematopoietic stem and/or progenitor cells or for increasing the efficiency of gene editing of haematopoietic stem and/or progenitor cells.

In one example, 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.

In one example, the inhibitor is a p53 dominant negative peptide, an ataxia telangiectasia mutated (ATM) kinase inhibitor, and/or an ataxia telangiectasia and Rad3-related protein (ATR) inhibitor.

In one example, the inhibitor is pifithrin-α or a derivative thereof. In one example, the inhibitor is pifithrin-α cyclic. In one example, the inhibitor is pifithrin-α p-nitro. KU-55933 or a derivative thereof; GSE56 or a fragment or 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.

In one example, the inhibition of p53 in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is transient.

In one example, the inhibitor is a transient inhibitor (e.g. has an inhibitory action lasting less than about 1, 2, 3, 4, 5, 6, 7 or 14 days), such as a reversible inhibitor. Preferably, the cells are exposed to the inhibitor for about 1-48 or 1-24 hours, preferably 1-24 hours. The cells may be, for example, exposed to the inhibitor at the same time as the viral vector or before the viral vector.

In one example, the agent is administered before, at the same time as, and/or after the gene editing machinery is introduced into the cell.

In one example, the transient inhibition of p53 occurs during gene editing of the haematopoietic cells, haematopoietic stem cells and/or progenitor cells.

In one example, the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells at a concentration of about 1-50 µM.

In one example, the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells (e.g. in an in vitro or ex vivo culture) at a concentration of about 1-50, 1-40, 1-30, 1-20 or 1-15 µM, preferably about 1-15 µM. In another example, the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells at a concentration of about 5-50, 5-40, 5-30, 5-20 or 5-15 µM, preferably about 5-15 µM.

In one example, the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells (e.g. in an in vitro or ex vivo culture) at a concentration of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45 or 50 µM, preferably about 10 µM.

In one example, the agent comprises at least one adenoviral protein.

In one example, the agent comprises a nucleic acid sequence encoding at least one adenoviral protein.

Preferably, the at least one adenoviral protein is E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76. The at least one adenoviral protein may be a variant or fragment of E4ORF1, when the variant or fragment substantially retains the biological activity of the full length E4ORF1, e.g. the ability to increase the survival and/or engraftment of gene edited haematopoietic stem/progenitor cells defined herein.

Preferably, the at least one adenoviral protein is E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107. The at least one adenoviral protein may be a variant or fragment of E4ORF6/7, when the variant or fragment substantially retains the biological activity of the full length E4ORF6/7, e.g. the ability to increase the survival and/or engraftment of gene edited haematopoietic stem/progenitor cells defined herein.

In one example, the agent comprises adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76; and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107.

In one example, the agent comprises a nucleic acid sequence encoding adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76; and a nucleic acid sequence adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107.

The adenoviral protein is not limited to a particular Adenovirus serotype. For example, in one example, the at least one adenoviral proteins is from an Adenovirus of serotype 4, Adenovirus of serotype 5, Adenovirus of serotype 7 and/or Adenovirus of serotype 9. In one example, the at least one adenoviral protein is from an Adenovirus of serotype 5.

In one example, the agent comprises two compounds as defined herein that promote homology directed repair. In one example, the agent comprises the inhibitor of p53 activation and the adenoviral protein, or a nucleotide sequence encoding therefor. Preferably, the inhibitor of p53 activation is GSE56 or a variant thereof.

In one example, the agent comprises:

(a) the inhibitor of p53 activation and adenoviral protein E4ORF1 (or a nucleotide sequence encoding therefor). In one example, the E4ORF1 has the amino acid sequence of E4ORF1 set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76.;

(b) the inhibitor of p53 activation and adenoviral protein E4ORF6/7 (or a nucleotide sequence encoding therefor). In one example, the E4ORF6/7 has the amino acid sequence of E4ORF6/7 set forth in SEQ ID No. 2; or SEQ ID Nos. 77-107.

(c) the inhibitor of p53 activation, adenoviral protein E4ORF1 (or a nucleotide sequence encoding therefor), preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76, and adenoviral protein E4ORF6/7 (or a nucleotide sequence encoding therefor), preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2; or

SEQ ID Nos. 77-107. Preferably, the inhibitor of p53 activation is GSE56 or a variant thereof.

In one example, the agent is a composition comprising the inhibitor of p53 activation and the adenoviral protein, or a nucleotide sequence encoding therefor.

In one example, the inhibitor of p53 activation is administered simultaneously, sequentially or separately in combination with the adenoviral protein, or a nucleotide sequence encoding therefor.

In one example, the adenoviral protein, or a nucleotide sequence encoding therefor, is administered simultaneously, sequentially or separately in combination with the inhibitor of p53 activation.

In one example, the disclosure provides a product comprising (a) the inhibitor of p53 activation of the disclosure; and (b) the adenoviral protein of the disclosure, as a combined preparation for simultaneous, separate or sequential use in therapy, wherein the use is a use of the disclosure.

The term “combination”, or terms “in combination”, “used in combination with” or “combined preparation” as used herein may refer to the combined administration of two or more entities simultaneously, sequentially or separately.

The term “simultaneous” as used herein means that the entities are administered concurrently, i.e. at the same time.

The term “sequential” as used herein means that the entities are administered one after the other.

The term “separate” as used herein means that the entities are administered independently of each other but within a time interval that allows the entities to show a combined, preferably synergistic, effect. Thus, administration “separately” may permit one entity to be administered, for example, within 1 minute, 5 minutes or 10 minutes after the other.

In one example, the nucleic acid encoding the adenoviral protein is an mRNA.

In one example, the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cell, preferably the transient expression occurs during gene editing of the haematopoietic cell, haematopoietic stem cell and/or progenitor cell.

In one example, the target of the gene editing is selected from the group consisting of CD40L, RAG-1, IL-2RG, CYBA, CYBB, NCF1, NCF2, and NCF4. In one example, the target of the gene editing is a gene mutated in chronic granulomatous disease or the gene mutated SCID, atypical SCID and Omenn syndrome, or Hyper IgM syndrome. Preferably the target of the gene editing is CD40L.

In another example, the present disclosure provides a method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells comprising introducing gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells; and

wherein prior to, at the same time as, or after the introducing of the gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is contacted with an agent which promotes homology directed DNA repair;

In another example, the present disclosure provides a 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;
(b) introducing gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells; and
(c) editing the genome of said haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

In another example, the present disclosure provides a method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells comprising the steps:

(a) introducing gene editing machinery to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells;

(b) contacting the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells with an agent which promotes homology directed DNA repair; and

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

In another example, the present disclosure provides a method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells comprising the steps:

(a) introducing gene editing machinery and an agent which promotes homology directed DNA repair to the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells;

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

In one example, the method steps (a) and (b) are carried out *ex vivo* or *in vitro*. Thus, in one example of the method of gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, steps (a) and (b) are carried out ex vivo or in vitro. In one example steps (a) and (b) may be carried out simultaneously. In another example, steps (a) and (b) are carried out sequentially, either step (a) before step (b) or step (b) before step (a).

In one example, the gene editing machinery may comprise a nuclease such as a zinc finger nuclease (ZFNs), a transcription activator like effector nucleases (TALENs), meganucleases, or the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system.

The gene editing machinery (e.g.CRISPR/Cas system) may comprise one or more guide RNAs complementary to at least one target gene in a cell, an RNA-guided DNA endonuclease enzyme or nucleotide sequence encoding said endonuclease (e.g. Cas9 protein or a nucleotide sequence encoding a Cas9). In one example, the gene editing machinery may be a CRISPR/Cas system.

In one example, the gene editing machinery may be provided by one or more nucleotide sequences. Suitably, the nucleotide sequences encoding the gene editing machinery may be introduced to the cell sequentially or simultaneously. In one example, the agent which promotes HDR may be contacted with the cell simultaneously with the introduction of gene

editing machinery to the cell. In one example, one or more nucleotide sequences encoding gene editing machinery is introduced to the cell by electroporation. In one example, one or more nucleotide sequences is introduced to the cell by transduction. Suitably, the nucleotide sequence may be introduced by transduction of a viral vector. For example, a Cas9 ribonucleprotein may be introduced to a cell by electroporation before AAV6 transduction for the delivery of the donor DNA template.

As used herein, the term “introducing” refers to methods for inserting foreign DNA or RNA into a cell. As used herein the term introduced includes both transduction and transfection methods. Transfection is the process of introducing nucleic acids into a cell by non-viral methods. Transduction is the process of introducing foreign DNA or RNA into a cell via a viral vector.

In one example, AAV transduction is used to deliver the donor DNA template.

In one example, AAV6 transduction is used to deliver the donor DNA template.

In one example, the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells (e.g. the population of cells in step (a) of the method of gene editing a population of said cells are contacted with the agent about 15 minutes to about 4 hours; about 15 minutes to about 3 hours; or about 15 minutes to about 2 hours before transducing the population of cells with the viral vector. In another example, the cells are contacted with the agent about 1-4 hours; 1-3 hours; or 1-2 hours before transducing the population of cells with the viral vector.

In one example, the haematopoietic cells, haematopoietic stem cells and/or haematopoietic cell progenitor cells (e.g. the population of cells in step (a) of the method of gene editing a population of cells of the disclosure) are contacted with the agent about 15 minutes, 30 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours or 4 hours, preferably about 15 minutes, before transducing the population of cells with the viral vector.

Suitably, the agent may be active (e.g. may promote homology directed DNA repair) during gene editing.

In one example, the contacting of step (a) is for about 12-60 h, such as 24-60, 36-60 or 42-54 h, preferably about 42-54 h, before step (b) is started. In one example, the

contacting of step (a) is for about 12, 18, 24, 30, 36, 42, 48, 54 or 60 h, preferably about 48 h, before step (b) is started.

In one example, step (b) is carried out about 12-60 h, such as 24-60, 36-60 or 42-54 h, preferably about 42-54 h, after beginning culture of the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells (e.g. beginning culture after the cells are thawed from a frozen state). In one example, step (b) is carried out about 12, 18, 24, 30, 36, 42, 48, 54 or 60 h, preferably about 48 h, after beginning culture of the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

In one example, step (b) is carried out about 12-60 h, such as 24-60, 36-60 or 42-54 h, preferably about 42-54 h, after thawing the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells (e.g. which had been stored in a frozen state). In one example, step (b) is carried out about 12, 18, 24, 30, 36, 42, 48, 54 or 60 h, preferably about 48 h, after thawing the population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

In one example, the agent which promotes homology directed DNA repair is an inhibitor of p53 activation.

In one example, 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.

In one example, the inhibitor of p53 activation is pifithrin-α or a derivative thereof, including pifithrin-α cyclic or pifithrin-α p-nitro; 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. Preferably, the inhibitor of p53 activation is a dominant negative peptide. Preferably the inhibitor of p53 activation is GSE56 or a variant thereof.

In one example, the inhibitor is added to the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells at a concentration of about 1-50 μM.

In one example, the agent comprises a nucleic acid sequence encoding at least one adenoviral protein.

Preferably, the at least one adenoviral protein is E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76. The at least one adenoviral protein may be a variant or fragment of E4ORF1, when the variant or fragment substantially retains the biological activity of the full length E4ORF1, e.g. the ability to increase the survival and/or engraftment of gene edited haematopoietic stem/progenitor cells defined herein.

Preferably, the at least one adenoviral protein is E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107. The at least one adenoviral protein may be a variant or fragment of E4ORF6/7, when the variant or fragment substantially retains the biological activity of the full length E4ORF6/7, e.g. the ability to increase the survival and/or engraftment of gene edited haematopoietic stem/progenitor cells defined herein.

In one example, the agent comprises adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76; and adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107.

In one example, the agent comprises a nucleic acid sequence encoding adenoviral protein E4ORF1, preferably wherein the amino acid sequence of E4ORF1 is set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76; and a nucleic acid sequence adenoviral protein E4ORF6/7, preferably wherein the amino acid sequence of E4ORF6/7 is set forth in SEQ ID No. 2, or SEQ ID Nos. 77-107.

The adenoviral protein is not limited to a particular Adenovirus serotype. For example, in one example, the at least one adenoviral proteins is from an Adenovirus of serotype 4, Adenovirus of serotype 5, Adenovirus of serotype 7 and/or Adenovirus of serotype 9. In one example, the at least one adenoviral protein is from an Adenovirus of serotype 5.

In one example, the agent comprises two compounds as defined herein that promote homology directed repair. In one example, the agent comprises the inhibitor of p53 activation and the adenoviral protein, or a nucleotide sequence encoding therefor. Preferably, the inhibitor of p53 activation is GSE56 or a variant thereof.

In one example, the agent comprises:

(a) the inhibitor of p53 activation and adenoviral protein E4ORF1 (or a nucleotide sequence encoding therefor). In one example, the E4ORF1 has the amino acid sequence of E4ORF1 set forth in SEQ ID No. 1, or SEQ ID Nos. 57-76.;

(b) the inhibitor of p53 activation and adenoviral protein E4ORF6/7 (or a nucleotide sequence encoding therefor). In one example, the E4ORF6/7 has the amino acid sequence of E4ORF6/7 set forth in SEQ ID No. 2; or SEQ ID Nos. 77-107.

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

Preferably, the inhibitor of p53 activation is GSE56 or a variant thereof.

In one example, the agent is a composition comprising the inhibitor of p53 activation and the adenoviral protein, or a nucleotide sequence encoding therefor.

In one example, the inhibitor of p53 activation is administered simultaneously, sequentially or separately in combination with the adenoviral protein, or a nucleotide sequence encoding therefor.

In one example, the adenoviral protein, or a nucleotide sequence encoding therefor, is administered simultaneously, sequentially or separately in combination with the inhibitor of p53 activation.

In one example, the nucleic acid encoding the adenoviral protein is an mRNA.

In one example, the adenoviral protein is expressed transiently in the haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cell.

In one example, 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 the target of the gene editing is CD40L.

In one example, 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.

In one example, the population of haematopoietic stem and/or progenitor cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

In one example, the method according to the present disclosure includes a further step of enriching the population for haematopoietic stem and/or progenitor cells.

In another example, the present disclosure provides a method of gene therapy comprising the steps:

a) gene editing a population of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells according to the method of the present disclosure; and
b) administering the gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells to a subject.

In one example, the gene edited cells are administered to a subject as part of an autologous stem cell transplant procedure or an allogeneic stem cell transplant procedure.

The method of gene therapy may be, for example, a method of treatment of a disease selected from the group consisting of mucopolysaccharidosis type I (MPS-1), chronic granulomatous disorder, Fanconi anaemia (FA), sickle cell disease, metachromatic leukodystrophy (MLD), globoid cell leukodystrophy (GLD), GM_2 gangliosidosis, thalassemia and cancer.

The method of gene therapy may be, for example, a method of treatment of diseases caused by Rag-1 mutations, eg SCID, atypical SCID and Omenn syndrome

The method of gene therapy may be, for example, a method of treatment of Hyper IgM syndrome (e.g. wherein the target of the gene editing is CD40L).

In one example, the subject is a mammalian subject, preferably a human subject.

In one example, the present disclosure provides a population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells prepared according to the method according to the present disclosure.

In another example, the present disclosure provides a pharmaceutical composition comprising the population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells according to the present disclosure.

In yet another example, the present disclosure provides a population of gene edited haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells according to the present disclosure for use in therapy, preferably for use in gene therapy.

In one example, the population of gene edited haematopoietic stem and/or progenitor cells for use according to the present disclosure is administered as part of an autologous stem cell transplant procedure or an allogeneic stem cell transplant procedure.

In another example the disclosure provides the use of an agent which promotes homology directed DNA repair for the manufacture of a medicament for haematopoietic cell, haematopoietic stem cell and/or haematopoietic progenitor cell gene therapy, where in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells are gene edited.

In another example the disclosure provides the use of agent which promotes homology directed DNA repair for the manufacture of a medicament for increasing survival and/or engraftment of haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells, where in the haematopoietic stem and/or progenitor cells are gene edited.

In another example the present disclosure provides an AAV vector comprising a barcode sequence.

In another example the present disclosure provides an AAV vector comprising a barcode sequence, for use in gene therapy, preferably haematopoietic gene therapy.

Preferred AAV vectors are those which are able to achieve a high transduction efficiency in human primary cells, such as HSPC cells. The AAV vector may be HDR-proficient. In one example, the vector is an AAV6 vector or a vector derived from an AAV6 vector. Preferably the vector is an AAV6 vector.

A barcode sequence is a random, highly degenerated DNA sequence that acts as a unique identifier. Preferably the barcode sequence is a random DNA sequence. The DNA sequence may comprise or consist of at least 15 bp, or at least 18, 19 or 20 bp, for example, 20-40 bp, 20-30 bp, 20-25 bp, or about 22 bp. Preferably the barcode sequence does not comprise any undesired restriction sites (i.e. restriction sites for cloning restriction enzymes).

The AAV vector may further comprise a nucleotide sequence encoding a gene of interest. The "gene of interest" may be any gene. The gene may be a marker gene and/or a gene involved in human diseases.

The AAV vector may comprise the gene of interest and the barcode sequence such that the barcode sequence is able to report on whether the gene of interest is correctly integrated into a host genome and/or for clonal tracking of cells. In other words, the gene of interest and barcode sequence are flanked by homology sequences, such that during homology directed DNA repair the genome of interest and the barcode sequence are inserted into the host genome together.

The gene of interest may be operably linked to a promoter. The promoter may be any suitable promoter known to those of skill in the art. In some examples, the promoter is hPGK promoter.

The gene of interest may be operably linked to a polyadenylation sequence. The polyadenylation sequence may be any suitable polyadenylation sequence known to those of skill in the art. In some examples, the promoter is bGH-pA.

In some examples the AAV vector targets a safe harbour locus, preferably the AAVS1 locus, intron 1 of IL2RG, CD40L or RAG-1, most preferably the AAVS1 locus.

The AAV vector may comprise the nucleotide sequence shown below, or a nucleotide sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequence below (barcode sequence underlined):

CCACTGAGAACCGGGCAGGTCACGCATCCCCCCCTTCCCTCCCACCCCCTGCCAAGCT
CTCCCTCCCAGGATCCTCTCTGGCTCCATCGTAAGCAAACCTTAGAGGTTCTGGCAAG
GAGAGAGATGGCTCCAGGAAATGGGGGTGTGTCACCAGATAAGGAATCTGCCTAACAG
GAGGTGGGGGTTAGACCCAATATCAGGAGACTAGGAAGGAGGAGGCCTAAGGATGGG
GCTTTTCTGTCACCAATCCTGTCCCTA<u>NNYNNNTNTNNNNRTNDNNNHH</u>CCATAGAGCC
CACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCC
CCACCCCACCCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCA
TTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGG

GGGCCAGCCGTAGAGGTGACCCAGGCCACAAGCTGCAGACAGAAAGCGGCACAGGC
CCAGGGGAGAGAATGCTGGTCAGAGAAAGCA

In another example the present disclosure provides for use of the AAV vector of the present disclosure for clonal tracking of cells that have been gene-edited using said vector, preferably wherein the cells are haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells. In one example, the use is *in vitro* use.

In another example the present disclosure provides a method for clonal tracking of cells using the AAV vector of the present disclosure, preferably wherein the cells are haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells.

The method may comprise the following steps:

(a) providing an isolated population of cells;

(b) gene editing the isolated population of cells using the AAV vector of the present disclosure; and

(c) clonal tracking of the gene-edited cells using the barcode sequence of the AAV vector.

Preferably, the method further comprises: optionally, expanding the isolated population of gene-edited cells; and transplanting the isolated population of gene-edited cells into a subject.

Thus, in a preferred example, the method comprises the steps of:

(a) providing an isolated population of cells;

(b) gene editing the isolated population of cells using the AAV vector of the present disclosure;

(c) optionally, expanding the isolated population of gene-edited cells;

(d) transplanting the isolated population of gene-edited cells into a subject; and

(e) clonal tracking of the gene-edited cells using the barcode sequence of the AAV vector.

More preferably, the method further comprises: harvesting blood and/or tissue samples from the subject; isolating genomic DNA from the blood and/or tissue samples; and quantifying the barcodes in the genomic DNA.

Thus, in a preferred example, the method comprises the steps of:

(a) providing an isolated population of cells;

(b) gene editing the isolated population of cells using the AAV vector of the present disclosure;

(c) optionally, expanding the isolated population of gene-edited cells;

(d) transplanting the isolated population of gene-edited cells into a subject;

(e) harvesting blood and/or tissue samples from the subject;

(f) isolating genomic DNA from the blood and/or tissue samples; and

(g) quantifying the barcode sequences from the AAV vector in the genomic DNA.

The cells may be any suitable cells, as described herein. The cells may be haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells. The cells may be e.g. human or mouse cells. Preferably the cells are human cells and the subject is human. The cells may be CD34+ cells. In some examples the cells are HSCs. In some examples the cells are HSPCs.

The AAV vector may be transduced into the cells. Preferably the transduction is performed with 1 $x10^4$ to $5x10^4$ vg/cell.

Blood and/or tissue samples may be harvested after at least 1, 2, 3, 4, 8, 12, 24, 36 or 48 weeks. For example, blood and/or tissue samples may be harvested after about 1-100 weeks, 1-50 weeks, 1-20 weeks, 2-20 weeks, or 5-20 weeks.

Preferably, blood and/or tissue samples are harvested at least about every day, about every week, about every two weeks, or about every month. For example, in some examples, blood and/or tissue samples are harvested about every day, about every week, about every two weeks, or about every month.

In preferred examples the sample is a blood sample.

Clonal tracking or quantification of the barcode sequences may comprise the steps of:

(i) PCR amplification of the barcode sequences; and

(ii) sequencing the PCR-amplified barcode sequences.

Quantification may allow the number and distribution of gene-edited clones to be identified i.e. to allow for clonal tracking.

In another example the present disclosure provides use of a barcode sequence for clonal tracking of cells.

DESCRIPTION OF THE DRAWINGS

Figure 1. Single cell transcriptional impact of gene editing in HSPC. (A) Schematic representation of the gene editing protocol and scRNA-Seq analyses. Briefly, ~3000 cells for each experimental condition were FACS-enriched for CD133+ and CD90+ surface markers and subsequently collected for Drop-seq analysis at 24hrs after electroporation. **(B)** Reactome analysis on the top 100 genes (ranked by log 2-fold change (2FC)) up-regulated in scRNA-Seq data comparing edited vs. mock treated CD34+ cells. **(C)** Heatmap showing the expression level of a list of p53-activated genes retrived from *M. Fischer 2017 Oncogene* 36, 3943-3956 in single cells edited with RNP+AAV6 or Mock-treated. **(D)** Fold expression of *CDKN1A* relative to mock control measured 24hrs after electroporation of CD34+ cells treated as indicated (RNP+ssODN n=3, other conditions n=5).

Figure 2. Transient p53 inhibition by pifithrins. (A) Schematic representation of the gene editing protocol and the timing of phithrins addition. **(B)** Percentage of GFP+ cells measured within the indicated subpopulations 3 days after gene editing in presence or not of different phfithin-α derivatives (n=2). **(C)** Percentage of GFP+ cells measured within the indicated subpopulations and of NHEJ measured on the bulk cells 3 days after gene editing in presence or not of phfithin-α ciclic (n=5) **(D)** Subpopulation composition of treated CD34+ cells from (C) (n=5).

Figure 3. Transient p53 inhibition by GSE56 mRNA. (A) Schematic representation of the gene editing protocol ± GSE56 and cell analyses. **(B)** Fold expression of the indicated p53-target genes (CDKN1A, PHLDA3, RPS27L, APOBEC3H) relative to untreated control (UT) measured 24hrs after electroporation of CD34+ cells treated as indicated (UT, RNP+AAV6, RNP+AAV6+GSE56 n=8; mock electro n=3) *P<0.05, **P<0.01; Mann-Whitney Test. **(C)** (Left) Percentage of HDR (measured by ddPCR) or NHEJ (measured by heteroduplex assay) 3 days after *AAVS1* or *IL2RG* gene editing in presence or not GSE56 mRNA (n=9) *P<0.05, **P<0.01; Wilcoxon matched-pairs signed-rank test. (Right) Percentage of GFP+ cells measured within the indicated subpopulations 3 days after *AAVS1* or *IL2RG* gene editing in presence or not GSE56 mRNA (n=15) **P<0.01; Wilcoxon matched-pairs signed-rank test. **(D)** Growth curve of AAVS1 edited cells in presence or not GSE56 mRNA (n=2)

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.

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.

3. 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.

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.

5. The use of claim 4, 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.

6. The use of claim 4 or 5, wherein the inhibition of p53 in the haematopoietic cells, haematopoietic stem cells and/or haematopoietic progenitor cells is transient.

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

8. The use of any one of claims 4 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.

9. The use of any one of claims 1-8, wherein the agent comprises a nucleic acid sequence encoding at least one adenoviral protein, preferably wherein the at least one adenoviral protein 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. The use of claim 9, 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.

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. The use of any one of claims 1-11, 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) 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.

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

14. The use of any one of claims 9 to 13, 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. The use of any one of claims 1 to 14, 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. 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;

(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. The method of any one of claims 16 to 25, 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. The method of any one of claims 16 to 26, wherein the population of haematopoietic stem and/or progenitor cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

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

29. The method of any one of claims 16 to 28, 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. 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; and

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

31. The use of claim 30, 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. 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.

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

34. 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 for use in therapy.

35. 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.