SUMMARY

In one aspect, the present invention refers to a method of preparing a therapeutic cell population and a support cell population for clinical use from a starting population of cells comprising haematopoietic stem cells, said method comprising separating a population of cells that substantially do not express CD38 but which express CD34 from the starting population of cells, and transducing the separated cell population with a vector to obtain the therapeutic cell population which has the $CD34^+CD38^-$ phenotype, and wherein the separated CD38-expressing cells or portion thereof are retained to form a support cell population which has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype.

In another aspect, the present invention refers to use of a therapeutic cell population for the manufacture of a medicament for improving recovery after myeloablative conditioning, wherein the therapeutic cell population has the $CD34^+CD38^-$ phenotype and is prepared according to the method of any preceding claim, wherein the therapeutic cell population is to be administered in combination with a support cell population which has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype and is prepared according to the method as disclosed herein.

In yet another aspect, the present invention refers to use of a support cell population for the manufacture of a medicament for improving recovery after myeloablative conditioning, wherein the support cell population has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype and is prepared according to the method of any one of claims 1-7, wherein the support cell population is to be administered in combination with a therapeutic cell population which has the $CD34^+CD38^-$ phenotype and is prepared according to the method as disclosed herein.

We have unexpectedly shown that $CD34^+CD38^-$ HSCs undergo more efficient transduction when they are present at high purity. We have developed a protocol for purifying and transducing these cells in a manner that is suitable for clinical use.

We have also found that prostaglandin E2 and derivatives thereof increase the efficiency of gene transfer into $CD34^+$ and $CD34^+CD38^-$ HSCs.

These unexpected findings result in increased transduction efficiency which allows reduction in the quantity of vector applied and minimisation of *ex vivo* culture.

Continuing from these findings, we have developed an innovative protocol which is based on the co-transplantation of transduced, highly-purified long-term repopulating cells (e.g. cells expressing CD34, but not CD38) with unmanipulated progenitor cells (e.g. cells that express CD38). This protocol may improve safety and efficacy of gene therapy by:

1. increasing transduction efficiency;
2. reducing the number of cells necessary to be transduced, which results in a lower integration load required to be infused into the subject;

3. guaranteeing a rapid hematologic recovery.

In addition, we have also developed an innovative protocol which is based on the transplantation of transduced haematopoietic progenitor cells.

In one example, there is disclosed a method of preparing a therapeutic cell population for clinical use from a starting population of cells comprising haematopoietic stem cells, said method comprising separating a population of cells that substantially do not express CD38 but which express CD34 from the starting population of cells, and transducing the separated cell population with a vector, preferably a viral vector, to obtain the therapeutic cell population.

In one example the method comprises the steps of:

a. separating CD38-expressing cells from a starting population of cells comprising haematopoietic stem cells;
b. separating CD34-expressing cells from the population of cells obtained in step (a) that do not express CD38;
c. transducing the CD34-expressing cell population obtained in step (b) with a vector to obtain the therapeutic cell population.

In another example the method comprises the steps of:

a. contacting a starting population of cells comprising haematopoietic stem cells with an agent reactive for CD38;
b. separating the CD38-reactive cells from the CD38-non-reactive cells;
c. contacting the CD38-non-reactive cells obtained in step (b) with an agent reactive for CD34;
d. separating the CD34-reactive cells from the CD34-non-reactive cells wherein the CD34-reactive cells form the transduction cell population;
e. transducing the transduction cell population with a vector to obtain the therapeutic cell population.

The therapeutic cell population may have the $CD34^{+}CD38^{-}$ phenotype.

In one example the vector comprises a nucleotide of interest or is itself a nucleotide of interest.

In one example the separated CD38-expressing cells or CD38-reactive cells or portion thereof are retained to form a support cell population. The support cell population may have the $CD34^{+}CD38^{int1}$, $CD34^{+}CD38^{int2}$ and/or $CD34^{+}CD38^{+}$ phenotype.

In one example there is disclosed the step of transducing a population of cells with a vector comprises culturing the cells for about 44 h or more. The "step of transducing a population of cells with a vector" is to be understood as the pre-stimulation and vector exposure phases. For example, the population of cells may be cultured for about 44-66 h, 44-60 h, 44-54 h or

44-48 h during the step of transduction with a vector. In one embodiment the population of cells is cultured for about 66, 60, 54, 48 or 44 h during the step of transduction with a vector.

In another example, there is disclosed the step of transducing a population of cells with a vector comprises culturing the cells (i.e. during pre-stimulation and vector contact) for less than about 44 h. For example, the population of cells may be cultured for about 12-42 h, 12-36 h, 12-24 h or 12-18 h during the step of transduction with a vector. In one embodiment the population of cells is cultured for about 42, 36, 30, 24, 18 or 12 h during the step of transduction with a vector. Preferably the population of cells is cultured for about 24 h during the step of transduction with a vector.

In one example prostaglandin E2 or a derivative thereof is used in the methods as disclosed herein to increase the transduction efficiency.

Prostaglandin E2 or a prostaglandin E2 derivative (e.g. 16,16-dimethyl prostaglandin E2 (dmPGE2)) may be added to the population of cells during the step of transduction with a vector, preferably during the pre-stimulation phase of this step. Prostaglandin E2 or the prostaglandin E2 derivative may be added at the start of the pre-stimulation phase, or during the pre-stimulation phase. For example, prostaglandin E2 or the prostaglandin E2 derivative may be added about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 h before the exposure of the population of cells to the vector. Preferably, the prostaglandin E2 or the prostaglandin E2 derivative is added to the population of cells during the pre-stimulation phase, about 2 h before the exposure of the cells to the vector. In another embodiment the prostaglandin E2 or the prostaglandin E2 derivative is added to the population of cells at the same time as exposure to the vector.

In one example the starting population of cells comprising haematopoietic stem cells is obtained from a tissue sample.

In another example the starting population of cells comprising haematopoietic stem cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

In another example there is discloseda therapeutic cell population prepared according to a method of the invention.

In another example there is discloseda support cell population prepared according to a method of the invention.

In another example there is discloseda pharmaceutical composition comprising the therapeutic cell population or support cell population of the invention, preferably in the presence of a pharmaceutically acceptable carrier, diluent or excipient.

In another example there is discloseda therapeutic cell population and/or a support cell population for use in medicine.

In one example the therapeutic cell population is administered in combination with the support cell population of the invention.

In another example the therapeutic cell population is administered to a subject prior to administration of the support cell population.

In another example the therapeutic cell population is administered to a subject contemporaneously with or simultaneously to administration of the support cell population.

In another example there is discloseda kit comprising the therapeutic and support cell populations of the invention.

Also disclosed is a method of treatment comprising administering the therapeutic cell population of the invention to a subject in need thereof.

Also disclosed is a method of treatment comprising administering the therapeutic cell population of the invention and the support cell population of the invention to a subject in need thereof.

Preferably, the treatment is a treatment by gene therapy.

In another example there is disclosed a haematopoietic progenitor cell population for use in gene therapy, wherein the haematopoietic progenitor cell population has been transduced with a nucleotide of interest.

The step of transduction with a nucleotide of interest may, for example, utilise any of the methods of cell transduction described herein.

In another example there is disclosed a haematopoietic progenitor cell population for use in gene therapy, wherein said cells have been separated from a population of cells comprising haematopoietic stem and progenitor cells and then transduced with a nucleotide of interest.

In one example the haematopoietic progenitor cell population has the $CD34^{+}CD38^{int}$ phenotype. In another embodiment, the haematopoietic progenitor cell population has a $CD34^{+}CD38^{int1}$, $CD34^{+}CD38^{int2}$ and/or $CD34^{+}CD38^{+}$ phenotype. Thus the haematopoietic progenitor cell population may, for example, not comprise cells of the $CD34^{+}CD38^{-}$ phenotype.

In another example the transduced progenitor cell population is administered in combination with a population of haematopoietic stem cells, for example a population of cells with the $CD34^{+}CD38^{-}$ phenotype.

Also disclosed is a method of gene therapy comprising administering a haematopoietic progenitor cell population to a subject in need thereof, wherein the haematopoietic progenitor cell population has been transduced with a nucleotide of interest.

In another example there is discloseda method of controlling the duration of transgene expression in a patient, wherein the duration of transgene expression is controlled by selectively administering transduced haematopoietic stem and/or progenitor cells based on CD38 expression level. Increased transgene expression duration may be achieved by administering cells with decreased levels of CD38 expression.

In another example there is disclosedthe use of prostaglandin E2 or a prostaglandin E2 derivative for increasing gene transfer efficiency when transducing haematopoietic stem or progenitor cells with a vector, preferably a viral vector.

In one example the prostaglandin E2 derivative is 16,16-dimethyl prostaglandin E2.

Prostaglandin E2 or a prostaglandin E2 derivative (e.g. 16,16-dimethyl prostaglandin E2 (dmPGE2)) may be added to the population of cells during the pre-stimulation phase. In one embodiment the prostaglandin E2 or the prostaglandin E2 derivative is added at the start of the pre-stimulation phase. In another embodiment the prostaglandin E2 or the prostaglandin E2 derivative is added during the pre-stimulation phase.

For example, prostaglandin E2 or the prostaglandin E2 derivative may be added about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 h before the exposure of the population of cells to the vector. Preferably, the prostaglandin E2 or the prostaglandin E2 derivative is added to the population of cells during the pre-stimulation phase, about 2 h before the exposure of the cells to the vector.

In another example the prostaglandin E2 or the prostaglandin E2 derivative is added to the population of cells at the same time as exposure to the vector.

In another example there is disclosed a method of transducing a population of cells with a vector, preferably a lentiviral vector, wherein the step of transducing a population of cells with a vector comprises culturing the cells for about 44 h or more. The "step of transducing a population of cells with a vector" is to be understood as the pre-stimulation and vector exposure phases. For example, the population of cells may be cultured for about 44-66 h, 44-60 h, 44-54 h or 44-48 h during the step of transduction with a vector. In one

example the population of cells is cultured for about 66, 60, 54, 48 or 44 h during the step of transduction with a vector.

In another example there is disclosed a method of transducing a population of cells with a vector, preferably a lentiviral vector, wherein the step of transducing a population of cells with a vector comprises culturing the cells (i.e. during pre-stimulation and vector contact) for less than about 44 h. For example, the population of cells may be cultured for about 12-42 h, 12-36 h, 12-24 h or 12-18 h during the step of transduction with a vector. In one embodiment the population of cells is cultured for about 42, 36, 30, 24, 18 or 12 h during the step of transduction with a vector. Preferably the population of cells is cultured for about 24 h during the step of transduction with a vector.

It will be appreciated that the steps of the method of preparing the therapeutic cell population described herein may be carried out in a different order. Thus, the method of preparing the therapeutic cell population may comprise the steps of:

a. separating CD34-expressing cells from a starting population of cells comprising haematopoietic stem cells;

b. separating CD38-expressing cells from the population of cells obtained in step (a) that express CD34;

c. transducing the cell population that does not express CD38 obtained in step (b) with a vector to obtain the therapeutic cell population.

The cells or portion thereof obtained in step (b) that do express CD38 may be retained to form a support cell population.

The present application refers to uses of a support cell population. In one aspect of the present invention, the support cell population referred to herein may be replaced with the support cell population as defined above.

Alternatively, the cells or portion thereof obtained in step (b) that do express CD38 may be transduced with a vector. Such transduced cells could be used in therapy (e.g. administered to a subject). This approach may allow transient delivery of a gene (e.g. a therapeutic gene) to a subject (such as for use in cancer gene therapy).

DESCRIPTION OF THE DRAWINGS

Figure 1

CLAIMS

1. A method of preparing a therapeutic cell population and a support cell population for clinical use from a starting population of cells comprising haematopoietic stem cells, said method comprising separating a population of cells that substantially do not express CD38 but which express CD34 from the starting population of cells, and transducing the separated cell population with a vector to obtain the therapeutic cell population which has the $CD34^+CD38^-$ phenotype, and wherein the separated CD38-expressing cells or portion thereof are retained to form a support cell population which has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype.

2. The method of claim 1, wherein the method comprises the steps of:

   a. separating CD38-expressing cells from a starting population of cells comprising haematopoietic stem cells;

   b. separating CD34-expressing cells from the population of cells obtained in step (a) that do not express CD38;

   c. transducing the CD34-expressing cell population obtained in step (b) with a vector to obtain the therapeutic cell population.

3. The method of claim 1 or 2, wherein the starting population of cells comprising haematopoietic stem cells is obtained from mobilised peripheral blood, bone marrow or umbilical cord blood.

4. The method of any preceding claim, wherein the CD38-expressing cells and/or CD34-expressing cells are separated using magnetic bead-based separation or flow cytometry.

5. The method of any preceding claim, wherein the vector is a viral vector, preferably a lentiviral vector.

6. The method of any preceding claim, wherein the step of transducing a population of cells with a vector comprises culturing the cells for less than about 44 h, preferably wherein the step of transducing a population of cells with a vector comprises culturing the cells for about 12-24 hours.

7. The method of any preceding claim, wherein the step of transducing a population of cells with a vector comprises treating the population of cells with prostaglandin E2 or 16,16-dimethyl prostaglandin E2, preferably wherein the cells are treated with

prostaglandin E2 or 16,16-dimethyl prostaglandin E2 before exposure of the cells to the vector.

8. Use of a therapeutic cell population for the manufacture of a medicament for improving recovery after myeloablative conditioning, wherein the therapeutic cell population has the $CD34^+CD38^-$ phenotype and is prepared according to the method of any preceding claim, wherein the therapeutic cell population is to be administered in combination with a support cell population which has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype and is prepared according to the method of any preceding claim.

9. Use of a support cell population for the manufacture of a medicament for improving recovery after myeloablative conditioning, wherein the support cell population has the $CD34^+CD38^{int/lo}$, $CD34^+CD38^{+/int}$ and/or $CD34^+CD38^+$ phenotype and is prepared according to the method of any one of claims 1-7, wherein the support cell population is to be administered in combination with a therapeutic cell population which has the $CD34^+CD38^-$ phenotype and is prepared according to the method of any one of claims 1-7.

10. The use of claim 8 or 9, wherein the number of $CD34^+$ cells to be administered to a subject in the support cell population is about 5-15× the number of $CD34^+$ cells to be administered in the therapeutic cell population when the subject has undergone myeloablative conditioning.

11. The use of any one of claims 8-10, wherein the therapeutic and support cell populations are to be administered as part of an autologous stem cell transplant procedure or an allogeneic stem cell transplant procedure.

12. The use of any one of claims 8-11, wherein the therapeutic cell population is to be administered to a subject prior to administration of the support cell population, or wherein the therapeutic cell population is to be administered to a subject contemporaneously with or simultaneously to administration of the support cell population.