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(54) Title: VECTOR PARTICLES FOR TARGETING CD34+ CELLS

(57) Abstract: The present invention relates to a vector particle for transferring biological material into cells, wherein said vector particle comprises at least: -a first protein which comprises the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein, and -a second protein which comprises a ligand of the c-Kit receptor.

VECTOR PARTICLES FOR TARGETING CD34+ CELLS

The present invention relates to vector particles intended for the specific delivery of biological material to cells.

For the correction by gene therapy of many inherited or acquired defects of the hematopoietic system, the therapeutic gene must be delivered to cells able both to self-renew and to differentiate into all hematopoietic lineages. As such, these gene therapies must be targeted to the "right" cells, *i.e.* hematopoietic stem cells (HSCs), without modifying their properties. The population of choice for targeting HSCs is constituted of $CD34^+$ progenitor cells, which are particularly enriched in these stem cells. However, $CD34^+$ cells only represent 0.001% of the total blood cells for instance. Accordingly, to avoid the cumbersome steps of cell extraction, culture in the presence of multiple growth factors or transduction adjuvants, and infusion into the patient, the vector particles have to display a very high specificity towards $CD34^+$ cells, in order to allow transduction of $CD34^+$ cells in non-purified bodily samples, such as blood samples, or to ensure an efficient *in vivo* transduction of $CD34^+$ cells despite dilution of the vector particles.

Thus, Sandrin *et al.* (2002) *Blood* **100**:823-832 have devised Simian Immunodeficiency Virus (SIV)-derived vector particles which display a chimeric envelope glycoprotein, RDTR, constituted of the fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of the Murine Leukemia Virus-A envelope glycoprotein. Such vector particles are also disclosed in WO 03/91442. When using a transduction adjuvant, such as RetroNectin®, the transduction rate obtained using vector particles displaying the chimeric RDTR protein is of approximately the same rate as that observed with SIV-derived vector particles displaying the Vesicular Stomatitis Virus (VSV) G envelope glycoprotein. However, in the absence of transduction adjuvant, the RDTR vector particles exhibit a much lower transduction of isolated $CD34^+$ cells than vectors displaying the VSV-G glycoprotein. Besides, no particular selectivity towards $CD34^+$ cells has been shown to be associated to RDTR, since vector particles displaying this chimeric protein transduce $CD34^+$ cells and peripheral blood lymphocytes with approximately the same efficiency.

In another attempt at targeting $CD34^+$ cells, Verhoeyen *et al.* (2005) *Blood* **106**:3386-3395 have devised HIV-1-derived vector particles which display the VSV-G envelope glycoprotein and so-called early acting cytokines, namely Thrombopoietin (TPO) and Stem Cell Factor (SCF). The authors have thus shown that these vector particles provided for efficient transduction of isolated $CD34^+$ cells. However, no targeting specificity of these vector particles could be evidenced.

Accordingly, it is an object of the present invention to provide vector particles which are more efficient than those of the prior art at specifically targeting $CD34^+$ cells.

Summary of the invention

The present invention arises from the discovery, by the inventors, that the co-display of RDTR and SCF on HIV-derived vector particles had unexpected synergic effects on the efficiency and the specificity of transduction of $CD34^+$ cells. Advantageously, such vector particles are not dependant on RetroNectin® to achieve transduction, can effect efficient transduction at low dosage, and are capable to transduce $CD34^+$ cells in fresh whole blood.

Thus, the present invention relates to a vector particle for transferring biological material into cells, wherein said vector particle comprises at least:

- a first protein which comprises the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein, and
- a second protein which comprises a ligand of the c-Kit receptor.

The present invention also relates to the use of (i) a first nucleic acid comprising a sequence encoding a first protein as defined above and of (ii) a second nucleic acid comprising a sequence encoding a second protein as defined above, for preparing a vector particle for transferring biological material into cells and in particular for preparing a vector particle as defined above.

The present invention also relates to a method for preparing a vector particle for transferring biological material into cells and in particular for preparing a vector particle as defined above, wherein (i) a first nucleic acid comprising a sequence encoding a first protein as defined above and (ii) a second nucleic acid comprising a sequence encoding a second protein as defined above, are

transferred in a producer cell, and the vector particle is recovered from said producer cell.

The present invention also relates to a medicament comprising a vector particle as defined above as active ingredient.

The present invention also relates to a method for treating an individual in need of gene therapy, wherein a therapeutically effective amount of a vector particle as defined above is administered to the individual.

The present invention further relates to the use of a vector particle as defined above, for transferring the biological material into cells *ex vivo*.

The present invention also relates to a method for preparing cells intended for treating an individual, wherein cells to be administered to the individual are contacted with a vector particle as defined above.

The present invention also relates to a method for treating an individual in need of gene therapy, wherein in a first step cells to be administered to the individual are contacted with a vector particle as defined above and in a second step said cells are administered to the individual.

The present invention also relates to a protein represented by SEQ ID NO: 4.

The present invention also relates to a nucleic acid encoding a protein of sequence SEQ ID NO: 4.

Brief description of the figures

Figure 1 represents the percentage of $CD34^+$ cells (vertical axis) transduced by GFP-encoding HIV-derived vector particles displaying RDTR only, in the presence of recombinant TPO (10 ng/ml) or recombinant SCF (50 ng/ml), or vector particles displaying RDTR and TPOHA, or RDTR and SCFHA, in the presence (widely hatched bars) or absence (closely hatched bars) of RetroNectin®.

Figure 2 represents the percentage of $CD34^+$ cells (vertical axis) transduced by GFP-encoding HIV-derived vector particles displaying RDTR only, in the presence of recombinant TPO (10 ng/ml) or recombinant SCF (50 ng/ml), or vector particles displaying RDTR and TPOHA, or RDTR and SCFHA, at a Multiplicity Of Infection

(M.O.I.) of 10 (widely hatched bars), 2 (white bars) or 0.2 (closely hatched bars) as determined on HeLa cells.

Figure 3 represents the percentage of GFP expressing cells (vertical axis) present in a PBMC population isolated from cord blood transduced by GFP-encoding HIV-derived vector particles displaying RDTR only in the presence of recombinant SCF (50 ng/ml), RDTR and SCFHA, VSV-G only in the presence of recombinant SCF (50 ng/ml), or VSV-G and SCFHA, wherein the cells are $CD34^+$ cells (closely hatched bars) or $CD3^+$ cells (widely hatched bars).

Figure 4 represents the percentage (vertical axis) of GFP expressing $CD34^+$ cells or $CD3^+$ cells present in whole cord blood transduced by GFP-encoding HIV-derived vector particles displaying RDTR only in the presence of recombinant SCF (50 ng/ml) (first bar), RDTR and SCFHA (second bar), VSV-G only and recombinant SCF (50 ng/ml) (third bar), or VSV-G and SCFHA (fourth bar).

Figure 5 represents the analysis by fluorescence-activated cell sorter (FACS) of the transduction (GFP^+) of total human cells in the bone marrow. The three histograms show respectively the results obtained on three different injected mice. The cells were sorted according to hCD45 expression ($hCD45^+$, vertical axis) and GFP expression (GFP^+, horizontal axis).

Figure 6 represents the analysis by FACS of the transduction (GFP^+) of early progenitors ($hCD34^+$), myeloid progenitors ($hCD13^+$), monocytes ($hCD14^+$) and pre- and pro B-cells ($hCD19^+$) in the bone marrow. The first histogram shows the results obtained with cells sorted according to hCD34 expression ($hCD34^+$, vertical axis) and GFP expression (GFP^+, horizontal axis). The second histogram shows the results obtained with cells sorted according to hCD13 expression ($hCD13^+$, vertical axis) and GFP expression (horizontal axis). The third histogram shows the results obtained with cells sorted according to hCD14 expression ($hCD14^+$, vertical axis) and GFP expression (horizontal axis). The fourth histogram shows the results obtained with cells sorted according to hCD19 expression ($hCD19^+$, vertical axis) and GFP expression (horizontal axis).

Figure 7 represents the analysis by FACS of the transduction (GFP^+) of human thymocytes in the thymus. The cells were sorted according to hCD45 expression ($hCD45^+$, vertical axis) and GFP expression (GFP^+, horizontal axis).

Figure 8 represents the analysis by FACS of the transduction (GFP^+) of B-cells ($hCD19^+$) and T-cells ($hCD3^+$) in the peripheral blood. The first histogram shows the results obtained with cells sorted according to hCD19 expression ($hCD19^+$, vertical axis) and GFP expression (GFP^+, horizontal axis). The second histogram shows the results obtained with cells sorted according to hCD3 expression ($hCD3^+$, vertical axis) and GFP expression (horizontal axis).

Figure 9 represents the analysis by FACS of the transduction (GFP^+) of human splenocytes ($hCD45^+$), B-cells ($hCD19^+$) and T-cells ($hCD3^+$) in the spleen. The first histogram shows the results obtained with cells sorted according to hCD45 expression ($hCD45^+$, vertical axis) and GFP expression (GFP^+, horizontal axis). The second histogram shows the results obtained with cells sorted according to hCD19 expression ($hCD19^+$, vertical axis) and GFP expression (horizontal axis). The third histogram shows the results obtained with cells sorted according to hCD3 expression ($hCD3^+$, vertical axis) and GFP expression (horizontal axis).

Detailed description of the invention

As intended herein, "vector particle" denotes any particle liable to display the first protein and the second protein at its surface and to reversibly bind to a biological material.

It is preferred that such a vector particle is a viral vector particle, in particular a lentiviral vector particle, such as a lentiviral vector particle selected from the group consisting of Human Immunodeficiency Virus (HIV), *e.g.* HIV-1 or HIV-2, and Simian Immunodeficiency Virus (SIV).

Lentiviral vector particles are well-known to the man skilled in the art and are notably described in Naldini *et al.* (2000) *Adv. Virus Res.* **55**:599-609 and Negre *et al.* (2002) *Biochimie* **84**:1161-1171. Usually, lentiviral vector particles according to the invention comprise at least the following components: (i) an

envelope component, which is constituted of a phospholipidic bilayer associated to envelope proteins, wherein the envelope proteins comprise at least the above-defined first and second proteins, said envelope surrounding (ii) a core component, constituted of the association of a gag protein, said core itself surrounding (iii) genome components, usually constituted of ribonucleic acids (RNA), and (iv) an enzyme component (pol). The biological material can be present within the envelope, within the core and/or within the genome components.

Lentiviral vector particles can be readily prepared by the man skilled in the art, for example by following the general guidance provided by Sandrin *et al.* (2002) *Blood* **100**:823-832. Briefly, the lentiviral vector particles may be generated by co-expressing the packaging elements (*i.e.* the core and enzyme components), the genome component and the envelope component in a so-called producer cell, *e.g.* 293T human embryonic kidney cells. Typically from three to four plasmids may be employed, but the number may be greater depending upon the degree to which the lentiviral components are broken up into separate units.

Generally, one plasmid encodes the core (gag) and enzymatic (pol) lentiviral components of the vector particle. The origin of the gag and pol genes gives its name to the lentiviral vector particle. For instance the expression "HIV-1-derived vector particle" usually indicates that the gag and pol genes of the vector particle are those of HIV-1. This plasmid is termed the packaging plasmid. One or several other plasmids encode the proteins which are part of the envelope. In the present case these plasmids may notably encode the first and the second protein. As will be clear to one of skill in the art, the above defined first and second nucleic acid may be either distinct or fused. Yet another plasmid encodes the genome.

As intended herein the expression "biological material" relates to one or more compounds liable to alter the structure and/or the function of a cell. Within the context of the present invention, it is preferred that the biological material is one or more nucleic acids, which in the case of lentiviral vector particles may be comprised within the genome of the vector particle. The genome typically comprises the one or more nucleic acids, preferably linked to genetic elements necessary for their expression in the target cell, such as promoters and terminators, flanked by cis-acting elements necessary for the inclusion of the

genome in the core element, its reverse transcription into deoxyribonucleic acid (DNA), the import of the retrotranscribed genome into the nucleus of the target cell and the integration of the retrotranscribed genome within the genome of the target cell.

As intended herein the recipient cells for the biological material to be transferred, or target cells, relate to any cell liable to be bound by the above-defined vector particle. Where the vector particle is a lentiviral vector particle the target cell relates to any cell liable to be transduced by the vector particle. These cells usually express the c-Kit receptor which binds to the c-Kit ligand of the first protein. As such, the cells preferably targeted by the vector particle of the invention are $CD34^+$ cells, in particular human $CD34^+$ cells, and more particularly Hematopoietic Stem Cells (HSCs), notably human HSCs.

As intended herein "transferring" relates to the capacity of the vector particle to initially deliver the biological material to the membrane or the cytoplasm of the target cell, upon being bound to the target cell. After delivery, the biological material can be translocated to other compartment of the cell.

The feline endogenous RD114 virus envelope glycoprotein is notably described in Cosset *et al.* (1995) *J. Virol.* **69**:7430-7436. By way of example, the RD114 virus envelope glycoprotein corresponds to GenBank accession number X87829. Portions of RD114 corresponding to the transmembrane and extracellular domains can be readily identified by the man skilled in the art.

As intended herein, the expression "transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein" relates to transmembrane and extracellular domains of a natural feline endogenous RD114 virus envelope glycoprotein or to any mutant thereof derived therefrom by deletion, insertion or substitution of one or several amino acids, provided that said mutant presents essentially the same properties as the transmembrane and extracellular domains of the natural feline endogenous RD114 virus envelope glycoprotein from which it derives.

As intended herein, a mutant will be said to present essentially the same properties as the transmembrane and extracellular domains of a natural feline endogenous RD114 virus envelope glycoprotein from which it derives, if, when replacing the transmembrane and extracellular domains of a natural feline

endogenous RD114 virus envelope glycoprotein in a reference vector particle according to the invention carrying a first protein of sequence SEQ ID NO: 2 and a second protein of sequence SEQ ID NO: 4, the mutant-carrying vector particle presents at least 30%, preferably at least 50%, more preferably at least 75%, of the transduction of $CD34^+$ cells which can be observed with the reference vector particle. Preferably, the transduction conditions are those set forth in **Example 2**.

By way of example, the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein are represented by SEQ ID NO: 5.

Preferably, the first protein comprises or consists in a fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of a retroviral envelope glycoprotein. In this fusion it is preferred that the C-terminus of the transmembrane domain of RD114 is fused to the N-terminus of the cytoplasmic domain of a retroviral envelope glycoprotein.

More preferably, the first protein comprises or consists in a fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of the Murine Leukemia Virus-A envelope glycoprotein. In this fusion it is preferred that the C-terminus of the transmembrane domain of RD114 is fused to the N-terminus of the cytoplasmic domain of MLV-A envelope glycoprotein.

The Murine Leukemia Virus-A envelope glycoprotein is notably described in Ott *et al.* (1990) *J. Virol.* **64**:757-766. Preferably, the Murine Leukemia Virus-A envelope glycoprotein is that of strain 4070A. The portion of Murine Leukemia Virus-A envelope glycoprotein corresponding to the intracellular domain can be readily identified by the man skilled in the art. By way of example the intracellular domain of Murine Leukemia Virus-A envelope glycoprotein is represented by SEQ ID NO: 6.

Most preferably, the first protein is represented by SEQ ID NO: 2 and is in particular encoded by SEQ ID NO: 1. A preferred plasmid for expressing the first protein in a producer cell is represented by SEQ ID NO: 11.

The c-Kit receptor is well known to the man skilled in the art. It is notably described by Ashman (1999) *Int. J. Biochem. Cell. Biol.* **31**:1037-1051. By way of

example, the human c-Kit receptor is encoded by SEQ ID NO: 8. Accordingly, it is well within the reach of the man skilled in the art to identify, design or select ligands of the c-Kit receptor.

The natural ligand of the c-Kit receptor is the Stem Cell Factor (SCF) cytokine. The SCF cytokine is notably described by Ashman (1999) *Int. J. Biochem. Cell. Biol.* **31**:1037-1051. As such, in the above-defined vector particle, the ligand of the c-Kit receptor is preferably the SCF cytokine. As intended herein the expression SCF cytokine relates to a natural SCF cytokine or to any mutant of a natural SCF cytokine derived from said natural SCF by deletion, insertion or substitution of one or several amino acids, wherein said mutant retains the ability of the natural SCF cytokine to bind to the c-Kit receptor. Preferably, the SCF cytokine is the human SCF cytokine. By way of example the human SCF cytokine corresponds to GenBank reference number P21583. It is most preferred that the SCF cytokine used herein is deprived of its signal peptide and of its transmembrane and cytoplasmic domain (*i.e.* only the extracellular domain of the SCF cytokine is used), *e.g.* as represented by SEQ ID NO: 9.

More preferably, the second protein of the above-defined vector particle comprises or consists in a fusion of the SCF cytokine and (i) the N-terminal domain of an hemagglutinin glycoprotein, or (ii) a retroviral envelope glycoprotein. In this fusion it is preferred that the C-terminus of SCF is fused to the N-terminus of the N-terminal domain of the hemagglutinin glycoprotein or to the N-terminus of the retroviral envelope glycoprotein.

Preferably, the hemagglutinin glycoprotein is that of an influenza virus, more preferably of the Fowl Plague Virus.

Preferably, the N-terminal domain of the hemagglutinin glycoprotein comprises or consists in the contiguous amino acids from the N-terminus of the glycoprotein to the C-terminus of the HA1 subunit.

The subunit structure of the hemagglutinin glycoprotein is well known to one of skill in the art. The Fowl Plaque Virus hemagglutinin is notably described in Hatziioannou *et al.* (1998) *J. Virol.* **72**:5313-5317.

By way of example the N-terminal domain of the Fowl Plague Virus hemagglutinin is represented by SEQ ID NO: 10.

Preferably, in the second protein, the retroviral envelope glycoprotein is Murine Leukemia Virus-A envelope glycoprotein.

As will be apparent to anyone of skill in the art, the second protein may also preferably comprise a signal peptide intended for promoting endoplasmic reticulum translocation of the second protein. In certain cases the signal peptide can be cleaved during or after insertion in the targeted membrane. Such signal peptides are well known to the man skilled in the art and can be found, for example, at the extremities of membrane proteins. By way of example the signal peptide can be that of the Murine Leukemia Virus-A envelope glycoprotein, which can be represented by SEQ ID NO: 7.

Thus, the second protein preferably comprises or consists in a fusion of the SCF cytokine, the N-terminal domain of an hemagglutinin glycoprotein, and a signal peptide. In this fusion it is preferred that the C-terminus of the signal peptide is fused to the N-terminus of SCF, and that the C-terminus of SCF is fused to the N-terminus of the N-terminal domain of the hemagglutinin glycoprotein.

When the second protein comprises or consists in a fusion of SCF and a retroviral envelope glycoprotein, it is preferred that the C-terminus of SCF is fused to the N-terminus of the retroviral envelope glycoprotein deprived of its signal peptide, and that the N-terminus of SCF is fused to the C-terminus of a signal peptide as defined above, which is preferably the signal peptide of the retroviral envelope glycoprotein to which it is fused.

Most preferably, the second protein is represented by SEQ ID NO: 4 and is in particular encoded by SEQ ID NO: 3. A preferred plasmid for expressing the first protein in a producer cell is represented by SEQ ID NO: 12.

In a particular embodiment of the above-defined vector particle, the first protein is represented by SEQ ID NO: 2 and the second protein is represented by SEQ ID NO: 4.

In another particular embodiment, the second protein as defined above is fused to the first protein as defined above. Preferably, when the first and second proteins are fused, the second protein consists of a SCF cytokine, optionally fused to a signal peptide as defined above. More preferably, when the first and second protein are fused, the C-terminus of a signal peptide is fused to the N-terminus of a SCF cytokine, the C-terminus of the SCF cytokine is fused to the N-terminus of

the extracellular domain of RD114, and the C-terminus of the transmembrane domain of RD114 is fused to the N-terminus of the cytoplasmic domain of a retroviral envelope glycoprotein.

The present invention also relates to the fused first and second proteins as defined above and to the nucleic acids which comprise sequences encoding them.

In another particular embodiment, the above-defined vector particle does not comprise the Vesicular Stomatitis Virus (VSV) G envelope glycoprotein.

The VSV-G envelope glycoprotein is notably described in Yee *et al.* (1994) *Methods Cell Biol.* **43**:99-112. By way of example the VSV-G envelope glycoprotein is represented by SEQ ID NO: 13.

As is apparent from the foregoing, the above-defined vector particle can be used for the *in vivo* or *ex vivo* transfer of biological material to cells, in particular to $CD34^+$ cells, and among them to HSCs.

Accordingly, the vector particle is particularly indicated for treating hematopoietic cells-related diseases either by direct administration of the vector particle to the individual afflicted by such a disease, or by administering cells, in particular cells originating from the individual afflicted by such a disease, which have been contacted *ex vivo* with the vector particle.

In this frame, it is preferred that the vector particle is a lentiviral vector particle as defined above and/or that the target cells are transduced by one or more nucleic acids, preferably intended for treating the disease.

The vector particle would thus be indicated for treating myelosupression and neutropenias which may be caused as a result of chemotherapy, immunosupressive therapy, infections such as AIDS, genetic disorders of hematopoietic cells, cancers and the like.

Exemplary genetic disorders of hematopoietic cells that are contemplated include sickle cell anemia, thalassemias, hemaglobinopathies, Glanzmann thrombasthenia, lysosomal storage disorders (such as Fabry disease, Gaucher disease, Niemann-Pick disease, and Wiskott-Aldrich syndrome), severe combined immunodeficiency syndromes (SCID), as well as diseases resulting from the lack of systemic production of a secreted protein, for example, coagulation factor VIII and/or IX.

In such cases, one would desire to transfer one or more nucleic acids such as globin genes, hematopoietic growth factors, which include erythropoietin (EPO), the interleukins (especially Interleukin-1, Interleukin-2, Interleukin-3, Interleukin-6, Interleukin-12, etc.) and the colony-stimulating factors (such as granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, or stem-cell colony-stimulating factor), the platelet-specific integrin αIIbβ, multidrug resistance genes, the gp91 or gp 47 genes which are defective in patients with chronic granulomatous disease (CGD), antiviral genes rendering cells resistant to infections with pathogens such as human immunodeficiency virus, genes coding for blood coagulation factors VIII or IX which are mutated in hemophiliacs, ligands involved in T cell-mediated immune responses such as T cell antigen receptors, B cell antigen receptors (immunoglobulins), the interleukin receptor common γ chain, a combination of both T and B cell antigen receptors alone and/or in combination with single chain antibodies (ScFv), IL2, IL12, TNF, gamma interferon, CTLA4, B7 and the like, genes expressed in tumor cells such as Melana, MAGE genes (such as MAGE-1, MAGE-3), P198, P1A, gp100 etc.

Exemplary cancers are those of hematopoietic origin, for example, arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). Lymphoid malignancies which may be treated using a vector particle as defined above include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas contemplated as candidates for treatment utilizing the lentiviral vector particles of the present invention include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T-cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF) and Hodgkin's disease.

Where the vector particle is used as a medicament and is administered to an individual in a therapeutic method, administration through the intravenous route or by the medullar route, in particular the femur or humerus medullar route, is

preferred. For intravenous administration a unit dose from about 5.10^8 to about 10^9 vector particles as defined above can be used, whereas for medullar administration a unit dose from about 10^8 to about 5.10^8 vector particles as defined above can be used.

Where the vector particle is used *ex vivo* the vector particle can be contacted, preferably *in vitro*, either with isolated or purified cells, such as $CD34^+$ cells, or with non-purified bodily samples.

The cells can be isolated or purified from various tissues, in particular taken from the individual, such as blood, in particular cord blood, or bone marrow.

Non-purified bodily samples can originate from the individual to be treated, and notably comprise blood samples, in particular whole cord blood samples.

The quantity of vector particle to be used for *ex vivo* transfers of biological material is for example from about 10^7 to about 5.10^7 for about 10^6 total white blood cells (where the cells to be transduced are comprised in total white blood cells from a whole blood sample).

EXAMPLES

Example 1

Production of lentiviral vector particles (LVs)

The inventors displayed two early acting cytokines, Thrombopoietin (TPO) and Stem Cell Factor (SCF), on a lentiviral vector particle (LV) surface.

A TPO truncated form of 171-amino acid long, shown to have a 3-fold higher biological activity than wild-type TPO, was fused to the N-terminus of the influenza hemagglutinin (HA) glycoprotein to form TPOHA. The second cytokine, SCF, was also fused to the N-terminus of HA glycoprotein to form SCFHA (SEQ ID NO: 4), which efficiently incorporates on LVs.

Since these chimeric HA glycoproteins demonstrated a reduced infectivity, an additional fusion competent glycoprotein was co-expressed. A chimeric feline endogenous RD114 virus envelope glycoprotein was chosen, in which the cytoplasmic tail of RD114 was exchanged for that of Murine Leukemia Virus-A (MLV-A) env glycoprotein resulting in a mutant RDTR (SEQ ID NO: 2), that allows high incorporation onto HIV as well as SIV vector particles (Sandrin *et al.* (2002) *Blood* **100**:823-832).

Thus, a transfection protocol was optimized to co-display SCFHA or TPOHA with RDTR on HIV-derived lentiviral vector particles.

Briefly, $2.5.10^6$ 293T cells were seeded the day before transfection in 10 cm plates in a final volume of 10 ml DMEM. The next day these cells were cotransfected with an HIV or SIV gag-pol construct (8.6 μg) with the lentiviral gene transfer vector particle (8.6 μg) and two glycoprotein-encoding constructs selected from: a) VSV-G (1.5 μg) (SEQ ID NO: 14) or RDTR (SEQ ID NO: 11) (7 μg) and b) TPOHA (SEQ ID NO: 15) or c) SCFHA (SEQ ID NO: 12) (1.5 μg), using the Clontech calcium-phosphate transfection system. 4 μg of a neuraminidase-encoding plasmid was also co-transfected to allow efficient release of vector particle from the producer cell since the HA (SCFHA and TPOHA) envelope otherwise binds the vector particles to the producer cells because of the expression of sialic acid by the producer 293T cells. 15 h after transfection, the

medium was replaced with 6 ml of fresh CellGro® medium (CellGenix) and 36 h after transfection, vector particles were harvested, filtrated through 0.45 μm pore-sized membrane and stored at -80°C. The vector particles can be further concentrated via ultracentrifugation or polyethylene-glycol mediated concentration at low-speed centrifugation.

Titers of 5.10^5-10^6 IU/ml were thus obtained, that were comparable to RDTR single pseudotyped vector particles.

Functional co-display of TPO on TPOHA/RDTR co-displaying vector particles was demonstrated on BAF3-Mpl cells, which are dependent on TPO for survival and growth, essentially as described by Geddis *et al.* (2001) *J. Biol. Chem.* **276**:34473-34479. Similarly, functional co-display of SCF on SCFHA/RDTR vector particles was confirmed since they sustained survival of BAF3-cKit cells which depend on SCF for survival (Bayle *et al.* (2004) *J Biol Chem.* **279**:12249-12259), even at low multiplicity of infection (M.O.I.)

Example 2

Transduction of isolated $CD34^+$ cells

The vector particles were first tested on the transduction of $CD34^+$ cells isolated from human cord blood (CB). CB $CD34^+$ cells are very immature hematopoietic cells containing hematopoietic stem cells.

Briefly, $CD34^+$ cells were isolated by positive selection using anti-$CD34^+$ beads (Miltenyi Biotech) from cord blood and were cultured on uncoated or RetroNectin® (Takara) coated plates. Subsequently, the cells were incubated with Green Fluorescent Protein (GFP) encoding HIV derived vector particles displaying RDTR, in the presence of human recombinant cytokines (TPO= 10 ng/ml; SCF = 50 ng/ml) (Preprotech, Rocky Hill, US), or co-displaying RDTR and TPOHA or RDTR and SCFHA, at a multiplicity of infection (M.O.I.) of 10, essentially as described by Verhoeyen *et al.* (2005) *Blood* **106**:3386-3395.

As shown in **Figure 1**, the resulting RDTR/SCFHA pseudotyped HIV vector particles were far more efficient in transducing cord blood-derived $CD34^+$ cells, than the LV pseudotyped with RDTR and TPOHA, or with RDTR only in the presence of the corresponding cytokines in their soluble form. In addition, in

contrast to the RDTR/SCFHA pseudotyped HIV vector particles, the RDTR-only pseudotyped vector particles are completely dependent on RetroNectin® for the transduction of $CD34^+$ cells (RetroNectin® is a chimeric peptide of human fibronectin produced in *Escherichia coli* which is thought to link vector particles and target cells).

Thus, the above results indicate that an unexpected synergistic mechanism is taking place, between RDTR, allowing vector particle and cell fusion, and SCFHA, allowing specific binding and stimulation of c-Kit^+ / $CD34^+$ cells, which results in the high transduction efficiency observed.

Example 3

Multiplicity of infection for $CD34^+$ cells

An important issue for the *in vivo* use of the vector particles of the invention is that they should allow high transduction efficiency into $CD34^+$ cells even at very low vector particle dosage, since a systemic administration of a therapeutic vector particle would result in an important dilution of vector particle concentration. Thus, the Inventors tested the minimal effective dosage of the vector particles according to the invention.

Briefly, $CD34^+$ cells were isolated by positive selection using anti-$CD34^+$ beads (Miltenyi Biotech) from cord blood and were cultured on uncoated culture plates (*i.e.* in the absence of RetroNectin®). Subsequently, the cells were incubated with Green Fluorescent Protein (GFP) encoding HIV derived vector particles displaying RDTR, in the presence of human recombinant cytokines (TPO= 10 ng/ml; SCF = 50 ng/ml), or co-displaying RDTR and TPOHA or RDTR and SCFHA at a M.O.I. of 10, 2, or 0.2, essentially as described by Verhoeyen *et al.* (2005) *Blood* **106**:3386-3395. At day 3 post initiation of transduction, cells were evaluated for GFP expression by fluorescence-activated cell sorter (FACS).

As shown in **Figure 2**, the RDTR/SCFHA vector particle of the invention enabled a reduction of vector particle dosage to a M.O.I. of 0.2, without observing a significant drop in transduction efficiency of $CD34^+$ cells. Thus, a 50-fold decrease in RDTR/SCFHA vector particle dosage resulted on average only in a

1.4-fold reduction of $CD34^+$ cell transduction. In contrast, the RDTR/TPOHA vector particle resulted in a significantly lower $CD34^+$ transduction when an M.O.I. of 0.2 was used.

Example 4

RDTR/SCFHA targets transduction to $CD34^+$ cells in a peripheral mononuclear blood cell population

A vector particle intended for *in vivo* gene therapy notably needs to be highly discriminative between target and non-target cells. Thus, after having demonstrated the ability of the vector particle according to the invention to transduce isolated $CD34^+$ cells, its selectivity was evaluated by adding vector particle to a whole peripheral blood mononuclear cell (PBMC) population at low M.O.I. In this respect, it is important to highlight that no more than 1% $CD34^+$ cells are contained in such a population.

Briefly, PBMCs were isolated from fresh cord blood by ficol gradient, as is well-known to the man skilled in the art, and cultured in the absence of RetroNectin®. Transduction of PBMCs was performed with Green Fluorescent Protein (GFP) encoding HIV derived vector particles displaying RDTR or VSV-G in the presence of human rSCF (50 ng/ml), or co-displaying RDTR and SCFHA or VSV-G and SCFHA, without adding exogenous cytokines, at a M.O.I. of 0.2, essentially as described by Verhoeyen *et al.* (2005) *Blood* **106**:3386-3395. At day 3 post initiation of transduction, $CD34^+$ and $CD3^+$ cells were evaluated for GFP expression by fluorescence-activated cell sorter (FACS).

As shown in **Figure 3**, the RDTR/SCFHA vector particle was able to preferentially target and transduce $CD34^+$ target cells (up to 19%), in sharp contrast to the vector particle pseudotyped with RDTR only, in the presence of soluble SCF, which provided for no transduction at all, or to the VSV-G/SCFHA vector particle, which allowed a transduction level of $CD34^+$ cells of 5% at the most. Importantly, the RDTR/SCFHA vector particle allowed to transduce $CD34^+$ cells within the PBMC population at a level equivalent to that obtained for the transduction of isolated $CD34^+$ cells (compare **Figures 2 and 3**). Furthermore, the T-cell population, which make up 80% of the whole PBMC population, was very

poorly transduced by the RDTR/SCFHA vector particle (**Figure 3**). Worth noting, other cell lineages present in the PBMC population, such as monocytes, B-cells and NK-cells were not transduced at all.

Example 5

RDTR/SCFHA targets transduction to CD34$^+$ cells in *in vivo*-like conditions

The inventors then devised conditions as close as possible to *in vivo* settings for targeting gene transfer into CD34$^+$ cells. Thus, the inventors performed transduction of fresh total cord blood, which contains cells from each hematopoietic lineage: early progenitors, including Hematopoietic Stem Cells (HSCs), lymphocytes, monocytes, and erythrocytes. This allows, (i) evaluation of targeted gene transfer in the CD34$^+$ cells population, which represents only 0.001% of cells in whole blood, and (ii) exposure of the vector particle to an active human complement system, an obstacle encountered by viral vector particles *in vivo*.

Thus, fresh total cord blood (0.5 ml) was incubated with GFP encoding HIV vector particles pseudotyped with RDTR only or VSV-G only, in the presence of soluble SCF (50 ng/ml), or co-displaying RDTR and SCFHA or VSV-G and SCFHA, without adding exogenous cytokines, at a M.O.I. of 0.01 (calculated for the total amount of white and red blood cells present in the blood sample). After 6-8 h incubation with the vector particles, total PBMCs were separated from the blood by a ficol gradient.

Subsequently, the CD34$^+$ cells were isolated by positive selection using anti-CD34$^+$ beads (Miltenyi Biotech) and were further cultured in a serum-free medium in presence of soluble recombinant human SCF in order to sustain survival until FACS analysis.

In order to reveal possible non-target gene transfer, after removal of the CD34$^+$ cells, the residual PBMCs, consisting mainly of T-cells, were cultured in RPMI supplemented with anti-CD3 and anti-CD28 antibodies (BD Pharmingen, Le Pont de Claix, France) and recombinant human IL-2 (Preprotech Rocky Hill, US). This was done with a dual purpose: (i) to activate T-cells in order to enable transduction, since the majority of T-cells in the blood are in a quiescent state and accordingly are not permissive to lentiviral transduction, and (ii) to sustain survival

of these cells until analysis. Worth noting, very stringent conditions were thus used to reveal gene transfer in the non-target T-cell, which are most probably never met in *in vivo* conditions. In other words the experimental settings used most probably overestimate *in vivo* non-specific gene transduction of T-cell. At day 4 post initiation of transduction, $CD34^+$ and $CD3^+$ cells were evaluated for GFP expression by fluorescence-activated cell sorter (FACS).

As shown in **Figure 4**, the RDTR/SCFHA vector particle allowed a transduction of 4.5% $CD34^+$ cells versus 0.4% for the VSV-G/SCFHA vector particle, while transduction with vector particles displaying VSV-G only or RDTR only is negligible. Thus, the RDTR/SCFHA vector particle is 10 times more efficient than the VSV-G/SCFHA vector particle for transducing $CD34^+$ cells. In addition, the VSV-G/SCFHA vector particle readily transduced the non-target T-cell population, resulting in an 1.8 fold only selectivity for $CD34^+$ cells transduction as compared to T-cell. In contrast, the RDTR vector particle demonstrates up to 95-fold selectivity for $CD34^+$ cells as compared to T-cells. Thus, knowing that only 0.01% of the blood cells initially transduced are $CD34^+$ cells and that T-cells represent 1% of the blood cells, the RDTR/SCFHA vector particles efficiently target transduction to $CD34^+$ cells.

As regards the low transduction efficiency achieved with the VSV-G/SCFHA vector particles, it might be due to the vector's susceptibility to human complement, which, as a consequence, would impair its use *in vivo*.

Example 6

RDTR/SCFHA displaying LVs allow gene transfer into $hCD34^+$ cells in vivo

The inventors assessed targeted gene transfer into HSCs by the RDTR/SCFHA vector particles *in vivo* in a humanized murine model.

Briefly, full and functional reconstitution of all human haematopoietic lineages including B and T-cells was achieved in newborn $Rag2^{-/-}$; $\gamma c^{-/-}$ Balbc mice by injection with human umbilical cord blood (UCB) $CD34^+$ cells. After 13 weeks of reconstitution the inventors detected on average 35% of human cells ($hCD45^+$) in the bone marrow of these mice (**Figure 5**) of which 5 to 15 % expressed hCD34.

GFP-encoding RDTR/SCFHA vector particles were concentrated by low speed centrifugation over a filtration column to obtain titers up to 5.10^8 IU/ml. 1.10^5 infectious units of the RDTR/SCFHA vector particles were injected into the femural bone marrow of the humanized mice from 13 week of age on.

One week after the injection, three-colour marking was performed to measure GFP expression in the different haematopoietic lineages as well as in the target $hCD34^+$ cells in the bone marrow.

In the flushed bone marrow the inventors detected a transduction of up to 3% of the total human cells that had colonized the marrow of the mice (**Figure 5**). Taking into account that a femur contains $1.5.10^7$ cells, the inventors administered a very low vector dose (MOI= 0.006). However, a selective transduction of up to 3% of early human progenitors ($hCD34^+$ cells) and of 3% of the myeloid progenitors ($hCD13^+$) in the BM was detected (**Figure 6**). In contrast, monocytes and pre- and pro-B-cells were transduced to a low extent (hCD14 = 0%; hCD19 = 0.2 %). These results should be explained by the fact, that one week after the injection, differentiation of $hCD34^+$ cells, including transduced $hCD34^+$ cells, into early progenitors such as $hCD13^+$ myeloid progenitors and pre- and pro-B cells may have already occurred.

Of utmost importance, the inventors verified *in vivo* escape of vectors by analysing transduction of the other hematopoietic tissues. They did not detect GFP^+ human thymocytes (**Figure 7**), nor transduction of human $CD19^+$ B-cells and $CD3^+$ T-cells in the blood stream of these intrafemural injected mice (**Figure 8**). Additionally, they did not detect significant levels of transduced B-cells ($hCD19^+$ cells) and transduced T-cells in the spleen (**Figure 9**).

Summarizing, local administration of low doses of RDTR/SCFHA LV into the BM of humanized mice resulted in a selective transduction of $hCD34^+$ cells *in vivo*.

Sequence identifiers reference table:

SEQ ID NO:	Feature
1	Nucleic acid encoding a fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of MLV-A envelope glycoprotein
2	Fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of MLV-A envelope glycoprotein
3	Nucleic acid encoding a fusion of the SCF cytokine, the N-terminal domain of an influenza virus hemagglutinin glycoprotein, and a signal peptide
4	Fusion of the SCF cytokine, the N-terminal domain of an influenza virus hemagglutinin glycoprotein, and a signal peptide
5	Transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein
6	Cytoplasmic domain of Murine Leukemia Virus-A envelope glycoprotein
7	Signal peptide of the Murine Leukemia Virus-A envelope glycoprotein
8	Human c-Kit receptor
9	Human SCF cytokine
10	N-terminal domain of the Fowl Plague Virus hemagglutinin
11	Plasmid encoding the fusion protein of SEQ ID NO: 2
12	Plasmid encoding the fusion protein of SEQ ID NO: 4
13	VSV-G envelope glycoprotein
14	Plasmid encoding VSV-G
15	Plasmid encoding TPOHA

CLAIMS

1. A vector particle for transferring biological material into cells, wherein said vector particle comprises at least:

- a first protein which comprises the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein, and
- a second protein which comprises a ligand of the c-Kit receptor.

2. The vector particle according to claim 1, wherein the ligand of the c-Kit receptor is the Stem Cell Factor (SCF) cytokine.

3. The vector particle according to claim 1 or 2, wherein the vector particle does not comprise the Vesicular Stomatitis Virus (VSV) G envelope glycoprotein.

4. The vector particle according to any of claims 1 to 3, wherein the vector particle is a lentiviral vector particle.

5. The vector particle according to claim 4, wherein the lentiviral vector particle is selected from the group consisting of HIV and SIV.

6. The vector particle according to any of claims 1 to 5, wherein the vector particle is intended for transferring biological material into $CD34^+$ cells.

7. The vector particle according to any of claims 1 to 6, wherein the biological material is one or more nucleic acids.

8. The vector particle according to any of claims 1 to 7, wherein the first protein comprises or consists in a fusion of the transmembrane and extracellular domains of the feline endogenous RD114 virus envelope glycoprotein and the cytoplasmic domain of a retroviral envelope glycoprotein.

9. The vector particle according to claim 8, wherein the cytoplasmic domain of a retroviral envelope glycoprotein is that of Murine Leukemia Virus-A.

10. The vector particle according to any of claims 1 to 9, wherein the first protein is represented by SEQ ID NO: 2.

11. The vector particle according to any of claims 1 to 10, wherein the second protein comprises or consists in a fusion of a SCF cytokine and (i) the N-terminal domain of an hemagglutinin glycoprotein, or (ii) a retroviral envelope glycoprotein.

12. The vector particle according to any of claims 1 to 11, wherein the second protein comprises or consists in a fusion of a SCF cytokine and the N-terminal domain of an influenza virus hemagglutinin glycoprotein.

13. The vector particle according to any of claims 1 to 12, wherein the second protein is represented by SEQ ID NO: 4.

14. The vector particle according to any of claims 1 to 10, wherein the first and the second proteins are fused.

15. The vector particle according to claim 14, wherein the second protein consists of a SCF cytokine, optionally fused to an endoplasmic reticulum translocation signal peptide.

16. The use of (i) a first nucleic acid comprising a sequence encoding a first protein as defined in any of claims 1 and 8 to 10, and of (ii) a second nucleic acid comprising a sequence encoding a second protein as in any of claims 1, 2 and 11 to 13, for preparing a vector particle for transferring biological material into cells.

17. A medicament comprising a vector particle as defined in any of claims 1 to 15 as active ingredient.

18. The use of a vector particle as defined in any of claims 1 to 15, for transferring the biological material into cells *ex vivo*.

19. The use according to claim 18, wherein the cells are $CD34^+$ cells.

20. The use according to claim 18 or 19, wherein the cells are comprised in a blood sample.

21. A method for preparing cells intended for treating an individual, wherein cells to be administered to the individual are contacted with a vector particle as defined in any of claims 1 to 15.

22. The method according to claim 21, wherein the cells are $CD34^+$ cells.

23. The method according to claim 21 or 22, wherein the cells are comprised in a blood sample.

24. The method according to any of claims 21 to 23, wherein the cells are transduced by one or more nucleic acids transferred from the vector particle.

25. A protein represented by SEQ ID NO: 4.

26. A nucleic acid encoding a protein according to claim 25.

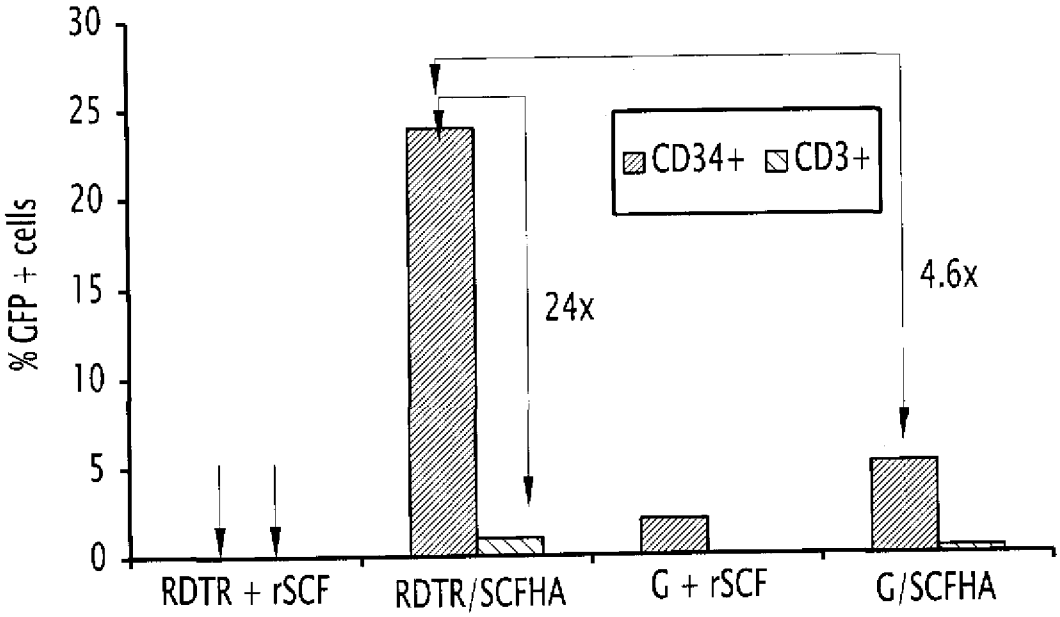
30
25
20
15
10
5
0
% GFP + cells
CD34+
CD3+
24x
4.6x
RDTR + rSCF
RDTR/SCFHA
G + rSCF
G/SCFHA

FIG.3

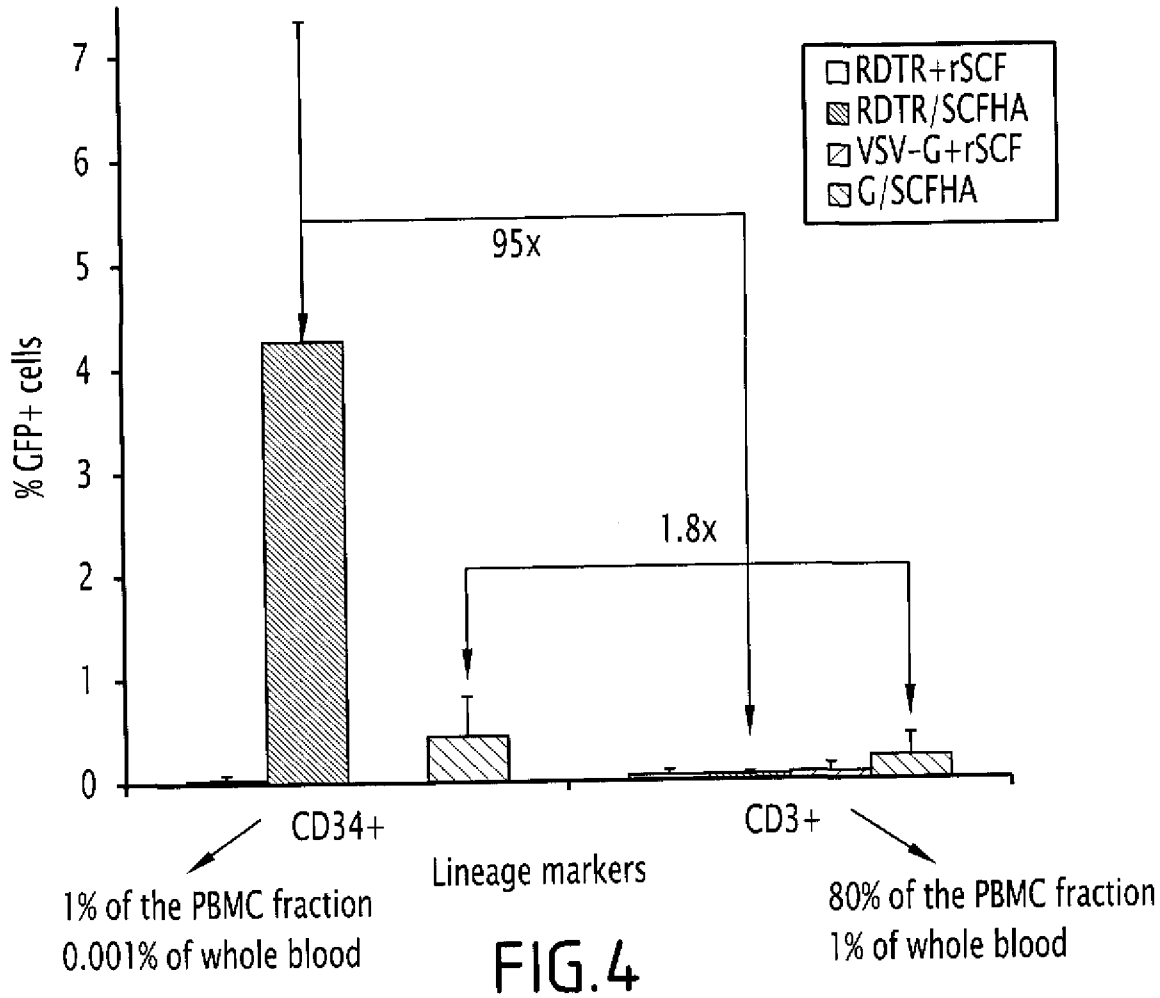
7
6
5
4
3
2
1
0
% GFP+ cells
RDTR+rSCF
RDTR/SCFHA
VSV-G+rSCF
G/SCFHA
95x
1.8x
CD34+
CD3+
Lineage markers
1% of the PBMC fraction
0.001% of whole blood
80% of the PBMC fraction
1% of whole blood

FIG.4

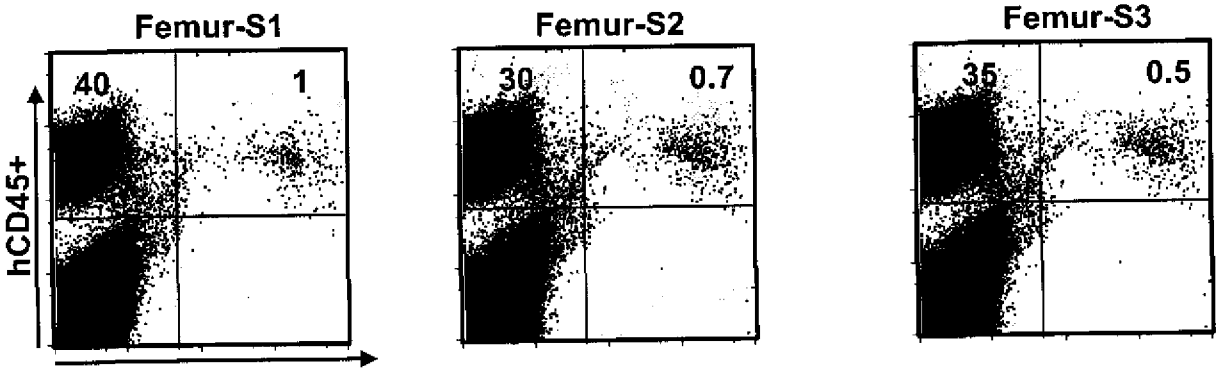

FIG.5

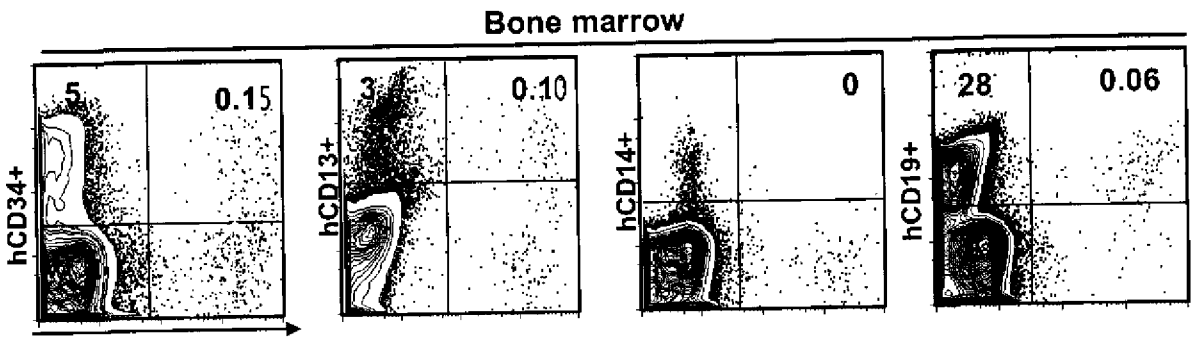

FIG.6

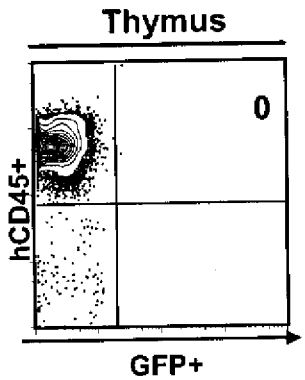

FIG.7

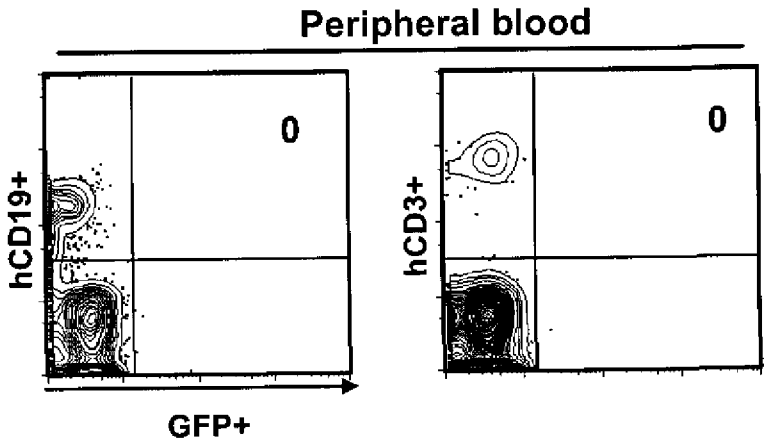
Peripheral blood
0
0
hCD19+
hCD3+
GFP+

FIG.8

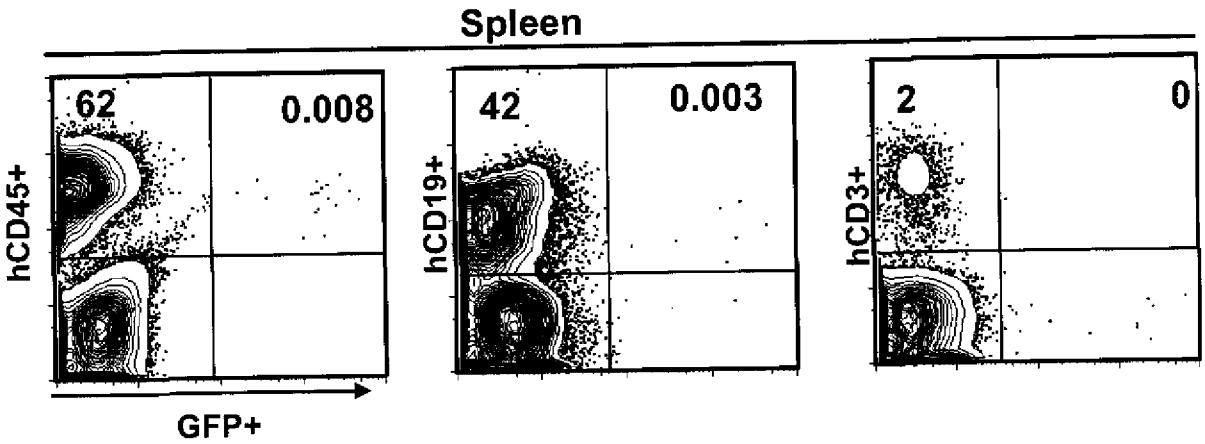
Spleen
62
0.008
42
0.003
2
0
hCD45+
hCD19+
hCD3+
GFP+

FIG.9

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/059674

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/867 C12N7/00 C07K14/52 C12N15/12 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VERHOEYEN E. ET AL.: "Novel lentiviral vectors displaying "early-acting cytokines" selectively promote survival and transduction of NOD/SCID repopulating hematopoietic cells." BLOOD, vol. 106, no. 10, November 2005 (2005-11), pages 3386-3395, XP002452869 cited in the application the whole document	25,26
A		1-24
A	WO 01/66150 A2 (ST JUDE CHILDRENS RES HOSPITAL [US]; KELLY PATRICK F [US]; VANIN ELIO) 13 September 2001 (2001-09-13) the whole document	1-26

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2008	24/10/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Galli, Ivo

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/059674

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SANDRIN V ET AL: "Lentiviral vectors pseudotyped with a modified RD114 envelope glycoprotein show increased stability in sera and augmented transduction of primary lymphocytes and CD34+ cells derived from human and nonhuman primates" BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, US, vol. 100, no. 3, 1 August 2002 (2002-08-01), pages 823-832, XP002242672 ISSN: 0006-4971 cited in the application the whole document	1-26
A	WO 03/091442 A (INST NAT SANTE RECH MED [FR]; INST CLAYTON DE LA RECH [CH]; TRONO DIDI) 6 November 2003 (2003-11-06) cited in the application the whole document	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/059674

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-28

A vector particle for transferring biological material into cells, comprising (pseudotyped) with the transmembrane and extracellular domains of RD114 and a ligand of the c-Kit receptor. Compositions and uses thereof.

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2. claims: 25-26

A fusion proteins of Stem Cell Factor with Haemagglutinin and a signal peptide.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/059674

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0166150 A2	13-09-2001	AU 4007801 A	17-09-2001
WO 03091442 A	06-11-2003	AU 2003226598 A1	10-11-2003
		CA 2484166 A1	06-11-2003
		CN 1659284 A	24-08-2005
		EP 1499736 A1	26-01-2005
		JP 2005523706 T	11-08-2005